



CONTINUOUS OZONATION OF POLYCYCLIC AROMATIC HYDRO- CARBONS IN OIL/WATER-EMULSIONS AND BIODEGRADATION OF OXIDATION PRODUCTS

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ABSTRACT

The continuous ozonation of polycyclic aromatic hydrocarbons (PAH) was studied in a two stage ozonation system followed by aerobic biological degradation. The highly condensed PAH benzo(e)pyrene and benzo(k)fluoranthene were oxidized selectively in synthetic oil/water-emulsions. The influence of the ozone mass transfer gas-liquid on the reaction rate of benzo(k)fluoranthene was studied for process optimization. The dissolved ozone concentration is influenced by temperature to a higher degree than the reaction rate of PAH. In dependence on pH, PAH oxidation occurs by a direct reaction with ozone inside the oil droplets. Two main ozonation products of benzo(e)pyrene were quantified at different retention times during ozonation and their transformation could be shown in the biological treatment step. © 1999 IAWQ Published by Elsevier Science Ltd. All rights reserved

KEYWORDS

Continuous ozonation; biodegradation; dodecane; oil/water-emulsion; oxidation products; polycyclic aromatic hydrocarbons (PAH).

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) can be considered to be ubiquitous and originate from incomplete combustion of organic material. Besides soil and water from hazardous waste sites associated with fuel, gas and wood-impregnation facilities, they can be found in different industrial wastewaters especially from hydrocarbon production, coke plants and metal processing industry. Considering their mutagenic and carcinogenic potential, the United States Environmental Protection Agency listed 16 PAH as priority pollutants. Due to their lipophilic behavior and low water solubility PAH are often dissolved in the dispersed phase of oil/water-emulsions or in hydrophobic organic material. Consequently, their concentration in the environment is very low in water, like levels of 0.045-0.51 µg/L for total PAH in groundwater (Harrison *et al.*, 1975), but high in soil and oils. Grimmer *et al.* (1983) found contents of the five ring condensed benzo(a)pyrene between 0.1-3.6 and benzo(e)pyrene between 1.2-28.9 mg/kg in eight crude oils of different origin. In used metalworking oil the greatest PAH-content was determined in quenching oil, e.g. with benzo(a)pyrene concentrations of 9.79 to 34.8 mg/kg (Eyes, 1981). While PAH with less than five rings,

like anthracene, phenanthrene and pyrene, are usually biodegradable under aerobic conditions (Rockne *et al.*, 1998) and have been studied frequently (e.g. McNally *et al.*, 1998), the use of highly condensed PAH (five and more rings) by bacteria as sole carbon and energy source has not been proved yet. Therefore, within the scope of the research center Sfb 193 "Biological treatment of industrial wastewater" the selective ozonation of highly condensed PAH was studied during batch and continuous ozonation followed by biological treatment of the oil and oxidation products formed.

