Combined Method for Degradation of Chlorophenols

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Abstract

A combined method is proposed for the degradation of chlorophenols in a flow-through photoreactor using an UV XeBr* excilamp (283 nm) and the subsequent processing of the photolysis products by a destructor microorganism *Bacillus cereus* culture isolated from the aeration pond of the Baikal Pulp and Paper Mill. The maximum efficiency for the degradation of chlorophenols amounts to 95 %, thus the utilization of main photolysis products is achieved.

Keywords: chlorophenols, degradation, excilamp, photolysis products, microorganisms

INTRODUCTION

Chlorophenols (CP) represent toxic chloroorganic contaminants either of a man-caused or natural origin, those are characterized by the capacity for bioaccumulation as well as by a relative stability with respect to decomposition in the environment. According the "List of hazardous substances detrimental to Baikal Lake ecosystem" developed, PC belong to the category of "especially dangerous" substances, whose content in the Baikal Lake's water and its tributaries is unacceptable [1]. Chlorophenols were found out during our earlier investigations in the superficial water of Selenga River, the tributary of the Baikal Lake, as well as in the Baikal water over the area of purified wastewater discharge from the Baikal Pulp and Paper Mill (BPPM) [2]. The total content of phenols including CP, in the BPPM wastewaters prior to biological purification could be as much as 31.9 mg/L [3]. In this connection the research and development of modern methods for CP degradation is of currently central value.

In order to decompose such ecotoxicants, as a rule, photochemical and biological methods and their various combinations are customary used. Combined oxidation technologies those superpose the advantages of various oxidizing processes (so called Advanced Oxidation Processes, AOP) and biological methods [4–8] are considered nowadays to be among the most promising methods. The biodegradation of chlorophenols under an increased CP concentration was shown to be accompanied by an inhibition of organisms' growth and a relatively low degradation process rate.

A prospective solution for to increase the CP biodegradation level could consist in the use of a combined method including a preliminary UV photolysis of CP to produce readily oxidized species with the subsequent biological treatment. As the sources of UV radiation, modern excilamps are of considerable interest which excilamps represent a rather new class of the sources spontaneous UV and VUV radiation generated due to the decay of excimer molecules (excited dimers of either noble gases R_2^* or halogens X_2^*)

or exciplex molecules (excited complexes of the halogenides of noble gases RX*). The basic advantage of the excilamps consists in the fact that up to 80 % and more of the total radiation power is concentrated in a rather narrow spectral band (the half-width value being less than 10 nm) of the corresponding molecule [9, 10].

Moreover, the excilamps are characterized by a high photon energy (within the range from 3.5 to 10 eV), high radiation power density, long-duration lifetime (1000–10 000 h), with the absence of elemental mercury that provides them with an advantage comparing to environmentally faulty mercury lamps [11, 12]. The efficiency of KrCl* (maximum radiation at 222 nm), XeBr* (283 nm) and XeCl* (308 nm) excilamps has been earlier demonstrated for the decomposition of phenol, 4-chlorophenol [13] and methylated phenols in aqueous solutions under static conditions [14]. For phenol, 4-chlorophenol and 2,4-dichlorophenol the kinetics of photolysis induced by VUV radiation of the Xe_2^* (172 nm) excimer lamp in aqueous media was studied under the conditions of a flow-through photoreactor [15].

In the present work for the first time a combined degradation of CF by UV radiation of XeBr* excilamp (283 nm) and by a destructor microorganism culture such as *Bacillus cereus* was studied.

EXPERIMENTAL

Reagents

2-Clorophenol (2-CP), 4-clorophenol (4-CP) and 2,4-diclorophenol (2,4-DCP) 99 % in purity purchased from Aldrich Chemical Co. were used in the experiments. For the identification of the intermediate species proposed to be formed during CP degradation process, the following compounds were used: resorcinol (99 % purity, Aldrich), hydroquinone (>99 % purity, Sigma), *p*-benzoquinone (98 % purity, Aldrich), pyrocatechin (>99 % purity, Sigma-Aldrich), 4-chlorocatechol (97 % purity, Aldrich), chlorohydroquinone (85 % purity, Aldrich), 4-chlororesorcinol (of 98 % purity, Aldrich), phenol (99.5 % purity, AnalaR).

