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# Comparative Assessment of the Photocatalytic Efficiency of TiO<sub>2</sub> Wackherr in the Removal of Clopyralid from Various Types of Water

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## 1. Introduction

Many pyridine derivatives have found widespread application as herbicides. Because of their frequent use, chemical stability and resistance to biodegradation, they are encountered in waste waters, and, due to their hazardous effects on ecosystems and human health, their removal is imperative (Stapleton et al., 2006). With this in mind, we have recently paid significant attention to the study of the model compounds (Abramović et al., 2003; Abramović et al., 2004a, 2004b) and pyridine containing pesticides (Abramović et al., 2007; Šojić et al., 2009; Abramović & Šojić, 2010; Abramović et al., 2010; Guzsány et al., 2010; Šojić et al., 2010a, 2010b; Banić et al., 2011).

Clopyralid (3,6-dichloro-2-pyridinecarboxylic acid, CAS No. 1702-17-6, C<sub>6</sub>H<sub>3</sub>Cl<sub>2</sub>NO<sub>2</sub>, M = 192.00 g mol<sup>-1</sup>) (CLP) is a systemic herbicide from the chemical class of pyridine compounds, i.e., pesticides of picolinic acid. It has been used effectively for controlling annual and perennial broadleaf weeds in certain crops and turf. It also provides effective control of certain brush species on rangeland and pastures. The acidic form of CLP and three CLP salts (triethylamine, triisopropylamine, and monoethanolamine), which are very soluble in water, are commonly used in commercial herbicide products. Its chemical stability along with its mobility allows this herbicide to penetrate through the soil, causing long-term contamination of the ground water, as well as surface water supplies (Cox, 1998; Huang et al., 2004; Donald et al., 2007; Sakaliene et al., 2009). Due to these properties, CLP has recently been reported to occur in drinking water at concentrations above the Permitted Concentration Value of 0.1 µg L<sup>-1</sup> for an individual pesticide (EU directive 98/83/EC). Although the occurrence of CLP in surface, ground and drinking waters has been widely reported, there are only a few studies concerning with its photocatalytic removal from water (Šojić et al., 2009; Šojić et al., 2010a, 2010b; Tizaoui et al., 2011). These studies showed that the degradation of this herbicide takes place most effectively in the presence of Degussa P25 as photocatalyst. However, several recent studies of photocatalytic activity reported that some cosmetic pigments (TiO<sub>2</sub>, Wackherr's

“Oxyde de titane standard”) are even more efficient than TiO<sub>2</sub> Degussa P25 in the photodegradation of phenol (Rossatto et al., 2003; Vione et al., 2005) and herbicides with a pyridine ring (Abramović et al., 2011).

The aim of this work was to study the effect of water type (double distilled (DDW), tap and river water) on the efficiency of TiO<sub>2</sub> Wackherr toward photocatalytic degradation of CLP. First of all, the study is concerned with the transformation kinetics and efficiency of photocatalytic degradation of CLP in DDW. The study encompasses the effects of a variety of experimental conditions such as the effect of the type of irradiation, catalyst loading, the initial concentration of CLP, temperature, pH, presence of electron acceptors, and hydroxyl radical (<sup>•</sup>OH) scavenger on the photodegradation kinetics in DDW. The results were compared to the most often used TiO<sub>2</sub> Degussa P25. An attempt has also been made to identify the reaction intermediates formed during the photo-oxidation process of CLP, using the LC-ESI-MS/MS method. The cell growth activity of CLP alone or in the mixture with its photocatalytic degradation intermediates was evaluated *in vitro* in rat hepatoma and human fetal lung cell line, using colorimetric Sulphorhodamine B assay. Finally, the matrix effect of river and tap water on photocatalytic removal of CLP was also studied.