Ozone reacts with organic substances in two main mechanisms. At neutral to low pH an electrophilic addition takes place at electron rich parts of organic molecules like C-C double bonds. At alkaline conditions ozone decays mostly to hydroxide radicals and by chain reactions to other radicals, which effect an unspecific radical reaction with organic substances. The reaction rate of PAH with ozone is relatively high and the oxidation products formed are depending on the molecular structure, pH and type of solvent. Apart from secondary ozonides, the stable oxidation products are quinoids in case of an ozone attack at an atom of PAH or formyl- or carboxyl-products at a double bond attack of ozone. The early studies on PAH-ozonation were predominately performed in organic solvents at low temperature for preparative reasons (Bailey *et al.*, 1958). Studies in water at low concentrations were seldom and conducted under the aspect of contaminated ground and drinking water (Butkovič *et al.*, 1983; Corless *et al.*, 1990). Later biodegrading oxidation products and toxicological effects became more important accompanied by better analytical facilities. Eberius *et al.* (1997) used ^{14}C labeled pyrene and benzo(a)pyrene for batch ozonation in a very small fixed-bed reactor (24 mL) filled with soil or silica. Reaction products of pyrene found could mostly be explained by bond-attack pathway, while the oxidation products of benzo(a)pyrene followed from atom-attack. In general, an enhanced biodegradability is assumed by previous ozonation due to an introduction of oxygen in PAH. A heterogeneous system was used by Haeseler *et al.* (1993), where the two to four ring condensed PAH naphthalene, phenanthrene and pyrene were ozonated in crystalline suspensions followed by biological degradation to enhance mineralization. A complete oxidation was not achieved under the chosen conditions, but the oxidation rate of each PAH was correlated with their water solubility. Pyrene biodegradation was positively influenced by previous ozonation. High toxicity of oxidation products of phenanthrene was decreased by biodegradation. Burluson *et al.* (1979) studied the influence of ozonation on the mutagenesis of benzo(a)pyrene. The mutagenicity was destroyed after a short time of ozonation. A few studies focus on oil containing systems, like Marley *et al.* (1987), who ozonated naphthalene derivatives in kerosene films floating on top of the buffer solutions. Specht *et al.* (1996) treated lowly condensed PAH in anthracene oil by Fenton's reagent and hydrogen peroxide. Due to radical reaction in the water phase and the low water solubility of PAH a complete primary oxidation was not achieved. Most studies of PAH-ozonation are conducted in batch experiments and preferably with lowly condensed PAH, like Beltrán *et al.* (1995), who studied main parameters, mass transfer and kinetics in the ozonation. A continuous ozonation of highly condensed PAH followed by biological treatment is not known from literature.

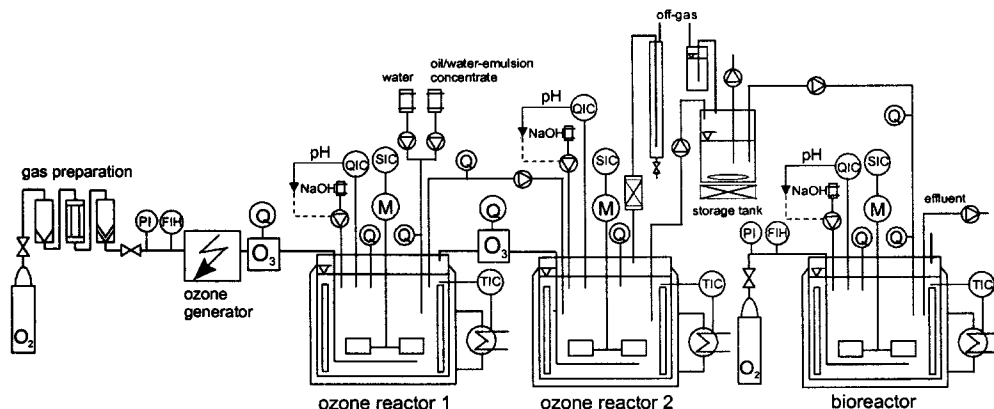


Figure 1. Experimental set-up for a two stage continuous ozonation followed by aerobic biological treatment.

MATERIAL AND METHODS

Experimental setup. Figure 1 presents the system for continuous experiments consisting of a two stage ozonation followed by a bioreactor. A two step system was used in order to get a more narrow residence time distribution. The ozonation was performed in two standard stirred glass reactors ($D=150$ mm) equipped with four equally spaced baffles and a stainless steel six blade turbine ($d=75$ mm). The liquid volume was 2 L in each ozone reactor.