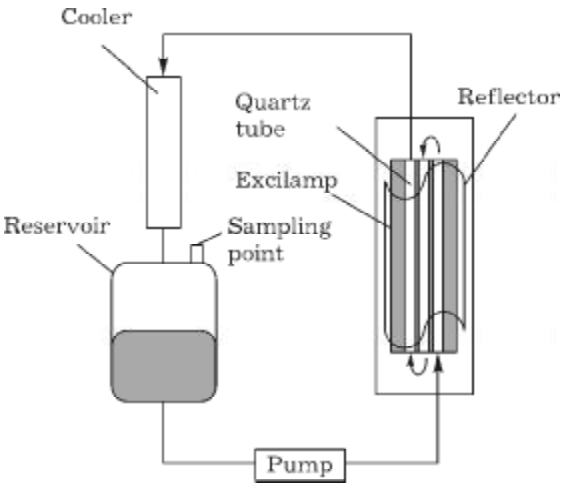

Fig. 1. Schematic diagram of the experimental set-up based on XeBr* excilamp.

Flow-through photoreactor

At the first stage the photolysis of CP in aqueous solution was carried out for initial CP concentration amounting to 10, 20, 50 and 100 mg/L and pH 5.5–6.2 in a flow-through photoreactor basing on an UV XeBr*excilamp (the wavelength being at 283 nm) with the excitation by the barrier discharge (an XeBr_BD_P excilamp from the High-Current Electronic Institute, SB RAS, Tomsk).

The CP solution under irradiation circulated at a flow rate of 66 L/h through three quartz tubes located near the exit window of the excilamp, and after cooling was fed into a reservoir from where the sampling was carried out (Fig. 1). The effective light intensity generated by the lamp in the exitt plane of the window in the zone of pipes arrangement, amounted to 18 mW/cm^2 ($6.63 \cdot 10^{-6}$ Einstein/(L · s)).

Biodegradation

At the second stage we studied the biologically induced degradation of CP before and after the UV treatment by the culture of *Bacillus cereus* that we had isolated earlier from the sludge of the BPPM aeration pond and identified as a result of 16S rDNA sequencing [16]. Aqueous solutions of CP were exposed to preliminary UV treatment during the periods of $t_{1/2}$ and $t_{1/10}$, that is for to achieve the

residual concentration CP equal to 50 and 10 %, respectively. The subsequent culturing of *B. cereus* was carried out within the irradiated solutions after adjusting the pH value up to 7.0 and adding the mineral salts as described in [16]. The degradation ability of the *B. cereus* culture before UV processing was also is tested in for a mineral medium containing either 2-CP, 4-CP or 2,4-DCP as the only source of carbon and energy.

Methods of analysis

The determination of CP during the process of degradation was carried out by means of HPLC assay using a Perkin Elmer LC290 UV-Vis detector. A Waters Spherisorb column (ODS-2 3u, 100 mm) was used, the eluent consisted of 60 % of methanol, 2.5 % of acetic acid and water; the elution rate amounting to 0.5 mL/min. For the identification of intermediate species and photolysis products a GC-MS technique was used. The sample preparing consisted in a triple extraction of an aqueous aliquot in acidic media with diethyl ether in the ratio of 1 : 1 during 1 min.

Further the organic phases joined together were evaporated until dry; a solid residue was then dissolved in 1 mL of diethyl ether. The extract obtained in amount of 1 mL was analysed by means of a Hewlett Packard HP 6890 gas chromatograph supplied with a mass-selective detector (a HP-5MS capillary column 30 m in length, internal diameter being 0.25 mm). The conditions of the gas chromatography analysis were as it follows: both the evaporator and detector temperature being at 200 °C, helium being used as a carrier gas, the detector nitrogen feed amounting to 60 mL/min, split ratio amounting to 4 : 1. The temperature of a column thermostat increased from 50 °C (hold time for 3 min) up to 200 °C (hold time for 3 min) with the rate of 10 °C/min. The pH value was determined with the help of a 3310 JENWAY pH meter supplied with a PHM-300-070E Russel electrode.