## 2. Experimental

### 2.1 Water samples, chemicals and solutions

All chemicals were of reagent grade and were used without further purification. CLP, 99.4%, pestanal quality, was manufactured by Riedel-de Haën; 85% H<sub>3</sub>PO<sub>4</sub> was obtained from Lachema (Neratovice, Czech Republic) and NaOH from ZorkaPharm (Šabac, Serbia). The other chemicals used, such as 30% H<sub>2</sub>O<sub>2</sub>, *cc* acetic acid and 96% ethanol, were obtained from Centrophem (Stara Pazova, Serbia), KBrO<sub>3</sub>, (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and 60% HClO<sub>4</sub>, from Merck, while 99.8% acetonitrile (ACN) and HPLC gradient grade methanol (MeOH) were products of J. T. Baker and humic acids (HUM) technical, of Fluka. All solutions were made using DDW. The pH of the reaction mixture was adjusted using a dilute aqueous solution of HClO<sub>4</sub> or NaOH. Aspirin<sup>®</sup> was purchased from Bayer, doxorubicin (Doxorubicin-Teva<sup>®</sup>) from Pharmachemie B.V. (Haarlem, Netherlands) and gemcitabine (Gemzar<sup>®</sup>) from Lilly France S.A. (Fegersheim, France), fetal calf serum (FCS) from PAA Laboratories GmbH (Pashing, Austria), penicillin and streptomycin from Galenika (Belgrade, Serbia), trypsin from Serva (Heidelberg, Germany), and EDTA, trichloroacetic acid (TCA), mercury(II) chloride from Laphoma (Skopje), and tris(hydroxymethyl)amino methane (TRIS) from Sigma Aldrich.

Wackherr's “Oxyde de titane standard” (100% anatase form, surface area 8.5±1.0 m<sup>2</sup> g<sup>-1</sup>, crystallite size 300 nm (Vione et al., 2005) hereafter “TiO<sub>2</sub> Wackherr”, and TiO<sub>2</sub> Degussa P25 (75% anatase and 25% rutile form, 50 m<sup>2</sup> g<sup>-1</sup>, about 20 nm, non-porous) were used as photocatalysts.

The tap water sample was taken from the local water supply network (Novi Sad, Serbia). River water, collected from the Danube (Novi Sad, Serbia) in May 2010, was filtered through Whatman filter paper 42 (diameter: 125 mm, pore size: 0.1 μm, ashless) before use. The physicochemical characteristics of the water samples, along with that of DDW are given in Table 1.

Parameter	Water type		
	DDW	Tap water	Danube river
pH	6.5	7.3	7.8
El. conductivity at 25 °C (µS mL <sup>-1</sup> )	2.9	516	365
TOC (mg L <sup>-1</sup> )	1.04	1.80	5.60
Carbonate hardness (°dH)	0.37	13.06	8.36
HCO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )		285	182

Table 1. The physicochemical characteristics of the analysed water types.

2.2 Photodegradation procedures

The photocatalytic degradation was carried out in a cell made of Pyrex glass (total volume of ca. 40 mL, liquid layer thickness 35 mm), with a plain window on which the light beam was focused. The cell was equipped with a magnetic stirring bar and a water circulating jacket. A 125 W high-pressure mercury lamp (Philips, HPL-N, emission bands in the UV region at 304, 314, 335 and 366 nm, with maximum emission at 366 nm), together with an appropriate concave mirror, was used as the radiation source. Irradiation in the visible spectral range was performed using a 50 W halogen lamp (Philips) and a 400 nm cut-off filter. The outputs for the mercury and halogen lamps were calculated to be ca.  $8.8 \times 10^{-9}$  Einstein mL<sup>-1</sup> min<sup>-1</sup> and  $1.7 \times 10^{-9}$  Einstein mL<sup>-1</sup> min<sup>-1</sup> (potassium ferrioxalate actinometry), respectively. In a typical experiment, and unless otherwise stated, the initial CLP concentrations were 1.0 mM, and the TiO<sub>2</sub> Wackherr loading was 2.0 mg mL<sup>-1</sup>. The total suspension volume was 20 mL. The aqueous suspension of TiO<sub>2</sub> Wackherr was sonicated (50 Hz) in the dark for 15 min before illumination, to uniformly disperse the photocatalyst particles and attain adsorption equilibrium. The suspension thus obtained was thermostated and then irradiated at a constant stream of O<sub>2</sub> (3.0 mL min<sup>-1</sup>). During the irradiation, the mixture was stirred at a constant speed. All experiments were performed at the natural pH (~ 3.5), except when studying the influence of the pH on the photocatalytic degradation of the substrate. In the investigation of the influence of electron acceptors, apart from constant streaming of O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, KBrO<sub>3</sub> or (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> was added to the CLP solution to make a 3 mM concentration. Where applicable, ethanol (400 µL) was added as a hydroxyl radical scavenger.