At a retention time of 10 min, a concentrate of the oil/water-emulsion (flow rate of 0.6 L/h) was mixed with tap water (flow rate 11.4 L/h), before they were fed together to the first ozone reactor. Ozone was produced from pure oxygen by a high-voltage discharge ozone generator (Sorbios, GSG 1.2). The pure oxygen was supplied to the ozone generator continuously at a flow rate of 12.5 L/h (0°C , $1.013 \cdot 10^5$ Pa). The ozone/oxygen gas was introduced to reactors via steel aeration rings (12 holes, 0.3 mm diameter) at the bottom below the stirrers and dispersed at a rotational speed of 380 rpm. The ozone gas inlet concentration to the first ozone reactor was normally 20 mg/L. The ozone containing off-gas of the first ozone reactor was introduced to the second. The ozone gas inlet and outlet concentrations of the first ozone reactor were measured continuously by two UV-photometers (BMT 961 TPC, $\lambda=254$ nm, 0°C , $1.013 \cdot 10^5$ Pa). The ozone parameters of the first reactor were identical to those used in batch experiments (Korrmüller *et al.*, 1997a). The ozone reactors were slightly pressurized at $+0.05 \cdot 10^5$ Pa. A data monitoring system was used for continuously measured parameters (ozone gas concentrations, pH, redox potential) with a time interval of 2 min. The effluent of the second ozone reactor was stored in a tank, until ozone was totally used. Afterwards it was pumped into the bioreactor (flow rate 66 mL/h), which was operated as a chemostat with the same dimensions. But the retention time was 30 h. Nutrients and trace elements were fed. The oxygen gas flow rate was 10 L/h to the bioreactor and the oxygen concentration was measured in the liquid phase. The temperature of all three reactors was controlled at $30 \pm 1^{\circ}\text{C}$. The pH was measured continuously (Mettler-Toledo 405-S7/200; WTW pH 160 meter) and controlled automatically by dosing sodium hydroxide (1 M or 0.1 M NaOH) to 6.7. During continuous treatment, samples were withdrawn by glass taps at the top of reactors for analysis. With this kind of reactor any sealing material in the fluid phase and therefore PAH-adsorption to it was avoided.

Oil/water-emulsion. Dodecane (> 99%, Merck-Schuchardt) was used as a model substance for a mineral oil. To produce the synthetic oil/water-emulsion, the PAH were dissolved in dodecane first. The five ring condensed PAH benzo(e)pyrene (BeP) and benzo(k)fluoranthene (BkF, both 99%, Sigma-Aldrich) were applied at different initial concentrations in oil/water-emulsion concentrates, which were comparable to real oil containing wastewaters. For the concentrate preparation, the internal oil phase was dispersed in tap water under defined energy input of 10,000 rpm in a toothed disc dispersing machine (Ystral X40/38) for one minute. The dispersion was stabilized by a nonionic emulsifier, the fatty alcohol ethoxylate Eumulgin ET5 (ET5; 3-9% C14, 25-28% C16, 60-70% C18, partially containing one or two double bonds, with five ethylene oxide groups (EO), Henkel) or Genapol UD-050 (GUD-50; C11 with 5 EO, Hoechst). The oil/water-emulsion contained dodecane in a final concentration of 750 mg/L and 150 mg/L emulsifier. The molecular structures of BeP, BkF, dodecane and nonionic emulsifiers are shown in Figure 2.

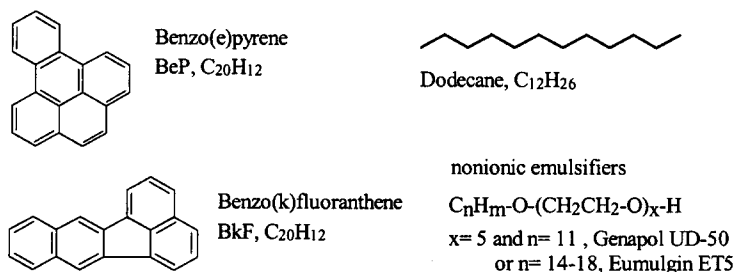


Figure 2. Molecular structures of PAH, dodecane and nonionic emulsifiers studied in continuous ozonation.

