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Leachate treatment by the combination of photochemical oxidation with biological process

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Abstract

Leachates are well known as a particular sort of problematic wastewater, both from the viewpoint of ecotoxicity and the treatment technique. Leachates from municipal waste deposits, biologically pre-cleaned, were treated subsequently by photochemical oxidation using three different UV sources. The reactor concept was based on a 'continuous circuit reactor' with back-mixing and thin film. The power was 84 kW/m^3 for the low pressure mercury lamp, 100 kW/m^3 for the middle pressure mercury lamp, and 30 kW/m^3 for the vacuum mercury lamp. The ratio of chemical oxygen demand (COD) and BOD_5 was reduced from ca. 230 to 3–4 (in the case of low pressure and vacuum mercury lamp) and to 6 (middle pressure mercury lamp). After the photochemical oxidation stage, the leachate was treated in an additional step using an activated sludge plant. After this biological stage, the values of COD, BOD, and AOX decreased further below the threshold values defined in the legislative regulation. The Ames-, umu-, and alkaline filter elution-tests were applied at all treatment stages. The treated water, independent on treatment stage, proved to be free of mutagens. The luminescent bacteria—green algae—as well as Daphnia-test were applied for each of the individual treatment stages.

Keywords: Leachate; UV irradiation; AOP; Biological treatment; Biological toxicity test; Mutagenicity test

1. Introduction

Strict monitoring of leachates is required according to legislative regulation in terms of efficient elimination of refractory pollutants [1]. Particularly, in wastewater purification processes which depend on the characteristics of the components and require specific discharge systems [2,3], intensive studies have been carried out to improve the efficiency for decreasing chemical oxygen demand (COD) and adsorptive organic halogen (AOX) as well as ammonium/nitrogen contents. For this purpose, the biological treatment is generally accepted as the economically most efficient method. Especially, the activated sludge process is applicable for the removal of the biodegradable organics and inorganic N-compounds, where high sludge contents and intensive air ventilation are necessary. Heavy metals play mostly a less significant role in leachate treatments. Nevertheless, the application of such processes resulted in residues, which must be further treated, concentrated, and if necessary, deposited as special refuse [4–6].

On the other hand, the photochemical oxidation shows specific advantages because it can eliminate non-biodegradable organic components and avoids a deposit of residues as a special waste. This technique is based on H_2O_2's catalytic reaction, which is decomposed into reactive radicals by UV light [7]. Depending on the concentration of added oxidation reagent and the intensity of radiation energy, any organic components of wastewater can be broken down, and completely mineralized into CO_2 and H_2O. Nevertheless, this process is rather costly, and mutagenic intermediate products could be formed during the UV-oxidation [8]. For these reasons, the coupling of photochemical oxidation process with a biological subsequent treatment was designed for the wastewater treatment [9–11], where the potential of leachate toxicity was reduced considerably by such a combination process.

In this study, the behavior of raw leachates and also biologically pre-treated leachates from three German refuse deposits were investigated thoroughly, which were treated by photochemical oxidation in an operating circuit reactor of pilot scale. The intensive color of wastewater inhibits an effective transmittance of UV light. Therefore, in view of accessible UV-light radiation, two types of reactor were examined, consisting of not only a common loop system,

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but also a thin film. Further-on, irradiated leachates were biologically post-treated in an activated sludge plant.

The treatment efficiency of each process, e.g. biological pre-treatment, UV/H_2O_2- and biological post-treatment was controlled by the detection of the typical sum parameters (such as COD-, AOX-, and BOD-values). In order to assess the ecological compatibility of the UV-oxidation in combination with the biological process, some ecotoxicity tests were applied on aquatic biological organisms, such as luminescent bacteria, Daphnia as well as green algae. Corresponding tests for genotoxic effects (Ames-, umu-test, and alkaline filtration) were run according to standard protocol in order to evaluate a potential human toxicity and mutagenicity at each treatment stage.

2. Material and method

2.1. UV-irradiation system with middle and low pressure mercury lamp module

The laboratory equipment of photochemical oxidation was constructed on the base of a loop system by two UV irradiation reactors, which consisted of a "middle pressure mercury lamp" and a "low pressure mercury lamp" module, optionally operated by a valve control switch. These radiation sources were purchased from Heraeus Noblelight (Hanau) company.

In the middle pressure mercury lamp (BEQ 1023) the power intake was 1.0 kW and a broad radiation spectrum was found with main emission lines at wavelengths of 254, 313, and 360 nm. In order to prevent the heating up of the reactor, the UV lamp module was enclosed by a cooling jacket.

In the low pressure mercury lamp an emission peak shows up mainly at the wavelength of 254 nm. The low pressure mercury reactor consisted of seven UV lamps (total intake capacity: 0.21 kW), which were disposed not only in a radial way around a quartz glass pipe (6×30 W), but also in a central position inside of the pipe (1×30 W), in order to guarantee an intensive irradiation into a relatively thin water layer.

The whole equipment was constructed of stainless steel for the purpose of an inhibition of corrosion caused by chlorine contents and an adsorption of chloroorganic substances.

The photolysis experiments were carried out with a recycling concept, by which a portion of irradiated leachates was continuously refluxed into the circuit reactor to accomplish a high efficiency in the chemical oxidative treatment process. The total treated volume of leachates per operation was 40 l.

2.2. UV-irradiation system with vacuum mercury lamp module

In this study, also a vacuum mercury lamp (VUV/UV), of which emission peaks occurred at wavelengths of 185 and 254 nm, was separately utilized for efficient photo-oxidative degradation of organic substances in leachates. The total intake of power was 15 W; this VUV lamp was generously provided by Institut für Niedertemperatur-Plasmaphysik e.V. (INP) in Greifswald, Germany. The U-formed vacuum mercury lamp tube was placed in the center of the reactor at a distance of 2 cm from a trickling thin film of the water flow, which permitted, especially, the passage of higher energy photons through a 1 mm thin layer to the target molecules. The investigated reactor was also operated in continuous circulation mode identical to the above oxidation system (see Section 2.1).

