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DEGRADATION OF PHENANTHRENE AND HYDRAULIC CHARACTERISTICS IN A CONSTRUCTED WETLAND

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Abstract—An artificial waste water containing phenanthrene and Tween 80® was treated in the horizontal–vertical flow macrophyte-based treatment system. The pilot plant consisted of a cascade of five steel tanks filled with lava, each tank planted with *Typha* spp. and *Scirpus lacustris*. The overall removal of phenanthrene in water was about 99.9%. Adsorption of phenanthrene was primarily observed in the upper lava layer. 1-Hydroxy-2-naphthoic acid (HNA) as a bacterial metabolite of phenanthrene was quantified. An MPN(most probable number)-method for the enumeration of phenanthrene-degrading bacteria was modified by staining degradation products formed in the culture media with diazonium salts. The number of phenanthrene-degrading bacteria was highest in the first tank of the cascade. The number of total bacteria remained nearly constant within the cascade. The amount of bacteria in lava exceeded that in water by a factor of 10^3. The number of phenanthrene-degrading bacteria in lava samples in a phenanthrene-loaded tank was approximately $10^6\ g^{-1}$, and $10^2\ g^{-1}$ in a non-loaded tank. Furthermore, tracer experiments were conducted in 1993 and 1994. The mean residence time was determined to be 1.18 days (1993) and 1.15 days (1994) in the first tank, and 6.66 days (1993) and 6.4 days (1994) in the whole cascade. The experimental values agreed approximately with the theoretical values of 1.27 days and 6.54 days. The Peclet numbers agreed with those of the dispersion characteristic of the lava filling as a porous medium.

Key words—constructed wetland, *Typha* spp., *Scirpus lacustris*, PAH-biodegradation, phenanthrene, 1-hydroxy-2-naphthoic acid, residence time, dispersion

INTRODUCTION

Due to its simplicity, low maintenance requirements and low construction costs, the concept of using macrophyte-based treatment systems for the purification of waste waters has received increasing international attention during the last two decades (Hammer, 1989; Thofern, 1994).

Practical applications have demonstrated the effectiveness of using wetland plants to treat household effluents and discharges from small communities. In these cases, the reduction of P-, N-, COD- and BOD-loads were the main points of interest (Bucksteeg, 1985, 1986; Brown, 1994).

In recent years, enhanced efforts have been undertaken to treat industrial waste waters through the use of aquatic plants (Altmann *et al.*, 1992). Umweltschutz Nord GmbH & Co. designed a horizontal–vertical flow macrophyte-based treatment system for the purification of waste water from the oil refining industry. During a 3 yr operating period, under practical conditions, the effectiveness of aquatic plants in removing COD and BOD was revealed (Altmann *et al.*, 1989). A similar treatment system was used for the purification of waste water containing PAHs, BTX and phenolic compounds from a wood impregnation factory (Hine and Pilidis, 1995).

The reduction of pollutants is brought about by various physical, biological and chemical factors. However, there is a lack of information concerning the degradation mechanisms and the fate of persistent compounds like PAHs. Understanding these problems will help to answer questions about the performance of constructed wetlands.

The behaviour of phenanthrene as a three-ring PAH was examined in a constructed wetland plant located in Munich, Germany.

Investigations outlined in this paper focus on the results obtained during the time of operation from August to December, 1994, and encompass microbial degradation of phenanthrene (formation of a metabolite, the population size of total

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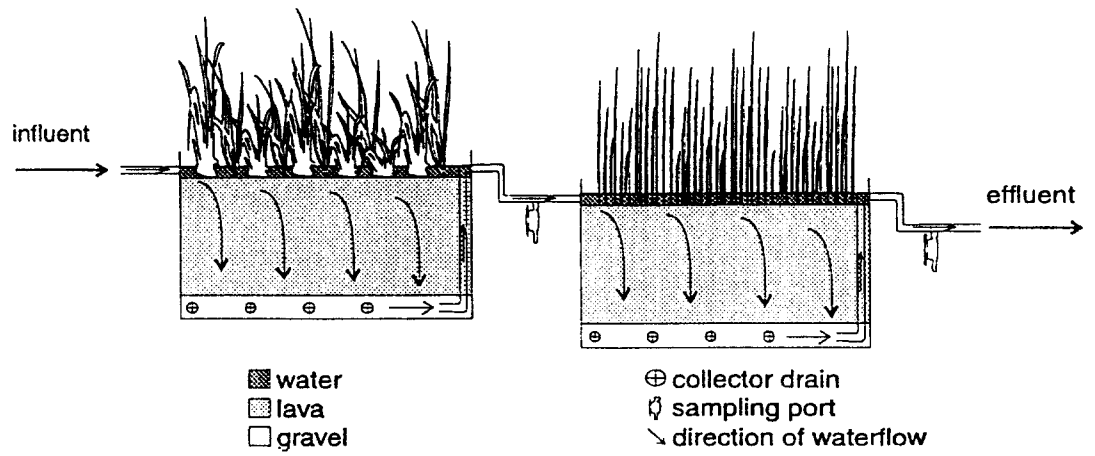

