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In Situ Biorestoration of a subsoil, contaminated with oil

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ABSTRACT

As part of a Research Programme on Biological Treatment of Contaminated Soil the feasibility (technical aspects as well as costs and time) of an in situ biorestoration is studied.

As experimental site a gasoline contaminated site, a petrol station, has been chosen. Besides the characterization of the soil, the spreading of the contamination is described.

From laboratory studies into the possible stimulation of the biodegradation rate in the soil of the site it has been concluded that by adjustment of the abiotic conditions (nutrients, water saturation, pH) and possibly the biotic conditions (increase of autochtonous or addition of allochtonos biomass) the biodegradation rate, determined as carbon dioxide production, could be increased from 1 to 10 mg $C.kg^{-1}.d^{-1}$. It was concluded that the biodegradation was limited by the availability of the gasoline components.

Leaching experiments have shown the slow release by dissolution of the gasoline. After an initial high leaching a maximum rate of 5 mg $C.kg^{-1}.d^{-1}$ was found, depending on the percolation rate.

Column experiments are in progress. The preliminary results confirm the technical feasibility and show the use of hydrogen peroxide as alternative oxygen source and neglect of nitrate as electron acceptor.

A preliminary hydrological design of the clean-up is shown.

1. INTRODUCTION

The past ten years several thousand cases of soil contamination have been discovered in the Netherlands. It is expected that in about 1600 of these cases the soil has to be cleaned.
An important part of the costs of clean-up is involved with excavation of the contaminated soil. These costs could be reduced considerably by an in-situ treatment of the location, especially in case of contamination at greater depth or under buildings.
In the framework of the Research Program on Biological Soil Clean-Up Techniques the Ministry of Housing, Physical Planning and Environment has assigned a research project to the RIVM in co-operation with the Division Technology for Society of TNO.

The aim of the research project is to study the feasibility of a biological in-situ treatment, including aspects of costs and time.

As contamination an oil pollution has been chosen, because a large number of sites are contaminated with oil and oil consists of components which are in principal biodegradable. Oil contamination of the subsoil might threaten eventually the quality of drinking-water.

The project includes three stages:

1. A literature study and the selection of the experimental site;
2. Laboratory research and detailed (geohydrological and chemical) investigation of the site;
3. Design and execution of the clean-up.

The literature study summarized the theoretical base of in-situ treatment and revealed four in-situ biorestoration experiments in the subsoil. Three of these experiments were carried out at sites contaminated with oil. The results of the treatments varied. In all cases the ground-water was clean after 0.5 to 2 years. However, in two cases the contaminant was still present in the soil at rather high concentrations. Concerning the technical aspects and the final results, it was concluded that biological in-situ treatment offered reasonable prospectives.

This paper describes the results of the site investigation, the laboratory research (biodegradation and leaching experiments) and the preliminary results of experiments in large stainless steel columns. A preliminary design of the clean-up is also given.

2. DESCRIPTION OF THE SITE

The site is situated at Asten, in the province of Noord-Brabant. It is a petrol-station, where the soil is contaminated with gasoline and a small quantity of diesel oil.
The contamination has been caused by a leaking tank of the petrol station. At least 30,000 liters of normal gasoline has been lost, and about 1500 m^3 of soil has been contaminated. The contaminated area is about 25x25 square meters. The horizontal spreading of the contamination is given by the dotted line in figure 1. This figure also shows the location of the monitoring wells, which have been placed to get a good impression of quantity and the composition of the contamination. In this wells the free product was measured up to 150 cm. About 20,000 liters of free product has been recovered, by pumping off after the discovery of the contamination.

At the location, the soil is generally sandy, nearly without any organic material (< 0,05%). Until 15-20 meters below surface level, some layers of clay and loam are present. At 20 meters, there is a thick layer of porous material. The ground-water level increased from 2.5 to 3.5 meter below surface level during the pumping off. Figure 2 shows the composition of the soil at the site.

Several samples of soil and ground-water have been analyzed. The concentrations of gasoline at different depths in the soil are also indicated in figure 2. Concentrations varied mainly between 1,000 and 5,000 mg/kg. The concentrations of some individual gasoline components in the ground-water are given in table 1.

TABLE 1. Concentrations of some individual gasoline components in ground-water at the site. ($ug.1^{-1}$)

Filter,	depth (cm)	BZ	Tol	EB	Napht
B1	400	550	405	1	13
C1	400	145000	200000	18000	2000
D1	400	250	680	30	18
E1	400	14000	14000	1490	338
A2	700	260	80	20	5
B2	700	10	40	10	4
C2	700	2	11	4	1
D2	700	4	8	4	2
E2	700	110	190	14	4
E3	1500	15	40	9	3

Reference values according to the guidelines of the Dutch government (Moen, 1986).

	BZ	Tol	EB	Napht
A	0,2	0,5	0,5	0,2
B	1	15	20	7
C	5	50	60	30

BZ - Benzene

Tol - Toluene

EB - Ethylbenzene

Napht - Naphthalene

3. BIODEGRADATION RESEARCH

The aim of this research was to determine the biodegradation capacity of the contaminated soil and the possibilities for stimulation of the biodegradation activity by changing the (a)biotic conditions.
The biodegradation experiments have been performed as batch experiments with contaminated soil from the site. The degradation has been determined by following the oxygen uptake (Sapromat test) or the carbon dioxide production. The parameters investigated with regard to the stimulation of the biodegradation were: C-N-P ratio, nitrogen source, addition of active oil degraders, addition of sodium acetate, moisture content, pH, and alternative oxygen sources (nitrate and hydrogen peroxide).
Because of the very low organic carbon content (< 0,05%) of the soil itself, the blank carbon dioxide production was very low with a mean rate less than 1 mg $C.kg^{-1}.d^{-1}$.
Figure 3 shows the results of research into the influence of the C-N-P ratio on the degradation with KNO_3 as N source. Other N sources as NH_4NO_3, NH_4Cl and $(NH_4)_2SO_4$ were also investigated. The C-N-P ratio as well as the kind of N source had a relative small influence on the degradation. However, as figure 4 indicates, at an increased phosphate dosage a higher degradation rate was found. This was due to the binding of phosphate to the soil. In spite of a good oxygen and nutrients supply, in all cases a relatively low microbial activity was observed in the Asten soil. Experiments with other types of soil confirmed this observation, as indicated in figure 5.

On the basis of the above mentioned results further research was focussed on the determination of the biodegradation limiting factors. Figure 6 summarizes the effect of different factors on the biodegradation rate. The degradation activity was enhanced most by the addition of seeding material from a landfarm. In case of the addition of sodium acetate an initial increase of the oil degradation rate was observed, probably caused by an initial higher biomass due to growth on sodium acetate. An increased degradation rate was also found by the saturation of soil with water and addition of phosphate and nitrate. A neutral pH and buffering also contributed to favourable conditions. Under these stimulated conditions the degradation rate varied between 5 and 40 mg $C.kg^{-1}.d^{-1}$ and the estimated average rate was about 10 mg $C.kg^{-1}.d^{-1}$.

Oxygen diffusion was not the limiting factor in the laboratory tests. Under field conditions however, biodegradation is probably restricted by oxygen transfer. Therefore, hydrogen peroxide and nitrate were investigated as alternative oxygen sources. The results, shown in figure 7 indicated that hydrogen peroxide could be a suitable oxygen source. Nitrate seemed to be inefficient because a decrease of the biodegradation rate was observed. Hydrogen peroxide had no toxic effect on the micro-organisms up to a concentration of 200 mg $H_2O_2.kg^{-1}$.

The following conclusions were drawn:

- The biodegradation activity of Asten soil was stimulated mainly by inoculation, working under saturated and neutral pH conditions and addition of nitrogen and especially phosphate. The biodegradation rate did not increase during the experiments, but remained constant in time. This may imply that an unknown limiting factor consists which restricts the biodegradation.
- From mass balance studies it appeared that the degradation rate of the gasoline contamination in the Asten soil was probably determined by the rate at which the oilcomponents became available. In the laboratory experiments the availability was determined predominantly by the dissolution and volatilization processes, but under field conditions only the dissolution process prevails. This aspect was investigated further by leaching experiments.

4. LEACHING EXPERIMENTS

The key question in the clean-up of an oil contaminated soil is the removal of the immobile rest-saturation of oil.
The aim of the leaching experiments was to determine the availability of the oil components for the microbial degradation and to try to enhance the availability.

The leaching test has been performed by applying a continuous flow of a nutrient solution through a soil column which had an gasoline rest-saturation just above the ground-water level. The bioavailability of the gasoline was determined by comparison of the dissolved organic carbon concentrations in the leachates of contaminated and clean columns. In the leaching experiments the effect of the percolation rate, the concentration of the gasoline contaminant in the soil and the addition of detergents was determined.

The experiments have shown that in test set-up the leaching of the gasoline, which was present as a rest saturation in the soil, is small and hardly influenced by the concentration of the contaminant. At a rest saturation of gasoline of 5% pore volume the concentration of leached carbon is about 20-30 mg $C.l^{-1}$ after an initial high concentration of 20-200 mg $C.l^{-1}$ (figure 9). The leaching rate after the initital phase is calculated for 5 mg $C.kg^{-1}.d^{-1}$ at the highest percolation rate (0,4 $m.d^{-1}$).
A linear relationship was determined for the total quantity of organic carbon leached from the column and the flow rate (figure 10). An immediate equilibrium was suggested for the partition of the oil components to the aqueous phase.
The mobility of the gasoline components could not be promoted by the application of detergents in the nutrient solution because of clogging of the soil. This clogging might be caused by biomass grown on the biodegradable detergents or what seemed more probable, by an interaction between the oil, water, detergent and solid phase. In contrast to what was expected a decreased leaching of gasoline was found when detergents were applied (figure 11). Negative leaching in course of time is explained by the leaching of more organic carbon from clean soil columns than from contaminated soil columns.