The cellular growth was monitored through the measurement of optical density at 600 nm using a JENWAY 6300 spectrophotometer.

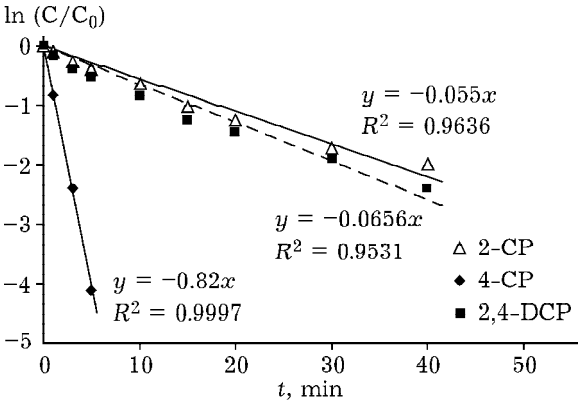

Fig. 2. Dependence of ln (C/C_0) on the duration CP photolysis at the initial concentration of 50 mg/L. CP – chlorophenol, DCP – dichlorophenol.

RESULTS AND DISCUSSION

UV photolysis

The fact that ln (C/C_0) value demonstrates a linear dependence on time with the correlation coefficient amounting to 0.95–0.99 indicates that the reaction of PC photolysis can be described by the equation the first order reaction (Fig. 2). The maximum decomposition rate was observed for 4-CP, which is in a good agreement with the data from the literature [17–19]. As one can see from Table 1, for our experiment the maximal initial concentrations of 4-CP and 2,4-DCP at those the reaction rate constant and the half-life period represented almost constant values, amounted to 20 and 50 mg/L, respectively. For 2-CP this concentration threshold was lower and amounting to about ~10 mg/L.

The further increase in initial concentration was accompanied by a regular decrease of the reaction rate constant and increase in the half-life value. Thus, the rate of photolysis increased in the order such as 2-CP < 2,4-DCP < 4-CP, which is in a good agreement the data available from the literature concerning the reduction of decomposition reaction rate for CP either as the number of clorine atoms in the benzene ring increased [17], or due to the chlorine atom location at the ortho position [18, 20].

The photolysis reaction rate constants obtained are almost three orders of magnitude higher than those for the direct photolysis

TABLE 1

First-order rate constants and half-life time for clorophenols (CP) in theprocess of direct UV photolysis