In addition, the photolysis experiment with vacuum UV lamp was carried out independently under nitrogen stream supply to detect how the degradation process was changed under oxygen deficient conditions.

2.3. Activated sludge system for biological treatment

For the purpose of optimizing the degradation efficiency with the help of suitable microorganisms, which were injected from a biological pre-treatment stage of a deposit site, the required nutrients were added by means of a pump into an activated sludge reactor. In addition, the air pump provided filtered, oil-free air, which was uniformly distributed by a frit installed at the bottom of the reactor generating small bubbles into the system. The sludge contents were adjusted to about 3–4 g/l dry substances. In order to examine the efficiency of the activated sludge system the degradation rate of "synthetic wastewater" was determined first.

The activated sludge plant was constructed to treat 4 l volume of effluent from the above UV/H_2O_2 process, operated with low and middle pressure mercury lamp reactor (34 l).

In addition, a smaller reactor, of 0.7 l (diameter = 7 cm) volume, was constructed, that was treating effluent from the photochemical oxidation operated with the vacuum mercury lamp reactor (6 l) (see Table 1).

The scheme of all leachate treatment process is depicted in Fig. 1.

2.4. Determination of sum parameter such as BOD_5, COD, AOX

In order to control the general sum parameters, the H_2O_2-residue was eliminated by the addition of 100 μl catalase to each 100 ml test solution after the neutralization with NaOH or HCl, which was prepared by adding 500 μl of catalase (cow liver Sigma C-100) in 100 ml of 0.05 M potassium phosphate buffer (pH 7). The concentration of residual hydrogen peroxide in the test solution was controlled by use of test sticks (Merck Peroxid-Test 10011).

The precise determination of hydrogen peroxide in the test solution, which was added to the photolytical experiment, was performed on the basis of a photometrical determination (DIN 38409 part 15) [12].

Table 1
Efficiency (by determination of sum chemical parameters) of various treatment processes and operation parameters

Parameter	Leachate treatment process															
	Biological pre-treatment	0.21 kW low pressure mercury lamp				1.0 kW middle pressure mercury lamp				15 W vacuum mercury lamp (aerobic)				15 W vacuum mercury lamp with N_2 (almost anaerobic)			
		UV/H_2O_2 stage		Biological post-treatment		UV/H_2O_2 stage		Biological post-treatment		UV/H_2O_2 stage		Biological post-treatment		UV/H_2O_2 stage		Biological post-treatment	
		Det.	Deg.[a]	Det.	Deg.[b]	Det.	Deg.[a]	Det.	Deg.[b]	Det.	Deg.[a]	Det.	Deg.[b]	Det.	Deg.[a]	Det.	Deg.[b]
COD[c] (mg/l)	920	326[d] 245[e]	64%[d] 73%[e]	183	60.5%	328	58%	138	62%	463	47%	184	65.8%	489	40%	240	57%
BOD[c] (mg/l)	4.5	72.3	16[f]	6	96.8%	54	10[f]	3	98.1%	71	16[f]	3	98.3%	67	15[f]	3	98%
AOX[c] (μg/l)	1100	500[d]	55%[d]	500	0	684	38%	215	69%	715	51%	320	55%	600	59%	350	42%
	–	488[e]	56%[e]														
Throughput	–	2.5 l/h		0.4 l/h		20 l/h		0.4 l/h		0.5 l/h		0.07 l/h		0.5 l/h		0.07 l/h	
Circulation rate	–	300 l/h			–	300 l/h			–	180 l/h			–	180 l/h		–	
Stationary total volume	–	34 l and 20 l		4 l		34 l		4 l		6 l		0.7 l		6 l		0.7 l	
Energy demand	–	84 kW/m^3		–		100 kW/m^3		–		30 kW/m^3		–		30 kW/m^3		–	

Det.: determined value; Deg.: degradation efficiency.

[a] Calculated by the relation of effluent of UV/H_2O_2 stage and influent from biological post-treatment.

[b] Calculated by the relation of effluent of biological post-treatment and influent from UV/H_2O_2 stage.

[c] Sum parameter at the end of each process (UV stage, $t = 8$ h; biological stage, $t = 40$ days).

[d] Experiment with 34 l stationary total volume of reactor.

[e] Experiment with 20 l stationary total volume of reactor.

[f] Factor by which the BOD value was increased in relation to its initial value.

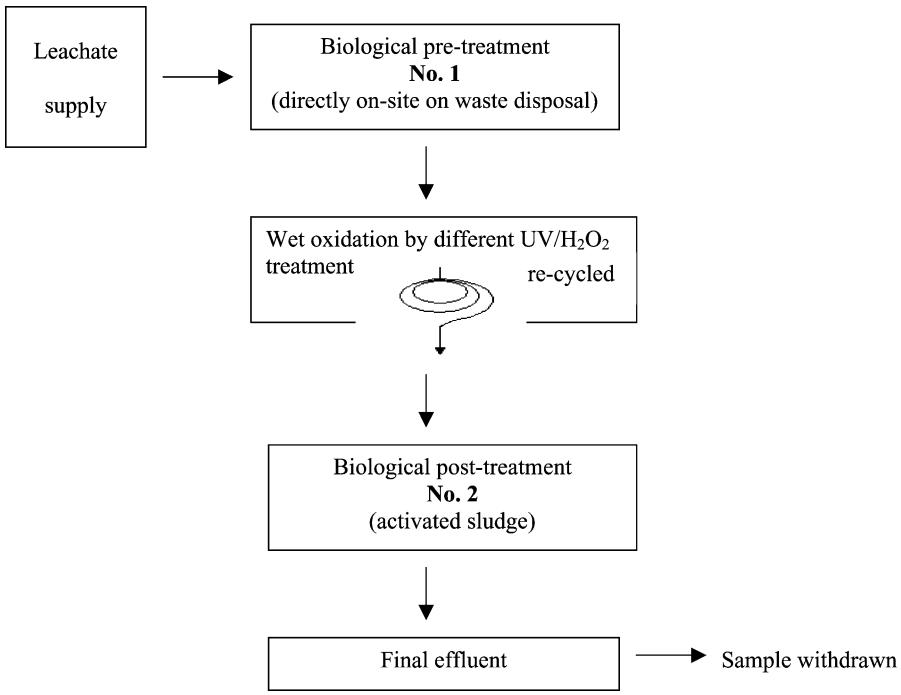

Fig. 1. The scheme of leachate treatment processes.