Analysis. PAH and dodecane were prepared for analysis by liquid-liquid extraction. To a sample volume of 5 mL, 1 g magnesium sulfate and 1 drop sulphuric acid were added for breaking the emulsion and 5 mL toluene for extraction. Samples from the bioreactor were extracted with 10 mL toluene in order to secure disintegration of bacteria cells. The extracts were analyzed for dodecane by a Hewlett-Packard 5890 gas-chromatograph equipped with a flame ionization detector. The details of this analysis are described elsewhere (Kornmüller *et al.*, 1997a). High pressure liquid chromatography (HPLC) on reversed phase (Phenomenex Envirosep-PP-column, 125 x 4.6 mm, thermostated at 40°C) was run for the analysis of PAH from toluene extracts. A Shimadzu fluorescence detector RF 551 was used for detection of BeP at the excitation 279 nm and emission 388 nm and of BkF at 302 nm/ 429 nm. Methanol/water-mixture 90:10 (v:v) was applied as mobile phase with a flow rate of 1 mL/min at isocratic operation. The oxidation products of BeP were analyzed at isocratic conditions with acetonitrile/water 85:15 (v:v) by HPLC using the fluorescence detection at 265 nm/ 377 nm. The previous 10 mL sample was extracted by addition of 1 g magnesium sulfate and 10 mL ethylacetate. Parallel samples were extracted with pentane in the ratio 1:1 or 1:2 and scanned at 200-900 nm in the spectrometer UV 1202 (Shimadzu). Protein concentration as a biomass parameter was determined according to Lowry *et al.* (1951).

Ozone consumption. Due to the problems of measuring the dissolved ozone concentration in oil/water-emulsions (see Kornmüller *et al.*, 1997a), the ozone consumption was calculated from the ozone gas balance for the first ozone reactor. The overall ozone consumption rate $r_{O_3,\Sigma}$ contains the ozone mass used by physical absorption, decomposition and reaction of ozone related to the liquid volume V_L and was calculated by Equ. (1) with \dot{V}_g : gas flow rate, c_{g0} : ozone gas inlet concentration and c_{g1} : ozone gas outlet concentration.

$$r_{O_3,\Sigma} = \frac{\dot{V}_g(c_{g0} - c_{g1})}{V_L} \quad (1)$$

PAH reaction rate. The PAH reaction rate was calculated by Equ. (2) with \dot{V}_L : liquid flow rate, c_{s0} : PAH influent concentration and c_{s1} : PAH effluent concentration.

$$r_s = \frac{\dot{V}_L(c_{s0} - c_{s1})}{V_L} \quad (2)$$

RESULTS AND DISCUSSION

First studies were focused on the two-stage ozonation system to examine main parameters influencing the oxidation rate of BeP and BkF, which were already determined in batch experiments (Kornmüller *et al.*, 1997a, b), and to show the general feasibility of a continuous PAH ozonation.

Improvement of mass transfer rate gas-liquid. The influence of the mass transfer gas-liquid on the PAH ozonation was the first main parameter to be studied. One possibility of enhancement was the use of increasing stirrer speeds, which leads to a better dispersion of the oxygen/ozone air bubbles. The BkF oxidation rate increased with rising stirrer speed, but no improvement was achieved at higher than 380 rpm. Only the ozone consumption still increased. For further continuous ozonation a stirrer speed of 380 rpm was used. In the context of increasing the ozone mass transfer gas-liquid, the influence of the ozone gas inlet concentration was studied.

Subsequently to Henry's law, an increase in ozone gas inlet concentration effects a higher equilibrium concentration at the interface gas-liquid and consequently a stronger gradient between the equilibrium and dissolved ozone concentration in water. The mean BkF reaction rate r_s and the overall ozone consumption rate $r_{O_3,\Sigma}$ at stable operation are shown in Fig. 3 versus the ozone gas inlet concentration of the first reactor. There is a strong influence on the ozone gas inlet concentration, but above 20 mg/L the BkF elimination decreases compared to the still rising overall ozone consumption rate due to the very low residual BkF concentration in the first reactor. This is a comparable behavior to the increase of the stirrer speed.

The ozone gas inlet concentrations of the second ozone reactor, which are not shown, amount to 29% of the first reactor. This lower ozone gas inlet concentrations have only a small contribution to the total BkF oxidation under the condition chosen. Subsequently a ozone inlet concentration of 20 mg/L was used further, where a residual BkF concentration of 20 µg/L was obtained in the first ozone reactor. The dodecane concentration stayed almost constant during ozonation; no oxidation but a small loss of dodecane by stripping and adsorption to reactor glass walls occurred. Consequently BkF, which is dissolved in the dodecane droplets due to its low water solubility (0.0006 mg/L at 25°C; Sims *et al.*, 1983), was lost under aeration with pure oxygen by 7% in the first and 9% in the second reactor compared to the influent.