Fig. 1. Cross sectional view of the AQUAPLANT® system.

bacteria and phenanthrene-degrading bacteria) and the adsorption onto the substratum. Hydraulic investigations were performed to obtain information on the mean residence time and dispersion characteristics in the pilot plant within an 18-month period (1993–1994).

SITE DESCRIPTION

The pilot plant was installed in 1991 (Fig. 1). It consisted of a cascade of five steel tanks, each measuring 2.5 × 2.3 × 1.2 m (length × width × height). The tanks were filled up to a 1 m height with a coarsely graded lava material (nominal diameter 2–8 mm). At the bottom of the tanks, collector drains were installed to discharge the water into the next tank. Waste water was introduced into the first, most elevated tank by means of a metering pump and passed through the cascade by gravity. Plants were placed into the tanks in the autumn of 1991 and the summer of 1992. The first and second tank were planted with cattails (*Typha* spp.) and the remaining with bullrushes (*Scirpus lacustris*). The plants were fertilized in 1993 with an inorganic fertilizer (NPK fertilizer from Maier-Samen, Bodenkirchen, Germany; composition: 12% N, 12% P_2O_5, 17% K_2O, 2% MgO, 0.02% B, 0.01% Zn; 200 g per tank). The wetland plant was fed with tap water until the start of the degradation studies.

MATERIALS AND METHODS

Chemicals and media

Phenanthrene and 1-hydroxy-2-naphthoic acid (purity > 98%) were purchased from Aldrich (Steinheim, Germany), *p*-nitroaniline (purity > 98%) from Sigma (Deisenhofen, Germany) and sodium nitrite (p.a.), methanol (gradient grade), tetrahydrofurane (p.a.), 2-propanol (p.a.) and Tween 80® (for synthesis) from Merck (Darmstadt, Germany). Dichloromethane and cyclohexane were each of Pestanal® quality and purchased from Riedel de Haën (Seelze, Germany). All chemicals were used as supplied. Water for HPLC-analysis was produced by a Millipore® purifying system. Mineral salt medium (MSM) adjusted to pH 7.25 with orthophosphoric acid prior to sterilization was used for microbiological tests (Lockhead and Chase, 1973). R2A medium was used for obtaining a total bacterial count (Reasoner and Geldreich, 1985). A 1:5 mixture of *p*-nitroaniline (0.5% in 2M hydrochloric acid) and sodium nitrite (0.5%) in deionized water) was used as the reaction mixture for detecting microbial phenanthrene degradation products.

Preparation of artificial waste water

Artificial waste water was produced continuously by mixing an aqueous stock solution, containing 500 mg L^{-1} phenanthrene and 30,000 mg L^{-1} Tween 80® (a non-ionic detergent to enhance the solubility of phenanthrene), with tap water under vigorous stirring. The resulting phenanthrene concentration of the artificial waste water was 0.385 mg L^{-1}. It was continuously applied to the pilot plant at a constant flow rate of 3 L min^{-1}.

Microbiological methods

The extraction of microorganisms from lava material was performed by shaking mixtures of 10 g of wet lava and 90 mL of MSM for 1 h. After sedimentation of the suspended particles, 1 mL of the supernatant was used for subsequent serial dilution. Water samples were used as obtained from the plant.

For the enumeration of phenanthrene-degrading bacteria a modified MPN(most probable number)-method according to Stieber *et al.* (1994) and used. 25 μL portions of a phenanthrene solution in methanol (10 g L^{-1}) were distributed into each well of a sterile polyethylene microtiter plate (Nunc 8 × 12, Nunclon Delta, Wiesbaden, Germany) by means of an eight channel dosing pipette. The solvent was allowed to evaporate under sterile conditions. Tenfold dilutions in MSM were performed from 10^{-1} to 10^{-4} with water samples and from 10^{-1} to 10^{-8} with lava extract. The different dilutions were transferred (200 μL/well^{-1}) into the prepared microplates. Twelve wells were inoculated with the same dilution. The plates were incubated at 25°C for 10 days. Then 25 μL of reaction mixture and 25 μL of 1M NaOH were added to each well. All plates were checked for coloured products according to the MPN-method (De Man, 1975, 1977). A computer program based on a Newton–Raffson iteration (Clarke and Owens, 1983) was used to calculate the MPN.