The determination of BOD_5 was carried out according to standard test protocol of DIN 38409 part 51. The oxygen demand was determined after an incubation time of 5 days at 20 °C in the dark [13].

For the determination of the COD the reagent kit of Hach company was used. The result was expressed as an extinction of potassium dichromate at 420 nm, which was regularly calibrated with given standards according to DIN 38409, part 41 [14].

The concentration of anions, such as chloride, nitrite and nitrate ion, which were contained in the leachate, was determined by ion chromatography based on DIN 38405 part 19 [15].

The quantitative detection of AOX was carried out according to DIN 38409 part 14 [16].

2.5. Sample preparation and operation parameter

The leachates were sampled in three municipal waste deposits located in North Germany, at which house- and industrial-refuses were deposited in addition to various other sources (building site waste, building rubble, clay, and mixed soil).

The biological pre-treated leachates (COD: 790–920 mg/l, BOD: 4.5–6 mg/l and AOX: 1100–1470 μg/l) from municipal deposits were introduced as influent into a preliminary reactor, where they were mixed with previously photo-treated leachates. Here, COD of the diluted influent was reduced to 270–500 mg/l and BOD increased to 65–85 mg/l. H_2O_2 was added to the solution at concentration of 1000 mg/l and adjusted to a pH 4. A stationary oxygen concentration was set up to 20 mg/l for the low pressure- and middle pressure-mercury lamp and to 10 mg/l for vacuum mercury lamp, which exceeded the saturation concentration (at 30 °C about 7.5 mg/l) in the solution. In another experiment a continuous N_2 stream was supplied in connection with vacuum mercury lamp keeping the oxygen concentration of the medium at about 1.6 mg/l. The transmittance of UV light that was an important factor in the UV/H_2O_2 process was about 90% at 1 cm path length.

After the given treatment time with different UV-reactors a part of irradiated leachates was withdrawn as the effluent, and the common chemical sum parameters, such as oxygen concentration, temperature, and pH values were again controlled in a prepared detection chamber.

Thereafter the pH value of the effluent from the UV/H_2O_2-process was adjusted to 7.5 ± 0.1 and the remaining H_2O_2 concentration was eliminated by an addition of catalase. At the last treatment stage with an activated sludge plant the reaction time of leachate was set to 10 h and the O_2-content was set in the range of 7.5–8.5 mg/l. The volume of leachate was stepwise increased by 10–20%. The adaptation phase of microorganisms lasted 3 months.

The overall results of each process are summarized in Table 1, where significant degradation rates of treated leachates were achieved in terms of COD, BOD and AOX reduction.

2.6. Toxicity and mutagenicity experiments

In order to conduct the toxicity tests, the leachate samples were neutralized and existing residual concentrations

of hydrogen peroxide were eliminated by the addition of catalase as described above.

The luminescent bacteria test was carried out according to DIN 38412 part 34 [17] with *Photobacteria phosphoreum* as a selected organism of the strain *Vibrio fischeri*. Light emission of the luminescent bacteria after an incubation time of 30 min was determined at 585 nm by means of an "Illuminometer", available under the trade name Lumistox® of Dr. Lange GmbH.

The leachate samples were also investigated with respect to the response of freshwater green alga, *Scenedesmus subspicatus*, in the cell growth inhibition test according to DIN 38412 part 33 [18]. The cell growth inhibition rate after an incubation time of 72 h was determined by means of a fluorescent and particle counter device in order to generate reproducible results.

The toxicity of leachates upon the motility of *Daphnia magna Straus* was investigated in the 24 and 48 h immobilization test according to DIN 38412 part 30 [19].

The Ames test was carried out following to DIN UA 12 [20] based on "Revised methods for the Salmonella mutagenicity test" [21,22]. Experiments were run for 24 h at 37 °C with two bacteria strains of *Salmonella typhimurium*, TA 98 and TA 100 that were generously supplied by the laboratory of Dr. Ames, University of California. In a separate but essentially identical histidine-deficient petri dish, another batch of Salmonella bacteria was incubated together with S9 fraction (Organon Technika company in Eppelheim) which consisted of mammalian enzymes (obtained from liver cell extracts of rats) required for mammalian metabolism.

The umu test was applied according to DIN 38415 (DIN UA 12) (ISO/DIS 13829, 2000) [23] and based on incubation of bacteria *S. typhimurium* TA 1535/pSK 1002 for 2 h at 37 °C. Genotoxicity effects of test solution were quantified by measuring the induction of specific umuC-genes and their analogue of muc AB, which were coupled with bacterial mutagenese. The activation of the mutagen response in the bacteria was then defined by recording the activity of β-galactosidase, which was determined photometrically by using micro-titer plates. For standard applications of the umu test, parallel controls of metabolic activation using S9 were run.

The alkaline filter elution test as well as the above described umu test was carried out in cooperation with the research group "Molecular Mechanisms of Environmentally Responding Gene Toxicity" at University of Mainz. Alkaline filter elution test [24,25] was applied according to Waldmann [26] to detect the specific and quite distinctive DNA transformation as single strand breakage, cross-linking and endonuclease sensitive positions. *Corbicula fluminea* (river shellfish) utilized as the test organism was incubated between 2 and 24 h with test solutions. After the addition of the alkaline solution, any damaged DNA short fragments eluted through the filter were quantified by using a fluorometric measurement.

