Bioremediation of a Light NAPL (Toluene) Contaminated Soil under Different Environmental Conditions

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Abstract

LNAPLs are a common contaminant in the environment. When a LNAPL infiltrates the soil, it leaves behind a trail of immobile ganglia in the pore spaces. When the groundwater table is reached, the LNAPL forms a film on top of the groundwater, where it generally migrates laterally, subjected to the groundwater flow. LNAPLs are toxic to humans by ingestion, adsorption through skin and inhalation. The combination of mobility and toxicity classify the contamination as a high risk. However, soil microorganisms are able to remediate LNAPLs. The rate of this remediation depends on several factors like temperature, soil moisture content, initial concentration and soil type. The objective of this study was to investigate the impacts of the environmental factors on the rate of bioremediation of a LNAPL. The BTEX component toluene was used as a representative for this group of contaminants. Numerous batch experiments were performed under controlled conditions to reach these objectives. The batches contained soil, groundwater spiked with toluene stock and sufficient headspace to prevent oxygen deficiency. The biodegradation rate of toluene increased with increasing temperature up to 21° C. No significant increase in biodegradation rate was observed at 30° C. The biodegradation rate also increased with increasing moisture content, when sufficient headspace was present. As for the dependence on initial toluene concentration, the initial biodegradation rate increased with increasing toluene concentration reaching its optimum around 100 mg/L. The soil type comparison showed that the biodegradation rate of toluene was high in a soil containing organic matter compared to clean sand.

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Table of content

1. Introduction

Nonaqueous phase liquids (NAPLs) are hydrocarbons that exist as a separate, immiscible phase when in contact with water and/or air. These volatile hydrocarbons can be classified as either light nonaqueous phase liquids (LNAPLs), having densities less than that of water or dense nonaqueous phase liquids (DNAPLs), which have densities greater than that of water (Newell 1995).

An example of a LNAPL is BETX (benzene, toluene, ethylbenzene, xylene), which is a common contaminant in the environment (Fitts 2002). The main sources for these contaminations are leakages of gasoline from underground storage tanks and pipelines. The BTEX component toluene (C_6H_5-CH_3) is used as a solvent for paints, coatings, gums, oils and resins (www.egr.msu.edu).

When a LNPAL infiltrates a soil, it leaves behind a trail of immobile ganglia trapped in the pore spaces. When water percolates through these LNAPL containing pores, it will dissolve some amount of the pollutant, resulting in a continuous delivery of dissolved LNAPL to the groundwater. When the volume of the LNAPL is large enough, it will be able to travel to the groundwater table. There it will form a layer on top of the groundwater (Fitts 2002). After reaching the water table, the LNAPL will continue to migrate laterally, influenced by the groundwater flow. Evaporation takes place from the LNAPL, in pure phase as well as dissolved in the groundwater, when it is in contact with the air phase (Newell 1995, Mayer 2000). The process of LNAPL transport through porous media is shown in figure 1.1.

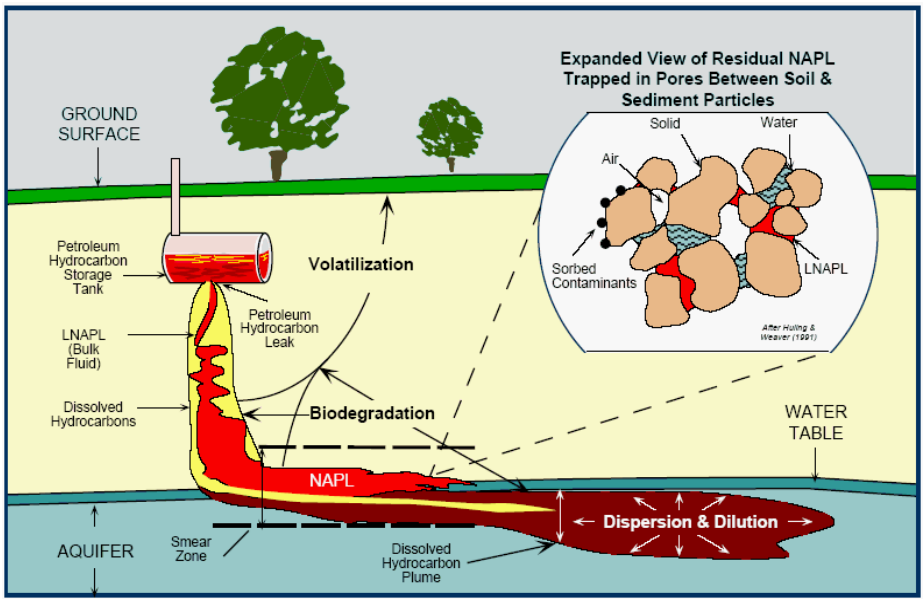

Figure 1.1: LNAPL distribution in soil (http://oceanworld.tamu.edu). Infiltration of the LNAPL leaves a trail of immobile ganglia. The volume of LNAPL that reaches the groundwater table forms a layer on top of the groundwater and will continue to migrate laterally (Fitts 2002, Newell 1995, Mayer 2000).

Because of this groundwater flow induced mobility and volatilization, LNAPLs can form a health risk to the environment. The LNAPLs are toxic to the human being by ingestion, adsorption through skin and inhalation. High risk exposure to LNAPLs can result from contaminated drinking water when the LNAPL is transported to a drinking water well by the groundwater flow or when residing on contaminated soil (http://www.egr.msu.edu). These risks yield the necessity to remediate LNAPL contaminations.

Remediation of soils contaminated with LNAPL can be done by means of for example excavation or pump and treat. However, these methods are often not sufficient and can be very expensive (Newell 1995). A more efficient and eco-friendly alternative is bioremediation, which uses the natural capability of microorganisms to degrade these organic contaminants. Though biodegradation occurs naturally at most sites, the overall rate of the reaction may be limited by several factors like temperature, moisture content, soil organic matter and initial concentration of the contaminant (Newell 1995, Davis 1996, DeVaull 1997). Therefore, the objectives of this proposed study are: to investigate the impact of temperature, soil moisture content, initial concentration and soil type on toluene biodegradation. Toluene was used in this study as a representative for the LNAPL group of contaminants.

1.1 Research questions

The research on bioremediation of a LNAPL contaminated soil under different environmental conditions was done to address the following research questions:

- What is the effect of temperature on the biodegradation rate of toluene?
- How does soil moisture content influence the biodegradation rate of toluene?
- How does the initial toluene concentration affect the toluene biodegradation rate?
- What is the effect of soil organic matter on the biodegradation rate of toluene?

1.2 Hypothesis

- Higher temperatures are associated with higher enzymatic activity and faster biodegradation rates, up to an optimum value that is species specific (Alvarez 2006);

- The biodegradation takes place in the liquid phase (Zhang 1998). Therefore the expectation is that the biodegradation rate increases with increasing moisture content, as long as there is no oxygen limitation;

- The biodegradation rate is expected to increase with increasing initial substrate concentration, up to the concentration that is toxic to the toluene degrading bacteria (Davis 1996).

- Since microorganisms are generally unable to degrade sorbed compounds, the overall biodegradation rate will be inhibited by the presence of organic matter (Weissenfels 1991, Davis 1996, Zhang 1998, Breedveld 2000, Polymenakou 2004).

1.3 Methodology

Several batch experiments were performed at three different temperatures. Batches were partially filled with soil and groundwater and a certain volume of toluene stock was added. Measurements were done in the pore water. Another set of batch experiments was performed at five different soil moisture contents, varying from residual to saturated. For these batches, the headspace was sampled. The before mentioned experiments were performed in triplicate and two sterile batches ran simultaneously. Lastly, several different batches were assembled having different concentrations of toluene being obtained by adding different volumes of toluene stock solution to the groundwater. Measurements were done in the pore water. These experiments were ran in duplicates. Sufficient headspace at atmospheric pressure was provided in all batch experiment to prevent oxygen limitation. Airtight sealing of the system has prevented any leakages. All samples were analyzed using gas chromatography. For the comparison of biodegradation rates in different soil types, results from another research were used.

The research framework in figure 1.2 is an overview of the project. The results of the different laboratory experiments give insight into the values of biodegradation rates of toluene and the optimal temperature, soil moisture content, initial concentration and soil type for the biodegradation of toluene.

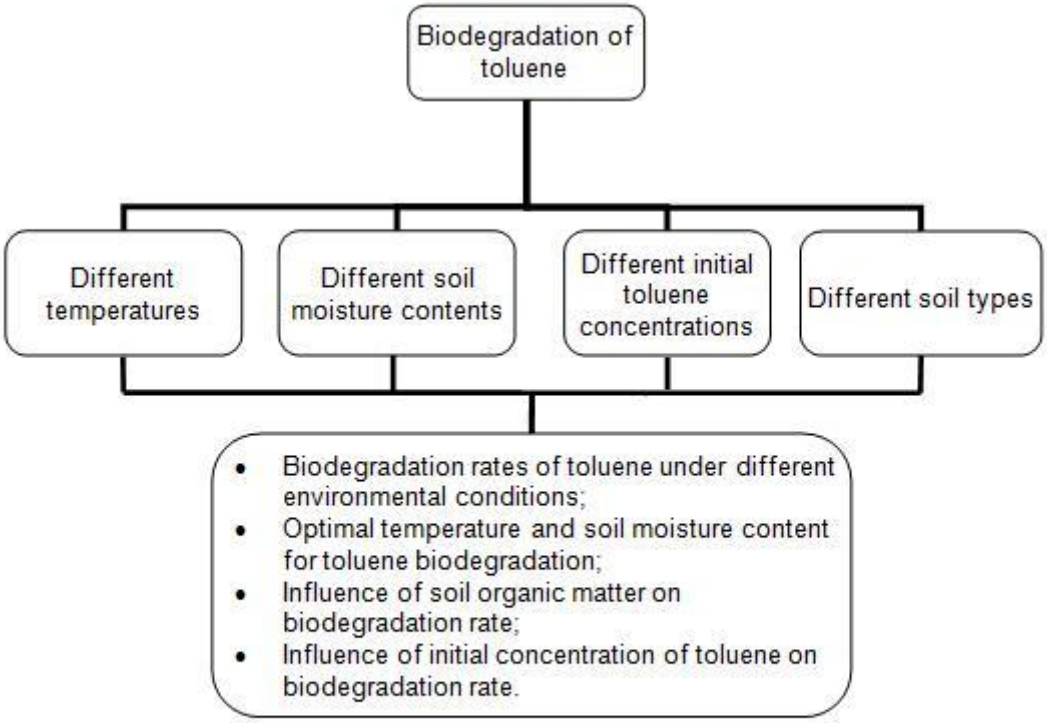

Figure 1.2: Schematic presentation of the research framework for bioremediation of a LNAPL

2. Literature review

In order to formulate a hypothesis and decide on methodology, a literature study has been done. However, the research done on toluene biodegradation alone is not sufficient for a solid theoretical basis for this study. Therefore, papers on other organic contaminants and mixtures of organic contaminants are also included. The behavior of these substances can be compared to that of toluene, provided that there are slight differences in behavior.

2.1 Temperature

Biodegradation can be affected by temperature through two mechanisms; (1) desorption rates increase and distribution coefficients decrease with increasing temperature and (2) the growth and activity of bacteria is enhanced by increasing temperature (as long as an upper threshold is not exceeded) (Zhou 1994, Zhang 1998, Eriksson 2002, Torres 2005). A rule of the thumb for the magnitude of the influence of temperature states that for every 10°C decrease in temperature, the biodegradation rates decreases by a factor of two (Zhang 1998, Polymenakou 2004).

Polymenakou (2004) found that phenol biodegradation rates are somewhat similar at 10°C and 40°C and slightly higher at 20°C. A significantly higher maximal biodegradation rate is shown for the batches incubated at 30°C. However, these biodegradation experiments are conducted in batches containing liquid medium and a bacterial culture only.

Torres (2005) conducted biodegradation experiments on diesel at 19°C, 28°C and 35° C. For the experiments at 19°C, the microcosms were kept under environmental conditions, resulting in a temperature fluctuation in the range of 10.8°C to 26.6°C, giving an average of 19°C. Experimental results show that higher temperatures correspond with higher biodegradation rates.