C_0, mg/L	pH	I, Einstein/(L · s)	λ, nm	k_0, min^{-1}	$t_{1/2}$, min	Ref.
			2-CP			
50	2.5	$6.45 \cdot 10^{-7}$	250	$8.76 \cdot 10^{-3}$	25	[18]
50	9.5	$6.45 \cdot 10^{-7}$	250	$4.04 \cdot 10^{-2}$	n/d	[18]
100	6.0	$5.97 \cdot 10^{-5}$	320–400	$1.52 \cdot 10^{-3}$	12.0	[18]
100	9.0	$5.97 \cdot 10^{-5}$	320–400	$9.00 \cdot 10^{-4}$	8.5	[18]
100	7.1	no data	250–370	$2.85 \cdot 10^{-2}$	24.3	[21]
10	5.6	$6.63 \cdot 10^{-6}$	283	$1.00 \cdot 10^{-1}$	6.9	p/w
20	5.9	$6.63 \cdot 10^{-6}$	283	$6.70 \cdot 10^{-2}$	10.3	p/w
50	5.7	$6.63 \cdot 10^{-6}$	283	$5.89 \cdot 10^{-2}$	11.8	p/w
100	5.9	$6.63 \cdot 10^{-6}$	283	$4.51 \cdot 10^{-2}$	15.4	p/w
			4-CP			
50	2.5	$6.45 \cdot 10^{-7}$	250	$2.52 \cdot 10^{-2}$	27.3	[18]
50	9.5	$6.45 \cdot 10^{-7}$	250	$5.08 \cdot 10^{-2}$	13.4	[18]
100	6.0	$5.97 \cdot 10^{-5}$	320–400	$9.32 \cdot 10^{-3}$	8.6	[18]
100	9.0	$5.97 \cdot 10^{-5}$	320–400	$5.10 \cdot 10^{-4}$	7.4	[18]
10	6.2	$6.63 \cdot 10^{-6}$	283	$7.77 \cdot 10^{-1}$	0.9	p/w
20	6.0	$6.63 \cdot 10^{-6}$	283	$8.09 \cdot 10^{-1}$	0.9	p/w
50	5.8	$6.63 \cdot 10^{-6}$	283	$7.29 \cdot 10^{-1}$	0.9	p/w
100	5.9	$6.63 \cdot 10^{-6}$	283	$5.07 \cdot 10^{-1}$	1.4	p/w
			2,4-DCP			
50	2.0	$4.80 \cdot 10^{-4}$	185–436	$3.80 \cdot 10^{-2}$	17.5	[18]
50	2.0	$3.52 \cdot 10^{-5}$	185–436	$3.80 \cdot 10^{-2}$	no data	[18]
50	9.0	$3.52 \cdot 10^{-5}$	185–436	$1.73 \cdot 10^{-1}$	no data	[18]
100	4.5–6	$2.50 \cdot 10^{-6}$	350	$5.00 \cdot 10^{-5}$	>90	[22]
10	5.6	$6.63 \cdot 10^{-6}$	283	$8.63 \cdot 10^{-2}$	8.1	p/w
20	5.6	$6.63 \cdot 10^{-6}$	283	$8.46 \cdot 10^{-2}$	8.1	p/w
50	5.5	$6.63 \cdot 10^{-6}$	283	$6.29 \cdot 10^{-2}$	11.0	p/w
100	5.5	$6.63 \cdot 10^{-6}$	283	$4.69 \cdot 10^{-2}$	14.7	p/w

Note. p/w – the data of the present work.

through UV irradiation (see Table 1). It is obvious that the narrow-band UV radiation at the wavelength of 283 nm is more efficient as compared to either a wide-range wavelength radiation, or a radiation with higher wavelength and intensity values. It should be noted that the reaction rate constant obtained for the photolysis of 2,4-DCP with no participation of oxidizing agents at the initial concentration of 50 mg/L ($6.29 \cdot 10^{-2}$ min^{-1}) is quite comparable with the reaction rate constants for the photolysis in the following AOP systems (pH 2.0, λ = 185–436 nm): UV/H_2O_2 ($4.40 \cdot 10^{-2}$ min^{-1}), UV/Fenton ($8.80 \cdot 10^{-2}$ min^{-1}), UV/O_3 ($6.5 \cdot 10^{-2}$ min^{-1}) [17].

For the initial concentration of 2,4-DCP at 100 mg/L the reaction rate constant for the direct photolysis ($4.69 \cdot 10^{-2}$ min^{-1}) is quite comparable to that for the system such as UV/Fenton ($5.7 \cdot 10^{-2}$ min^{-1}), being two orders of magnitude higher than the reaction rate constants found out earlier for the systems such as UV/H_2O_2 ($2.6 \cdot 10^{-4}$ min^{-1}) and UV/Fe(III) ($6.2 \cdot 10^{-4}$ min^{-1}) (pH 4.5–6, λ = 350 nm, $I = 2.5 \cdot 10^{-6}$ Einstein/(L · s)) [22]. The reaction rate constant for photocatalytic decomposition of 2-CP with the use of TiO_2 at the wavelength λ = 365 nm [23] is 2.46-fold less than the rate constant obtained from the direct photolysis at the wavelength

TABLE 2

Intermediate species of the photolysis of chlorophenols (CP) identified by GC-MS method

Duration of UV treatment	Intermediate species
	2-CP
$t_{1/2}$	Catechol, 2-chlorohydroquinone
$t_{1/10}$	Phenol, benzyl alcohol, catechol, 2-phenoxyethanol, 4-chlororesorcinol, phthalic acid
	4-CP
$t_{1/2}$	*p*-Benzoquinone, hydroquinone
$t_{1/10}$	*p*-Benzoquinone, hydroquinone, benzyl alcohol, 5-chlororesorcinol, 4-propoxyphenol, 2,5-cyclohexadiene-1,4-dione, benzoic acid, phthalic acid
	2,4-DCP
$t_{1/2}$	Hydroquinone, 2-chlorohydroquinone
$t_{1/10}$	Hydroquinone, benzyl alcohol, 2-chlorohydroquinone, 4-chlorocatechol, phthalic acid

$\lambda = 283$ nm given the same values of the solution initial concentration and pH.