The mutagenicity tests (Ames-, umu-, and alkaline filter elution test) were normally carried out with samples dissolved in distilled water. In order to determine any gene toxicity of treated leachate samples at high sensitivity, some selected samples were concentrated by means of a solid phase extraction with RP C18 material or by enrichment on XAD resin.

Potential response to all mentioned toxicity and mutagenicity test of the additives catalase, NaOH, and HCl were recorded and taken into account.

3. Results and discussion

3.1. Sum parameter-analysis of COD, AOX, BOD and pH

In Table 1, the efficiencies of four different UV/H_2O_2 processes and subsequent biological post-treatment at the end of each process are summarized.

In the photochemical treatment with UV lamps after 8 h reaction time an efficient COD (40–64%) and AOX (38–59%) reduction was accomplished, although BOD_5 increased up to between 70 and 80 mg/l. The ratio of COD/BOD_5 was generally decreased from 195–230 to 4–6, so its ratio was drastically enhanced by the above photochemical treatment.

In all test series, it was consistently shown that COD degradation could be achieved at low pH value (pH < 4). This could be explained on the basis that the pH has influenced the oxidation potentials of OH-radicals. In addition, pH could play an important role in the reduction of carbonate concentration and a consistent raise of transmission. In order to test a pH effect for the photochemical reaction, in one experiment HCl was added to the solution. At this very low pH, such additives caused an undesired salination of leachates. But this disadvantage could be compensated with the reduced energy demand for their degradation by UV irradiation.

Another photolysis experiment (turnover rate: 10 l/h) was carried out at pH 7. Surprisingly the COD increased here from 250 mg/l to over 500 mg/l during the experiment and a degradation of leachate could not be achieved. In this case (pH 7), an efficient reaction was possible only when the turnover rate of leachate was reduced to about 5 l/h and the concentration of H_2O_2 was raised to 2000 mg/l. Consequently, this procedure resulted in a duplication of energy demand of about 200 kW/m^3.

The initial transmittance of UV light of 89%, that was an important factor in the UV/H_2O_2 process, was significantly decreased to about 80% after 1 h, and then remained constant until the end of the treatment of 8 h. The oxygen concentration was held constant until the end of irradiation. The initial temperature of the solution was from 19 °C (low pressure mercury lamp) to 30 °C (vacuum mercury lamp) and it was held 33 °C, continuously remaining here until the

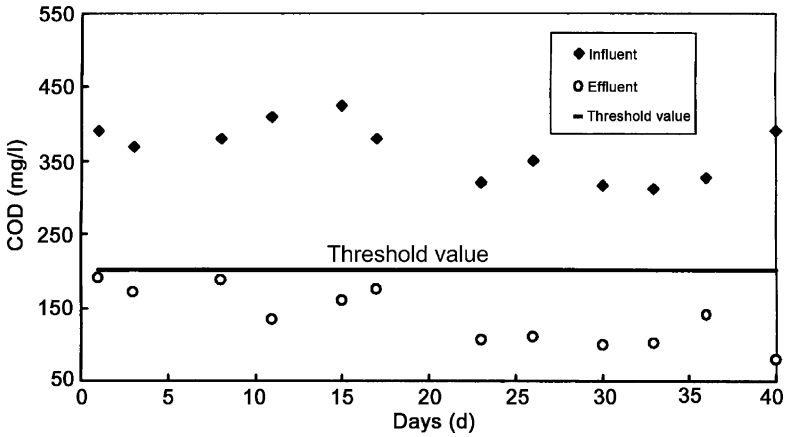

Fig. 2. COD of effluent of biological stage (with an activated sludge plant) which was treated by UV/H_2O_2 with 1.0 kW middle pressure mercury lamp (threshold value for discharge into "canal").

end of experiment. The temperature of the whole irradiation system of the middle pressure mercury lamp was kept constant at 42 °C by means of a cooling jacket.

The energy demand of the middle pressure mercury lamp (100 kW/m^3) was larger than that of low pressure mercury lamp (84 kW/m^3). The "positive effect" of low pressure mercury lamp could be interpreted by the factor that the sole emission at 254 nm as radiation source was very effective for a degradation of organic compounds. The COD degradation efficiency of vacuum mercury lamp was inferior to that of the two other UV reactors (see Table 1), but it required less electrical power (30 kW/m^3) for the operation of the UV reactor. In this case, a photolysis of oxygen occurred on the basis of the formation of active neutral O_2/O_3-radicals and O_2/O_3-anions that were produced by UV light at 185 nm. The reduction of O_2-content with the vacuum mercury lamp under nitrogen supply led to the lowest COD degradation, so it was concluded that the COD degradation efficiency was higher in the case of at O_2-saturation phase than at O_2-deficiency.

The COD value of leachates after the biological post-treatment step (after 40 days) was observed to be below 200 mg/l, the threshold value for a direct discharge of wastewater according to the legal restriction. In particular a BOD value close to zero was achieved (reduction by 98%) and the rather small values of the chemical parameters (BOD, AOX) enabled the direct discharge of leachate into the sewage line. It could be interpreted that the photons in photochemical oxidation were not sufficient for a complete degradation of large molecules (humic acids, proteins, and carbohydrates, etc.) and ultimately the partial degraded hydroxylic fragments were mineralized at the end by using microorganisms in an activated sludge plant.