Similarly, experimental data from Eriksson (2002) shows that generally higher temperatures yield higher biodegradation rates for polycyclic aromatic hydrocarbons (PAH's). The soil material used for the experiments came from four different, though all cold environments (< 10°C for the majority of the year). The difference in biodegradation rates was specifically significant in the first 20 hours of the incubation time.

Chablain (1997) conducted research on toluene biodegradation at four different temperatures (10°C, 15°C, 20°C and 30°C). The results show a general trend of higher biodegradation rates corresponding with higher temperatures. However, after an estimated lag time of 18 hours, the batches incubated at 10°C show degradation rates similar to those incubated at 30°C. The batches incubated at 10°C (~18 hours) as well as 15°C (~7hours) show a significant lag time.

Zhou (1994) conducted experiments on biodegradation of gasoline. However, results for the separate components present in gasoline are presented in the paper, allowing consideration of toluene as a sole contaminant. Noted should be that the behavior of toluene in a biodegradation experiment will be slightly different when present in a mixture like gasoline from when toluene is present as a pure component. Chang (2001) shows that toluene

degrades faster when present as single component than when present in a BTEX mixture. Zhou (1994) did not only conduct the experiments at different temperature, but also allowed the soil samples an incubation time of 1.5 month at the different temperatures before starting the experiment. The soil samples are taken from a contaminated site, implying that the soil contains microorganisms that are able to degrade gasoline. However, by allowing a incubation time at a certain temperature, the present microorganisms will adapt to the temperature and the lag time in the actual experiments therefore is short or even nonexistent. The experimental results show that the degradation rates for toluene were somewhat similar at 11°C and 25°C, but significantly higher at 37°C.

The model proposed by Zhang (1998) suggests that a moderate increase of temperature, substantially reduces the extend of sorption. This implies that a relatively small increase in temperature, increases the biodegradation rate significantly. Zhang (1998) also suggests that the influence of temperature on the distribution coefficient increases with increasing hydrophobicity. This implies that for toluene, that has a high solubility compared to petroleum hydrocarbons (Chang 2001), the temperature influence of the distribution coefficient is smaller than for BTEX mixtures.

Generally, the literature shows that the biodegradation rate increases with increasing temperature. Solely Polymenakou (2004) concludes that the biodegradation rate has an optimum around 30°C to 37°C. However, this research is conducted in batches containing only liquid medium and a bacterial culture. Lag times are shown to be shorter at higher temperatures.

2.2 Soil moisture content

Soil is a porous media that consist of a solid matrix with pore spaces that are generally filled with water and/or air. The volume of water in reference to the volume of soil is the soil moisture content (Domenico 1990, Mayer 2000). The water will form a film around the surface of the soil grains. The thickness of the water film is soil specific and controls the nutrient and gas diffusion through the soil pores (Mayer 2000, Holden 2001). Due to the bacterial metabolism, which utilizes water, soil moisture content has a significant effect on biodegradation rates (Zhou 1994, Davis 1996). Rainwater (1993) therefore states that aerobic degradation may be most active in the capillary fringe of a soil, though it can be limited by several local conditions. The benefits of the improved oxygen availability can be downsized by the disadvantage of the low water content, including aqueous phase nutrient limitations and physiological impairment (Holden 2001).

Holden (2001) conducted biodegradation experiments on toluene at moisture contents ranging from 10% - 30%. The experimental results show that the toluene depletion rate increases with increasing water content.

Similarly, Davis (1996) conducted biodegradation experiments using toluene. The experiments are conducted at moisture contents of 100%, 14% (~50% of field capacity) and 2% (air dry). The saturated batches show the highest degradation rate, while the degradation seized completely at a moisture content of 2%. After saturating those slurries, biodegradation started to occur again, but at a rate that is four fold lower than that of the slurries that were continuously at saturation. Davis (1996) also considered the length of the lag time for the

different moisture contents. The slurries at 14% moisture content have a 1,5 times longer lag time than the saturated slurries.

In conclusion, the biodegradation rate is reported higher for high moisture content. Also, a lower moisture content leads to a longer lag time.

2.3 Initial substrate concentration

The presence of a contaminant provides energy and carbon for bacterial growth. Therefore, a threshold concentration of the contaminant may exist below which biomass growth cannot be sustained. On the other hand, at high concentrations the contaminant is likely to inhibit bacterial growth and in some cases will be toxic to the microorganisms. The initial concentration of the contaminant therefore has a significant influence on the biodegradation rate (Alvarez 1991, Zhou 1994, Zhang 1998, Choi 2008).

The biodegradation experiments on gasoline conducted by Zhou (1994) show that biodegradation rates increase with increasing initial concentration up to 900 ppm. Complete biodegradation occurs in all the batches that were made. Highest initial concentration in a batch was 1780 ppm.

Davis (1996) conducted biodegradation experiments on toluene with initial concentrations ranging from 0.5 to 200 µg/g soil. No biodegradation was observed in the microcosms with a initial substrate concentration of 250 µg/g soil or higher. This leads to the conclusion that degradation rates increase proportionally with increasing initial concentrations. The experimental results also show that the length of the lag time increases with increasing initial substrate concentration.

Alvarez (1991) performed biodegradation experiments on toluene and benzene to characterize the biodegradation kinetics. The experimental data shows that higher initial substrate concentrations lead to higher biodegradation rates.

Biodegradation experiments on phenol were done by Polymenakou (2004). Both temperature (10 to 40° C) and initial concentration (80 to 400 mg/L) were varied in the conducted batch experiments. At all considered temperatures, the biodegradation was highest for the batches with the initial concentration of 80 mg/L, except at 20° C, where the batch with the initial concentration of 175 mg/L was depleted from phenol fastest. At both 10 and 40° C, no significant biodegradation was shown for the batches with initial concentrations of 280 and 400 mg/L.

Generally, it can be concluded that the biodegradation rate increases with the increase of the initial concentration and reaches a maximum value. Thereafter, the biodegradation rate starts to decrease. The optimal concentration varies for different contaminants.

2.4 Soil type

The biodegradation rate of an organic compound is known to vary with soil type. The main factor in this is the bioavailability of the compound, that is influenced by soil matrix and more importantly, the organic content of the soil (Weissenfels 1991, Zhou 1994, Davis 1996, Zhang 1998, Breedveld 2000, Polymenakou 2004).

Natural soils consist of particles of different sizes, which implies that the pores in the soil matrix are of different sizes. Some of the pores in soil materials are smaller than the indigenous bacteria and therefore inaccessible for them. In the pores that are accessible for bacteria, bacterial growth can be limited by slow mass transfer of nutrients and organic substrate (Zhang 1998).

The organic content of a soil determines to a large extend how much of the present contaminant will adsorb to the soil particles (Weissenfels 1991, Davis 1996, Zhang 1998, Breedveld 2000, Polymenakou 2004). Another important factor is the hydrophobicity of the contaminating compound (Weissenfels 1991). Since bacteria are commonly not able to degrade the sorbed contaminant, the organic content influences the biodegradation rate significantly. Even when the bacteria are in direct contact with the sorbed contaminant, biodegradation remains insignificant. The 'age' of the contamination is also an important aspect, as long sorption time is associated with irreversible binding of contaminants to organic matter (Weissenfels 1991, Zhang 1998).

Davis (1996) conducted toluene biodegradation experiments on three different soils (sand, sandy loam and clay) from different locations. The soils are treated equally before and during the experiments. The experimental results show that the biodegradation rates are similar for the sandy loam soil and clay. The biodegradation rate for the sand however, is nearly half as large compared to those for the sandy loam soil and clay. The soil type also had an influence on the lag time for biodegradation; the lag time increased with decreasing soil organic content.

Biodegradation in two different soils, with different initial concentrations of PAHs were studied in the percolation experiments conducted by Weissenfels (1991). The organic carbon content was considered to be the most significant difference between the two soils. In the subsurface sand, with a contamination of 1.8 g PAH/kg soil, biodegradation to an overall average of 62% removal occurred. While in the soil consisting of heterogeneous material (rubble, stones and loam), with a contamination of 1.0 g PAH/kg soil, no significant biodegradation took place. Even after inoculating the heterogeneous soil with a bacterial culture able to degrade PAHs, no biodegradation was observed. As viable microorganisms were observed, Weissenfels (1991) concludes that this is due to adsorption of the PAHs to the soil material. In an additional experiment, a known quantity of PAHs, as well as a bacterial culture, was added to the subsurface sand and to the heterogeneous soil. In the case of the subsurface sand, biodegradation is completed in seven days. In the case of the heterogeneous soil, the biodegradation rate was lower and there was a fraction of 23% that is considered non-biodegradable.

Breedveld (2000) conducted column experiments with three types of soil containing a PAH contamination. The fraction of contamination prone to bioremediation is clearly depending on the organic content of the soil. In the aquifer material (0.38% organic matter, 95% PAH removal) and the topsoil (4.17% organic matter, 70% PAH removal) the majority of the contamination is removed. However, only 18% of the PAH contamination is degraded in the organic rich soil (16.6% organic matter).

According to the model of Zhang (1998), naphthalene biodegrades 2,8 times faster in an all aqueous environment compared to an environment containing an aqueous phase and soil with an organic content of 1.07%.

Manilal (1990) shows that the biodegradation rate of phenanthrene is significantly slower in soils with a high organic content. In contrast to other studies, Manilal (1990) finds that even in muck soil, where all phenanthrene is considered sorbed, phenanthrene is biodegraded.

The literature discussed above generally concludes that the fraction of the contaminant that is adsorbed to the soil will not be degraded. Both Weissenfels (1991) and Manilal (1990) conclude that the biodegradation rate decreases with an increasing organic content. However, Davis (1996) contradicts this conclusion. All discussed literature agrees that the lag time decreases for increasing soil organic content.

3. Materials and methods

This chapter gives an overview of the materials and methods that are used during this research.

3.1 Materials

The materials that were used in the laboratory experiments of this research are discussed in this paragraph.

3.1.1 Soil

The organic soil was collected from Wageningen University and originates from a site in Kielekamp, The Netherlands. It was characterized in 1993 by 'Bedrijfslaboratorium voor grond- en gewasonderzoek'. Additional tests were done at Wageningen University. The soil properties are summarized in table 3.1.

Table 3.1 Organic soil characteristics

Organic content (%)	2.2	Characterization done by 'Bedrijfslaboratorium voor grond- en gewasonderzoek' (appendix i).
Dry bulk density (ρ_b)	1.7 kg/L	Measured at Wageningen University by S. Picone and B.K. Yadav
Soil density (ρ_s)	2.7 kg/L	Fitts 2002, p. 28
Porosity (Φ)	0.46	Measured at Utrecht University
Field capacity	0.10	Ward 2000, p.194

The results of some of the performed experiments were compared to results obtained by Rajbhandari (unpublished) and Morée (2010). Both Rajbhandari and Morée used quartz sand H31, obtained from Sibelco (Belgium) in their experiments. This material consists of clean quartz sand grains, without any organic material present.

3.1.2 Groundwater

The groundwater was collected from a pump and treat site in Haarlem, The Netherlands, that is contaminated by organic constituents. Toluene was depleted from the groundwater by oxygen exposure before its use to prevent that concentrations in the experiments were higher than expected. A volume of groundwater was sterilized by adding 100 mL $HgCL_2$ stock (10 g/L) to 1 liter of groundwater.

3.1.3 Chemicals and Milli-Q water

All used chemicals were of analytical grade. Toluene, mercury chloride, ethanol and methanol were all obtained from Merck. Toluene was used as the contaminant that was biodegraded. Mercury chloride was used for sterilization of groundwater to create sterile controls as well as for the sterilization of the samples to ensure that biodegradation was stopped at the moment of sampling. Ethanol and a gas mixture (99.5% N_2 and 0.5% CO_2) were used to clean the used syringes after taking liquid and headspace samples respectively. Methanol was used as a solvent to make a 1 mg/L toluene stock for liquid phase calibrations. All aqueous solutions and calibration lines were prepared with Milli-Q water. Also, when necessary, samples were diluted using Milli-Q water.

3.1.5 Batches

The batches were 120 mL or 250 mL glass bottles (Alltech). The batches were capped with a viton stopper (Rubber B.V., Hilversum, The Netherlands) and a 20 mm aluminum seal (Wheaton, The Netherlands). Toluene stock was injected using gas tight syringes (100 μL, Hamilton and other volumes, SGE) and 16 mm disposable needles (Φ 0.5 mm, Terumo).