5. EXPERIMENTS IN LARGE STAINLESS STEEL COLUMNS

The combined leaching and biodegradation processes were studied in large stainless steel columns to confirm the findings from the former experiments in continuous flow experiments and to investigate possible oxygen limitations and alternative oxygen sources. The stainless steel columns had a 20 cm diameter and a length of 70 cm. Six columns were filled with undisturbed profiles at depths from 2,5 to 3,2 m below surface level. In the laboratory they where build-up in a climate room (10 °C). All columns were percolated with aerated artificial rainwater in the up-flow mode and all presented different modes of action.

Column 1: has no other features and is used as a blank.

Column 2: addition of nitrate (12,5 mg $N.1^{-1}$ as NH_4NO_3) and phosphate (15 mg $P.1^{-1}$ as a buffer of KH_2PO_4 and Na_2HPO_4, pH 6,9 at 10 °C) in the rainwater.

Column 3: as column 2 with H_2O_2 as an additional oxygen source (conc. up to 200 $mg.1^{-1}$)

Column 4: as column 2 with an initial addition of sodium acetate to stimulate the biomass

Column 5: as column 2 with potassium nitrate as an additional oxygen source (conc. up to 100 mg $N.1^{-1}$)

Column 6: as column 2 but the effluent is recirculated after a treatment step.

The column experiments were started at the first of July.
To follow the processes in the columns the following parameters were measured: nitrate, nitrite, ammonium, phosphate, oxygen, dissolved organic carbon, gasoline and its individual components (GC-analysis) and colony count.

The percolation rate through the columns was reasonably constant and was increased after 100 days from 0,4 $m.d^{-1}$ to 0,8 $m.d.^{-1}$ for the necessary input of dissolved oxygen to meet the mean degradation rate of 10 mg $C.kg^{-1}.d^{-1}$.
Although no final results can be given, the following preliminary results are reported.

In all columns, except the recirculated one, about 20% of the gasoline present (GC-analysis) was leached in the first week (figure 12) at concentrations upto 400 mg gasoline.l^{-1}. Table 2 shows the total quantities and the percentages leached of three of the columns after 110 days. After 110 days 39% was leached in the blank column (no. 1) and 27% in column no. 3 (hydrogen peroxide). The behaviour of the various gasoline components could be different, considering percentages and time course. Toluene (figure 13) was leached for 70% in the blank column and 50% in the hydrogen peroxide column, but in both columns no longer after 40 days, while 1,3,5-trimethylbenzene (figure 14) was still present in the leachate after 110 days with a cumulative leaching of 50% in column no. 1 and 30% in column no. 3. Octane was only leached for less than 2% in all columns.

As table 2 shows, generally lower leaching percentages were observed for column no. 3. However, the total leached quantities were comparable except for ethylbenzene. The leaching of the gasoline was calculated for about 100 mg C.kg^{-1}.d^{-1} during the first week and 5 mg C.kg^{-1}.d^{-1} in the next period, during which no decrease in the leaching was observed. These leaching results corresponded well with those of the laboratory leaching experiments.

The oxygen profiles measured over the column at several sampling dates (figure 15) showed oxygen to be consumed in the first few centimeters of the column. The addition of hydrogen peroxide provided much more oxygen to the microbial population as shown in the oxygen profile. Nitrate was not used in any of the columns as alternative oxygen source (figure 16).

Recirculation of the effluent after a deironization step over a sand and gravel filter, gave no technical problems and offers a possibility to reduce the necessary water-volume.

The experiments are continued until a considerable biodegradation of the gasoline is calculated, based on oxygen input, or the oxygen profile indicates a decrease in the biodegradation. However, the actual biodegradation and clean-up of the soil has to be determined from analysis of the soil after the columns have been dismantled.

TABLE 2. Leaching of gasoline and individual components out of three of the columns (numbers 1 to 3)

	Total leached quantity (g)			Leached percentage		
	column 1	column 2	column 3	column 1	column 2	column
gasoline	47,9	43,8	40,1	39	35	27
benzene	7,4	8,1	7,0	738	579	463
toluene	4,8	4,4	4,8	71	61	46
ethylbenzene	1,4	1,6	0,85	55	69	17
naphthalene	0,8	1,2	0,85	50	70	47
octane	0,02	0,01	0,02	1,8	1,2	1,5
1,3,5-trimethylbenzene	1,1	0,8	0,75	49	35	28
m + p-xylene	4,1	4,2	4,4	40	44	35

6. DESIGN FOR THE CLEAN-UP OPERATION

Based on a degradation rate of 10 mg C.kg^{-1}.d^{-1}, a daily throughput of about 1850 m^{3} water in the area of 625 m^{2} has been calculated and used for the hydrological design of the clean-up operation.

It has been decided to infiltrate the water by drains, which will be installed at a depth of about 1 meter below surface. Withdrawal of the infiltrated water will take place by pumping wells which are spread on the contaminated area. The rate of withdrawal will be larger than the rate of infiltration.

For the optimal design of the drains and the pumping well the computer program CONTOUR is used. As a first non-optimal example a design has been made which is presented in figures 17 and 18.

Boundary conditions for the design are the hydrological isolation of the contamination in vertical and horizontal sense and the possible increases and decreases of the ground-water in the area. Special attention has to be paid to the percolation under the buildings on the site.

7. SUMMARIZING CONCLUSIONS

From the laboratory experiments it was found that a stimulated biological treatment gives rise to a clean-up period of 1.5 years, based on a degradation rate of 10 mg $C.kg^{-1}.d^{-1}$, measured under laboratory conditions. The started column experiments show the oxygen limitation and possible use of hydrogen peroxide as alternative oxygen source. Nitrate seems no suitable oxygen source. Results of the residual gasoline components analysis are necessary to confirm all findings of the laboratory tests.
A preliminary hydrological design seems to confirm the technical feasibility of this project.
It is expected to start the execution of the clean-up in the spring of next year. Our experiments until now have learned that in-situ biological treatment offers a good alternative with some advantages over excavation combined with physical, chemical or biological treatment.

8. REFERENCES

Berg, R.van den, Eikelboom, D.H., en Verheul, J.H.A.M., 1987. In situ biorestauratie van een met olie verontreinigde bodem. Resultaten van het laboratoriumonderzoek. RIVM rapport 728518002 (RIVM, Bilthoven).

Eikelboom, D.H., 1985. In situ biorestauratie van een met olieprodukten verontreinigde ondergrond. Een literatuurstudie. TNO rapport R85/320 (TNO, Delft).

Eikelboom, D.H., and Verheul, J.H.A.M., 1986. In situ biological treatment of a contaminated subsoil. Pp. 686-692. In: Contaminated Soil (J.W.Assink and W.J.van den Brink, Eds.). Martinus Nijhoff, Dordrecht.

Moen, J.E.T., Cornet, J.P., and Evers, C.W.A., 1986. Soil protection and remedial actions: Criteria for decision making and standardization of requirements. Pp. 441-448. In: Contaminated Soil (J.W.Assink and W.J.van den Brink, Eds.). Martinus Nijhoff, Dordrecht.

Verheul, J.H.A.M., Berg, R.van den, en Eikelboom, D.H., 1987. In situ biorestauratie van een met olie verontreinigde bodem. Nader onderzoek rapport. RIVM rapport 728518001 (RIVM, Bilthoven).

Figure 1. Map of the selected site at Asten (N.Br.). The dotted line gives the contour of the horizontal spreading of the gasoline. The leaking tank is indicated by N.T.