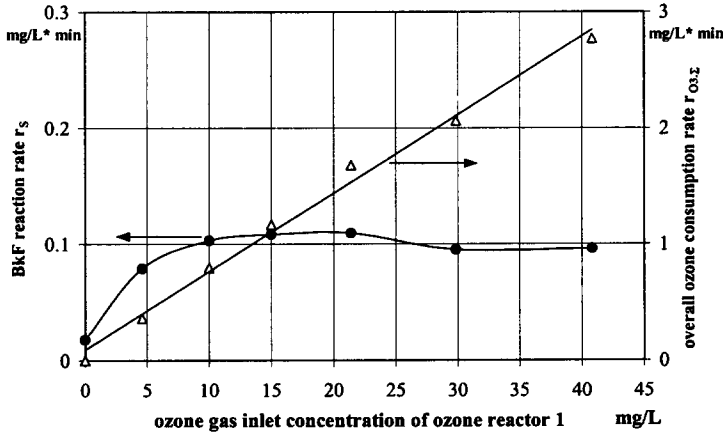


Figure 3. Mean reaction rate r_s of benzo(k)fluoranthene and overall ozone consumption rate $r_{O_3, \Sigma}$ versus ozone gas inlet concentration c_{O_3} of the first reactor ($c_{BkF} = 1$ mg/L BkF, $t_r = 10$ min, pH 6.7, 150 mg/L GUD-50, 750 mg/L dodecane).

Influence of temperature. While in batch experiments no significant influence of temperature in the PAH-ozonation could be detected (Kornmüller *et al.*, 1997a), in continuous treatment a dependency on the temperature was observed. Between 14 - 48°C the normalized PAH concentrations in both ozone reactors increased with rising temperature (Fig. 4). The decreasing dissolved ozone concentration predominates the reaction with rising temperature compared to the influence of temperature on the BkF reaction rate constant.

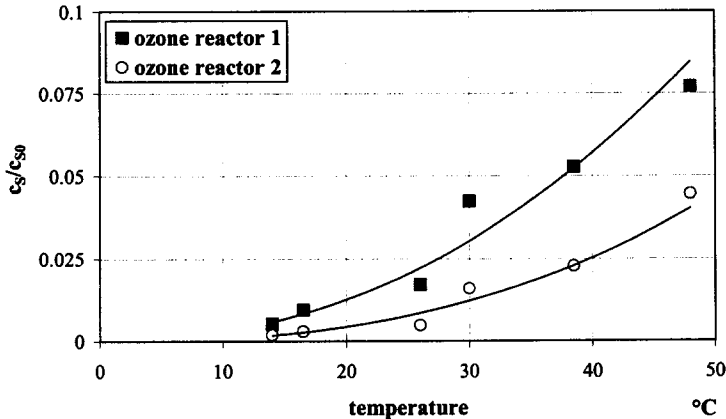


Figure 4. Mean concentration of benzo(k)fluoranthene in the first and second ozone reactor related to the influent concentration to the first ozone reactor ($c_{B0} = 2.3$ mg/L BkF) versus temperature ($t_r = 10$ min, pH 6.7, 150 mg/L GUD-50, 750 mg/L dodecane, $c_{O_3} = 20$ mg/L ozone).

Influence of pH. The pH value is an important parameter for the type of ozone reaction and oxidation products formed. The results of experiments with different pH confirmed a direct reaction mechanism for $\text{pH} \leq 8$, where molecular ozone is the reacting agent. But above pH 8 the BkF concentrations in the first and second ozone reactor increased strongly (see Fig. 5). At pH 11.8 no elimination of BkF could be achieved anymore by ozonation, which can be explained by the high decay of molecular ozone in the water by hydroxide ions and consequently the lack of dissolved ozone inside the oil droplets, where the direct reaction with PAH takes place. At alkaline pH a great influence of the ozone mass transfer fluid-fluid on the reaction has to be assumed.