On the basis of the results obtained the duration of the preliminary UV treatment $t_1/_2$ was found to amount to 10 min for 2-CP and 2,4-DCP and 1 min for 4-CP. The $t_1/_{10}$ value for 2-CP, 2,4-DCP and 4-CP amounted to 40, 35 and 3 min, respectively. After the irradiation the media acidity reached the value of pH 3.50 because of the formation of intermediate products acidic in character [24]. Table 2 demonstrates the results of the identification of the intermediate species formed after UV photolysis during the periods of $t_1/_2$ and $t_1/_{10}$. These data indicate that the role of the key stages of CP decomposition was played by the reactions of the benzene ring dechlorination and hydroxylation. The photolysis during the period of $t_1/_{10}$ resulted in the formation of some aromatic organic acids and alcohols.

By means of HPLC technique we have confirmed the formation of 2-chlorohydroquinone and hydroquinone as the result of 2-CP and 2,4-DCP photolysis, respectively. The other intermediate species proposed for 2,4-DCP photolysis (hydroquinone, 4-chlorocatechol, 2-chlorohydrquinone) were not identified with the help of the HPLC. Nevertheless, hydroquinone and *p*-benzoquinone were identified by this technique as the intermediate species of 4-CP photolysis, which is in a good agreement with the data from the literature [18, 19].

Biodegradation of CP before and after UV photolysis

From the experiments on the biodegradation of CP before UV treatment it was established that that the maximum degradation capability the *B. cereus* cells demonstrated with respect to 2-CP in the series of 4-CP < 2,4-DCP < 2-CP (Fig. 3). For mono-substituted CP it can be caused by the fact that the biodegradation ability for CP in aerobic conditions is apt to vary in the order such as *meta* < *para* < *ortho* [18, 25]. The maximum concentration change was registered for 2-CP being at 15.5 mg/L.

As it is known, polychlorinated phenols can be decomposed by microorganisms much more difficultly than monochlorinated phenols. In this

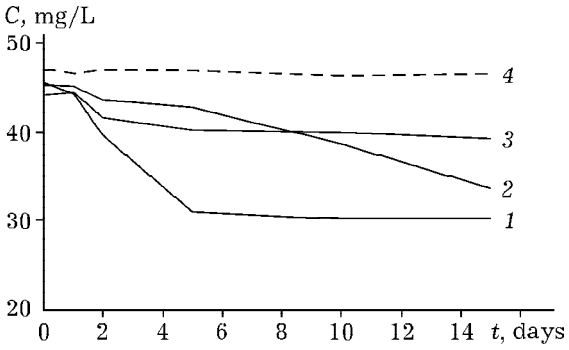

Fig. 3. Dynamics of the decomposition of clorophenols (CP) by *B. cereus* culture without UV treatment: *1* – 2-CP, *2* – 2,4-DCP, *3* – 4-CP, *4* – reference.