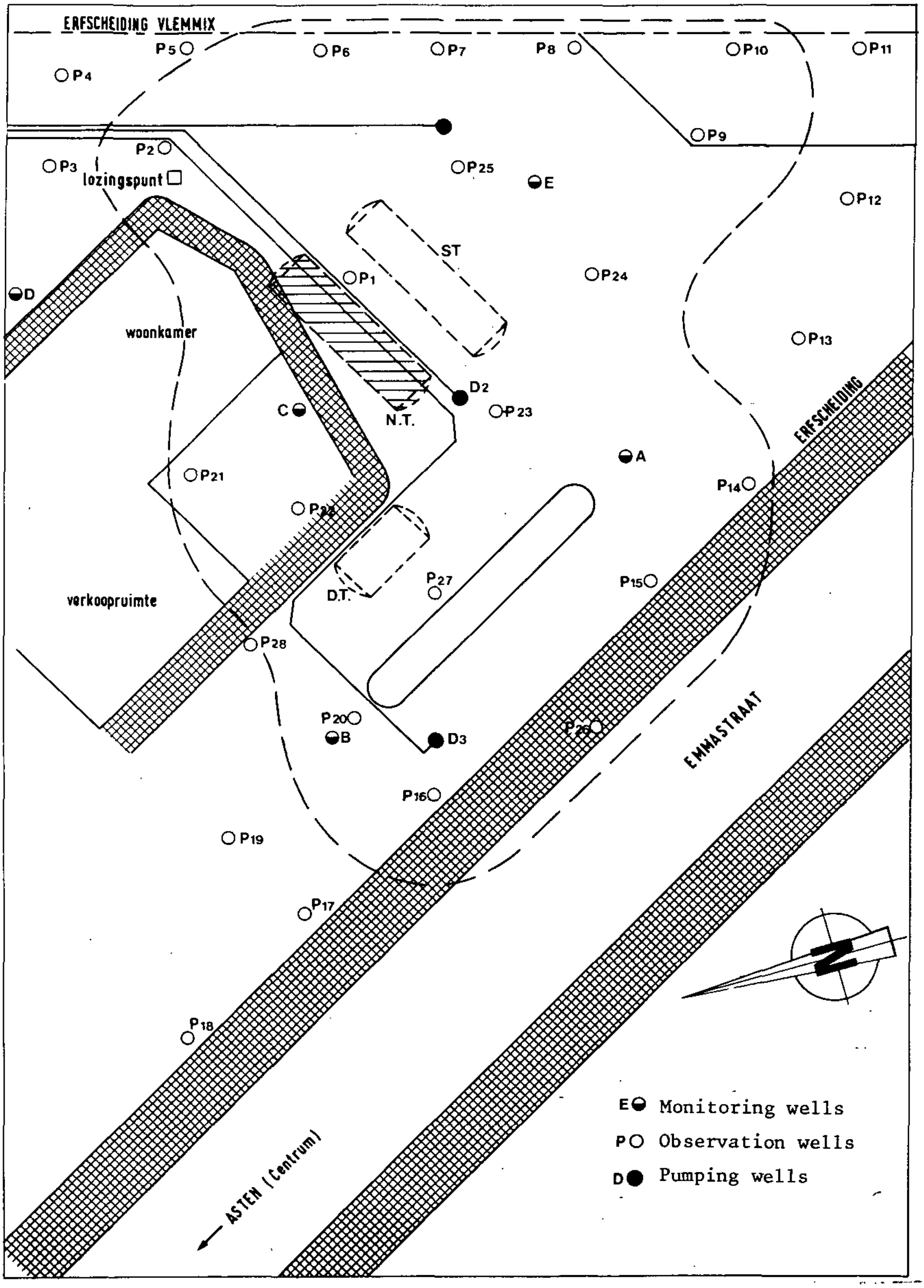

Figure 2. Soil profile and average concentrations of gasoline at the contaminated site (Asten), compared with the A-B-C reference values of the Dutch government.

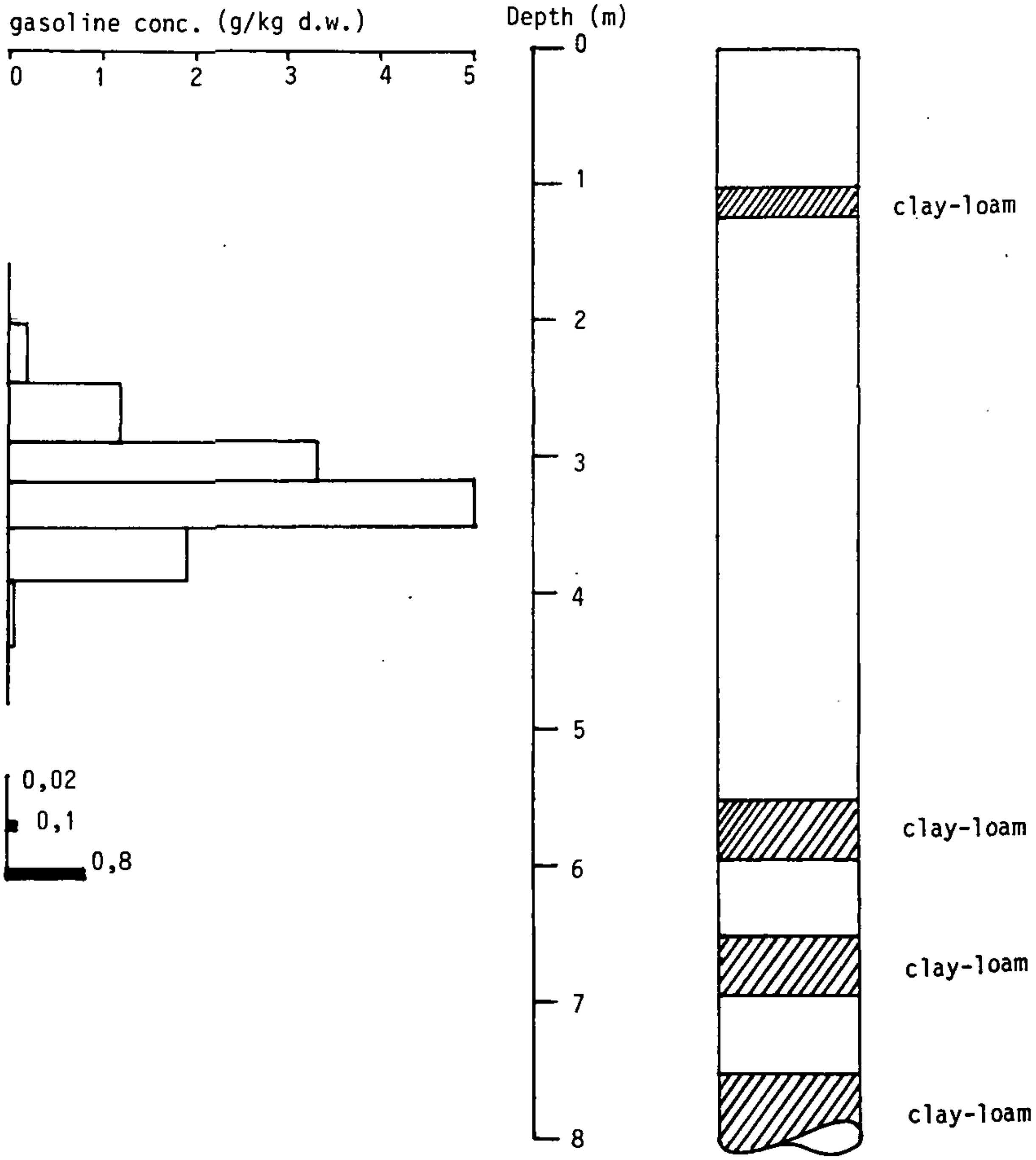

Figure 3. Influence of different CNP-ratio's on the biodegradation of gasoline, measured as CO_2-production (mg C/kg). Nitrogen source: KNO_3. Gasoline concentration: 800 mg C/kg.

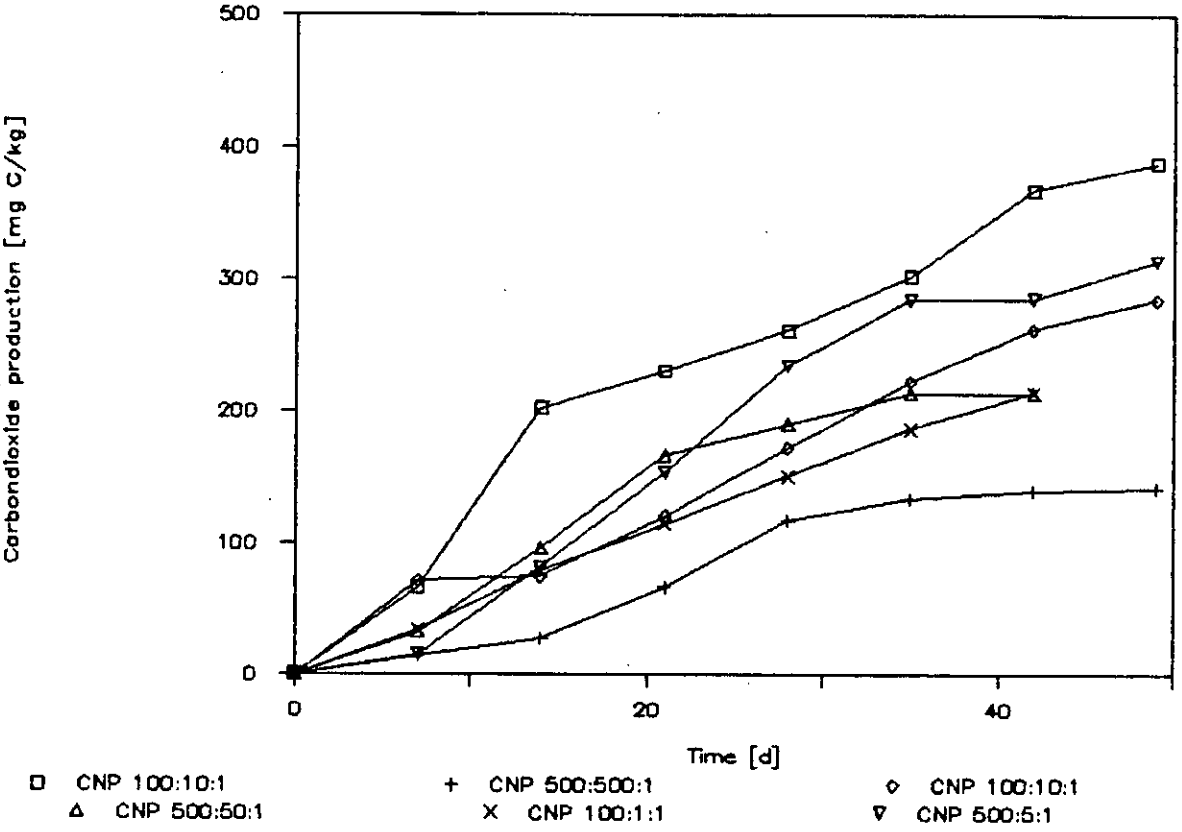

Figure 4. Influence of different C-P ratio's on the biodegradation of gasoline, measured as CO_2-production (mg C/kg). C-N ratio: 100:10. Gasoline concentration 2600 mg C/kg.