3.1.6 Sampling materials

The groundwater was sampled by using a 1 mL gas tight syringe (SGE) and 25 mm disposable needles (0.6 mm diameter, Terumo). The headspace samples were taken using a 1 mL luer locksyringe (SGE) equipped with a gastight valve and 25 mm disposable needles (0.6 mm diameter, Terumo).

3.2 Experimental methods

Batches are filled with a certain volume of soil and groundwater. Toluene stock is added to obtain the required concentration. Methods and volumes are discussed per set of experiments in this paragraph.

3.2.1 Batches at different temperatures

The soil was sieved (mesh size 0.3 mm) to ensure that larger organic matter was excluded from the batches. The sieved soil (10 g) was weighed in the 120 mL glass bottles and 15 mL groundwater (live or sterilized) was added. The bottles were capped with a viton stopper and aluminum seal. The toluene stock (0.032 mL at solubility limit, 490 mg/L) was injected through the stopper. For every temperature, a set of three live and two sterile batches were run. Since there was only limited groundwater in the batch that was available for sampling, an arbitrary maximum of 6 samples per batch was considered. Therefore, the experiments were cut up in two or three (for 10°C) parts in order to obtain sufficient data point for an accurate analysis. The batches were shaken by hand for approximately 10 seconds before taking the first sample. The batches were put horizontally on an orbital shaker at approximately 100 rpm to ensure complete mixing of the system. Also, the batches were covered with black polythene to shield them from light to prevent growth of photosynthetic organisms. The batches were incubated in a fridge (10°C), at room temperature (21±1°C) or in a water bath at 30°C. Sampling times were based on experiments performed by S. Rajbhandari (2010, unpublished).

3.2.2 Batches at different moisture contents

The sieved soil (mesh size 0.3 mm, 50 g) was weighed in the 120 mL glass bottles and a volume of groundwater (live or sterilized) was added (table 3.2). After that, the bottles were capped with a viton stopper and aluminum seal. The toluene stock (at solubility limit, 490 mg/L, volumes in table 3.2) was injected through the stopper. The concentration in all batches was 25 mg/L in the liquid phase. For each moisture content, a set of three live and two sterile batches were run. The batches were shaken by hand for approximately 10 seconds before taking the first sample. Also, the batches were covered with black polythene to shield them from light to prevent growth of photosynthetic organisms. The experiment was performed in a laboratory that was at a nearly constant 24°C. Sampling times were based on preliminary experiments (appendix ii).

Table 3.2: Volumes of groundwater and toluene stock added to the batches at different moisture contents.

θ	Volume of groundwater (mL)	Volume of toluene stock (μL)
0.10	1.85	95
0.22	4.07	210
0.34	6.30	320
0.46	8.52	435

3.2.3 Batches with different initial concentrations

The soil was sieved through a mesh size of 2.0 mm. The soil (10 g) was weighed in the 120 mL or 250 mL glass bottles (see table 3.3) and 15 mL groundwater (live or sterilized) was added. After that the bottles were capped with a viton stopper and aluminum seal. The toluene stock (at solubility limit, 490 mg/L, volumes in table 3.3) was injected through the stopper. For every initial concentration, a set of two batches were run. The batches were shaken by hand for approximately 10 seconds before taking the first sample. The batches were put horizontally on an orbital shaker at approximately 100 rpm to ensure complete mixing of the system. The batches were covered with black polythene to shield them from light to prevent growth of photosynthetic organisms. The experiment was performed in a laboratory that was at a nearly constant 22°C. The batches are sampled every two hours for a period of eight hours.

Table 3.3: Volumes of groundwater and toluene stock of pure toluene added to the batches at different initial concentrations.

Initial concentration(mg/L)	Batch capacity (mL)	Volume of groundwater (mL)	Volume toluene stock added (µL)	Volume of pure toluene added (µL)
1.0	120	14.905	95	
5.3	120	14.530	480	
10.4	120	14.050	950	
20.8	120	13.100	1900	
52.2	120	10.240	4760	
104.2	120	5.490	9510	
122.3	250	14.990		10

The batches contained soil and a certain volume of groundwater spiked with toluene. To ensure the right initial concentration, the groundwater was spiked with a toluene stock or pure toluene for the highest initial concentration.

3.3 Analytical methods

All samples were analyzed using gas chromatography. This was done with two different gas chromatographs: 1) Varian Star 3600 CX, and 2) Agilent Technologies 6850. Both gas chromatographs and the calibration method are discussed below.

3.3.1 Agilent Technologies 6850

The samples from the experiments at different temperatures and with different moisture contents were analyzed using a Agilent Technologies 6850. This gas chromatograph (GC) is equipped with an Agilent J&W gas chromatograph column (Φ 0.32 mm, 30 m length, film thickness 0.25 µm) and a FID detector (250°C). The Stableflex SPME fiber has a polydimethylsiloxane/divinylbenzene coating. The GC has two trays available, which both can be set for 98 liquid samples (1,5 mL vials) or 32 headspace samples (10 mL vials).

3.3.2 Varian Star 3600 CX

The samples from the experiment with different initial concentrations were analyzed using a Varian Star 3600 CX. It is equipped with a Stabilwax DB column (Φ 0.32 mm, 30 m length, film thickness 1 µm), UV lamp and PID detector (200°C). The SPME fiber has a 85 µm polyacrylate coating. The autosampler used for the liquid samples is a Varian 8200 CX with 48 positions for 2 mL vials.

3.3.3 Calibration

Both GCs were calibrated by analyzing a calibration line of seven vials having toluene concentrations ranging from 0 to 1000 µg/L (table 3.4). Though the volumes of the vials used were different, the assembly of the calibration vials were equal, because both GC's measure the total concentration in the vials. For the Agilent Technologies 6850, a calibration line was run once a week. For the Varian Star 3600 CX, a calibration line was run before each time

liquid samples were analyzed. After every ten samples, a calibration vial 3 was analyzed to check if the GC was still calibrated properly (Mizab 2008).

Table 3.4: Schedule for making a liquid calibration line (Mizab 2008).

Calibration vial	Milli-Q water (mL)	Toluene stock (2 mg/L) (mL)	Concentration in vial (µg/L)
0	1	0	0
1	0.975	25	50
2	0.950	50	100
3	0.900	100	200
4	0.850	150	300
5	0.750	250	500
6	0.500	500	1000

4. Mass balance

To form an expectation of the concentrations measured in the experiments, mass balances were calculated for each experiment. The method and results are presented in this chapter.

4.1 Data and formulas

The main soil properties are listed in table 3.1. The remainder of the data used for calculating the mass balance is shown in table 4.1.

Table 4.1: Data used in the calculation of the mass balance.

Adsorption coefficient	log 2.06
Henry's constant (air-water)	0.201
Volume of batch	120 mL
Mass of soil	10 g
Concentration of stock	0.49 mg/mL
Volume of stock added	0.032 mL

The used symbols and meanings are listed in table 4.2.

$$C_t V_t = C_w V_w + C_a V_a + C_s V_s$$

$$C_w = H_c C_a$$

$$C_s = f_{oc} K_{oc} C_w$$

These formulas combined give;

$$C_t V_t = C_w \left(V_w + \frac{V_a}{H_c} + f_{oc} K_{oc} V_s \right)$$

Table 4.2: Symbols and meanings used in the calculation of the mass balance.

C	Concentration (mg/L)
V	Volume (L)
Hc	Henry's costant air-water
f_{oc}	Organic carbon fraction
K_{oc}	Adsorption coefficient for toluene

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4.2 Mass balances of the batch experiments

The batches that were run at different temperatures had the same composition. The mass balance was calculated using the formulas given in paragraph 4.1.

Table 4.3: Concentrations and masses in the different phases of the batches at different temperatures.

C_w (mg/L)	0.315
C_s (mg/L)	0.795
C_a (mg/L)	0.036
$m_{toluene,\ w}$ (mg)	0.005
$m_{toluene,\ s}$ (mg)	0.005
$m_{toluene,\ a}$ (mg)	0.006

The composition of the batches with different moisture contents are listed in table 3.2. The mass balance was calculated using the formulas given in paragraph 4.1. The results are shown in table 4.4.

Table 4.4: Concentrations and masses in the different phases of the batches with different moisture contents.

	θ1 (r) = 0.10	**θ2 = 0.22**	**θ3 = 0.34**	**θ4 (s) = 0.46**
C_w (mg/L)	0.670	1.444	2.171	2.868
C_s (mg/L)	1.693	3.647	5.483	7.243
C_a (mg/L)	0.135	0.290	0.436	0.576
$m_{toluene,\ w}$ (mg)	0.001	0.006	0.014	0.024
$m_{toluene,\ s}$ (mg)	0.031	0.068	0.102	0.134
$m_{toluene,\ a}$ (mg)	0.013	0.028	0.041	0.053

The composition of the batches with different initial concentrations are listed in table 3.3. The concentrations and masses in the different phases are shown in table 4.5. The mass balance was calculated using the formulas given in paragraph 4.1.

Table 4.5: Concentrations and masses in the different phases of the batches with different initial conditions.

	1 mg/L	**5 mg/L**	**10 mg/L**	**20 mg/L**	**50 mg/L**	**100 mg/L**	**122 mg/L**
C_w (mg/L)	1.041	5.260	10.410	20.820	52.160	104.211	122.336
C_s (mg/L)	2.630	13.286	26.295	52.591	131.754	263.231	309.013
C_a (mg/L)	0.209	1.057	2.092	4.185	10.484	20.946	24.590
$m_{toluene,\ w}$ (mg)	0.016	0.076	0.146	0.273	0.534	0.572	1.834
$m_{toluene,\ s}$ (mg)	0.010	0.049	0.097	0.195	0.488	0.975	1.144
$m_{toluene,\ a}$ (mg)	0.021	0.107	0.212	0.424	1.062	2.122	5.687

5. Results

The results for the different batch experiments are presented in this chapter. All the results can be found in appendices iii, iv and v.

5.1 Temperature dependency

The results of the performed batch experiments at different temperatures are shown in figures 5.1, 5.2 and 5.3. Because each graph is constructed from more than one experiment, the concentration is expressed as the relative concentration (C / C_0) in order to cancel out small differences that could originate from the assembly of the batches.

There is complete biodegradation at all temperatures. At 10°C, there is a lag phase of approximately 55 hours. All toluene is biodegraded after 100 hours. The batches at 21°C show a lag phase of approximately 20 hours. The graph does not show completion of the biodegradation, but the concentration in the batches is very low after 32 hours. At 30°C, the batches show a lag phase of approximately 20 hours and completion of the biodegradation after 30 hours..

Notice that the sterile batches at 21°C and 30°C show a drop in concentration at the beginning of the experiment. Whereas all the batches at 10°C show a rise in concentration with respect to the initial concentration. From 72 to 96 hours, the batches at 10°C show a large standard deviation.