Fig. 2 shows the decrease of COD of effluent having passed the biological processing as a function of treatment time, which was previously treated by photolysis with the middle pressure mercury lamp. The BOD decrease was plotted similarly versus the treatment time of leachate in an activated sludge plant in Fig. 3. The studied UV/H_2O_2 process vacuum UV lamp in combination with biological stage

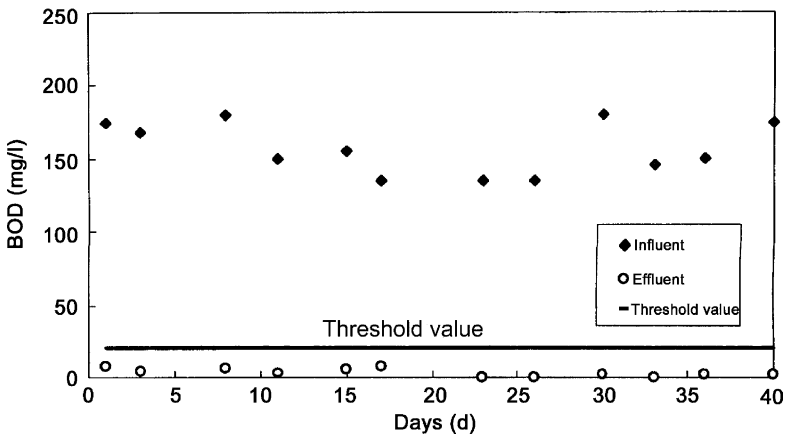

Fig. 3. BOD of effluent of biological stage (with an activated sludge plant) which was treated by UV/H_2O_2 with 1.0 kW middle pressure mercury lamp (threshold value for discharge into "canal").

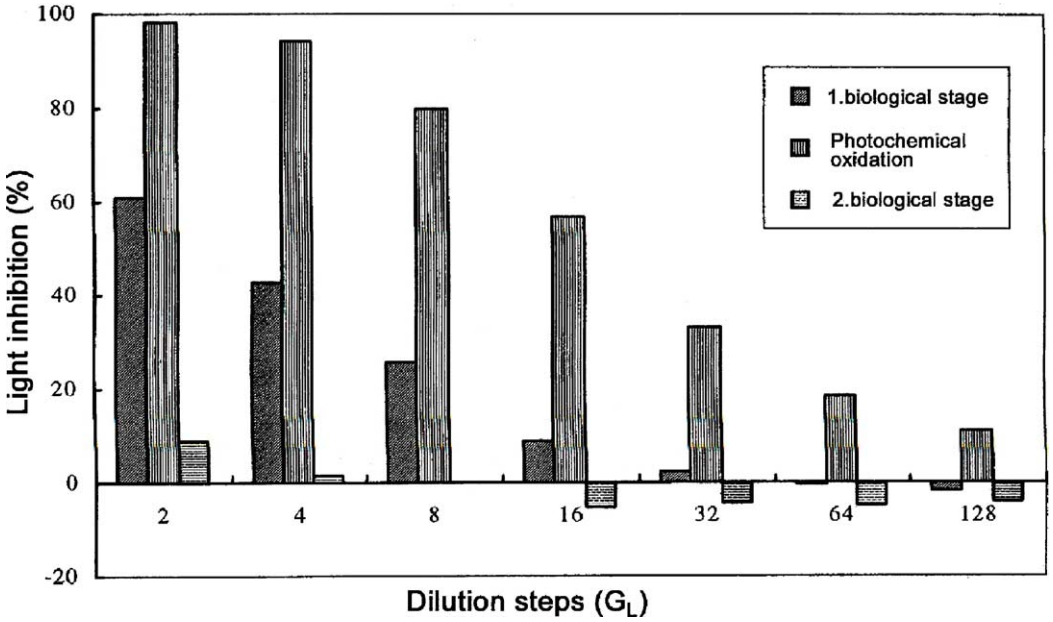

Fig. 4. Luminescent bacteria toxicity of various leachate treatment stages after 30 min incubation time: photochemical oxidation with low pressure mercury lamp (pH 7; H_2O_2 was eliminated by the addition of catalase).

showed the most effective reduction of chemical parameters (COD, BOD, AOX). With regard to the electric power demand the coupling of the photo-oxidation with vacuum mercury lamp followed by a biological post-treatment suggests a very suitable solution for a novel leachate treatment process of high efficiency.

In the photo-oxidation with vacuum mercury lamp under nitrogen supply, a somewhat higher de-halogenation reaction (AOX: 59%) at (nearly) anaerobic state was observed compared to that of aerobic state. Hence, the vacuum mercury lamp technique could be applied at (nearly) anaerobic conditions for the treatment of leachate of high AOX-contents.

The combination of UV/H_2O_2 (with vacuum mercury lamp) with a final second biological treatment step initiated the fragmentation of a part of organic molecules as indicated by the change of the sum parameters COD, BOD, and AOX. Nevertheless, it may be concluded that UV/H_2O_2 treatment, under rather costly nitrogen current, represented no real alternative for the aerobic leachate treatment in practice. Moreover, a sufficient oxygen content increased the degradation rate of lechate not only in the UV/H_2O_2 process, but also in biological treatment stage.

It is summarized that a combination of photolysis with biological treatment presents itself as a good option for the treatment of leachate as an alternative for conventional wastewater processes, i.e., first biological pre-treatment (nitrification and de-nitrification) $\Rightarrow$ UV/H_2O_2 treatment with a low pressure mercury UV lamp $\Rightarrow$ second (and final) biological post-stage (activated sludge plant).

3.2. Toxicity tests

3.2.1. Luminescent bacteria test

The blank sample containing only hydrogen peroxide showed a considerable acute luminescent bacteria toxicity of 20–100% compared to the pure water control when the concentration of hydrogen peroxide exceeded 1.25 mg/l. In addition, the light emission of luminescent bacteria was dependent on the pH value of the effluent of UV/H_2O_2 stage, of which data an overall inhibition was determined to be about 35% at pH 4 and only 7% at pH 7. The higher the pH value of the leachate sample was, the lower toxicity on luminescent bacteria was observed.