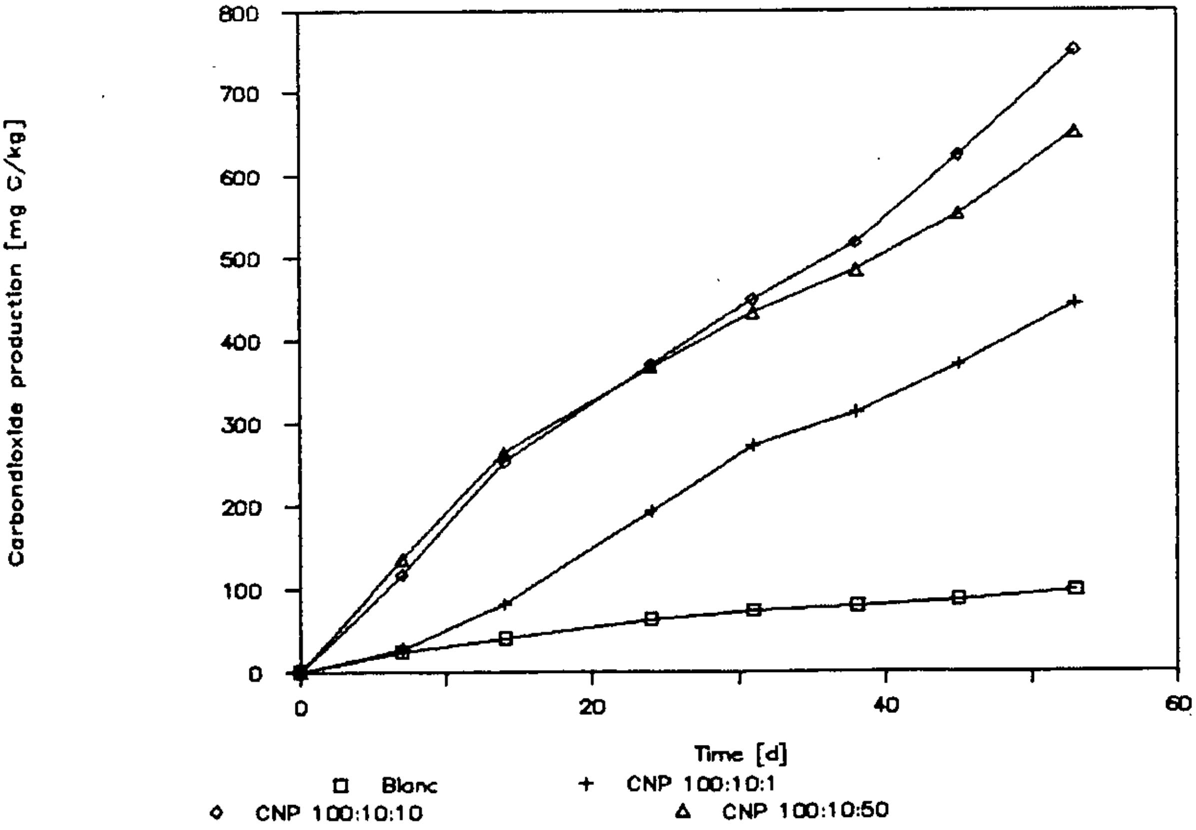

Figure 5. Biodegradation , measured as CO_2-production (mg C/kg) of gasoline, diesel and gasoil (concentrations 1000 mg C/kg) in Asten soil and a topsoil (TNO).

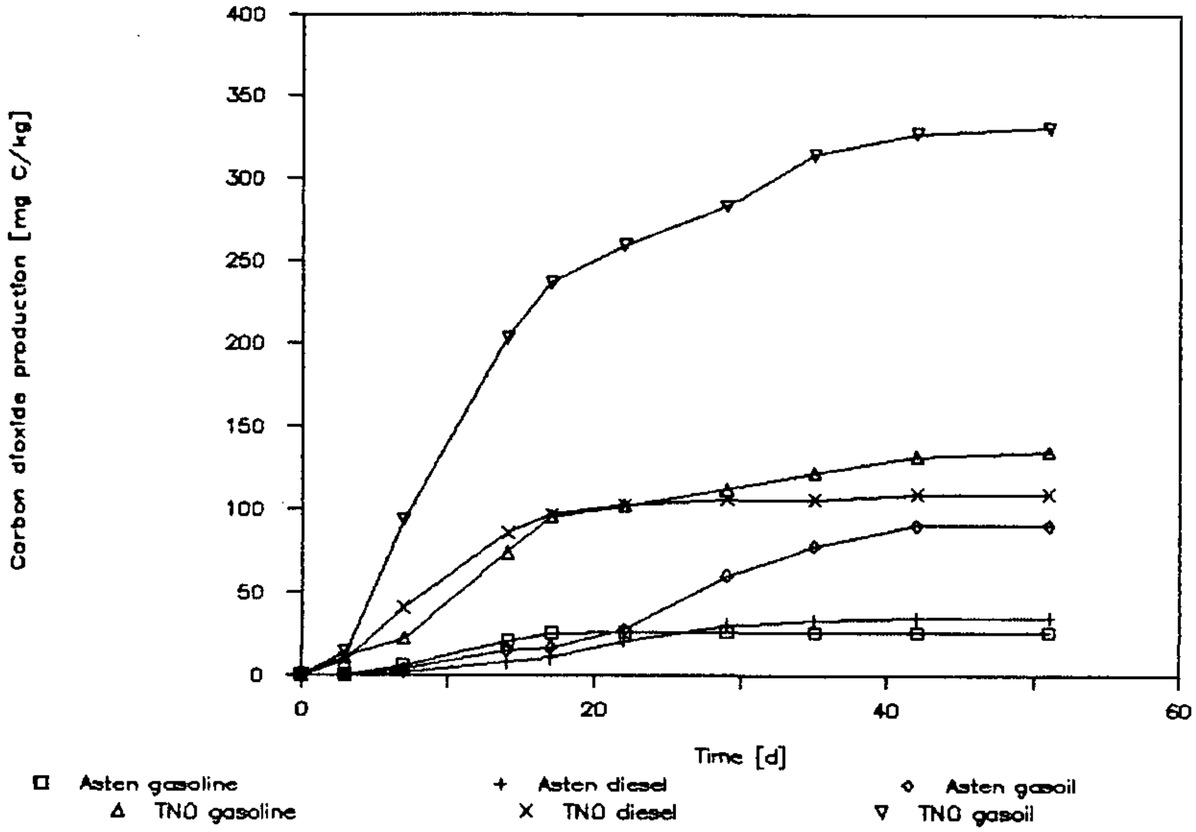

Figure 6. Biodegradation of gasoline measured as CO_2-production (mg C/kg). Gasoline concentrations: 3000-5700 mg C/kg. Effects of water saturation, nutrient addition (CNP ratio 100:10:1 and 100:10:5) and seeding with KONI soil: 50 g/kg.

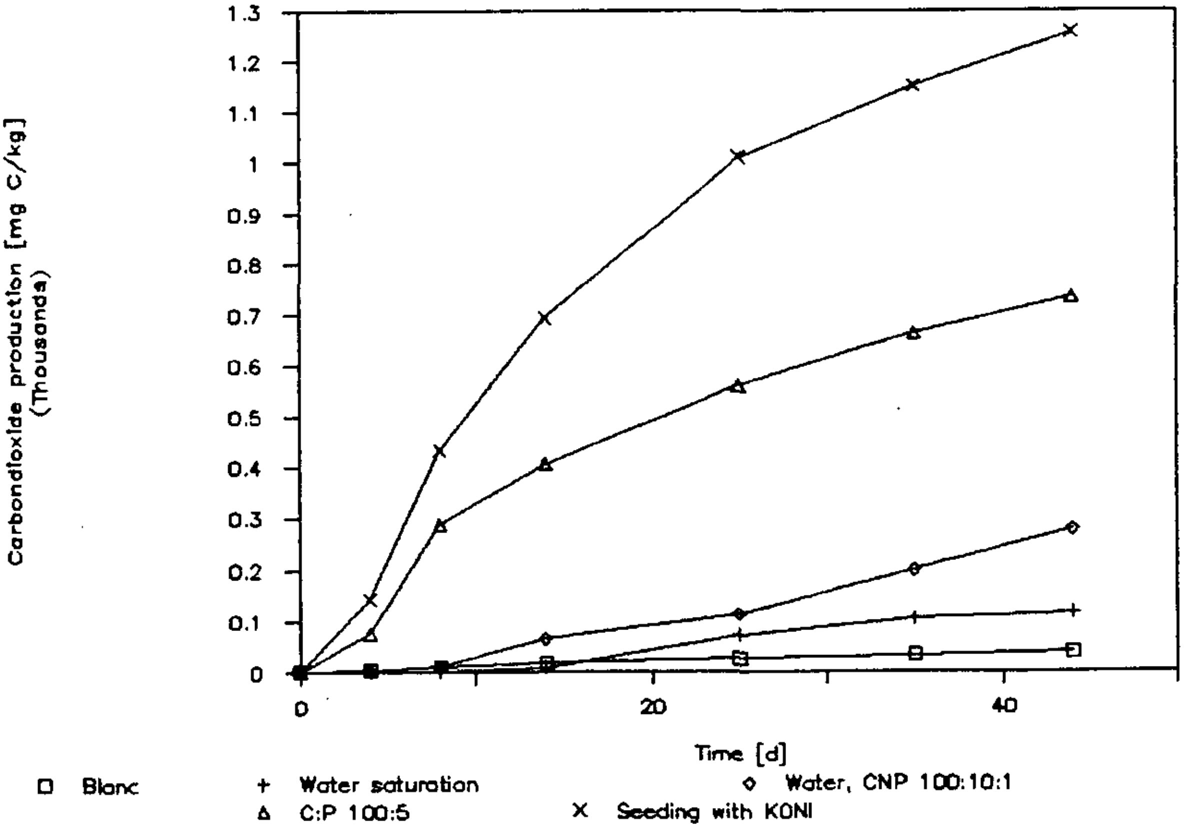

Figure 7. Influence of different oxygen sources on the biodegradation of gasoline , measured as CO_2-production (mg C/kg). When hydrogen peroxide was used the soil was seeded with KONI soil (50 g/kg). Gasoline concentration: 800 mg C/kg.