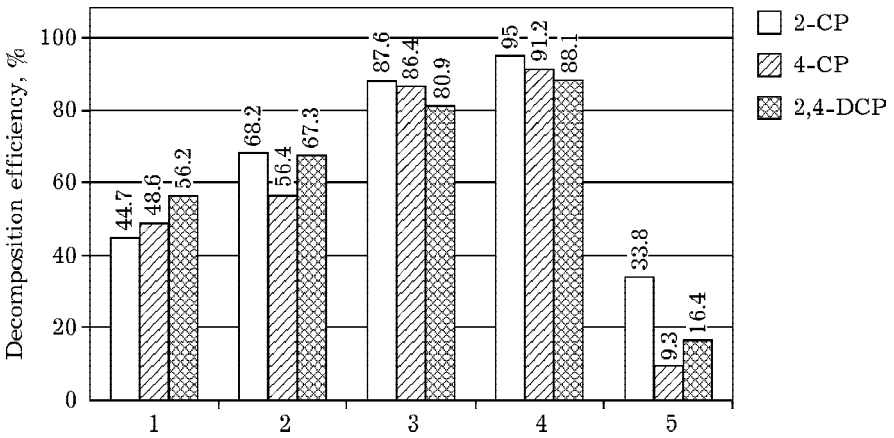

Fig. 4. Efficiency of the decomposition of chlorophenols as a result of UV and biologic treatment at the initial concentration of 50 mg/L: *1, 3* – after UV treatment during $t_1/_2$ (*1*) and $t_1/_{10}$ (*3*); *2, 4* – after the combined treatment during 10 days; *5* – after biotreatment during 10 days.

case a higher destructive activity is established for *B. cereus* culture with respect to 2,4-DCP, which, to all appearance, could be connected with a greater adaptation to this CP. According to HPLC data, after culturing for 2 days in the environment with 2-CP, three anew-formed compounds were observed, presumably 2-chlorohydroquinone, hydroquinone and phenol. Possible metabolites of 4-CP and 2,4-DCP were not identified reliably.

After preliminary UV treatment of CP during the period of $t_1/_2$, which resulted in the decomposition of ~50 % of the parent substance, and the subsequent treatment by *B. cereus* culture during 10 days the efficiency of parent CP decomposition was observed to be insignificant (Fig. 4). Nevertheless, an active growth of cells after the culturing during 2 days was observed. It was revealed that the products of 2-CP and 2,4-DCP photolysis can be completely utilized during 5 and 10 days of the culturing, respectively.

Parabenzoquinone was not found out among the products of 4-CP decomposition after the culturing for 1 day, whereas the concentration of hydroquinone remained at a previous level in the experiment with bacteria (4.96 mg/L), being increased up to 7.98 mg/L for the reference experiment with no bacteria contained in the solution. It could be caused by the hydration of *p*-benzoquinone in aqueous media and thus the formation of hydroquinone. After 2 days of culturing hydroquinone was completely utilized, whereas in the reference experiment its concentration changed insignificantly and amounting to 7.54 mg/L.

As evident from Fig. 4, that under the combined CP processing consisted in the UV irradiation during the period of $t_1/_{10}$ and the subsequent biologic treatment for 10 days, the maximum efficiency of 2-CP decomposition was as high as 95 %. The residual 4-CP and 2,4-DCP underwent the biodegradation to an insignificant extent. The maximal cellular growth was observed after 5 days of culturing, which, to all appearance, could be caused by a somewhat greater toxicity of the solution after profound UV treatment and, hence, more long lag phase. Nevertheless, the main products of profound photolysis of 2-CP after biotreatment during 5 days were not revealed. As the result of the present combined treatment, the basic products of profound photolysis of 2,4-DCP were utilized. In the case of 4-CP two other metabolites stable with respect to biodegradation could be formed.

CONCLUSION

At the initial concentration of CP less than 20 mg/L the CP solutions under investigation could be utilized directly using the biological method under aerobic conditions with the use of *B. cereus* culture.

At the initial concentration of CP >20 mg/L the solutions under purification were shown to be difficult to oxidize biologically. It was demonstrated that in this case the most efficient to

use is a combined method of degradation. At the first stage CP are oxidized with the help of UV radiation of XeBr* excilamp to be transformed into rather readily oxidized species for the subsequent returning back to the biological cycle. At the initial concentration of 50 mg/L and more a profound preliminary UV treatment may be recommended for the removal of 80–90 % of the initial substrate. It has been revealed that 4-CP exhibit a relative stability with respect to *B. cereus,* but it is apt to be readily decomposed due to UV irradiation.

As the result of the subsequent biotreatment during 10 days the basic photoreaction products of CP under investigation can be utilized. The proposed duration of the preliminary UV treatment is much shorter than the duration value found earlier in [26] with half-life periods for parent CP amounted to 2.2–54 h at the same initial concentration, thus the latter process being connected with an increased energy consumption.