2.3 Analytical procedures

2.3.1 Kinetic studies

For the LC-DAD kinetic studies of the CLP photodegradation, samples of 0.50 mL of the reaction mixture were taken at the beginning of the experiment and at regular time intervals. Aliquot sampling caused a maximum volume variation of ca. 10% in the reaction mixture. Each aliquot was diluted to 10.00 mL with DDW. The obtained suspensions were filtered through a Millipore (Millex-GV, 0.22 µm) membrane filter. The absence of the CLP adsorption on the filters was preliminarily checked. After that, a 20 µL sample was injected and analysed on an Agilent Technologies 1100 Series liquid chromatograph, equipped with a UV/vis DAD set at 225 nm (absorption maximum for CLP), and a Zorbax Eclipse XDB-

C18 (150 mm × 4.6 mm i.d., particle size 5 µm, 25 °C) column. The mobile phase (flow rate 1 mL min<sup>-1</sup>, pH 2.56) was a mixture of ACN and water (3:7, v/v), the water being acidified with 0.1% H<sub>3</sub>PO<sub>4</sub>. Reproducibility of repeated runs was around 5–10%.

The total organic carbon (TOC) analysis was performed on an Elementar Liqui TOC II according to Standard US EPA Method 9060A. In studying the influence of the initial pH on the photocatalytic degradation use was made of a combined glass electrode (pH-Electrode SenTix 20, WTW) connected to a pH-meter (pH/Cond 340i, WTW).

### 2.3.2 Identification of the reaction intermediates

For the LC-ESI-MS/MS evaluation of intermediates, a 1.0 mM of CLP solution was prepared. Aliquots were taken at the beginning of the experiment and at regular time intervals during the irradiation. Then, a 20 µL sample was injected and analysed on an Agilent Technologies 1200 series liquid chromatograph with Agilent Technologies 6410A series electrospray ionisation triple-quadrupole MS/MS. The mobile phase (flow rate 1.0 mL min<sup>-1</sup>) consisted of 0.05% aqueous formic acid and MeOH (gradient: 0 min 30% MeOH, 10 min 100% MeOH, 12 min 100% MeOH, post time 3 min). Components were separated on an Agilent Technologies XDB-C18 column (50 mm × 4.6 mm i.d., particle size 1.8 µm) held at 50 °C; UV/vis signal of the eluate was monitored at 210 nm, 225 nm and 260 nm (bandwidth 16 nm for each); continuous spectrum in the range from 200 to 400 nm (2 nm step) was also recorded. The eluate was forwarded to the MS/MS instrument without flow splitting. Analytes were ionised using the electrospray ion source, with nitrogen as drying gas (temperature 350 °C, flow 10 L min<sup>-1</sup>) and nebuliser gas (45 psi), and a capillary voltage of 4.0 kV. High-purity nitrogen was used as the collision gas. Full-scan mode (*m/z* range 100–800, scan time 100 ms, fragmentor voltage 100 V), using positive or negative polarity (depending on compound), was used to select precursor ion for CLP and each degradation product, as well as to examine isotopic peaks distribution (Table 2). Then, product ion scan MS<sup>2</sup> mode (fragmentor voltage 100 V, scan time 100 ms, collision energy 0–40 V in 10 V increments) was used for structure elucidation of each degradation product.

### 2.4 Cell growth activity

**Cell lines.** For the estimation of cell growth effects, the cell lines H-4-II-E (rat hepatoma) and MRC-5 (human fetal lung) were grown in RPMI 1640 (H-4-II-E) and DMEM medium (MRC-5) with 4.5% glucose, supplemented with 10% heat inactivated FCS, 100 IU mL<sup>-1</sup> of penicillin and 100 µg mL<sup>-1</sup> of streptomycin. Investigated cell lines grew attached to the surface. They were cultured in 25 mL flasks (Corning, New York, USA) at 37 °C in atmosphere of 5% CO<sub>2</sub> and 100% humidity, sub-cultured twice a week and a single cell suspension was obtained using 0.1% trypsin with 0.04% EDTA.

**Samples and controls used in cell growth experiments.** For the analysis of cell growth effects, serial dilutions in distilled water were used. Samples were filtered through a 0.22 µm micro filters (Sartorius) to obtain sterility. The final concentrations of CLP before beginning the irradiation as well as in the CLP solution that was not irradiated were in the range from 6.25 to 100 µM, i.e. the dilution was from 10 to 160. Solution of CLP, filtered suspension of TiO<sub>2</sub> Wackherr catalyst and Aspirin<sup>®</sup> were used as negative controls, while cytotoxic drugs doxorubicin and gemcitabine, as well as HgCl<sub>2</sub> were used as positive controls.