In Fig. 4, the luminescent bacteria toxicity of leachate treated in three different stages is presented graphically as a function of G_L-values of various dilution steps. The biological pretreated leachate at $G2$ (1:2 dilution) showed a significant toxic effect of 60%, varying between 45 and 60% (depending on sampling date) decreasing to below 20% inhibition only at $G_L = 16$. The toxicity of the test solution increased drastically after photochemical oxidation with low pressure mercury lamp (<20% at $G_L = 128$). This phenomenon could be explained by the fact that the partial oxidation through UV-irradiation of pollutants in leachate enhanced their ecotoxicity due to rather stable toxic intermediates of a somewhat radical character formed. Following the UV-treatment, the subsequent operation of the biological process (activated sludge process) could eliminate completely any toxic by-products formed by the prior photochemical oxidation ($G_L = 1$), so that metabolized intermediates led obviously to zero toxicity for luminescent bacteria. In the photo-oxidative treatment of both reactors

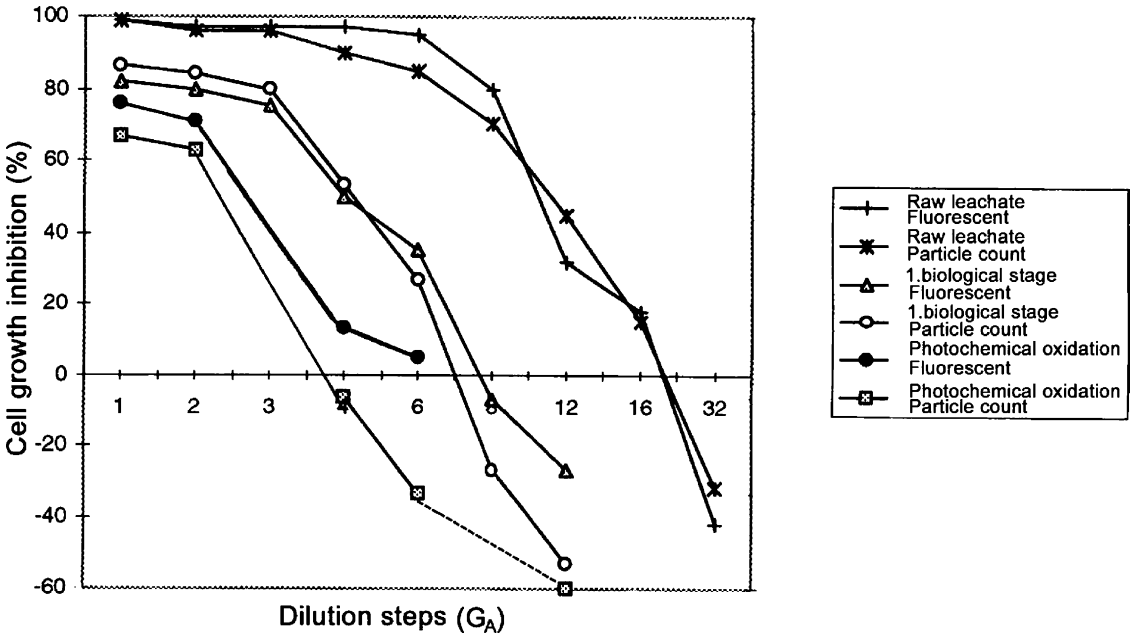

Fig. 5. Algae toxicity (recorded as fluorescent intensity and particle count) of various leachate treatment stages after 72 h incubation time: photochemical oxidation with batch reactor (0.15 kW middle pressure mercury lamp) (pH 7; H_2O_2 was eliminated by the addition of catalase).

of middle pressure- and vacuum mercury lamp, a significant acute toxicity of luminescent bacteria was also found similar to the low pressure mercury lamp, which decreased again drastically after the biological post-processing.

3.2.2. Algae test

Untreated leachates generally contain high contents of chloride and ammonia. Aliquots of raw leachate examined in this study were treated for 3 h in a 0.8 l batch reactor of middle pressure mercury lamp (0.15 kW), where hydrogen peroxide was added at a concentration of 1000 mg/l. As illustrated in Fig. 5, the raw leachate showed (dilution step $G_A = 16$) a rather high acute toxicity of algae. The G_A-value describes the dilution step at which a cell growth was inhibited by less than 20% compared to the control. The initial toxicity of raw leachate decreased considerably after the first biological pre-treatment at $G_A = 8$, at which the major part of ammonia was eliminated by the nitrification. A significant reduction in cell growth inhibition was observed after UV/H_2O_2 process at $G_A = 4$. At this stage, it is speculated that the high concentration of chloride caused the toxicity toward algae. A "negative inhibition" effect <0% of biological post-treatment stage is explained as the activation of cell growth through increase of suitable nutrients in the treated leachate.

In order to examine the phytotoxicity in each leachate treatment process, the effect of treated leachates on algae growth is compared with luminescent bacteria test (3.3.1). A significant growth inhibition of algae was found in the biologically pre-treated leachate. The undiluted samples showed an inhibition of activity of more than 70% compared to the control; even in dilution steps of *G*2–*G*4 the algae growth was still inhibited by about 40%. The toxicity against algae decreased drastically after the photochemical oxidative treatment, i.e., any intermediates formed could play only a minor role in overall toxicity and contributed to a much lower inhibition as compared with the above luminescent bacteria test. Consequently, the G_A-value decreased from $G_A = 8$ (first biological process) to $G_A = 4$ (photochemical oxidation). The biologically post-treated samples in the activated sludge plant showed obviously no toxicity at all ($G_A = 1$), so that the final biological treatment did reduce the toxicity towards algae very efficiently.

3.2.3. Daphnia test

In a 24-h test, all undiluted test solutions independent on prior leachate treatment stages showed a lethal effect to daphnial in all test series—the immobility amounted to 100%. The interpretation of these results is not straightforward though; nevertheless, it seemed to be that neither a photochemical process nor a biological treatment led to a considerable reduction rate in the immobilization rate of daphnids. Yet, an exposure to all samples in dilution steps from 1:2 to 1:8 resulted in a complete disappearance of acute toxicity of daphnids, independent on photo-oxidative treatment or biological pre- and post-treatment process.