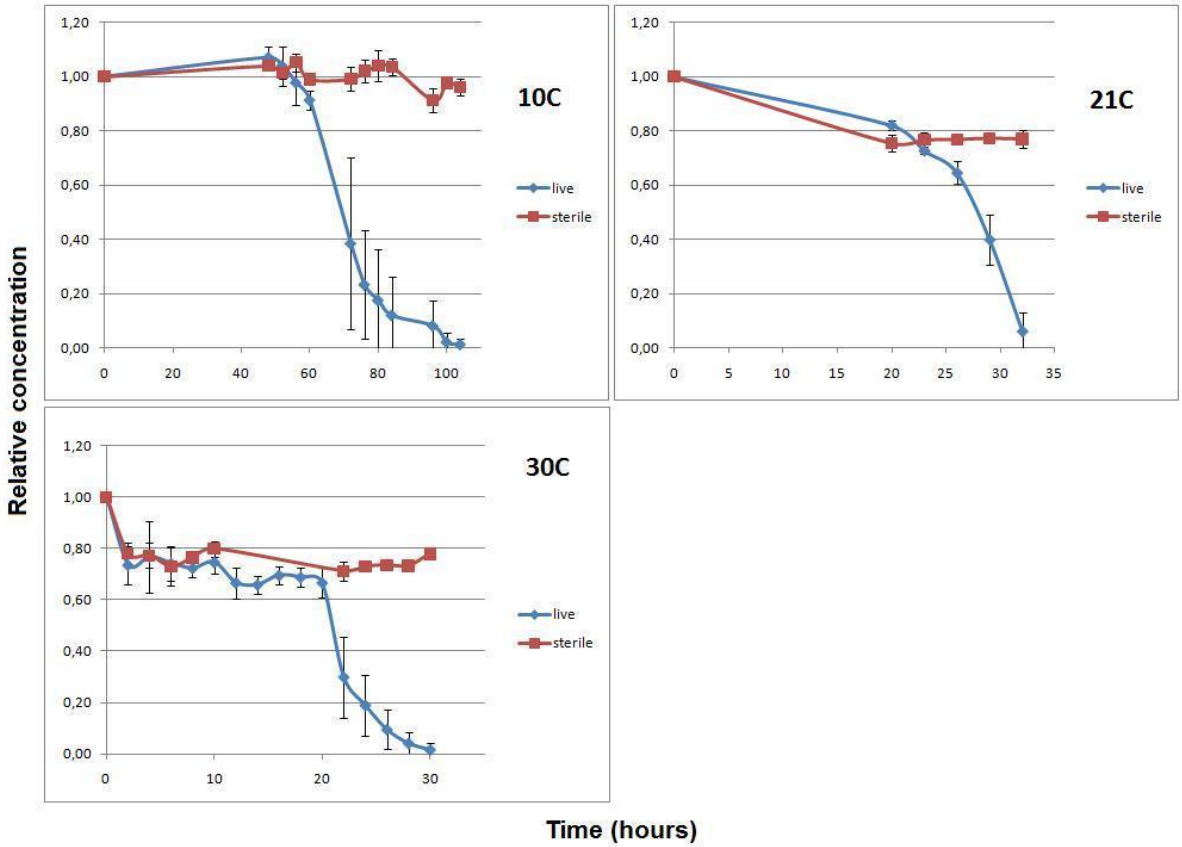

Figure 5.1: The variation of toluene concentration versus time for batches at 10° C, 21° C and 30° C.

Figure 5.2 shows how the biodegradation curves at different temperatures are proportionate to each other. In figure 5.2a is seen that the batches at 30°C have an equal lag time as the batches at 21°C. The total biodegradation completes faster at 30°C than at 21°C. The same trend is seen when the batches at 21° and 10° C are compared. However, the differences are significantly larger between those sets of batches. Also, the batches at 10°C show a longer lag time than those at 21°C and 30°C.

What stands out in figure 5.2b is that at 10°C, the concentration in the sterile batches shows a slight rise at some data points. At both 21°C and 30°C, a decrease in concentration is seen at the beginning of the experiments.

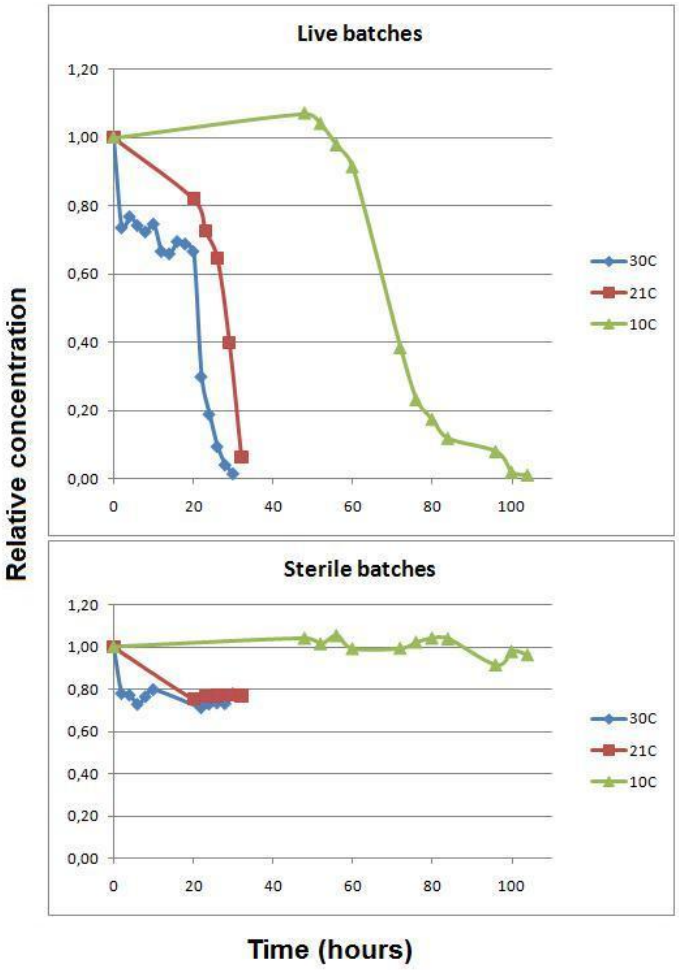

Figure 5.2a: Decrease in concentration versus time of the live batches at 10, 21 and 30° C.
Figure 5.2b: Decrease in concentration versus time of the sterile batches at 10, 21 and 30° C.

Figure 5.3 shows that the biodegradation rate increases with increasing temperature. Also, it confirms that the difference in rate is much larger between 10°C and 21°C than the difference between 21°C and 30°C.

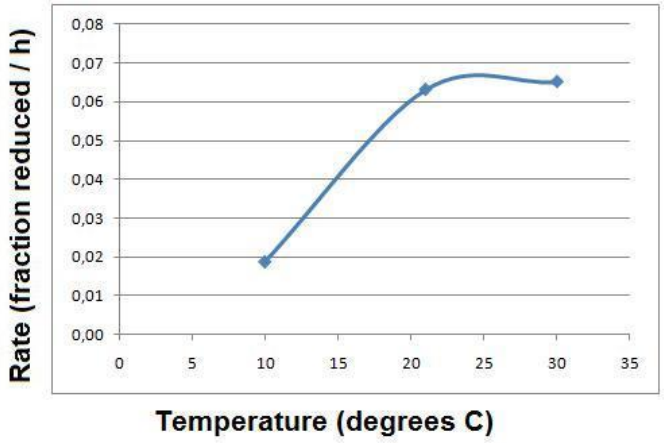

Figure 5.3: Biodegradation rate versus temperature.

5.2 Moisture content dependency

Batch experiments were performed with soil at four different moisture contents in the range of residual moisture content to saturated soil. For each moisture content, three live batches and two sterilized batches were run. The results are shown in figures 5.4 and 5.5.

All batches have a lag time of approximately 20 hours, after which biodegradation sets in. Notice that in the sterile batches, except for the batches at $\theta_r = 0.10$, the concentration rises above the initial concentration at 44 hours after start of the experiment.

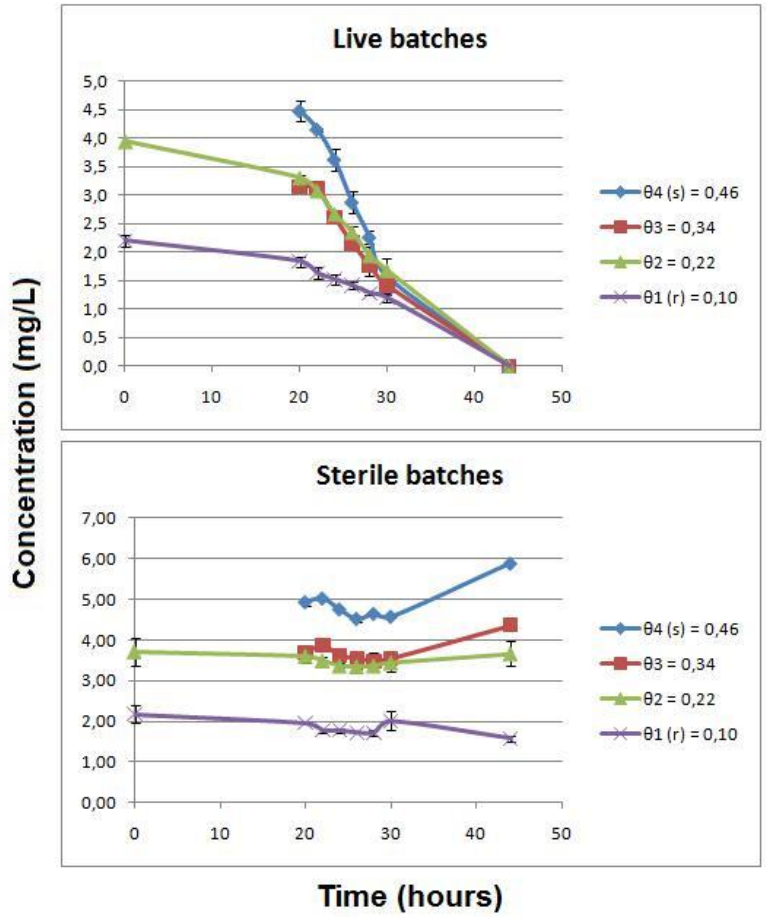

Figure 5.4: Concentration versus time for batch experiments with different moisture contents.

The biodegradation rate is plotted against the moisture content in figure 5.5. The rate increases with increasing moisture content in a linear trend.

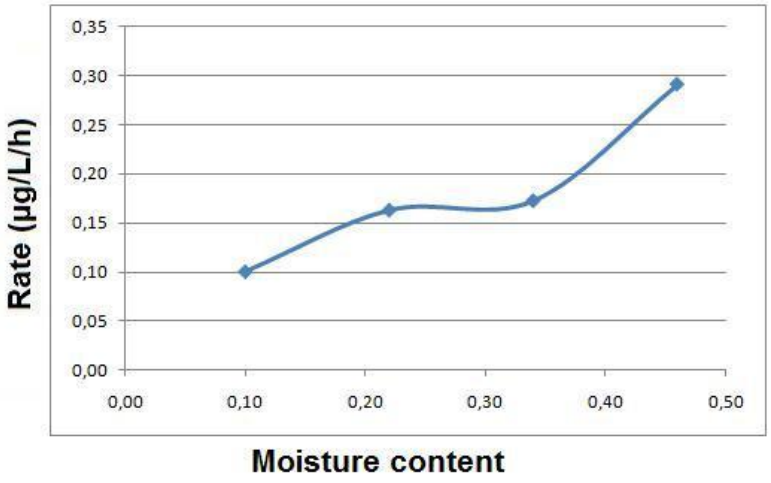

Figure 5.5: Degradation rate versus moisture content for batch experiments with different moisture contents.

5.3 Dependence on initial substrate concentration

Batch experiments were done for different initial concentrations, ranging from 1 mg/L to 122 mg/L in the liquid phase. The results are shown in figures 5.6 and 5.7.

All batches show degradation. Notice that all the batches show a lower concentration at the start of the experiment than expected from calculations (see individual graph headings). Also, in several batches, the concentration seems to increase after a period of time.