Compound	<i>t<sub>R</sub></i> (min)	<i>M<sub>M</sub></i> (g mol <sup>-1</sup> )	UV max. (nm)	Mode	Precursor ion <i>m/z</i>	<i>V<sub>col</sub></i> (V)	Product ions <i>m/z</i> , rel. abundance
1 CLP	1.16	191	222, 281	NI	190	0	190 (82), 146 (100) 10 146 (100) 20 146 (100)
2 3,6-Dichloro-4,5-dihydropyridine-2-carboxylic acid	0.81	223	216, 278	NI	222	0	222 (51), 178 (100) 10 178 (100), 142 (48), 106 (11) 20 178 (71), 142 (100), 106 (40) 30 142 (29), 106 (100), 78 (61), 66 (50)
3 3,6-Dichloro-pyridin-2-ol*	2.31	163	235, 315	PI	164	0	164 (100) 10 164 (100), 146 (5), 124 (31) 20 164 (43), 146 (86), 128 (100), 110 (37), 100 (26), 73 (78) 30 146 (37), 128 (10), 110 (93), 75 (9), 73 (100), 62 (6) 40 110 (79), 75 (34), 73 (100), 62 (11) 0 162 (100) 10 162 (100)
4 3,6-Dichloro hydroxypyridine-2-carboxylic acid*	1.39	207	204, 248, 295	PI	208	0	208 (3), 190 (100) 10 190 (100), 162 (42) 20 190 (14), 162 (100), 107 (25) 30 162 (45), 134 (9), 107 (100), 98 (15) 40 107 (100), 98 (15) 0 206 (100), 162 (36) 10 206 (12), 162 (100), 90 (11) 20 162 (100), 126 (12), 90 (95), 62 (11) 30 162 (7), 90 (100), 62 (94) 0 172 (100), 128 (9) 10 172 (54), 128 (100) 20 128 (100)
5 6-Chloro-3-hydroxypyridine-2-carboxylic acid or 3-Chloro-6-hydroxypyridine-2-carboxylic acid	1.57	173	233, 307	NI	172	0	

Table 2. MS/MS fragmentation data of CLP photodegradation intermediates (Part I).



Compound	<i>t<sub>R</sub></i> (min)	<i>M<sub>MI</sub></i> (g mol <sup>-1</sup> )	UV max. (nm)	Mode	Precursor ion <i>m/z</i>	<i>V<sub>col</sub></i> (V)	Product ions <i>m/z</i> , rel. abundance
6 3,6-Dichloro pyridinediol	1.65	179	~227sh, 310, ~350sh	PI	180	0	180 (100)
						10	180 (100), 162 (6), 144 (11), 88 (12)
						20	180 (69), 162 (52), 144 (29), 107 (50), 98 (7), 89 (12), 88 (100)
						30	162 (9), 107 (100), 89 (9), 88 (53)
						40	107 (100), 98 (10), 89 (15), 88 (39), 72 (7), 53 (7), 52 (13)
						0	178 (100), 142 (16)
7 3,6-Dichloro hydroxypyridine-2-carboxylic acid*	0.85	207	220, 265, 312-320**	PI	208	10	178 (100), 142 (51), 78 (21)
						20	78 (100)
						0	208 (100), 190 (24)
						10	190 (100), 162 (57)
						20	190 (12), 162 (100), 134 (11), 107 (59)
						30	162 (18), 107 (100)
						40	134 (10), 107 (100), 98 (7), 83 (8)
				NI	206	0	206 (92), 162 (100)
						10	162 (100), 126 (23), 90 (7)
						20	162 (45), 126 (76), 98 (52), 90 (100)
						30	126 (5), 98 (75), 90 (100), 66 (8), 62 (49)

\* previously identified using TiO<sub>2</sub> Degussa P25

\*\* wide spectral band

*M<sub>MI</sub>* – monoisotopic weight

Table 2. MS/MS fragmentation data of CLP photodegradation intermediates (Part II).