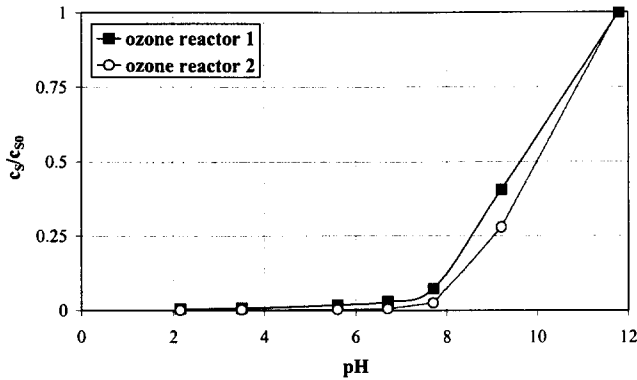


Figure 5. Mean concentration of benzo(k)fluoranthene in the first and second ozone reactor related to the influent concentration to the first ozone reactor ($c_{S0} = 2.3 \text{ mg/L BkF}$) versus pH value ($t_r = 10 \text{ min}$, 150 mg/L GUD-50 , $750 \text{ mg/L dodecane}$, $T = 30^\circ\text{C}$).

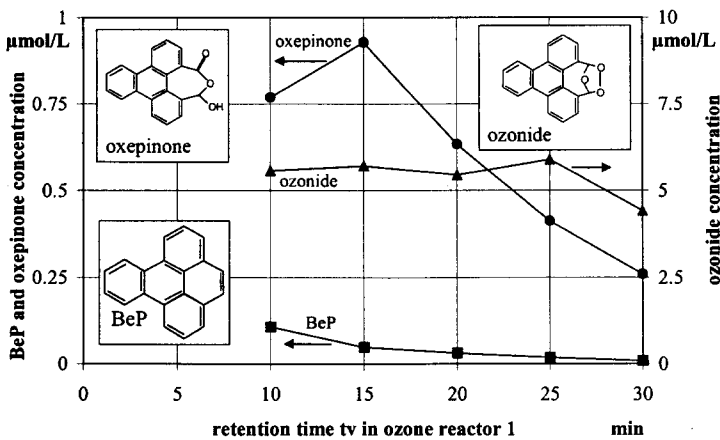


Figure 6. Mean molar concentration of benzo(e)pyrene, ozonide and oxepinone ($c_{S0} = 6.2 \mu\text{mol/L BeP}$) versus retention time in the first ozone reactor ($\text{pH } 6.7$, 150 mg/L GUD-50 , $750 \text{ mg/L dodecane}$).

Ozonation products. Schulz (1998) detected a number of lowly condensed oxidation products formed from BkF in similar oil/water-emulsions, while by ozonation of BeP only two main products: a secondary ozonide and oxepinone (hydroxytriphenylenol[4,5-cde]oxepin-6(4H)-one) could be identified and isolated. Both oxidation products correlate with a direct reaction mechanism of ozone. The purified oxidation products were used for analysis and quantification. Fig. 6 shows the molar concentrations of BeP, ozonide and oxepinone in dependence on retention times in the first ozone reactor. While the further oxidation of oxepinone starts at retention times above 15 min, an elimination of the ozonide by oxidation needs a retention time above 25 min. The molar mass balance fits to 85-115% with an average of 101%. Compared to the first reactor the oxepinone and ozonide concentrations in the second reactor were lowered by 15 % at

the mean of all retention times. The simultaneous formation and further oxidation of both oxidation products was shown in batch experiments too (Kornmüller *et al.*, 1996), which results in ring opening.