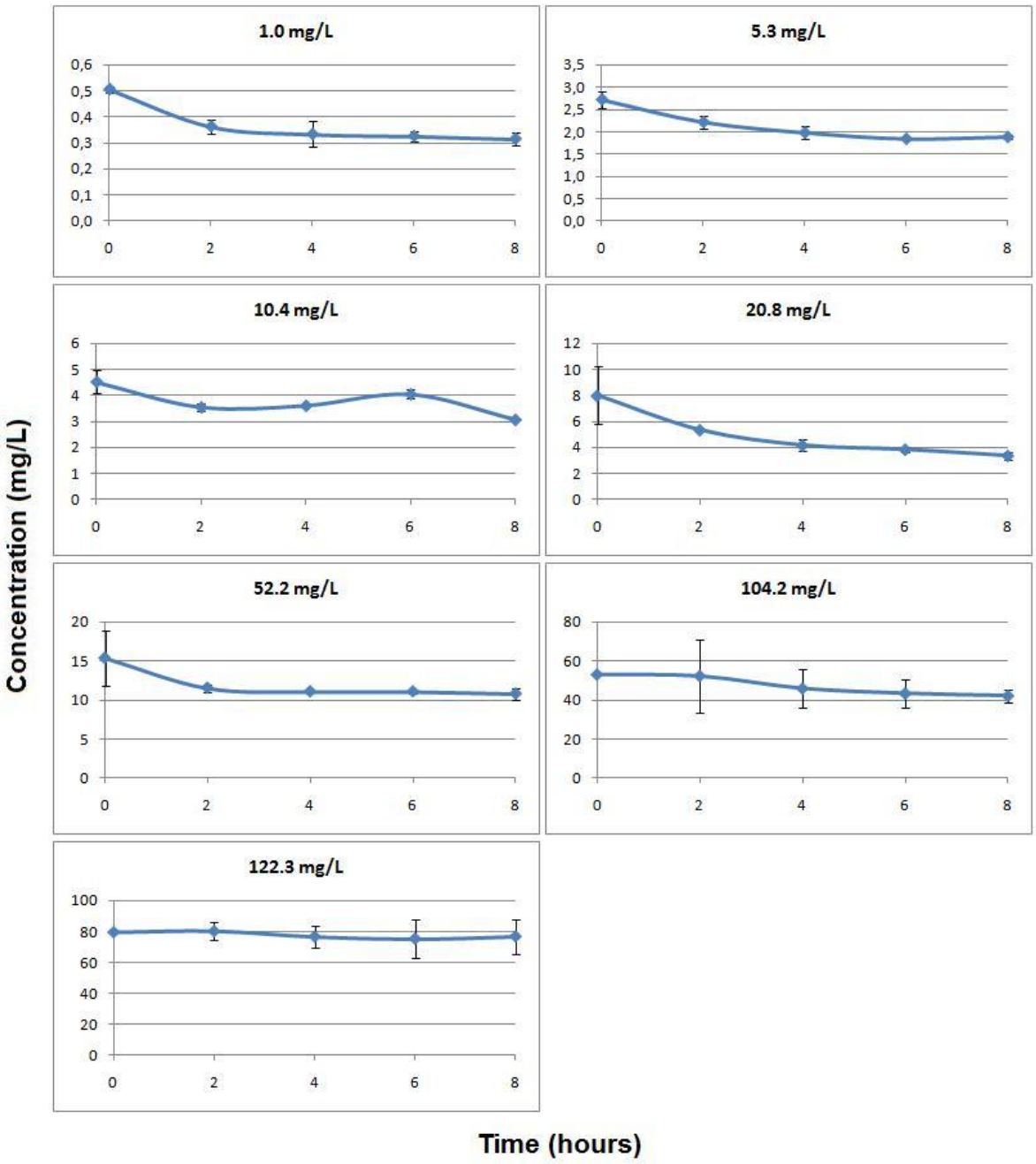

Figure 5.6: Concentration versus time for batch experiments with different initial concentrations of toluene in the liquid phase.

Figure 5.7 shows the initial degradation rate versus the initial concentration. The degradation rate increases with increasing concentration, up to a threshold value. At initial concentrations higher than this threshold value, the degradation rate diminishes significantly.

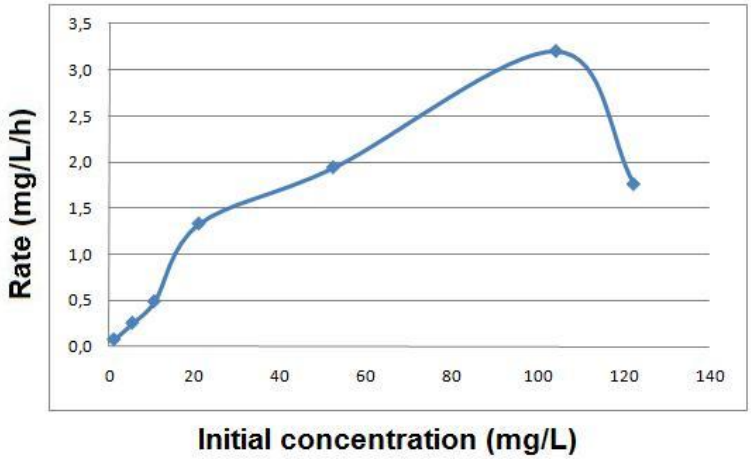

Figure 5.7: Initial rate versus initial concentration for batch experiments.

5.4 Dependence on soil type

The results of the batch experiments at different temperatures (paragraph 5.1) are compared with results from Rajbhandari (unpublished) and Morée (2010) in table 5.1. The sand and soil referred to in table 5.1 are characterized in paragraph 3.1.1.

At 10°C, the biodegradation rate of toluene in the organic soil is larger than in the clean sand. For 21°C, there is a large difference between the two sets of results for the clean sand. A comparable difference in rate is shown by the results for 30°C.

Table 5.1: Experimental data per temperature and soil type.

Temperature (°C)	Soil type	Lag phase (hours)	Total degradation time (hours)	Rate (mg/L/h)
10	Clean sand	50	105	0.015 [1]
	Organic soil	55	100	0.019
21	Clean sand	17	45	0.029 [1]
	Clean sand	0	15	6.667 [2, 3]
	Organic soil	20	32	0.063
30	Clean sand	0	21	4.762 [1, 3]
	Organic soil	20	30	0.065

1. Rajbhandari 2010, 2. Morée 2010, 3. Batches were not shielded from light.

6. Discussion

The results that are presented in chapter 5 are interpreted and discussed in this chapter.

6.1 Temperature dependency

All batches at 10°C show an increase in concentration with respect to the initial concentration (figure 5.1). As the batches were assembled at approximately 22°C, this could be due to the decrease in temperature after taking the first sample. A decrease in temperature equals a lowering in volatility, causing an increase in the partitioning to the liquid phase.

The batches at 10°C show a large standard deviation from 72 to 96 hours. Since the triplicates don't show one general trend, no explanation can be found.

The lag phase for biodegradation is approximately 55 hours at 10°C. The lag times of the batches at 21°C and 30°C can be considered equal (figure 5.2a).

Considering the sterile controls, one can conclude that approximately 20% of the initial concentration of toluene adsorbs to the soil at 21°C (figure 5.2b). The same is shown for the batches at 30°C. However, since the batches were assembled at 22°C, this drop in concentration could be due to the rise of temperature, causing an increase in partitioning to the air phase. The sterile batches at 10°C show no drop in concentration, indicating that no significant adsorption is taking place.

The biodegradation rate of toluene increases with increasing temperature. However, the difference between the rate at 21°C and 30°C is relatively small (figure 5.3). This leads to believe that the optimum temperature range for the toluene degrading bacteria starts at 21°C and the rate will not increase significantly at higher temperatures.

These findings do not correspond with the general conclusion found in the literature (Zhou 1994, Zhang 1998, Eriksson 2002, Torres 2005, Polymenakou 2004, Chablain 1997, Chang 2001). No explanation can be found for this difference.

6.2 Moisture content dependency

The batches were started with a concentration of 1 mg/L in the liquid phase, but since the quantity of groundwater differed for the different moisture contents, the initial concentrations were not equal. However, the batches at $\theta = 0.22$ and $\theta = 0.34$ show nearly equal concentrations throughout the whole biodegradation course (figure 5.4), which could indicate an error in the assembly of the batches. Therefore, the results of the batches at $\theta = 0.34$ are excluded in the further discussion.

For all batches, except those at $\theta_r = 0.10$, the concentration in the sterile batches was higher after 44 hours than it is at the start of the experiment (figure 5.4). This might be due to sample storage. While the other samples were stored in the fridge for a some minutes to hours, these samples were measured directly after collection.

The biodegradation rate increases linearly with increasing moisture content, when leaving the result for θ = 0.34 out of consideration (figure 5.5). Since the biodegradation takes place in the liquid phase (Zhang 1998), this is a plausible theory. However, it is based on merely three data points and therefore should be considered with great care.

It should be considered that in the natural situation, there is generally no headspace present. In a natural system, pore spaces are filled with either moisture or air. A higher moisture content therefore equals a smaller quantity of available oxygen in the pore spaces. Thus, it is likely that oxygen becomes limiting in a system with saturated soil, changing the relation between moisture content and biodegradation rate.

The observed increase of the biodegradation rate with increasing water content corresponds with the conclusions found in the literature (Domenico 1990, Mayer 2000, Holden 2001, Zhou 1994, Davis 1996, Rainwater 1993). However, in these experiments, no variation in lag time is observed unlike mentioned in the literature.

6.3 Dependence on initial substrate concentration

All the batches that were run show degradation. Since the batches were not allowed to run longer than eight hours, it is unknown whether complete biodegradation could have taken place or not. However, it is likely that for higher concentrations, oxygen had become limiting after a longer period.

Batches were made with initial concentrations ranging from 1 to 122 mg/L in the liquid phase. However, at the start of all experiments, the concentration in the liquid phase was lower than calculated in the mass balance (see figure 5.6 and paragraph 4.4). This would mean that the partitioning of the toluene deviates from the expectation. The absorption to the soil could be higher than expected due to the presence of large particles of organic material. The soil was sieved through a 2.0 mm mesh size (instead of 0.3 mm for the other experiments), because no other sieve was available at that time.

In some batches, an increase of toluene concentration was observed after six or eight hours (figure 5.6). Although this does not always pair with a large standard deviation, the only knowingly possible explanation is that these are dilution or measurement errors.

The biodegradation rate shows an nearly linear increase with increasing concentration, up to a concentration of 100 mg/L (figure 5.7). At concentrations higher than this threshold, the degradation rate diminishes significantly. This is likely due to the toxicity of the high toluene concentration to the microorganisms (Alvarez 1991, Zhang 1998, Choi 2008).

These findings coincide with the general conclusion found in the literature (Alvarez 1991, Zhou 1994, Zhang 1998, Choi 2008, Davis 1996, Polymenakou 2004).

6.4 Dependence on soil type

Both experiments for the clean sand at 21°C were performed in a laboratory which was assumed to be temperature controlled at a constant 21°C. No temperature measurements were done during the experiments. However, later measurements showed that the temperature in the laboratory was not constant and often above 21°C. Though the results for the organic soil at different temperatures show that the biodegradation rate does not increase

significantly in the range of 21°C to 30°C (figure 5.3), this should not be assumed without care to be equal for the clean sand. Therefore, comparison of the results for the clean sand and the organic soil at 21°C should be done with care.

The rates for the clean sand at 21°C by Morée (2010) and 30°C (Rajbhandari 2010) are two orders of magnitude higher than the other rates that are found (table 5.1). Also, these experiments show no lag phase, whereas the other experiments had a lag phase of at least 17 hours. This is probably due to the fact that the batches in those experiments were not shielded from light, which enables the growth of photosynthetic organisms. This seems to increase the biodegradation rate significantly. However, these two sets of results can be compared. As mentioned, there was no lag phase at both temperatures. The biodegradation was complete after 15 hours at 21°C, while at 30°C, this took 21 hours. In other words the biodegradation rate is higher at 21°C than at 30°C.

Without considering the results of the experiments that were not shielded from light, the results for the clean sand and the organic soil at 10°C and 21°C (table 5.1) can be compared. At both temperatures, the organic soil shows a longer lag phase, but the degradation completed in a shorter period of time. In other words, the biodegradation rate of toluene is higher in a soil with organic content compared to clean sand. This does not correspond with the general conclusion in the literature (paragraph 2.4), which states that organic matter inhibits biodegradation by adsorption. However, the organic soil may provide a better habitat for microorganisms than clean sand, leading to a larger population and therefore a higher biodegradation rate.

The conclusion considering the lag time, an increase for increasing organic content, does not coincide with the literature (Weissenfels 1991, Zhou 1994, Davis 1996, Zhang 1998, Breedveld 2000, Polymenakou 2004, Manilal 1990). This could be explained by the use of clean sand in these experiments, while in the researches discussed in the literature, generally used a natural soil with a lower organic content.

7. Conclusions

Biodegradation of toluene under varying temperature, soil moisture content, initial substrate concentration and soil organic content were investigated using batch experiments. All four environmental conditions have a significant influence on the biodegradation rate of toluene.

The optimal temperature range for biodegrading bacteria started around 21°C and the biodegradation rate did not increase significantly at higher temperatures. The lag phase before the biodegradation set in around 20 hours at 21°C to 30°C. At lower temperatures, the lag phase increased significantly. Approximately 20% of the initial toluene concentration adsorbed to the soil at 21°C to 30°C. At 10°C, no significant adsorption seemed to have taken place.

The relation between soil moisture content and toluene biodegradation rate seemed to be linear. However, this presumption was based on merely three data points and therefore should be investigated further. In a natural situation, oxygen may become limiting at high moisture contents and disrupt this relationship.