**Sulphorhodamine B (SRB) assay.** Cell lines were harvested and plated into 96-well microtiter plates (Sarstedt, Newton, USA) at a seeding density of  $4 \times 10^3$  cells per well (Četojevic-Simin et al., 2011), in a volume of 180  $\mu$ L, and preincubated in complete medium supplemented with 5% FCS, at 37 °C for 24 h. Serial dilutions and solvent were added (20  $\mu$ L/well) to achieve the required final concentrations and control. Microplates were then incubated at 37 °C for additional 48 h. Cell growth was evaluated by the colorimetric SRB assay according to Skehan et al. (1990). Cells were fixed with 50% TCA (1 h, +4 °C), washed with distilled water (Wellwash 4, Labsystems; Helsinki, Finland) and stained with 0.4% SRB (30 min, room temperature). The plates were then washed with 1% acetic acid to remove unbound dye. Protein-bound dye was extracted with 10 mM TRIS base. Absorbance was measured on a microplate reader (Multiscan Ascent, Labsystems) at 540/620 nm. The effect on cell growth was expressed as a percent of the control, and calculated as: % Control =  $(A_t/A_c) \times 100$  (%), where  $A_t$  is the absorbance of the test sample and  $A_c$  is the absorbance of the control.

**Statistical analysis.** The results of cell growth activity were expressed as mean  $\pm$  SD of two independent experiments, each performed in quadruplicate ( $n = 8$ ). Differences between control and treated groups were evaluated using one-way analysis of variance at the significance level of  $p < 0.05$  (Microsoft Office Excel 2003 software). IC<sub>50</sub> values were calculated using Calcsyn for Windows (Version 1.1.0.0.; Biosoft).

### 3. Results and discussion

#### 3.1 Effects of the type of TiO<sub>2</sub>

The photocatalytic activity of TiO<sub>2</sub> Wackherr was compared to that of the most often used Degussa P25 under UV and visible irradiation. As can be seen from Figure 1, practically no degradation was observed under the visible light irradiation, either in the presence or absence of TiO<sub>2</sub>. The lack of CLP disappearance in the presence of TiO<sub>2</sub> under these conditions also allows the exclusion of a significant adsorption of CLP on the catalyst surface during the course of the irradiation. In contrast, significant CLP removal could be observed under UV, and the process involving TiO<sub>2</sub> Wackherr was slightly faster compared to that observed in the presence of Degussa P25. This insignificant acceleration of the degradation of CLP in the presence of TiO<sub>2</sub> Wackherr is noteworthy, considering that this TiO<sub>2</sub> specimen has much larger particles (average radii in solution are 3–4 times larger) than Degussa P25 and a surface area that is almost six times lower (Vione et al., 2005).

The direct photolysis of CLP was also checked under the adopted irradiation conditions, in the absence of a catalyst (Figure 1). It appears that CLP can be degraded by direct photolysis in the near UV region, but at a significantly lower rate compared to the photocatalytic process.

Under the relevant experimental conditions, the reaction followed a pseudo-first order kinetics. On the basis of the kinetic curves  $\ln c$  (substrate concentration) vs.  $t$ , the values of the pseudo-first order rate constant  $k'$  were calculated. The degradation rate of CLP was calculated for all the investigated as the product  $k' c_0$ , where  $c_0$  is the initial concentration of CLP.



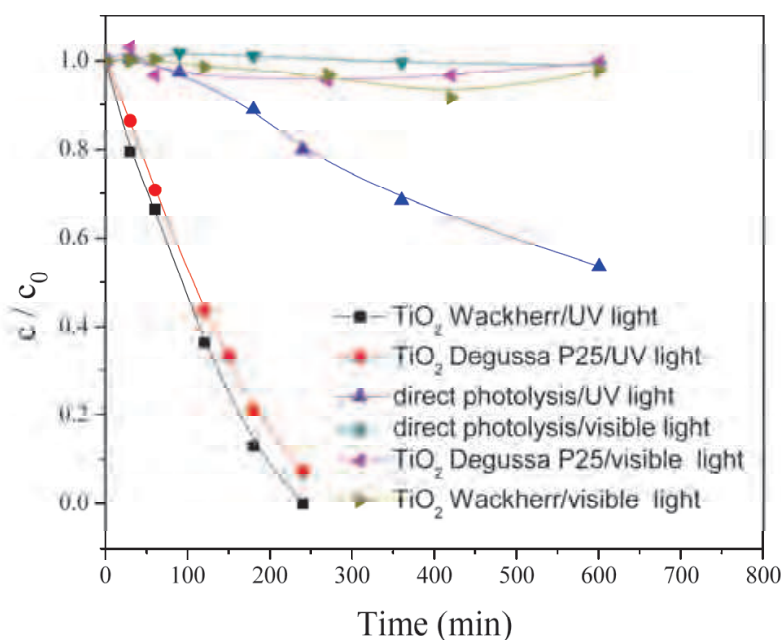


Fig. 1. Kinetics of the photolytic and photocatalytic degradation of CLP (1.0 mM). When present, the TiO<sub>2</sub> loading was 2.0 mg mL<sup>-1</sup>. Operation conditions:  $t = 40\text{ }^{\circ}\text{C}$  at pH  $\sim 3.5$ .