The total bacteria counts were also determined using the MPN-method on microplates. Water samples were diluted in R2A medium from 10^{-1} to 10^{-8} and lava extract from 10^{-3} to 10^{-10}. The microplates were inoculated with the different dilutions (200 μL/well^{-1}), 12 wells with the same

dilution. After incubation at 25°C for 10 days, the wells were evaluated for turbidity. The MPN was calculated as described above.

Analytical procedure

Water samples were collected twice a week at sampling ports located at the outlet of each tank. They were adjusted to pH < 2 by acidifying with hydrochloric acid. Enrichment of the analytes was performed by means of solid phase extraction (SPE). Prior to application, the 1-mL octadecyl (C_{18}) cartridges were preconditioned sequentially using cyclohexan, methylene chloride, 2-propanol and deionized water (1 mL of each). Water samples were drawn through the cartridges at a flow rate of about 10 mL min^{-1}. After drying the cartridges under ambient air the analytes were eluted with 1 mL methanol and twice with 1 mL dichloromethane:methanol (5:1, v/v). The solvent was evaporated using a gentle stream of nitrogen. Samples were adjusted to 2 mL with methanol.

The analysis were performed on a Perkin Elmer modular liquid chromatograph (pump 200 LC, auotosampler ISS 200 and a fluorescence detector LC 240 with variable wavelength detection). The column used was a Merck Lichrocart 125-4 RP-18 at 30°C, with a mobile phase of methanol and water (pH 2.3) 85:15 (v/v) and a flow rate of 0.8 mL min^{-1}. UV spectra were recorded during HPLC analysis by a Perkin Elmer diode array detector 235 C.

HNA was identified by comparing the retention time and UV spectrum with that of a standard material. Phenanthrene, as well as HNA, was quantified using fluorimetry (phenanthrene Ex.:248 nm, Em.:365 nm; HNA Ex.:248 nm, Em.:410 nm). Recovery rates of phenanthrene and HNA were found to exceed 80% within the required concentration range.

Samples of lava were taken from the center of the tanks at three depths (10, 50 and 90 cm). Prior to extraction, the lava was separated from residual plant and root material. The water content was determined by drying 10 g of sample material at 100°C to weight constancy, and was found to be 10–23% (w/w).

Ten grams of wet lava material were extracted with 10 ml of tetrahydrofurane in a sonification bath (Bandelin RK 510 S) for 1 h. Five replicates were performed for each sample type. After sedimentation of suspended particles for several hours in the dark, 10 μL of the clear supernatant were analyzed by HPLC. The recovery rates of phenanthrene and HNA in a concentration range of 10–5000 $\mu g\ kg^{-1}$ were found to be 80% and 66%.

Hydrodynamic investigatons

For the experiments, bromide was used as a tracer. 10 g of KBr dissolved in 10 L water were injected into the system inlet for 20 min. The first experiment was carried out in April 1993, the second in September 1994. Samples were collected automatically after defined time intervals, from the outlet of tank 1 and tank 5 of the cascade. The concentration of bromide was determined by ion chromatography.

RESULTS AND DISCUSSION

During the microbial degradation of phenanthrene, 1-hydroxy-2-naphthoic acid (HNA) was found as an initial conversion product in pure and mixed microbial cultures (Cerniglia, 1984; Kiyohara and Nagao, 1978; Guerin and Jones, 1988). With regard to our investigations, HNA provides a suitable indicator for bacterial phenanthrene metabolism. This compound is a naphthalene derivative and clearly originates from phenanthrene. Furthermore, it is formed in large amounts (Thiem, 1994).

Stieber *et al.* (1994) described a MPN-method using microplates for the quantification of PAH-degrading bacteria. The method is based on the formation of coloured metabolites during the growth of bacteria using PAHs as the sole source of carbon and energy. However, a weak or intermediary colorization of the culture liquid rendered the indication of bacterial growth difficult. To circumvent this problem the plates were supplemented with a diazonium salt-containing reaction mixture after 10 days of incubation. The instantaneously formed azo dyes indicated the presence of degradation products.