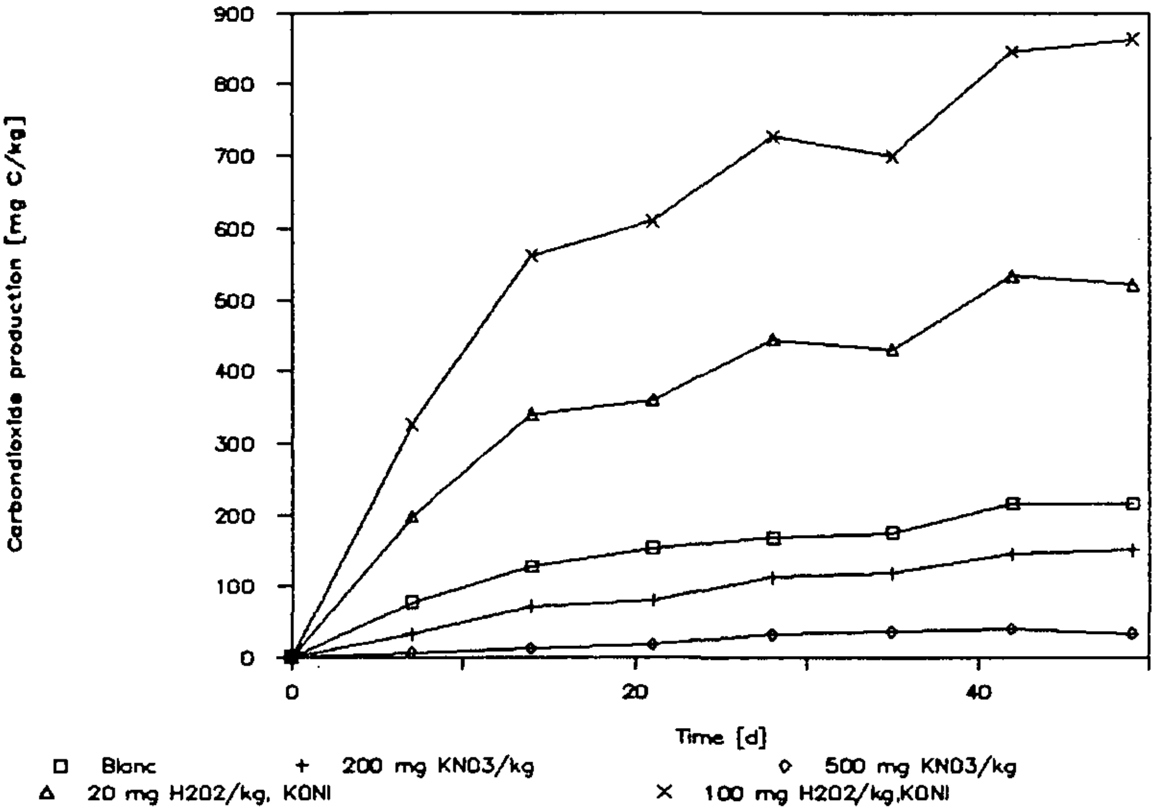

Figure 8. Influence of hydrogen peroxide addition (25 - 200 mg H_2O_2/kg) on the biodegradation of gasoline, measured as CO_2-production (mg C/kg). Gasoline concentration: 2600 mg C/kg. H_2O_2 was added at t=0 and at t=7 days.

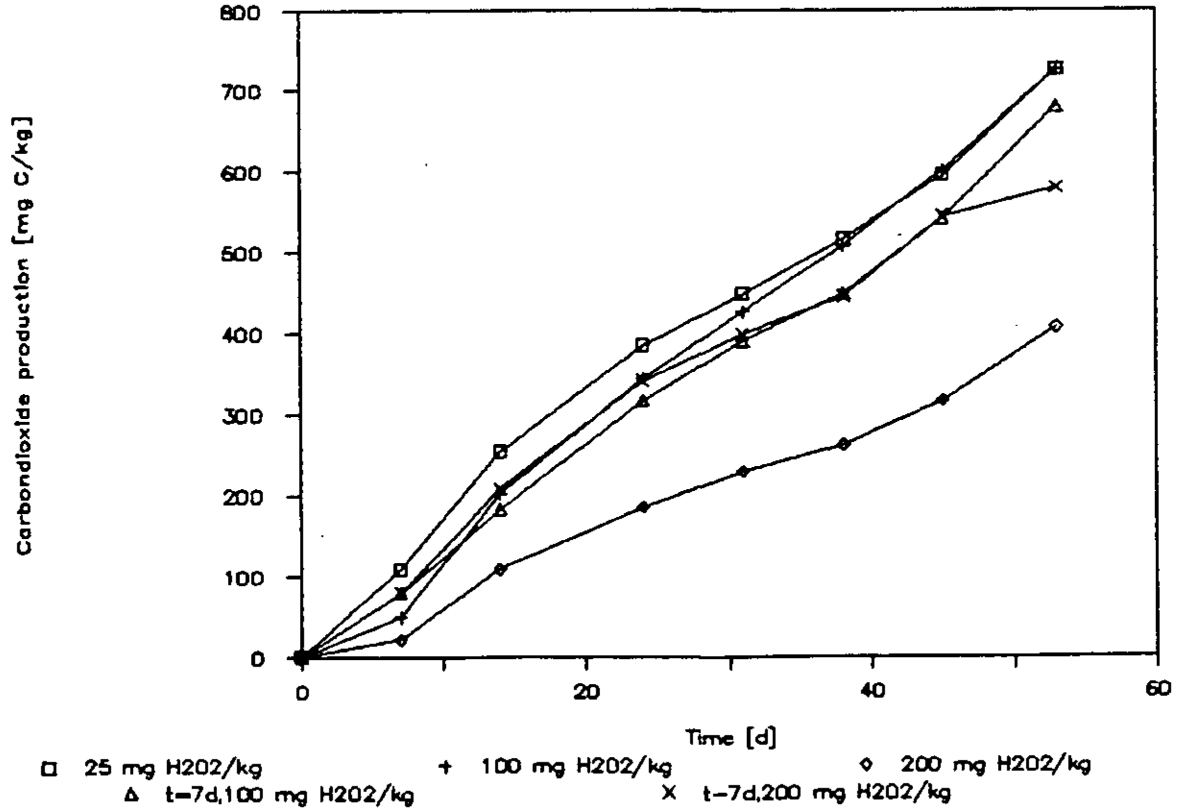

Figure 9. Concentration of the leached organic carbon (mg C/l) from continuous flow through columns as a function of the percolation time (h). Gasoline concentration 5% pore volume.

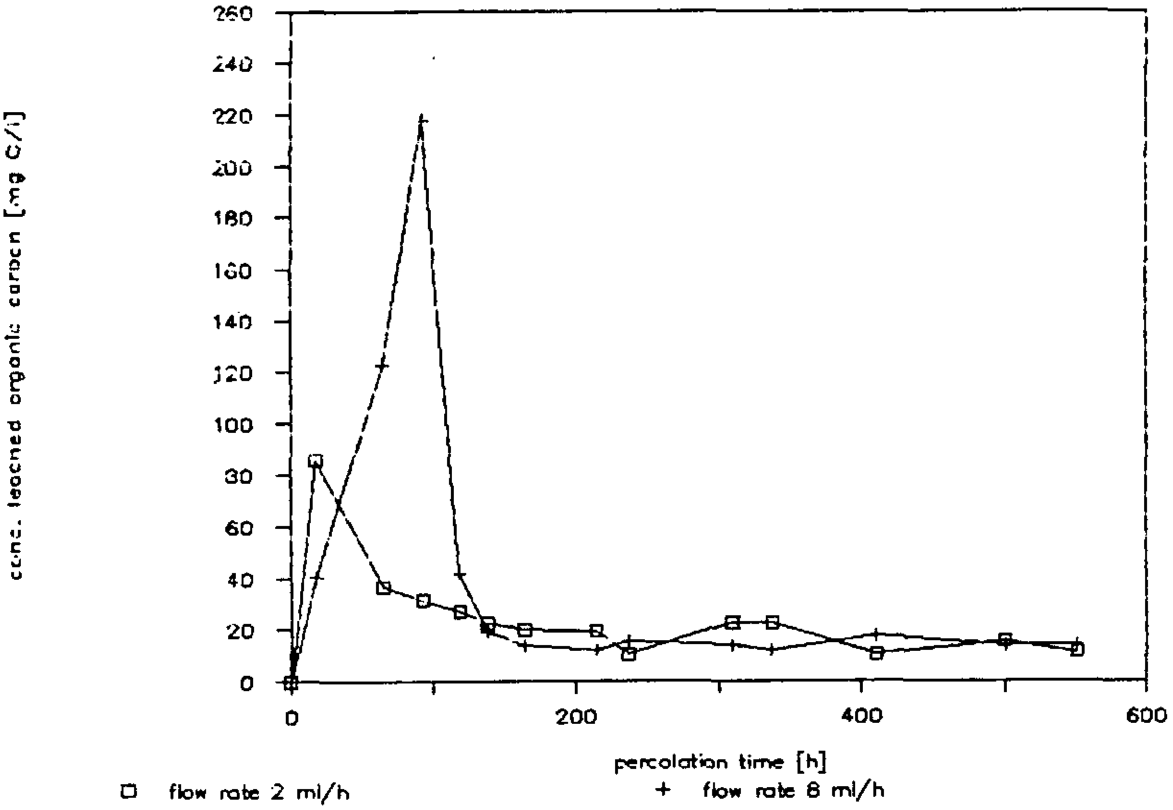

Figure 10. Influence of the flow on the leached organic carbon (mg C) from continuous flow through columns with 5% or 15% pore volume gasoline concentration.