Because of a possible increase of mutagenicity by ozonation, Ames-tests were done by the Department of Environmental Microbiology and Technical Hygiene of TU Berlin. The Ames test was performed in microtiter plates with *Salmonella typhimurium TA 98*, which allows a high reproducibility. Parallel samples were taken from the influent and the first ozone reactor during the ozonation of 1.8 mg/L BeP at a retention time of 10 min. A significance of less than 30% compared to the control, but no difference between the influent and the first ozone reactor was detected. In the original concentration no great sensitivity to mutagenicity could be stated and therefore no further dilution rows of the samples could be used to determine a concentration-effect-relationship, which is necessary to declare a mutagenicity. Even with the purified BeP oxidation products no significant mutagenicity could be determined (Schulz, 1998).

Biological treatment. Optimization of the whole treatment process affords, that the oxidation is only conducted until the primary PAH oxidation. The formed ozonation products should be treated biologically. Therefore, the two stage ozonation was performed with a retention time of 10 min. Instead of GUD-50, ET5 was used as emulsifier in the oil/water-emulsion. Besides the HPLC analysis, both BeP oxidation products can be followed easily by qualitative photometrical measurements, because of their absorption maxima at 265 nm. In Fig. 7 the differences in absorption spectra between „influent bioreactor“ and „effluent“ demonstrates transformation effects between 230 and 300 nm. None of the three absorption spectra (BeP, ozonide, oxepinone) could be observed in the effluent of the bioreactor.

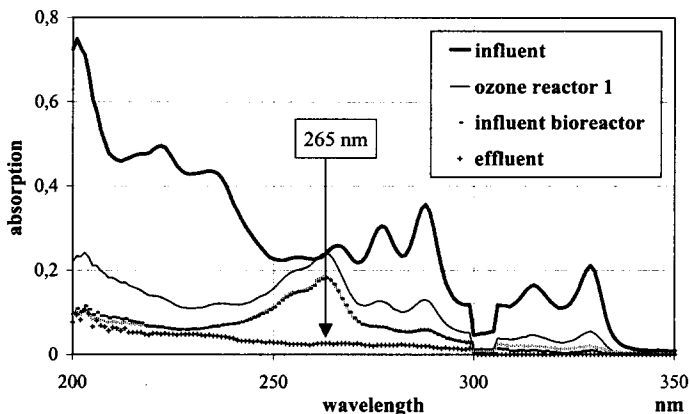


Figure 7. Absorption spectra during the two stage ozonation followed by biological treatment (c_{50} = 2 mg/L BeP, pH 6.9, 150 mg/L ET5, 750 mg/L dodecane, t_r = 10 min in ozone reactors, t_r = 30 h in bioreactor, c_{biomass} = 400 mg/L protein).

Dodecane was completely biodegraded. A better biodegradation of the nonionic emulsifier treated previously by ozonation was observed by batch experiments. Because of the low concentrations of BeP oxidation products, their biodegradation could not be proved by biomass growth or DOC measurements. An increase of protein concentration followed by dodecane and emulsifier mineralization, which was about 400 mg/L protein during continuous biotreatment. The retention time of 30 h in the continuously operated bioreactor was necessary for a total mineralization of dodecane and emulsifier (Cuno, 1996). In batch experiments the ozonide was eliminated during biodegradation after one hour.

CONCLUSIONS

By continuous ozonation in a two step reactor cascade the primary oxidation of the highly condensed BeP and BkF inside the dodecane droplets of oil/water-emulsions was demonstrated. Comparable to earlier batch experiments no ozonation of the biodegradable dodecane could be observed. Most of these PAH were transformed by ozonation inside the first step, so a simpler reactor design using only one ozone reactor can be realized. By improving the ozone mass transfer rate gas-liquid the PAH oxidation rate could be increased, but further improvement at very low residual PAH concentrations only effects an increasing ozone

consumption. By variation of pH and as a result of the type of ozonation products found the ozone reaction with the PAH studied can be attributed to a direct reaction mechanism. Two main ozonation products of BeP could be quantified and their further, slow ozonation as well as their biotransformation could be demonstrated. A mutagenicity of these oxidation products could not be proved finally. Furthermore this treatment process can be optimized by using a very small ozone reactor compared to the bioreactor and a sedimenter to increase the biomass concentration by recycling.

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