The number of phenanthrene-degrading bacteria present and the formation of HNA indicated a bacterial degradation of the applied PAH during passage through the wetland plant. Figure 2 shows the phenanthrene and HNA concentrations and bacterial population sizes detected in water samples of the wetland plant after 14 days of exposure. The removal of phenanthrene within the cascade was found to be greater than 99.9%. A significant portion of this reduction took place in the first tank (98%). In addition, the highest concentration of HNA was found in the first tank. In the second tank the phenanthrene concentration decreased from 8 $\mu g\ L^{-1}$ (effluent from tank 1) to about 0.2 $\mu g\ L^{-1}$. In the last three tanks no further reduction in phenanthrene concentration took place and a lower number of phenanthrene-degrading bacteria was detected. A similar distribution of phenanthrene, HNA and phenanthrene-degrading bacteria in the cascade (as shown in Fig. 2) was found throughout the entire period of operation. However, besides microbial degradation several other processes such as photodegradation, adsorption onto lava material or plants, translocation into the plants, etc., are assumed to play a role in the removal of phenanthrene. The total cell count remained nearly constant within the cascade.

Ambient temperature strongly affects microbial degradation rate (Shrimp *et al.*, 1993). Hence, temperature was considered when analyzing the change in population size and concentrations of phenanthrene and HNA in the first tank of the wetland (Fig. 3). The concentrations and population counts, obtained on the sampling dates, exhibited fluctuations due to varying ambient conditions. The concentration range of phenanthrene and HNA was found to be 1–30 $\mu g\ L^{-1}$ and 5–17 $\mu g\ L^{-1}$, respectively, during the first stage of operation (before interruption in November). During the second stage the HNA concentration did not exceed 3 $\mu g\ L^{-1}$ and only traces of phenanthrene were detected. It is evident, that both the mean number of total bacteria and phenanthrene-degrading bacteria remained at a nearly constant level over the total period of operation. This might be explained by a shift in the bacterial population towards more psychrophilic bacteria caused by a decreasing

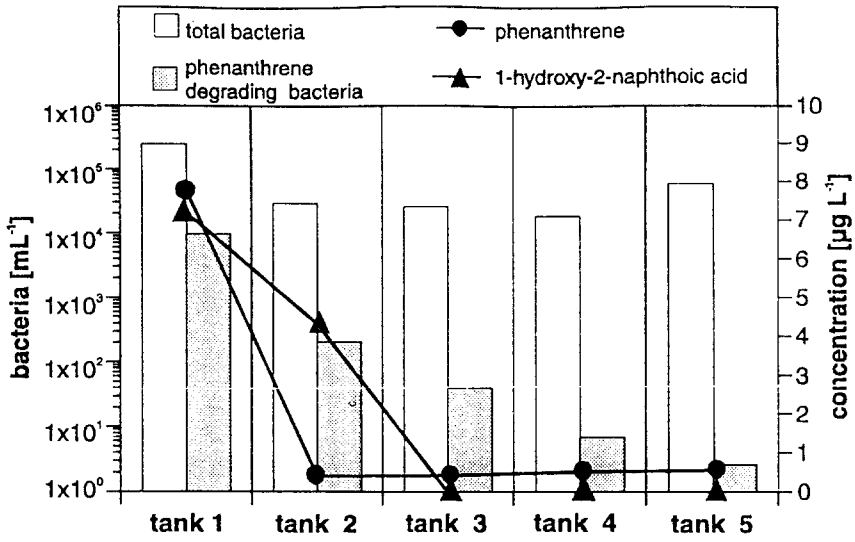

Fig. 2. Bacterial population and concentration of phenanthrene and HNA after 14 days of phenanthrene exposure.