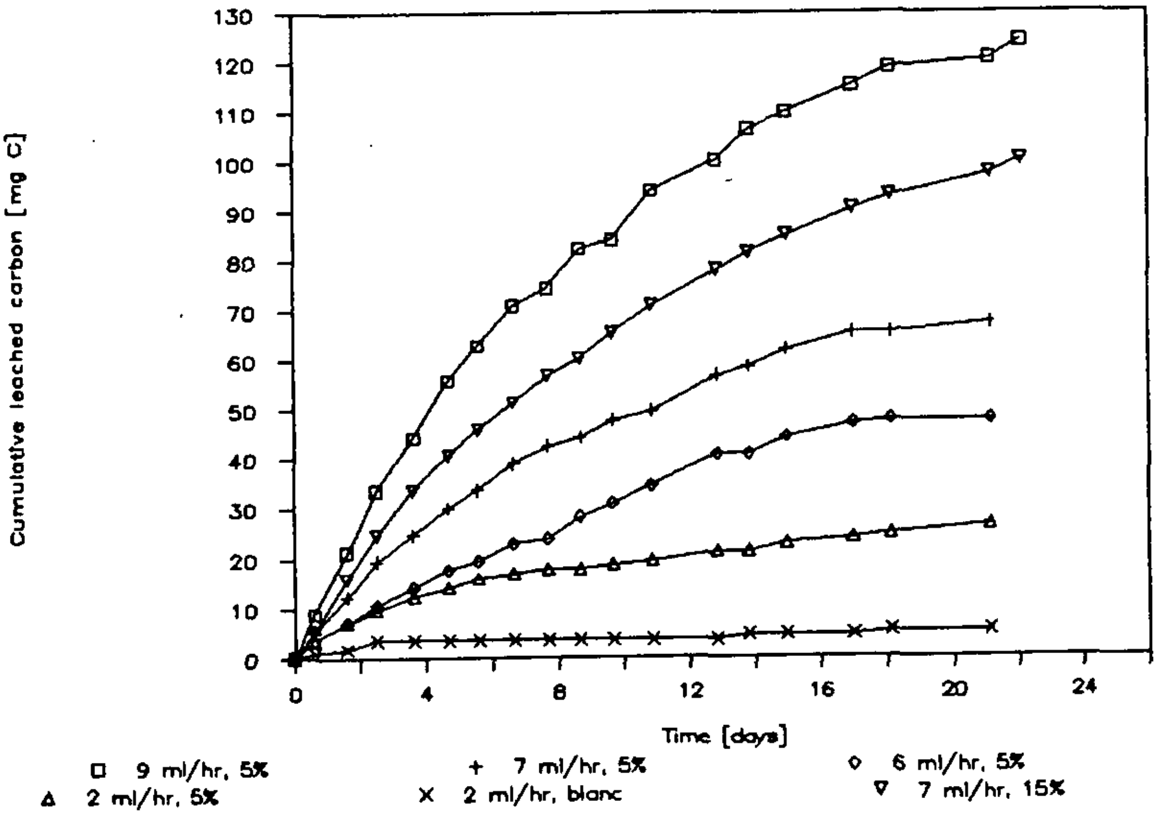

Figure 11. Influence of the detergents Brij 35, Tween 80 and SLS (250 mg/l) on the leaching of gasoline from continuous flow through columns. Shown is the difference in cumulative leached organic carbon between a column with gasoline/detergent and a column with detergent only.

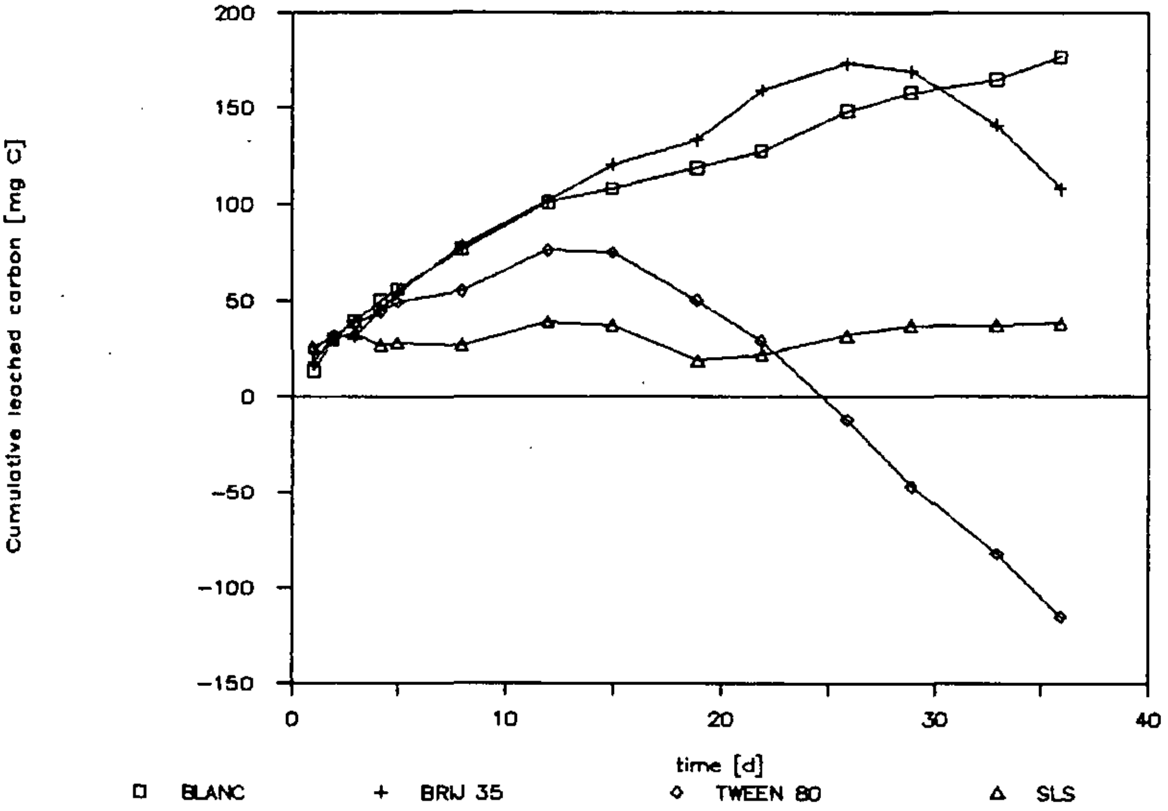

Figure 12. Cumulative leaching of gasoline, measured by GC-analysis (mg gasoline), as a function of the percolation time for three of the columns (numbers 1 to 3).