This can be possibly explained by the fact that only the high content of chloride at 2 mg/l in leachates caused the lethal activity, because *D. magna* is a typical fresh water inhabitant. It can be argued that in the diluted samples the concentration of chloride reached below the tolerance value and daphnids could again survive in toto. This organism in our investigation was obviously not sensitive enough to

Table 2
Induction rate of Ames test with native leachate samples and their corresponding treatment stages

Parameter (dilution steps)	Induction rate (test organism: TA 98)								
	Biological pre-treatment	0.21 kW low pressure mercury lamp		1.0 kW middle pressure mercury lamp		15 W vacuum mercury lamp (aerobic)		15 W vacuum mercury lamp with N_2 (almost anaerobic)	
		UV/H_2O_2 stage	Biological post-treatment	UV/H_2O_2 stage	Biological post-treatment	UV/H_2O_2 stage	Biological post-treatment	UV/H_2O_2 stage	Biological post-treatment
Without S9									
1:1	0.9	1.0	1.3	1.7	1.5	1.7	1.4	1.3	1.4
1:2	0.9	1.2	1.0	1.6	1.1	n.d.	1.2	1.0	1.1
1:4	0.8	0.8	1.0	1.5	1.0	1.9	1.3	1.2	1.4
1:8	n.d.	1.2	1.2	1.0	1.3	1.0	1.1	1.0	1.1
With S9									
1:1	n.d.	1.8	*2.1*	0.8	0.9	0.8	*2.1*	1.5	1.0
1:2	n.d.	1.2	*2.0*	0.7	0.7	1.6	1.6	1.0	1.1
1:4	n.d.	1.0	0.9	1.2	1.1	1.2	1.6	0.9	1.0
1:8	n.d.	1.1	1.1	0.8	1.1	1.0	1.3	1.4	1.3

pH of test solution after photochemical oxidation was adjusted about 7; H_2O_2 residues were decomposed. n.d.: not determined.

organic pollutants as compared to the other aquatic organisms as luminescent bacteria or algae.

3.2.4. Ames mutagenicity test

The mutagenic effect of leachates on *S. typhimurium* was expressed as the "induction rate", which is calculated as the relation of the number of mutants in test sample to those in the control. The test solution was defined as "mutagenic" according to the DIN criteria which had to be met simultaneously by two characterizations; on the one hand, as the dose effect in the test sample to which were added a S9 mix or not, where the number of revertant mutants would be at least increased by a factor of 2 compared to a negative control ($I \geq 2$, $I =$ induction rate); on the other hand, additionally, in the case of TA 98 strain, the count of mutants should, at least, be increased at above 40 mutants per test plate in average. However, in the case of TA 100 strain, the same amounted to a minimum of 100 mutants per test plate. Several types of standard mutants are used to test different classes of mutagen as follows; TA 100 without S9: nitrofurantoine, TA 100 without S9: NPDA (nitro phenylene diamine) and, TA 100 and TA 98 with S9: BaP (benzopyrene) and amino anthracene.

All samples given to TA 98 and TA 100 strains proved not mutagenic. Induction rates of TA 98 in four different photo-oxidative processes and biological pre-, post-treatment steps are described in Table 2. Test solution of biological pre-treatment step showed definitely no significant mutagenic effect towards test organisms of TA 98 and TA 100. In TA 98 mixed with S9 there were only two exceptions when induction rates exceeded the values of 2.1 and 2.0 observed in the effluents of biological post-treatment in combination with low pressure- and vacuum-mercury lamp. Nevertheless, these values were near to the sensitivity limit and hence cannot be interpreted as showing a significant mutagenicity. The result could be interpreted in terms that possible by-products of photochemical oxidation caused the mutagenicity of TA 98 or that there was an experimental error, because a higher induction rate was determined in the effluent of biological stage (2.1) than in the photochemical oxidation (1.8) (with low pressure mercury lamp). With regard to the potential mutagenic effect of the leachate the application of biological and photo-oxidative treatment played only an insignificant role. It is emphasized that the surprisingly high toxicity of luminescent bacteria by photochemical oxidation, which may have generated some chemical compounds of radical character, could not be correlated with a corresponding high mutagenicity.

3.2.5. Umu test

The test solution was investigated on a *S. typhimurium* strain referring to blind, negative, and positive controls. The standard substance of 4-NQO (4-nitrochinolin-N-oxide) was used for a positive control. The umu test resulted in a "significant" effect, when the positive control with a reference substance under the given test conditions showed a minimum induction rate of $\geq$2.

For calculation of the "genotoxic damage potency" (GDP), calibration curves were performed with standard carcinogen, benzopyrene, in GDP equivalents. A native wastewater sample was regarded as mutagenic at an induction rate of >1.5 according to DIN, which is calculated with GDP values observed. A positive genotoxicity of wastewater in terms of DIN was defined if a minimum of 3800 umu-[GDP] was observed and the detection limit of concentrated sample was GDP at 3.5.