The initial toluene biodegradation rate increased linearly with the initial substrate concentration up to a threshold value around 100 mg/L toluene in the liquid phase. At higher concentrations, the biodegradation rate diminished.

Although the lag phase was longer for soil containing organic material compared to clean sand, the period of time needed for the biodegradation to complete was shorter for the organic soil. This means that the biodegradation rate of toluene was higher in organic soil compared to the clean sand.

8. Recommendations

The relation between temperature and biodegradation rate found in this research does not coincide with the information found in literature. To investigate this further, batch experiments should be done in a wider range of temperature.

As the temperature of an environment is never constant. For a better representation of the natural situation, batch experiments at fluctuating temperatures could be done.

The relation between soil moisture content and toluene biodegradation rate seemed to be linear. However, since this presumption was based on merely three data points, further investigation is necessary.

In a natural system, pore spaces are filled with either moisture or air. Therefore, a higher moisture content equals a lower quantity of available oxygen. To represent this accurately, experiments could be performed in microcosms as designed by prof. dr. ir. S. Majid Hassanizadeh, dr. B.K. Yadav, P. Kleingeld and myself (figure 8.1). These microcosms have a better representation of the natural situation and allow sampling of the soil including the soil moisture and pore space air.

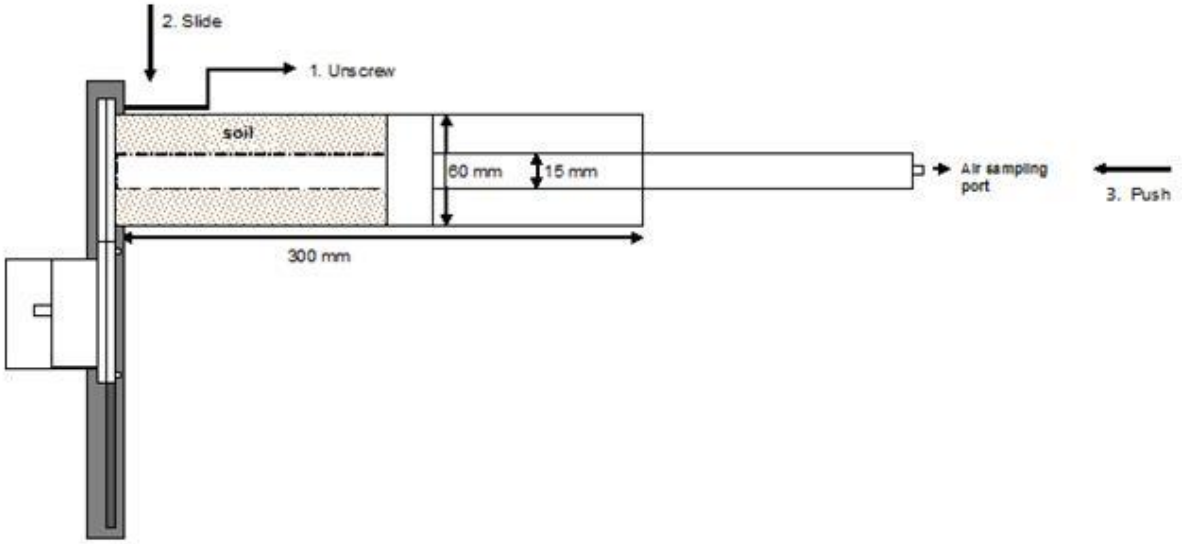

Figure 8.1: Schematic presentation of the designed microcosm and sampling mechanism. The outer column will contain soil at a certain toluene contaminated moisture content. The inner column will be filled with air at atmospheric pressure.

The influence of initial toluene concentration was only reviewed for the initial biodegradation rate in this research. To investigate the influence of initial concentration at the entire biodegradation process, longer running batch experiments should be done.

The data used for the analysis of the effect of soil type were from experiments performed by different people under, in some cases, slightly different circumstances. Comparison is therefore not straightforward. Additional research would give more insight in the dependence of toluene biodegradation rates on soil organic content.

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Appendices

Appendix i Soil properties

BEDRIJFSLABORATORIUM VOOR GROND- EN GEWASONDERZOEK
MARIËNDAAL 8
6861 WN OOSTERBEEK

Postbus 115
6860 AC OOSTERBEEK
Telefoon 085-346346
Postgiro nr. 89 09 96
Rabobank Oosterbeek nr. 10.10.54.777

ANALYSEVERSLAG
granulair onderzoek

Oosterbeek, 18-5-1994

332.605.5

Aan : dr. ir. J.T.C. Grotenhuis (LU), Vakgroep Milieutechnologie, Postbus 8129, 6700 EV WAGENINGEN

Onderzoeknummers : 872946

Datum bemonstering: Datum ontvangst: 16-03-1994

Herkomst : Kielekamp 10-11-1993

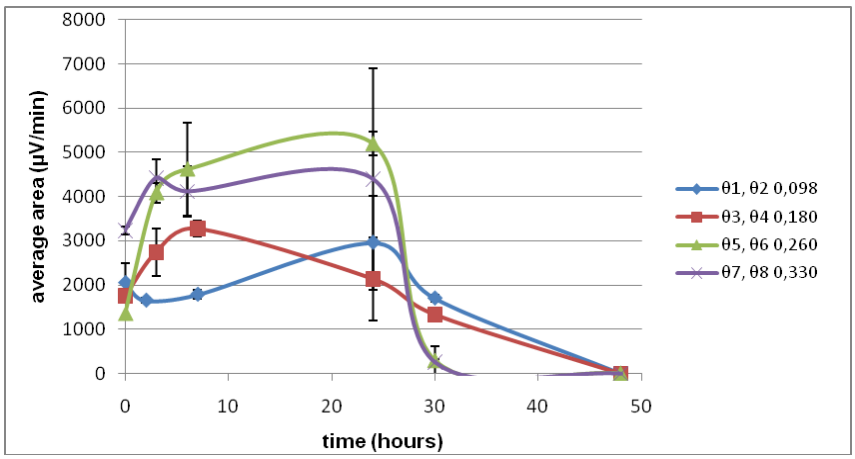
Onderzoeknummer	872946					
Gegevens van het monster	Kiele-kamp 1993					
Laag in cm						
pH-KCl	5,3					
In % v.d. stoof-droge grond						
Vocht 1) ~~Water~~ moisture	0,74					
Organische stof 2)	2,2					
$CaCO_3$	0,1					
Removable						
Afslibbaar 0-16	6,6					
Zand 16 - 2000	91,1					
(µm)						
Minerale delen in %			USDA 3 class			
0 - 2	4,2	clay	clay			
2 - 16	2,6					
16 - 50	9,4		silt			
50 - 105	16,8					
105 - 150	23,8	fine sand	SAND			
150 - 210	22,7					
210 - 2000	20,5	coarse sand				
1) g/100 g ldr.						
2) Gloeiverl.-meth.						

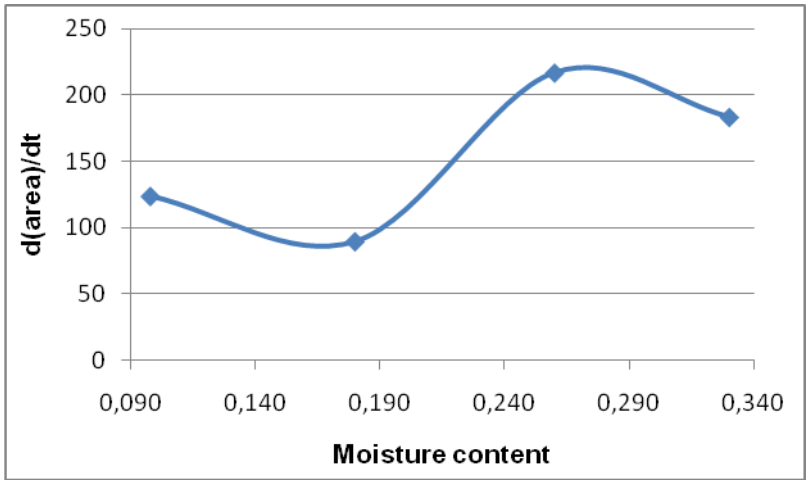
zand 200

Appendix ii Preliminary experiments

Preliminary batch experiments with sand at four different moisture contents, ranging from residual moisture content to saturated soil.

	t0	t3	t6	t7	t24	t30	t48
	area (µV/min)	area (µV/min)	area (µV/min)	area (µV/min)	area (µV/min)	area (µV/min)	area (µV/min)
θ1		2029,5		1657,6	3628,4	1672,6	0,0
θ2	2048,0	1274,7		1915,5	2290,3	1729,0	0,0
θ3	2064,8	2697,6		3345,2	2891,1	1384,5	0,0
θ4	1436,9	2778,7		3197,8	1384,5	1283,2	0,0
θ5		4247,8	5359,0		5007,8	521,9	0,0
θ6	1353,1	3924,5	3882,7		5387,5	56,4	0,0
θ7	3302,6	4127,4	3713,3		6170,4	206,8	
θ8	3169,0	4716,9	4520,2		2615,8	299,6	0,0



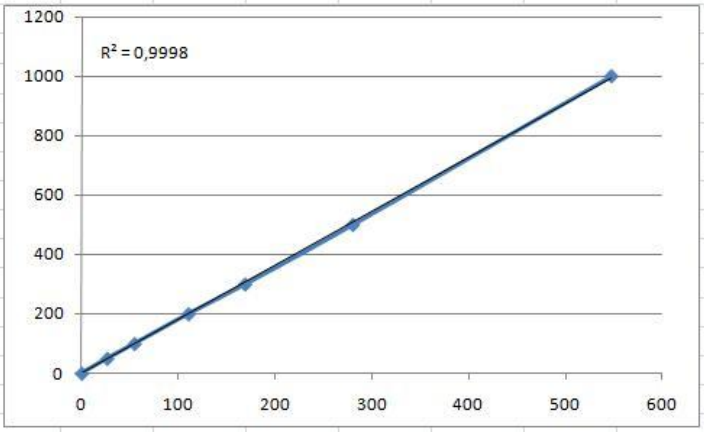


Appendix iii Results batches at different temperatures

Calibrations used for the experiments at different temperatures.

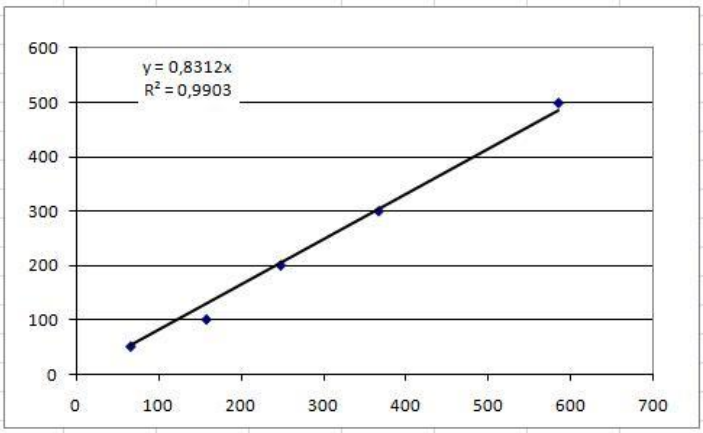
For STB3b and STB4b at t0

	area	concentration (µg/L)	slope
cal 0	0	0	0,549468
cal 1	26,3	50	
cal 2	54,6	100	
cal 3	110,4	200	
cal 4	168,9	300	
cal 5	280,3	500	
cal 6	547,5	1000	



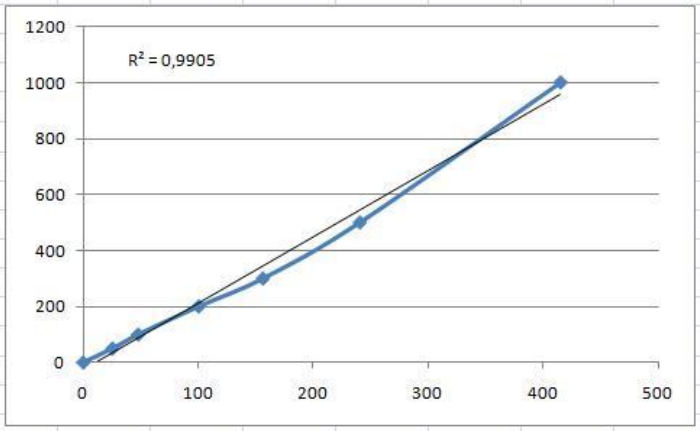
For TB1c, TB2c, TB3c, STB1c and STB2c
For STB3c and STB4c form t12 and on

No extra data available.