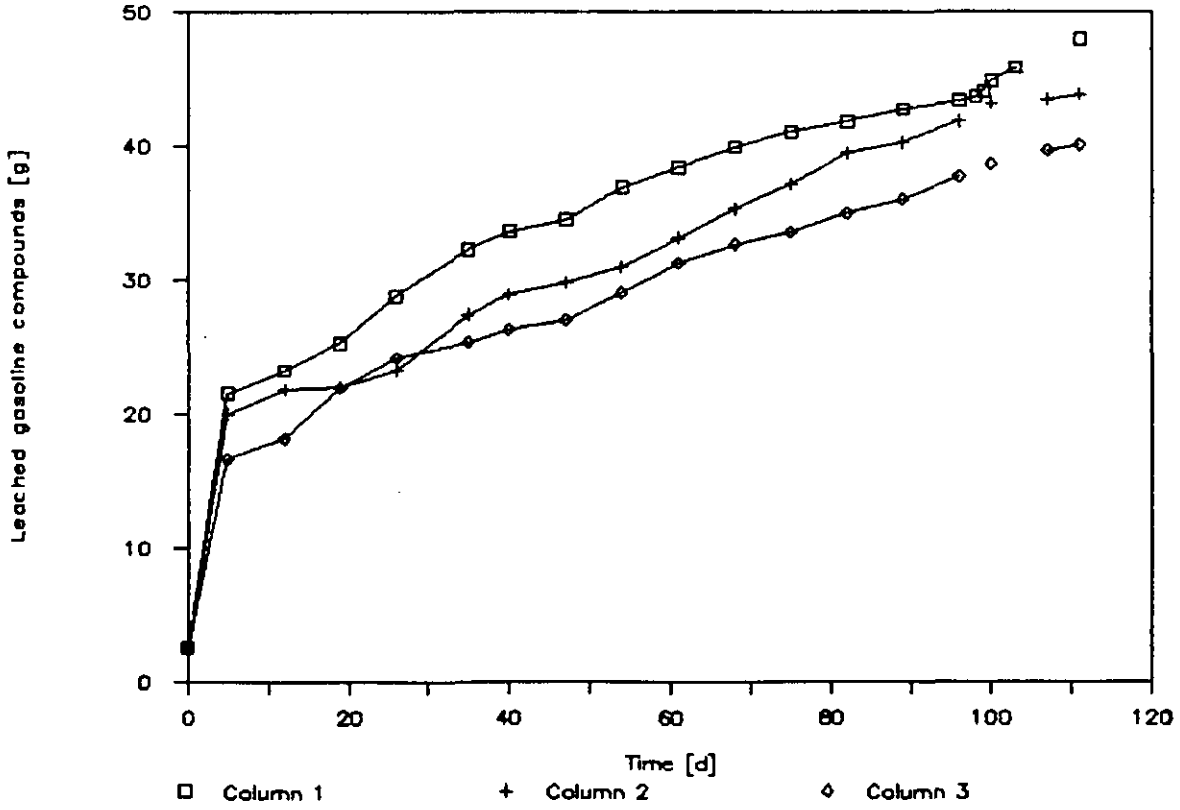

Figure 13. Leaching of toluene (mg/l) as a function of the percolation time for column no. 2 (nutrients addition and buffered). The latter part of the curve is shown enlarged.

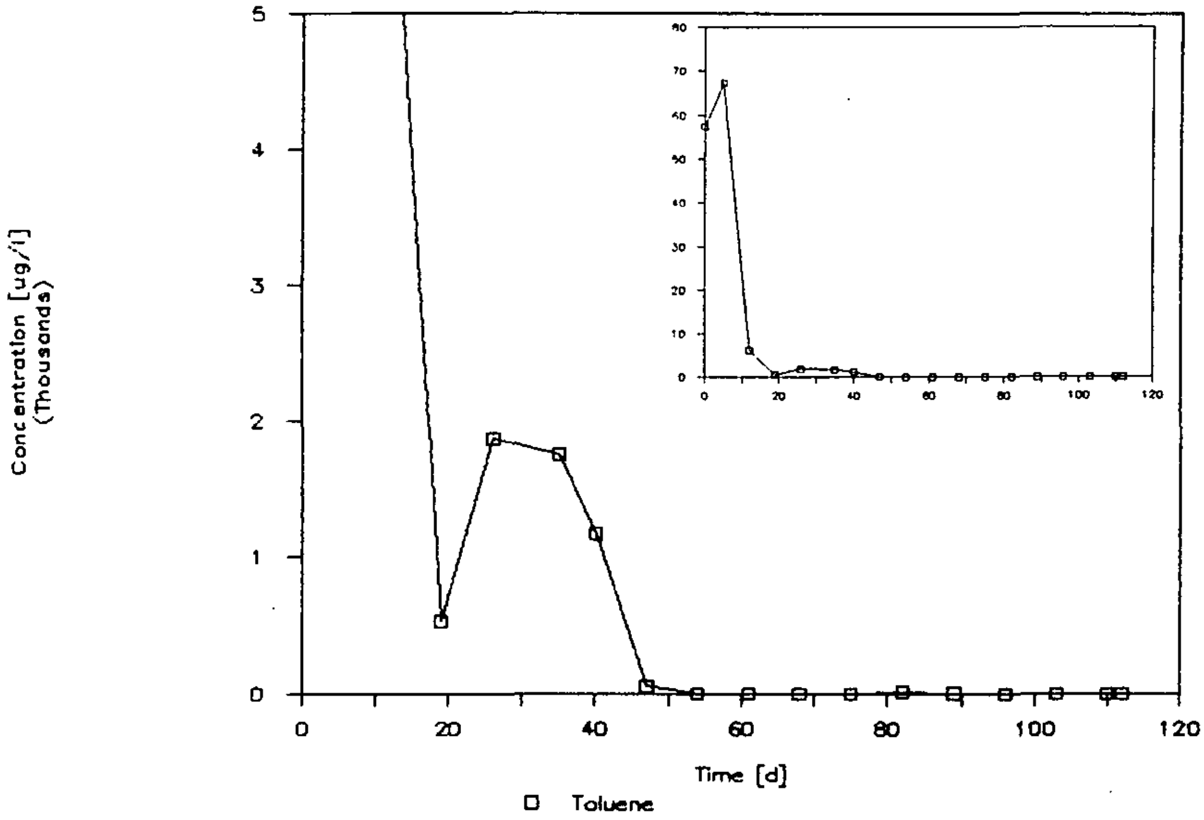

Figure 14. Leaching of 1,3,5-trimethylbenzene (mg/l) as a function of the percolation time for column no. 2 (nutrients addition and bufferd).

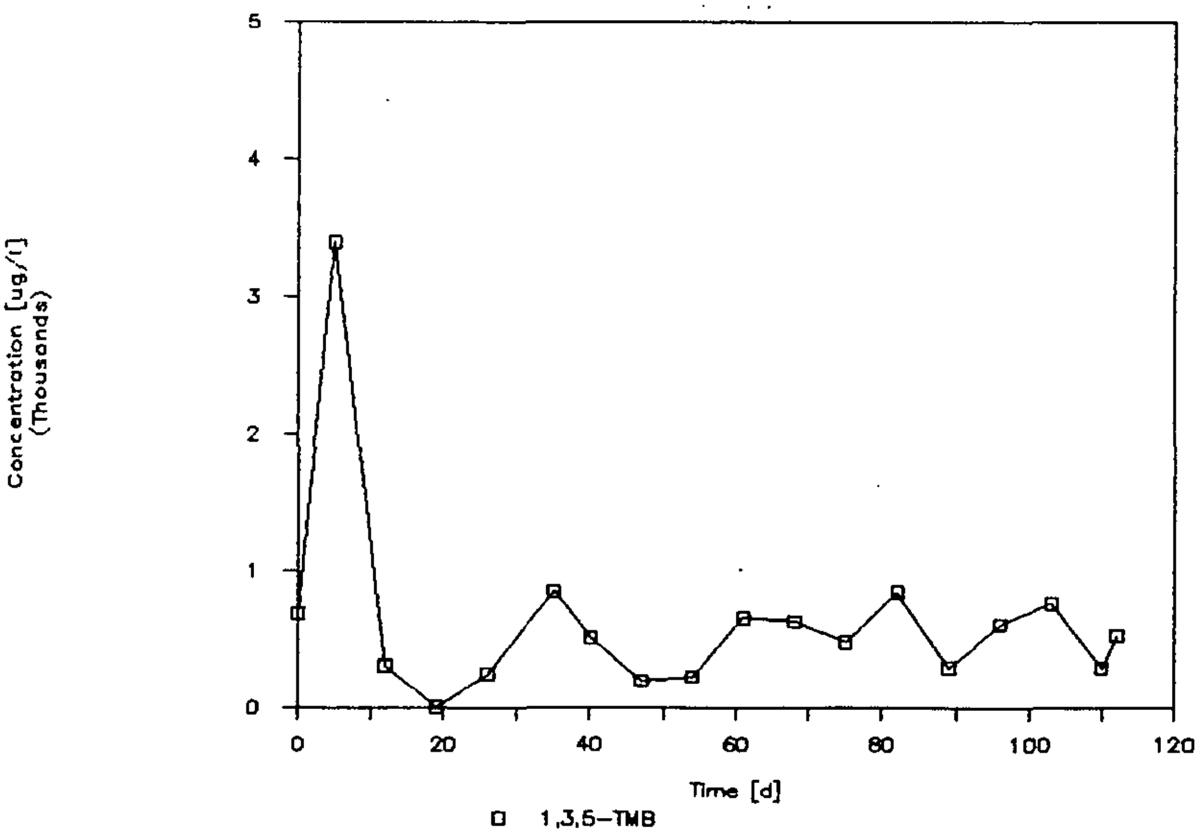

Figure 15. Oxygen profile of the soil column after 60 days of incubation for columns no. 2 (nutrients and buffered) and no. 3 (hydrogen peroxide).