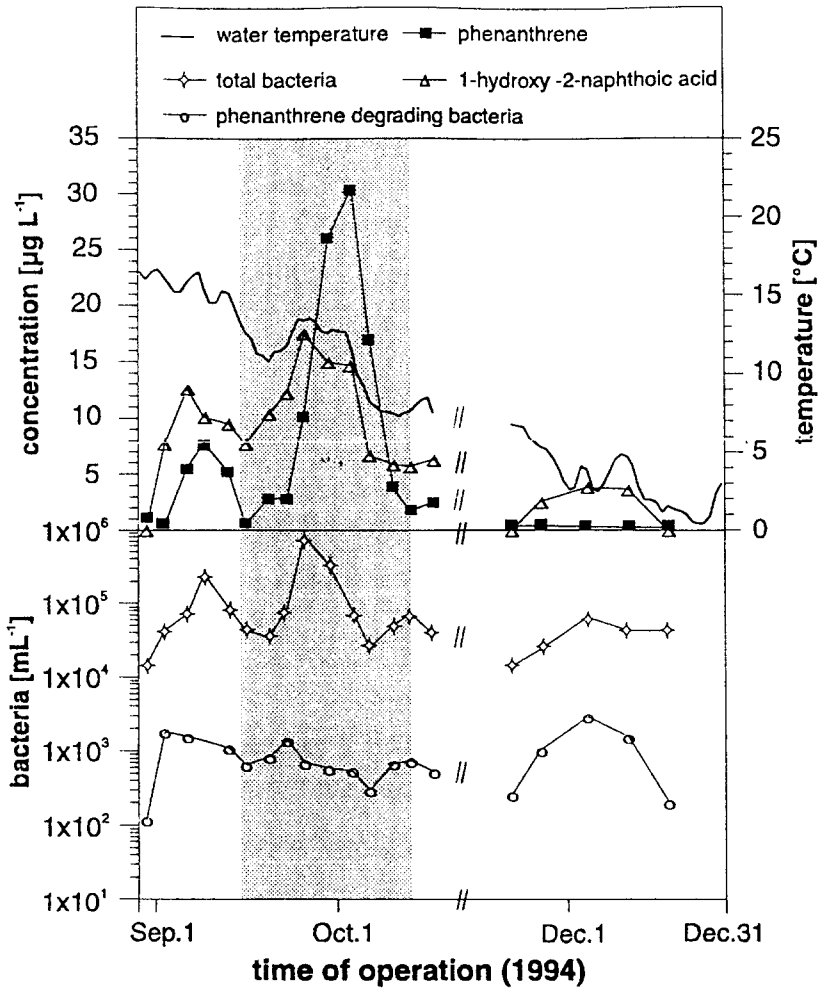

Fig. 3. Concentrations of phenanthrene and HNA, total bacteria and phenanthrene-degrading bacteria in water samples from tank 1 during the period of operation (//: interruption of operation for harvesting plants and several maintenance procedures).

temperature. A seasonal shift in the composition of a microbial community reflecting the rates of hydrocarbon metabolism was already reported by Atlas (1981). With regard to wetland plants, temperature was reported to have little effect on effluent quality and even increased total number of active bacteria in the soil and rhizosphere (Wood, 1990). In addition, BOD reduction in domestic sewage in a reed-bed system was not jeopardized during winter conditions (Bahlo and Wach, 1990).

As shown in Fig. 3 (shaded region), the concentrations of phenanthrene and HNA as well as the number of total bacteria exhibited a coincidence with respect to a short-term change in temperature during a period of 3 weeks. This fact is probably caused by several temperature dependent biological and physicochemical effects.

Lava samples taken from the wetland plant in November and December showed a phenanthrene concentration of 35.4 $\mu g\ kg^{-1}$ (ambient water temperature: 7°C) and 672 $\mu g\ kg^{-1}$ (ambient water temperature: 1.5°C) each in the upper lava layer of the first tank. The phenanthrene content in lava samples from 50 and 90 cm depth were below 10 $\mu g\ kg^{-1}$. A different adsorption behavior of phenanthrene as an aqueous solution with Tween 80® onto reference lava material at 20°C and 3°C in laboratory-scale experiments was not observed (data not shown). Hence, the presence of an effective sorbent in the upper layer of the lava filling might be assumed, e.g. humic substances, residual plant material, biofilm or algae. Tanner and Sukias (1994) determined an elevated content of organic matter, mainly in the upper 10 cm of the substratum layer in planted gravel-bed wetlands. In addition, organic coatings were reported to enhance the adsorption of hydrophobic organic compounds onto a soil grain surface (Murphy *et al.*, 1990).

Our findings suggest that temperature-dependent interactions take part in the adsorption of phenanthrene in the wetland plant and may give rise to the varying phenanthrene concentrations in the first tank, as shown in Fig. 3. It must be stated that the variability in concentrations of phenanthrene and HNA and bacterial counts is not restricted to temperature changes but also to several other parameters, e.g. plants, pH, oxygen concentration and nutrients.

The usual substratum in constructed wetland plants provides an ideal surface for microbial attachment, similar to trickling filters in waste water treatment (Williams *et al.*, 1992). Hence most of the microbial activity is assumed to be located on the lava grain surface, forming a biofilm. A comparison of the number of total bacteria and phenanthrene-degrading bacteria confirms this assumption. The amount of bacteria extracted from the lava of the first tank greatly exceeds that found in the water. The number of total bacteria in lava was found to be $10^8\ g^{-1}$, whereas in water only 2–6 $\times\ 10^4\ mL^{-1}$ were detected. Furthermore, the number of phenanthrene degrading bacteria found in lava ($10^6\ g^{-1}$) exceeded that found in water ($10^2\ mL^{-1}$). The number of phenanthrene-degrading bacteria on the lava was much higher in the phenanthrene-loaded than in the non-loaded tank. Lava samples from the latter contained only $10^2\ g^{-1}$ phenanthrene-degrading bacteria. In other words, the "outcome" of specific substrate degrading bacteria was obviously affected by the presence of a degradable carbon source. On the other hand, only a slight difference in the number of total bacteria was found in the lava samples of both tanks ($10^7\ g^{-1}$ in the non-loaded and $10^8\ g^{-1}$ in the loaded tank).