In all native leachate samples a genotoxic potential could not be observed and the induction rates of test solution extracted are summarized in Table 3. In order to examine a potentially weak genotoxicity, the test solution was concentrated up to a factor of 75. The induction rates of enriched samples of biological pre-treatment step were in the range from 1.4 to 2.9, while that of biological post-treatment step ranged between 2.2 and 3.0. These demonstrated a very

Table 3
Induction rate of umu test with extracts of leachate samples and their corresponding treatment stages

Enrichment factor	Induction rate (without S9)								
	0.21 kW low pressure mercury lamp			1.0 kW middle pressure mercury lamp			15 W vacuum mercury lamp		
	(1) Biological pre-treatment	UV/H_2O_2 stage	(2) Biological post-treatment	(1) Biological pre-treatment	UV/H_2O_2 stage	(2) Biological post-treatment	(1) Biological pre-treatment	UV/H_2O_2 stage	(2) Biological post-treatment
75	1.4	*4.9*	*3.0*	2.9	*6.5*	2.2	n.d.	n.d.	n.d.
38	1.2	2.5	2.3	2.6	*4.3*	1.9	n.d.	*4.8*	2.3
19	1.1	1.7	1.7	1.4	2.3	1.4	n.d.	2.6	1.8
9.4	1.1	1.3	1.5	1.2	1.4	1.1	n.d.	1.8	1.4
4.7	1.0	1.1	1.2	1.1	1.1	1.0	n.d.	1.4	1.2
2.3	1.0	1.1	1.0	1.1	1.0	1.0	n.d.	1.3	1.2
1.2	1.1	1.1	1.1	n.d.	n.d.	n.d.	n.d.	1.1	1.1
0.6	1.1	1.1	1.1	n.d.	n.d.	n.d.	n.d.	1.0	1.0

n.d.: not determined; pH of test solution after photochemical oxidation was adjusted about 7; H_2O_2 residues were decomposed.

Table 4
The results of alkaline filter elution test of leachate samples and their corresponding treatment stages

Dilution steps	Alkaline filter elution [AFE] quotient								
	0.21 kW low pressure mercury lamp (exposition time: 2 h)			1.0 kW middle pressure mercury lamp (exposition time: 24 h)			15 W vacuum mercury lamp (exposition time: 24 h)		
	(1) Biological pre-treatment	UV/H_2O_2 stage	(2) Biological post-treatment	(1) Biological pre-treatment	UV/H_2O_2 stage	(2) Biological post-treatment	(1) biological pre-treatment	UV/H_2O_2 stage	(2) Biological post-treatment
1:1	1.03	1.20	1.21	*2.10*	*2.50*	*2.32*	n.d.	*1.77*[a]	1.40
1:2	0.97	1.20	0.92	*1.71*	1.31	1.34	n.d.	1.30[a]	1.18
1:3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.17[a]	1.23
1:5	1.23	1.26	1.11	1.21	1.2	1.14	n.d.	n.d.	n.d.
1:10	1.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not determined; pH of test solution after photochemical oxidation was adjusted about 7; H_2O_2 residues were decomposed.
[a]Seize of mussel stem by closing mussel board.

low genotoxcity of the leachate. The 75-fold concentrated extract of leachate after photochemical oxidation showed a small mutagenetic effect, where induction rate was in the range of 4.9–6.5. (The absolute GDP values of effluent treated with different UV-lamp were as follows: with low pressure mercury lamp: 240, with middle pressure mercury lamp: 285, vacuum mercury lamp: 440. These results corresponded basically with that of luminescent bacteria test. Here it was a noticeable trend that the toxicities on the luminescent bacteria as well as the genotoxic potential were significantly increased after the photo-oxidative treatment, while the inhibition was again decreased after biological post-treatment. Nevertheless, the GDP blind value of the extract (at concentration factor of 75) was in the range of 140–175. Consequently, it cannot be completely excluded that a considerable part of genotoxicity potential found in the extract after photochemical oxidation after enrichment of the sample was caused by the various reagents and solvents used.

3.2.6. Alkaline filter elution

For the evaluation of the alkaline filter elution test, control cells of undamaged and negligibly damaged DNA were investigated in parallel. The genotoxic damage potential as the quotient of sample over control was calculated by the ratio of numbers of broken DNA strands of sample over that of control. Broken single strands were mainly eluted at an early elution stage. The alkaline elution of DNA obeyed nearly a kinetic first order law under experimental conditions. The elution curves of undamaged and damaged DNA strands could be differentiated by means of linear regression. A sample would show genotoxic character, if the "AFE-quotient" showed a statistically significant value of $\geq$1.3. (This corresponded with a 75 AFE-[GDP].)

As indicated in Table 4, it can be seen again that on the one hand, no significant genotoxicity was found in all leachate samples, on the other hand, a slightly increased quotient was observed in test solution of photochemical oxidation and biological post-treatment step.

4. Conclusion

The combination of a biological treatment process with UV/H_2O_2 is a technical suitable solution for leachate treatment with an efficient reduction of the sum parameters (COD, BOD, and AOX). Especially, the vacuum mercury lamp could be used as a good alternative for conventional degradation techniques of organic substances under economical aspects.

In addition diverse biological tests used in this investigation represented a very efficient and sensitive monitoring method of a leachate treatment in terms of ecotoxic- and mutagenic-effects following the various treatment stages. In the ecotoxicity test series the toxicity of algae and luminescent bacteria increased first by photochemical oxidation showing a questionable environmental compatibility. This was due partially to the production of radical intermediates, whereby luminescent bacteria reacted very sensitively to these radical substances. In the biological post-treatment process, the toxicity toward bio-organisms (luminescent bacteria, green algae, and Daphnia) was finally decreased again to a safe level in terms of degradable sum parameters. In the test series, the mutagenic potential played a significant role either in the Ames-Salmonella, umu-Salmonella, or alkaline filter elution test. Therefore, these positive results compensated somewhat for the partially increased eco-toxicity of luminescent bacteria and algae. The results show only a slight trend toward a small genotoxicity in the Ames- and umu test in the photo-treated samples as compared to the control, which could principally be correlated with the effect induced by photo-oxidatively generated by-products. In the subsequent post (second) biological treatment step, the mutagenicity was in most cases reduced again. However, these results of a slightly increased mutagenicity cannot be generated in the case of all leachate sources investigated here, because the quality of each leachate varies with the associated properties of wastes and deposits. Lechate sampled from other deposits could also show a certain mutagenic potential, as was reported in literatures. In further studies, refractive compounds of leachate should be investigated in detail in order to dispose specific industrial wastes more effectively and safeguard against ground water contamination.

It is essential to optimize the combination of photochemical oxidation and biological treatment for economic reasons by more systematic approaches.

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