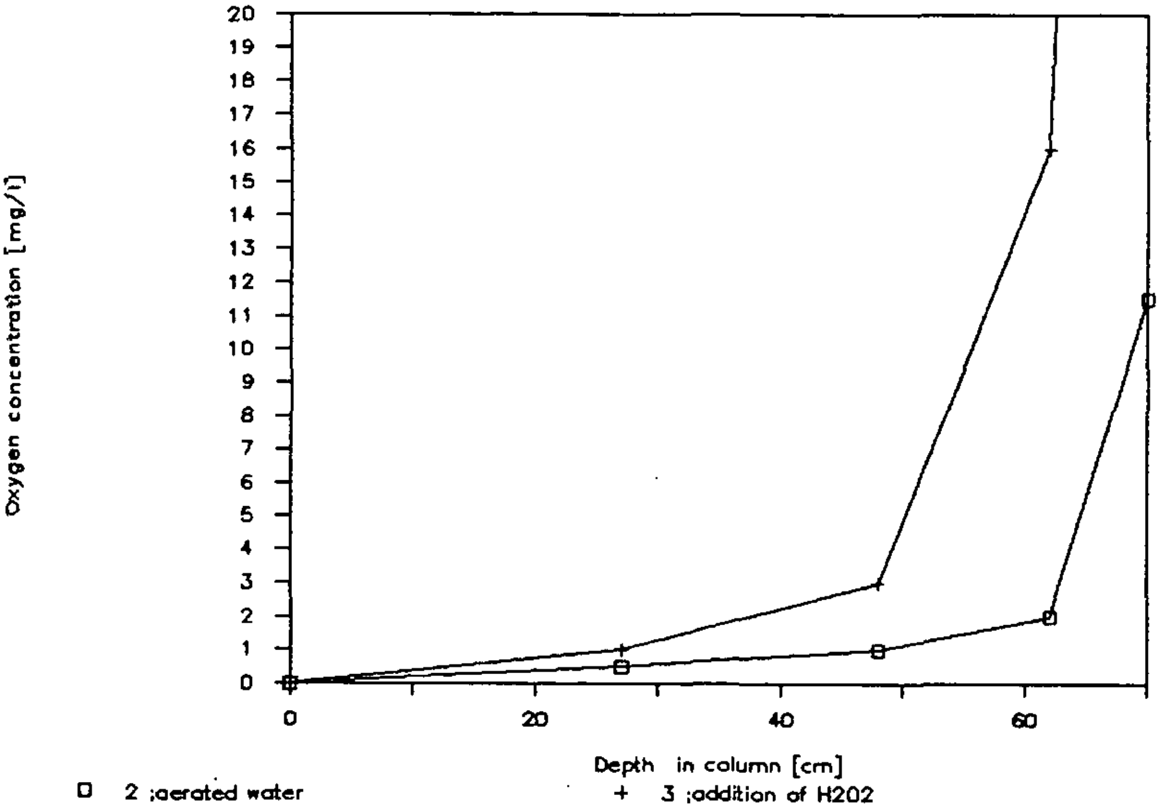

Figure 16. Nitrate concentrations in the effluent (mg N/l) of column no. 5 (nitrate as alternative electronacceptor) as a function of the percolation time.

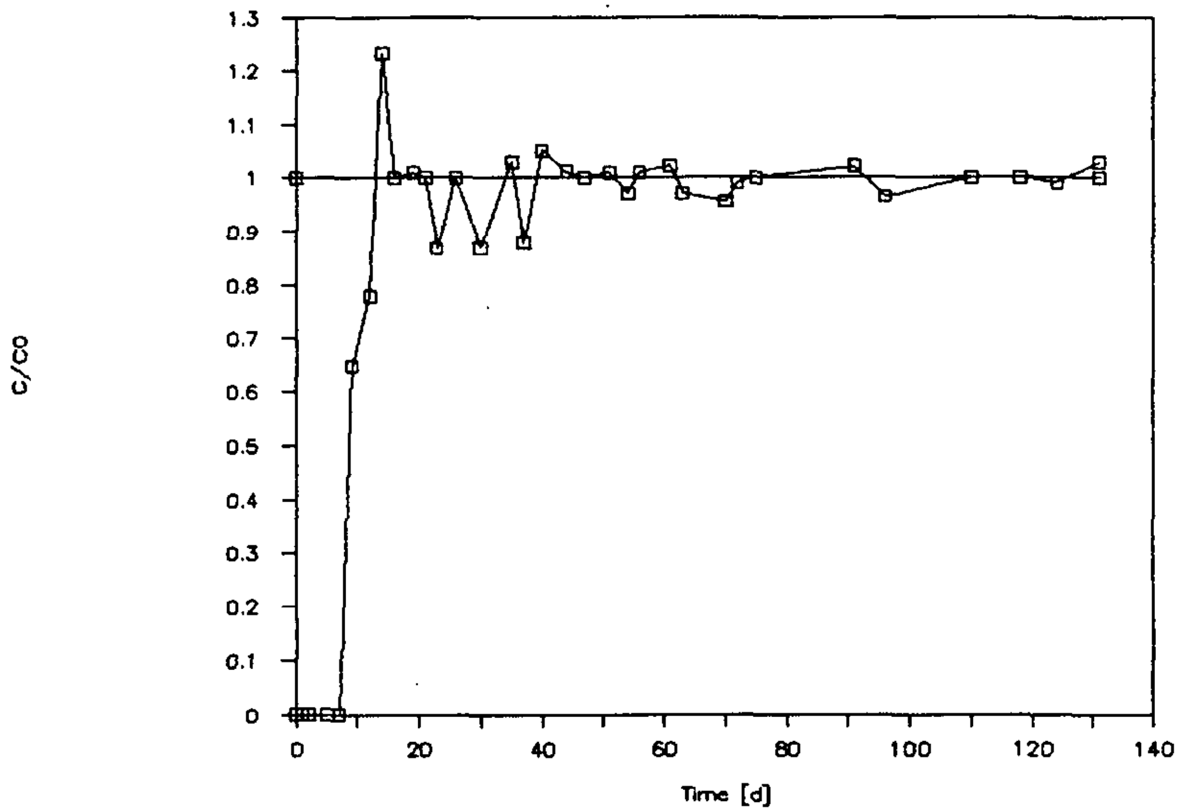

Figure 17. Design of the clean-up operation. The contours give the calculated heads. Design: 9 drains, parallel to each other; 25 pumping wells (x) divided over the area. Infiltration rate: 10 m^3/m drain/d with a total of 1850 m^3/d; withdrawal: 70 m^3/d per pumping well.

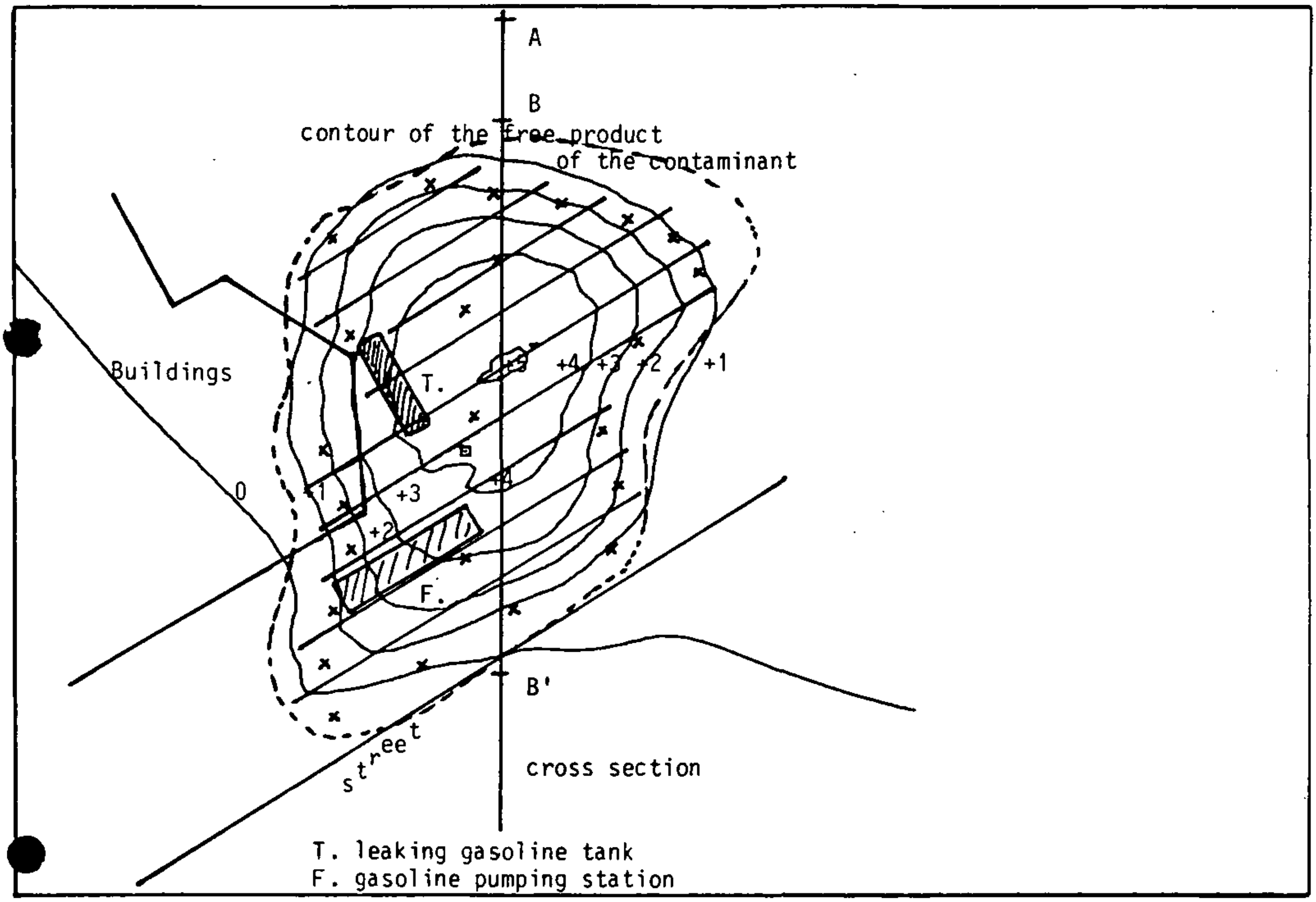

Figure 18. I. Vertical spreading of the contamination for the cross-section A.B.B' in figure 17.
II. Set-up of infiltration, withdrawal and treatment of water.