The efficiency of a biological treatment system is strongly affected by the contact time between pollutants and microorganisms (Hatano *et al.*, 1992; Portier and Palmer, 1989). Hence tracer tests were performed to examine the residence time and distribution of the tracer in the cascade. The theoretical residence time is defined as the net system volume divided by flowrate and may be calculated if the dimensions of the wetland plant, the hydraulic loading rate and the porosity of the substratum are known (Netter and Bischofsberger, 1990). The theoretical value, as obtained by a flow rate of 2 L min^{-1} and a porosity of 0.55, was determined to be 1.27 days for one tank and 6.54 days for the whole cascade.

In practice, due to dispersion processes a distribution of different residence times occurs with a mean residence time which is equal to the above theoretical residence time. Residence time distribution can be determined through the breakthrough curves of tracers which are injected into the system inlet. The mean residence time t_m is defined as $t_m = \int (ct)dt / \int c\,dt$ with c = concentration of the tracer at the system outlet and t = time difference after tracer injection. The width of the tracer breakthrough curve provides information on the dispersion characteristics (Klotz and Moser, 1974), e.g. if shortcuts exist which could negatively affect the degradation performance of the wetland plant.

The tracer breakthrough curves obtained (only data from 1994 shown in Fig. 4) generally correspond to those which are common to the flow in homogeneous porous media. Hydraulic shortcuts in the tanks were not found. However, especially in the outlet of the first tank secondary tracer peaks were observed which occurred with a 24-h delay. These peaks were more likely caused by circulations due to diurnal temperature fluctuations, than by branching flow paths. In the outlet of the fifth tank no such secondary peaks were observed. Further investigations of flow distribution within the tanks were performed based on these observations and are currently in process.

The experimental residence time in the first tank was found to be 1.18 days in April 1993 and 1.15 days in September 1994 and for the whole cascade to

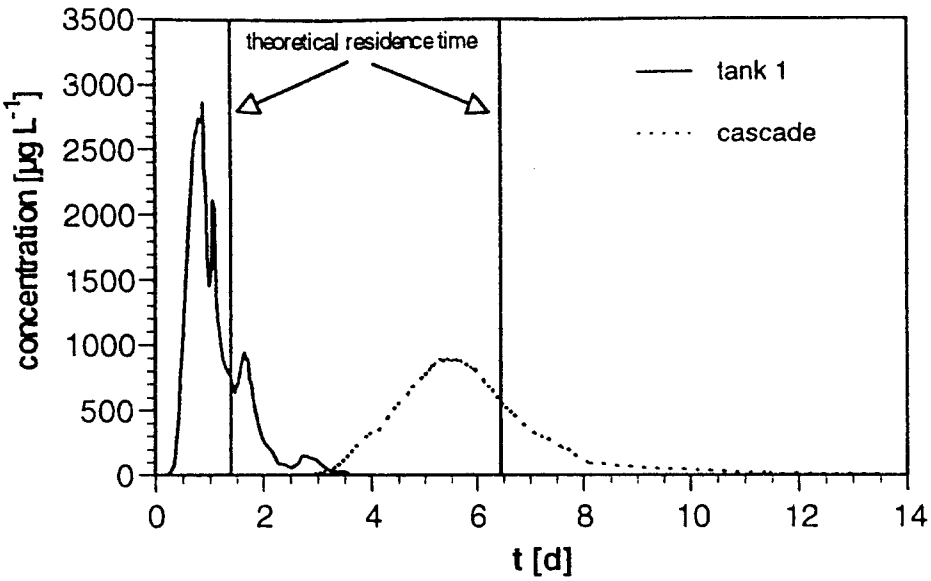

Fig. 4. Tracer response curves of bromide from tank 1 and the whole cascade in 1994.