The investigation carried out demonstrates a prospective application of the combined method with simultaneous use of XeBr* excilamp UV radiation and *B. cereus* culture for the efficient CP degradation at increased concentrations.

REFERENCES

1 Perechen' veshchestv, vrednykh dlya ekosistemy ozera Baikal, Moscow, 2004.
2 B. B. Batoev, G. G. Nimatsyrenova, G. S. Dabalaeva, S. S. Palitsyna, *Chem. Sustain. Dev.*, 13 (2005) 31.
URL: http://www.sibran/English/CSDE.HTM
3 A. M. Beim, G. V. Belyavtseva, V. G. Gorokhova *et al.*, *Khim. Ust. Razv.*, 5 (1997) 383.
4 C. Pulgarin, M. Invernizzi, S. Parra *et al.*, *Catal. Today*, 54 (1999) 341.
5 A. Marco, S. Esplugas and G. Saum, *Wat. Sci. Technol.*, 35 (1997) 321.
6 A. M. Amat, A. Arques, H. Beneyto *et al.*, *Chemosphere*, 53 (2003) 79.
7 P. Hörsch, A. Speck and F. Frimmel, *Wat. Res.*, 37 (2003) 2748.
8 F. Kastanek, Y. Maleterova and P. Kastanek, Proc. 1st Europ. Conf. on Environmental Applications of Advanced Oxidation Processes (EAAOP-1), Greece, 2006, p. 263.
9 E. A. Sosnin, T. Oppenländer and V. F. Tarasenko, *J. Photochem. Photobiol. C: Photochem. Rev.*, 7 (2006) 145. doi:10.1016/j.jphotochemrev.2006.12.002
10 T. Oppenländer and E. A. Sosnin, *IUVA News*, 7 (2005) 16.
11 M. I. Lomaev, V. S. Skakun, E. A. Sosnin *et al.*, *Usp. Fiz. Nauk*, 173 (2003) 201.
12 J.-Y. Zhang and I. W. Boyd, *Appl. Surf. Sci.*, 168 (2000) 296.
13 N. B. Sultimova, O. K. Bazyl', O. N. Tchaikovskaya *et al.*, *Atm. Ocean Opt.*, 15 (2002) 230.
14 T. V. Sokolova, O. N. Chaikovskaya, E. A. Sosnin, I. V. Sokolova, *Zh. Prikl. Spektrosc.,* 73 (2006) 566.
15 T. Oppenländer and S. Gliese, *Chemosphere,* 40 (2000) 15.
16 G. G. Matafonova, G. S. Shirapova, F. Giffhorn *et al.*, *Int. Biodeterioration & Biodegradation*, 58 (2006) 209.
17 F. J. Benitez, J. Beltran-Heredia, J. L. Acero, F. J. Rubio, *Chemosphere*, 41 (2000) 1271.
18 M. Pera-Titus, V. García-Molina, M. A. Bacos *et al.*, *Appl. Catal. B: Environmental*, 47 (2004) 219.
19 T. Pandiyan, O. Martinez Rivas, J. Orozco Martinez *et al.*, *J. Photochem. Photobiol. A: Chemistry*, 146 (2002) 149.
20 M. A. Barakat, J. M. Tseng and C. P. Huang, *Appl. Catal. B: Environmental*, 59 (2005) 99.
21 Zh. Shi, M. E. Sigman, M. M. Ghosh, R. Dabestani, *Env. Sci. Technol.*, 31 (1997) 3581.
22 F. Al Momani, C. Sans and S. Esplugas, *J. Hazard. Mat.*, B107 (2004) 123.
23 N. N. Rao, A. K. Dubey, S. Mohanty *et al.*, *Ibid.*, B101 (2003) 301.
24 Yu. I. Skurlatov, L. S. Ernestova, E. V. Vichutinskaya *et al.*, *J. Photochem. Photobiol. A: Chemistry*, 107 (1997) 207.
25 A. P. Annachhatre and S. H. Gheewala, *Biotechnol. Adv.*, 14 (1996) 35.
26 E. Tamer, Z. Hamid, Aly A. Magdy *et al.*, *Chemosphere*, 63 (2006) 277.