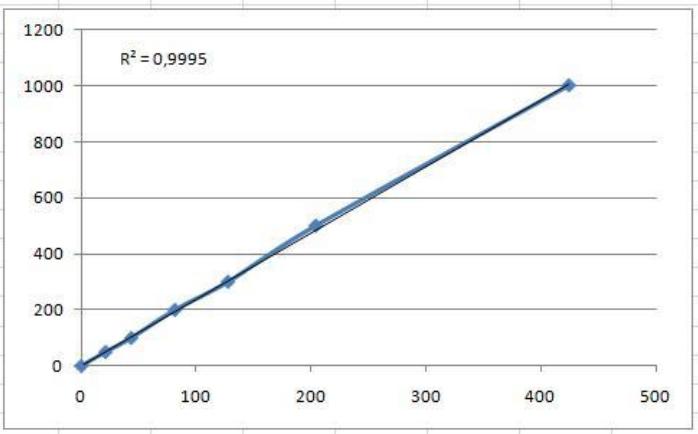
For TB1d,e, TB2d,e, TB3d,e, STB1d,e and STB2d,e at t0
For TB7(b), TB8(b), TB9(b), STB5(b) and STB6(b)
For TB4c,e, TB5c,d,e, TB6c,d,e, STB3c,d,e and STB4c,d,e

	area	concentration (µg/L)	slope
cal 0	0	0	0,417636
cal 1	25,4	50	
cal 2	47,8	100	
cal 3	100,4	200	
cal 4	156,3	300	
cal 5	240,6	500	
cal 6	415,2	1000	



For TB1e, TB2e, TB3e, STB1e and STB2e at t48 and on
For TB4f, TB5f, TB6f, STB3f and STB4f

	area	concentration (µg/L)	slope
cal 0	0	0	0,421964
cal 1	21,1	50	
cal 2	43,5	100	
cal 3	81,4	200	
cal 4	127,5	300	
cal 5	203,8	500	
cal 6	423,9	1000	



Results for the batches at 10°C.

	t0			t48			t52		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB1c	152,1	126,4	1,0						
TB1d	157,8	377,8	1,0						
TB1e	31,5	75,4	1,0	35,5	84,1	1,1	35,4	83,9	1,1
TB2c	146,1	121,4	1,0						
TB2d	147,8	353,9	1,0						
TB2e	42,1	100,8	1,0	44,8	106,2	1,1	41,1	97,4	1,0
TB3c	147,9	122,9	1,0						
TB3d	138,0	330,4	1,0						
TB3e	42,6	102,0	1,0	44,9	106,4	1,0	44,8	106,2	1,0
STB1b	146,5	121,8	1,0						
STB1c	145,4	120,9	1,0						
STB1d	125,8	301,2	1,0						
STB1e	70,2	168,1	1,0	74,3	176,1	1,0	72,8	172,5	1,0
STB2b	145,7	121,1	1,0						
STB2c	144,1	119,8	1,0						
STB2d	112,1	268,4	1,0						
STB2e	89,9	215,3	1,0	93,7	222,1	1,0	90,8	215,2	1,0

	t56			t60			t72		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB1c									
TB1d							114,7	274,6	0,7
TB1e	33,2	78,7	1,0	30,4	72,0	1,0			
TB2c									
TB2d							47,5	113,7	0,3
TB2e	37,8	89,6	0,9	38,3	90,8	0,9			
TB3c									
TB3d							14,4	34,5	0,1
TB3e	43,1	102,1	1,0	38,1	90,3	0,9			
STB1b							147,0	122,2	1,0
STB1c									
STB1d							122,6	293,6	1,0
STB1e	76,3	180,8	1,1	69,5	164,7	1,0			
STB2b							152,4	126,7	1,0
STB2c									
STB2d							105,6	252,9	0,9
STB2e	93,5	221,6	1,0	90,9	215,4	1,0			

	t76			t80			t84		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB1c									
TB1d	69,0	165,2	0,4	59,6	142,7	0,4	44,2	105,8	0,3
TB1e									
TB2c									
TB2d	32,9	78,8	0,2	21,7	52,0	0,1	11,5	27,5	0,1
TB2e									
TB3c									
TB3d	5,2	12,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TB3e									
STB1b	148,0	123,0	1,0	145,8	121,2	1,0	148,8	123,7	1,0
STB1c									
STB1d	129,4	309,8	1,0	141,5	338,8	1,1	130,7	313,0	1,0
STB1e									
STB2b	156,2	129,8	1,1	147,9	122,9	1,0	147,9	122,9	1,0
STB2c									
STB2d	109,0	261,0	1,0	115,1	275,6	1,0	120,8	289,2	1,1
STB2e									

	t96			t100			t104		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB1c	28,6	23,8	0,2	9,3	7,7	0,1	5,3	4,4	0,0
TB1d									
TB1e									
TB2c	4,9	4,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TB2d									
TB2e									
TB3c	3,0	2,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TB3d									
TB3e									
STB1b									
STB1c	137,1	114,0	0,9	144,1	119,8	1,0	136,5	113,5	0,9
STB1d									
STB1e									
STB2b									
STB2c	127,2	105,7	0,9	138,9	115,5	1,0	141,6	117,7	1,0
STB2d									
STB2e									

Results for the batches at 21±1°C.

	t0			t20			t23		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB7	118,0	282,5	1,0	94,8	227,0	0,8	86,2	206,4	0,7
TB8	142,8	341,9	1,0	119,9	287,1	0,8	101,7	243,5	0,7
TB9	116,6	279,2	1,0	95,4	228,4	0,8	86,2	206,4	0,7
STB5	126,5	302,9	1,0	92,8	222,2	0,7	94,9	227,2	0,8
STB6	122,4	293,1	1,0	95,0	227,5	0,8	96,2	230,3	0,8

	t26			t29			t32		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB7	80,80	193,47	0,68	47,10	112,78	0,40	6,20	14,85	0,05
TB8	85,90	205,68	0,60	70,30	168,33	0,49	19,40	46,45	0,14
TB9	75,80	181,50	0,65	35,80	85,72	0,31	0,00	0,00	0,00
STB5	96,20	230,34	0,76	96,30	230,58	0,76	94,60	226,51	0,75
STB6	95,30	228,19	0,78	96,30	230,58	0,79	97,20	232,74	0,79

Results for the batches at 30°C.

	t0			t2			t4		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB4c	128,60	307,92	1,00						
TB4e	131,20	314,15	1,00	91,70	219,57	0,70	101,00	241,84	0,77
TB4f	105,60	250,26	1,00						
TB5c	112,40	269,13	1,00						
TB5d	120,50	288,53	1,00						
TB5e	129,20	309,36	1,00	88,70	212,39	0,69	81,10	194,19	0,63
TB5f	70,40	166,84	1,00						
TB6c	106,70	255,49	1,00						
TB6d	121,20	290,20	1,00						
TB6e	96,30	230,58	1,00	79,20	189,64	0,82	87,40	209,27	0,91
TB6f	75,40	178,69	1,00						
STB3b	158,10	287,73	1,00						
STB3e	68,50	164,02	1,00	51,30	122,83	0,75	50,60	121,16	0,74
STB3f	87,10	206,42	1,00						
STB4b	170,10	309,57	1,00						
STB4e	76,50	183,17	1,00	62,10	148,69	0,81	61,80	147,98	0,81
STB4f	98,30	232,96	1,00						

	t6			t8			t10		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB4c									
TB4e	103,50	247,82	0,79	98,40	235,61	0,75	102,20	244,71	0,78
TB4f									
TB5c									
TB5d									
TB5e	89,90	215,26	0,70	90,10	215,74	0,70	92,30	221,01	0,71
TB5f									
TB6c									
TB6d									
TB6e									
TB6f									
STB3b									
STB3e	53,60	128,34	0,78	53,10	127,14	0,78	56,20	134,57	0,82
STB3f									
STB4b									
STB4e	51,70	123,79	0,68	57,80	138,40	0,76	59,50	142,47	0,78
STB4f									

	t12			t14			t16		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB4c	81,30	194,67	0,63	88,10	210,95	0,69	86,50	207,12	0,67
TB4e									
TB4f									
TB5c	66,50	159,23	0,59	71,20	170,48	0,63	75,90	181,74	0,68
TB5d	79,10	189,40	0,66	81,60	195,39	0,68	85,80	205,44	0,71
TB5e									
TB5f									
TB6c	76,00	181,98	0,71	65,10	155,88	0,61	73,50	175,99	0,69
TB6d	89,80	215,02	0,74	83,70	200,41	0,69	88,30	211,43	0,73
TB6e									
TB6f									
STB3b	115,80	96,25	0,33	114,90	95,50	0,33	123,90	102,99	0,36
STB3e									
STB3f									
STB4b	122,00	101,41	0,33	124,40	103,40	0,33	119,10	99,00	0,32
STB4e									
STB4f									

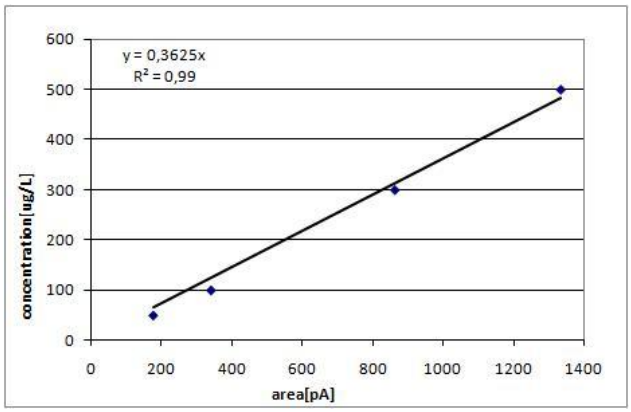
	t18			t20			t22		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB4c	91,30	218,61	0,71	93,90	224,84	0,73			
TB4e									
TB4f							22,00	52,14	0,21
TB5c	71,70	171,68	0,64	66,90	160,19	0,60			
TB5d	79,90	191,31	0,66	81,70	195,62	0,68			
TB5e									
TB5f							14,60	34,60	0,21
TB6c	75,40	180,54	0,71	75,80	181,50	0,71			
TB6d	87,70	209,99	0,72	75,00	179,58	0,62			
TB6e									
TB6f							36,30	86,03	0,48
STB3b	122,50	101,82	0,35	123,00	102,24	0,36			
STB3e									
STB3f							64,40	152,62	0,74
STB4b	128,80	107,06	0,35	123,80	102,90	0,33			
STB4e									
STB4f							67,40	159,73	0,69

	t24			t26			t28		
	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0	area	concentration (µg/L)	C/C0
TB4c									
TB4e									
TB4f	12,20	28,91	0,12	3,70	8,77	0,04	3,80	9,01	0,04
TB5c									
TB5d									
TB5e									
TB5f	8,90	21,09	0,13	4,70	11,14	0,07	0,00	0,00	0,00
TB6c									
TB6d									
TB6e									
TB6f	24,50	58,06	0,32	13,70	32,47	0,18	6,50	15,40	0,09
STB3b									
STB3e									
STB3f	63,20	149,78	0,73	63,30	150,01	0,73	63,80	151,20	0,73
STB4b									
STB4e									
STB4f	72,20	171,10	0,73	73,20	173,47	0,74	72,00	170,63	0,73

	t30		
	area	concentration (µg/L)	C/C0
TB4c			
TB4e			
TB4f	0,00	0,00	0,00
TB5c			
TB5d			
TB5e			
TB5f	0,00	0,00	0,00
TB6c			
TB6d			
TB6e			
TB6f	3,40	8,06	0,05
STB3b			
STB3e			
STB3f	66,80	158,31	0,77
STB4b			
STB4e			
STB4f	77,80	184,38	0,79

Appendix iv Results batches with different moisture contents

Calibration used for the batches with different moisture contents.