6.66 days and 6.4 days. The influence of evapotranspiration and rainfall could complicate a comparison of these results. It has to be assumed that a mean rainfall of 1.6 mm d^{-1} during the first experiment and 2.6 mm d^{-1} during the second experiment would compensate the loss of water by evapotranspiration (estimated values at 7°C and 13.7°C: 1.5–2.5 mm d^{-1} (Hofmann, 1992)). Based on these data only a negligible change in the residence times is expected. The shortening of the residence time in the cascade between 1993 and 1994 might be explained by the formation of a compact root mesh, which reduced the net system volume. However, the influence of a reduced void space by penetration of plant root on the experimental residence time was reported to be much higher than found in our experiments (Fisher, 1990; Stairs and Moore, 1994). With regard to the uncertainty of the porosity factor and the influent rate, as mentioned above, the theoretical residence times agreed with the practical values.

Peclet numbers (*Pe*) were derived from the tracer breakthrough curves to characterize the extent of hydraulic dispersion (low *Pe*: high dispersion, high *Pe*: low dispersion). They were found to be 12–15 after the first tank and approximately 35 after the cascade. These Peclet numbers are not significantly lower than those which can be expected for the lava fillings used as a porous medium (Dagan, 1989).

A slight decrease in the Peclet numbers from the first experiment (1993) to the second (1994) could be attributed to the formation of hydraulically active channels created by plant root growth.

CONCLUSIONS

The effectiveness of the wetland plant in treating an artificial waste water containing phenanthrene was demonstrated under both summer and winter conditions.

The occurrence of HNA as an intermediary metabolite of phenanthrene degradation indicated the involvement of bacterial microflora in the transformation of phenanthrene.

It was determined that the lava material played an important role as a support matrix for bacteria.

The mean residence time in the wetland plant was predicted by the hydraulic parameters, as influent rate, porosity and dimensions of the pilot plant.

Further investigations shall be performed in treating a highly contaminated groundwater, containing various PAHs and hydrocarbons.

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REFERENCES

Altmann B.-R., Heidmann T., Nagels G. and Schulz-Berendt V. (1992) Erfahrungsbericht über den Einsatz von Pflanzenkläranlagen zur Reinigung industrieller Abwässer. DGMK Bericht 453. Report about the Experiences made with Macrophyte-Based Purification Plants for Industrial Effluents. DGMK Projects 453, Hamburg, Germany.

Altmann B.-R., Lilie R. H., Nowak K.-E and Schulz-Berendt V. (1989) Pflanzenbiologische Reingung von Abwässern aus einem Mineralöltanklager. DGMK Bericht 388. Macrophyte-Based Biological Treatment of Effluents from a Petroleum Tank Farm. DGMK Project 388. Hamburg, Germany.

Atlas R. M. (1981) Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev.* **45**, 180–209.

Bahlo K. E. and Wach F. G. (1990) Purification of domestic sewage with and without faeces by vertical intermittent filtration in reed and rush beds. In *Constructed Wetlands in Water Pollution Control* (Edited by Cooper P. F. and Findlater B. C.), pp. 215–221. Proc. Int. Conf. on the Use of Constructed Wetlands in Water Pollution Control, Cambridge, U.K.

Brown D. S. (1994) Constructed wetlands in the U.S.A. *Wat. Qual. J.* **4,** 24–28.

Bucksteeg K. (1985) Erste Erfahrungen mit 12 Sumpfpflanzenkläranlagen. First Experiences with 12 Helophyte Beds. *Korr. Abw.* **5,** 376–385.

Bucksteeg K. (1986) Sumpfpflanzenkläranlagen—Verhfahrensvarianten, Betriebserfahrungen, Problem Bodenhydraulik. Helophyte Beds—process engineering—operating experience—problem soil conductivity. *gwf-Wasser Abwasser* **127,** 429–434.

Cerniglia C. E. (1984) Microbial metabolism of polycyclic aromatic hydrocarbons. *Adv. Appl. Microbiol.* **30,** 31–71.

Clarke K. R. and Owens N. J. P. (1983) A simple and versatile micro-computer program for the determination of 'most probable number'. *J. Microbiol.* **1,** 133–137.

Dagan G. (1989) *Flow and Transport in Porous Media*, Berlin.

De Man J. C. (1975) The probability of most probable numbers. *Eur. J. Appl. Microbiol.* **1,** 67–78.

De Man J. C. (1977) MPN tables for more than one test. *Eur. J. Appl. Microbiol.* **4,** 307–316.

Fisher P. J. (1990) Hydraulic characteristics of constructed wetland at Richmond, NSW, Australia. In *Constructed Wetlands in Water Pollution Control* (Edited by Cooper P. F. and Findlater B. C.), pp. 21–31. Proc. Int. Conf. on the Use of Constructed Wetlands in Water Pollution Control, Cambridge, U.K.