Results moisture content 1: θ(r) = 0.10

	t0		t20		t22		t24	
	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)
MCB10	6,4	2,3	5,3	1,9	4,8	1,7	4,5	1,6
MCB11	5,9	2,1	5,1	1,8	4,5	1,6	4,1	1,5
MCB12	5,9	2,1	4,8	1,7	4,2	1,5	4,0	1,5
SMCB7	5,6	2,0	5,4	2,0	5,0	1,8	5,0	1,8
SMCB8	6,4	2,3	5,4	2,0	4,8	1,7	4,8	1,7

	t26		t28		t30		t44	
	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)
MCB10	4,1	1,5	3,6	1,3	3,5	1,3	0,0	0,0
MCB11	3,8	1,4	3,5	1,3	3,2	1,2	0,0	0,0
MCB12	3,8	1,4	3,5	1,3	3,2	1,2	0,0	0,0
SMCB7	4,8	1,7	4,9	1,8	5,1	1,8	4,5	1,6
SMCB8	4,7	1,7	4,6	1,7	6	2,2	4,2	1,5

Results moisture content 2: θ = 0.22

	t0		t20		t22		t24	
	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)
MCB7	10,9	4,0	9,2	3,3	8,5	3,1	7,6	2,8
MCB8			9,2	3,3	8,5	3,1	7,3	2,6
MCB9			9,0	3,3	8,5	3,1	7,2	2,6
SMCB5	10,9	4,0	10,3	3,7	9,8	3,6	9,4	3,4
SMCB6	9,6	3,5	9,6	3,5	9,5	3,4	9,2	3,3

	t26		t28		t30		t44	
	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)
MCB7	6,2	2,2	4,9	1,8	4,0	1,5	0,0	0,0
MCB8	6,5	2,4	5,5	2,0	4,8	1,7	0,0	0,0
MCB9	6,8	2,5	5,7	2,1	5,1	1,8	0,0	0,0
SMCB5	9,4	3,4	9,5	3,4	9,9	3,6	10,7	3,9
SMCB6	9,1	3,3	9,1	3,3	9,1	3,3	9,5	3,4

Results moisture content 3: θ = 0.34

	t0		t20		t22		t24	
	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)
MCB4b	362,3	301,1	8,9	3,2	8,8	3,2	7,3	2,6
MCB5b	370,6		8,7	3,2	8,3	3,0	6,9	2,5
MCB6b	289,8		8,4	3,0	8,7	3,2	7,4	2,7
SMCB3b	286,6		10,4	3,8	10,8	3,9	10,1	3,7
SMCB4b	247,3		9,9	3,6	10,5	3,8	9,9	3,6

	t26		t28		t30		t44	
	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)
MCB4b	6,1	2,2	4,8	1,7	3,9	1,4	0,0	0,0
MCB5b	5,8	2,1	4,4	1,6	3,5	1,3	0,0	0,0
MCB6b	6,0	2,2	5,4	2,0	4,3	1,6	0,0	0,0
SMCB3b	9,7	3,5	10,0	3,6	10,0	3,6	11,9	4,3
SMCB4b	9,8	3,6	9,2	3,3	9,5	3,4	12,2	4,4

Results moisture content 4: θ(s) = 0.46

	t0		t20		t22		t24	
	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)
MCB1b	425,3	353,5	12,9	4,7	11,5	4,2	10,6	3,8
MCB2b	491,5	408,5	12,1	4,4	11,5	4,2	9,8	3,6
MCB3b	490,1	407,4	12,1	4,4	11,4	4,1	9,6	3,5
SMCB1b	469,5	390,2	13,8	5,0	13,8	5,0	13,1	4,7
SMCB2b	571,7	475,2	13,4	4,9	13,9	5,0	13,1	4,7

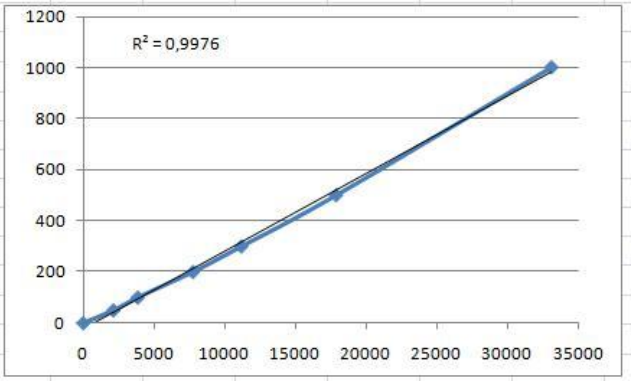
	t26		t28		t30		t44	
	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)	area	concentration (µg/L)
MCB1b	8,5	3,1	6,6	2,4	4,5	1,6	0,0	0,0
MCB2b	7,9	2,9	6,0	2,2	4,5	1,6	0,0	0,0
MCB3b	7,4	2,7	6,1	2,2	4,0	1,5	0,0	0,0
SMCB1b	12,3	4,5			12,6	4,6	16,3	5,9
SMCB2b	12,6	4,6	12,8	4,6	12,6	4,6	16,2	5,9

Appendix v Results batches at different initial concentrations

Calibrations used for the experiments at different initial concentrations.

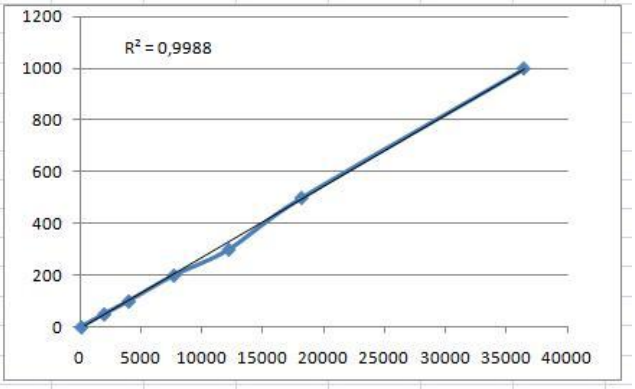
For KB1, KB2, KB3 and KB4

	area (uV/min)	concentration (ug/L)	slope
cal 0	50,6	0	32,88789
cal 1	2176,9	50	
cal 2	3894,0	100	
cal 3	7780,1	200	
cal 4	11208,0	300	
cal 5	17893,5	500	
cal 6	33085,2	1000	
cal 3	7420,8		
cal 3	7724,5		



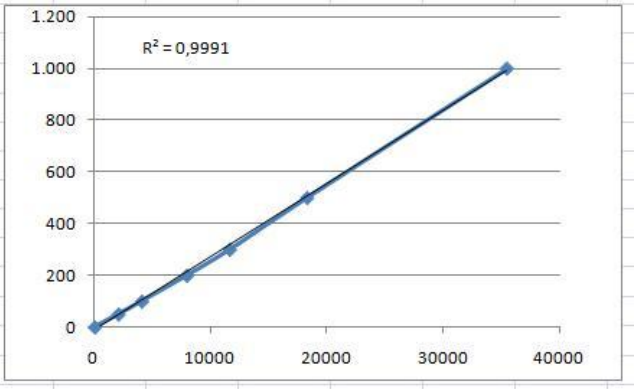
For KB7 and KB8

	area (uV/min)	concentration (ug/L)	slope
cal 0	67,8	0	36,1988
cal 1	1948,6	50	
cal 2	3960,2	100	
cal 3	7680,3	200	
cal 4	12189,7	300	
cal 5	18171,6	500	
cal 6	36443,8	1000	
cal 3	6802,1		
cal 3	7394,2		



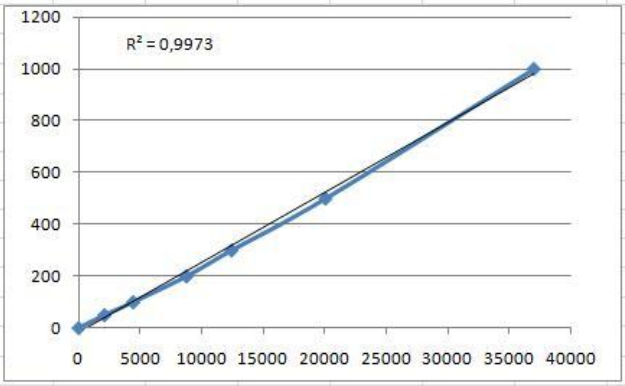
For KB5, KB6, KB9 and KB10

	area (uV/min)	concentration (ug/L)	slope
cal 0	36,2	0	35,21584
cal 1	2077,2	50	
cal 2	4085,2	100	
cal 3	7957,5	200	
cal 4	11646,8	300	
cal 5	18295,8	500	
cal 6	35478,9	1000	
cal 3	7514,2		
cal 3	7902,8		



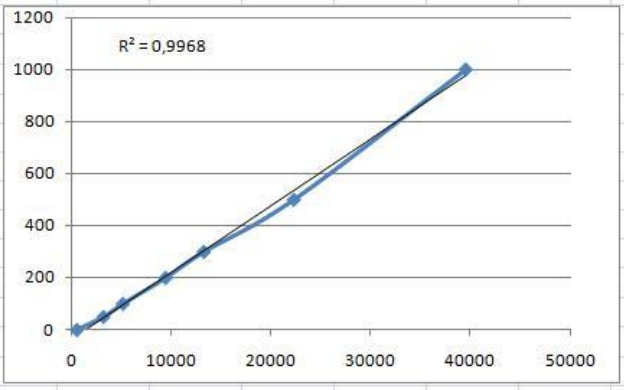
For KB11 and KB12

	area (uV/min)	concentration (ug/L)	slope
cal 0	32,2	0	36,85778
cal 1	2136,9	50	
cal 2	4466,4	100	
cal 3	8781,6	200	
cal 4	12448,2	300	
cal 5	20080,9	500	
cal 6	36987,1	1000	
cal 3	8368,8		
cal 3	8072,9		



For KB11+ and KB12+

	area (uV/min)	concentration (ug/L)	slope
cal 0	512,9	0	38,95806
cal 1	3181,1	50	
cal 2	5128,4	100	
cal 3	9411,3	200	
cal 4	13236,6	300	
cal 5	22270,3	500	
cal 6	39501,1	1000	
cal 3	9086,8		
cal 3	8987,0		



Results for the batches different initial concentrations.

initial	time (hours)	t0		t2		t4	
concentration (mg/L)	Batch	area (uV/min)	Concentration (mg/L)	area (uV/min)	Concentration (mg/L)	area (uV/min)	Concentration (mg/L)
1,0	KB1	16368,3	0,5	12579,7	0,4	12080,4	0,4
	KB2	16857,4	0,5	11241,7	0,3	9796,7	0,3
5,3	KB3	18788,3	2,9	13948,4	2,1	13681,8	2,1
	KB4	17021,8	2,6	15220,9	2,3	12333,7	1,9
10,4	KB5	17036,0	4,8	12865,4	3,7	12674,4	3,6
	KB6	14826,3	4,2	12156,1	3,5	12771,0	3,6
20,8	KB7	11670,5	6,4	9899,7	5,5	6922,4	3,8
	KB8	17305,0	9,6	9448,6	5,2	8155,2	4,5
52,2	KB9	12630,4	17,9	8360,3	11,9	7861,3	11,2
	KB10	9063,4	12,9	7864,6	11,2	7714,1	11,0
104,2	KB11			24166,2	65,6	19504,8	52,9
	KB12	19525,4	53,0	14336,1	38,9	14273,7	38,7
122,3	KB11+	10730,0		14817,4	76,1	13960,8	71,7
	KB12+	15487,8	79,5	16442,5	84,4	15928,8	81,8

initial	time (hours)	t6		t8	
concentration (mg/L)	Batch	area (uV/min)	Concentration (mg/L)	area (uV/min)	Concentration (mg/L)
1,0	KB1	11169,9	0,3	10911,6	0,3
	KB2	10215,0	0,3	9737,7	0,3
5,3	KB3	12130,6	1,8	12575,6	1,9
	KB4	12064,8	1,8	12169,3	1,9
10,4	KB5	14677,8	4,2	10872,6	3,1
	KB6	13859,6	3,9	10788,6	3,1
20,8	KB7	7137,5	3,9	5693,4	3,1
	KB8	6737,3	3,7	6447,8	3,6
52,2	KB9	7923,9	11,3	7970,7	11,3
	KB10	7639,5	10,8	7212,0	10,2
104,2	KB11	17794,5	48,3	16398,4	44,5
	KB12	14083,8	38,2	14572,5	39,5
122,3	KB11+	12963,5	66,6	13438,2	69,0
	KB12+	16356,5	84,0	16496,8	84,7