Guerin W. F. and Jones G. E. (1988) Two-stage mineralization of phenanthrene by estuarine enrichment cultures. *Appl. Environ. Microbiol.* **54,** 929–936.

Guerin W. F. and Jones G. E. (1988) Mineralization of phenanthrene by a mycobacterium sp. *Appl. Environ. Microbiol.* **54,** 937–944.

Hammer D. A. (1989) *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*. Lewis Publishers, Chelsea, MI.

Hatano K., Frederic D. J. and Moore J. A. (1992) Microbial ecology of constructed wetlands used for treating pulp mill wastewater. *Proc. Int. Conf. on Wetland Systems in Water Pollution Control*, Sydney, Australia, pp. 62.1–62.7.

Hine N. R. and Pilidis G. A. (1995)An assessment of efficiency of a macrophyte-based biological treatment plant to treat wastewater from a wood impregnation factory. *Fresenius Environ. Bull.* **4,** 630–635.

Hofmann K. (1992) Entwässerung und Vererdung von Kläschlamm in Schilfbeeten, Dissertation, Stuttgart. Sludge dewatering and mineralization in reed bed systems. Ph.D. Thesis.

Kiyohara H. and Nagao K. (1978) The catabolism of phenanthrene and naphthalene by bacteria. *J. Gen. Microbiol.* **105,** 69–75.

Klotz D. and Moser H. (1974) Hydrodynamic dispersions as aquifer characteristics—isotope techniques in groundwater hydrology, IAEA, Wien Vol. II, pp. 341–354.

Lockhead A. G. and Chase F. E. (1973) Quantitative studies of soil microorganisms. Nutritional requirements of the predominant bacterial flora. *Soil Sci.* **55,** 184–195.

Murphy E. M., Zachara J. M. and Smith S. C. (1990) Influence of mineral-bound humic substances on the sorption of hydrophobic organic compounds. *Environ. Sci. Technol.* **24,** 1507–1516.

Netter R. and Bischofsberger W. (1990) Hydraulic Investigations on planted soil filters. In *Constructed Wetlands in Water Pollution Control* (Edited by Cooper P. F. and Findlater B. C.), pp. 11–20. Proc. Int. Conf. on the Use of constructed Wetlands in Water Pollution Control, Cambridge, U.K.

Portier R. J. and Palmer S. J. (1989) Wetlands microbiology: form function, processes. In *Constructed Wetlands for Wastewater Treatment* (Edited by Hammer D. A), pp. 89–105. Lewis Publishers, Chelsea.

Reasoner D. J. and Geldreich E. E. (1985) A new medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* **49,** 1–7.

Shimp J. F., Traca J. C., Davis L. C., Lee E., Huang W., Erickson L. E. and Schnoor J. L. (1993) Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. *Crit. Rev. Environ. Sci. Technol.* **23,** 41–77.

Stairs D. B. and Moore J. A. (1994) Flow characteristics of constructed wetlands: tracer studies of the hydraulic regime. *Proc. 4th Int. Conf. on Wetland Systems for Water Pollution Control*, Guangzhou, China, pp. 742–751.

Stieber M., Haeseler F., Werner P. and Frimmel F. H. (1994) A rapid screening method for micro-organisms degrading policyclic aromatic hydrocarbons in microplates. *Microbiol. Biotechnol.* **41,** 753–755.

Tanner C. C. and Sukias P. (1994) Accumulation of organic solids in gravel-bed constructed wetlands. *Proc. 4th Int. Conf. on Wetland Systems for Water Pollution Control*, Guangzhou, China, pp. 617–627.

Thiem A. (1994) Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants. *Appl. Environ. Microbiol.* **60,** 258–263.

Thofern U. (1994) Pflanzenkläranlagen: Prinzipien—Verfahrensvariantent—Einsatzmöglichkeiten. Constructed wetlands: principles—design–applications. *Zbl. Hyg.* **196,** 197–226.

Williams J. B., May E., Ford M. G. and Butler J. E. (1992) Microbial mechanisms operating in gravel bed hydroponic beds used as a tertiary treatment stage for sewage effluents. *Proc. Int. Conf. on Wetland Systems in Water Pollution Control*, Sydney, Australia, pp. 5.1–5.9.

Wood A. (1990) Constructed wetlands for wastewater treatment—engineering and design considerations. In *Constructed Wetlands in Water Pollution Control* (Edited by Cooper P. F. and Findlater B. C.), pp. 481–494. Proc. Int. Conf. on the Use of Constructed Wetlands in Water Pollution Control, Cambridge, U.K.