A comparison of the mineralisation capacities of TiO<sub>2</sub> Wackherr and Degussa P25, presented in Figure 2, shows that the mineralisation efficiency in the presence of TiO<sub>2</sub> Wackherr is significantly higher – 90% of CLP was mineralised during 240 min, whereas in the case of Degussa P25 only about 60%. Also, the ratio of the removal rate of the parent compound and total mineralisation in the case of TiO<sub>2</sub> Wackherr is about 1.20, whereas in the case of P25 this ratio is significantly higher, amounting to even 2.80. If these results are compared with those of the photocatalytic degradation of pyridine pesticides such as picloram and triclopyr (Abramović et al., 2011) it can be seen that the ratios of the mineralisation rate to the rate of the parent compound degradation are different and that they depend on the type of the substituent in the pyridine ring. Namely, in the photocatalytic degradation of triclopyr, TiO<sub>2</sub> Degussa P25 showed a higher efficiency both in the process of mineralisation and degradation of the parent compound. In the case of picloram, TiO<sub>2</sub> Wackherr showed higher photocatalytic efficiency than Degussa P25. However, the ratio of the rates of removal of the parent compound and total mineralisation in the presence of TiO<sub>2</sub> Wackherr is much higher in the case of picloram than of CLP, and it amounts to about 9, whereas in the presence of Degussa P25 this ratio is about 3, which is similar to the value obtained also for CLP.

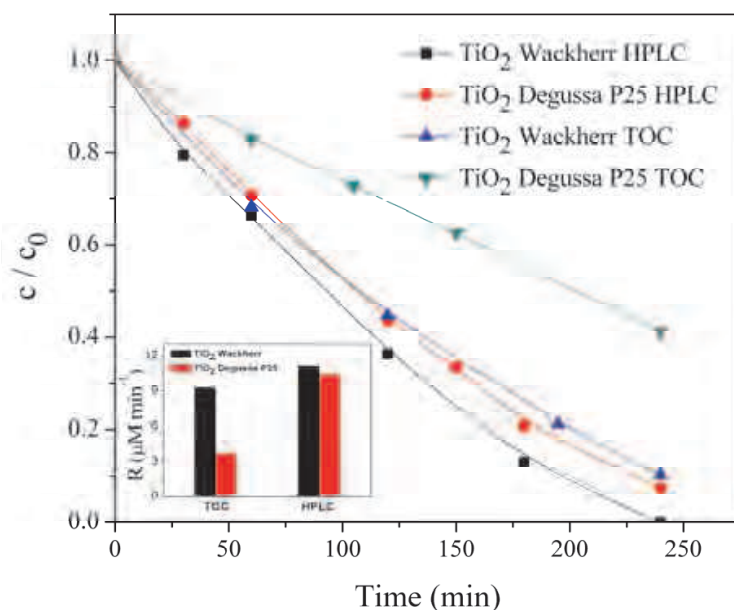


Fig. 2. Comparison of the photocatalytic removal parent compound and mineralisation of CLP. The inset shows the degradation rate ( $R$ ) calculated over 240 min of irradiation. Operation conditions:  $c(\text{CLP})_0 = 1.0 \text{ mM}$ ,  $\text{TiO}_2 = 2.0 \text{ mg mL}^{-1}$ ,  $t = 40^\circ\text{C}$ , at  $\text{pH} \sim 3.5$ .

### 3.2 Effect of catalyst loading

Due to the inherent nature of heterogeneous photocatalytic systems, there is always an optimum catalyst concentration at which the removal rate is at its maximum. In this study, the optimum was determined by changing the concentration of TiO<sub>2</sub> Wackherr over the loading range from 0.2 to 2.0 mg mL<sup>-1</sup>, as shown in Figure 3. As can be seen from the figure inset, the increase in TiO<sub>2</sub> loading up to 1.0 mg mL<sup>-1</sup> was accompanied by an increase in the degradation rate, but a further increase caused an opposite effect. Theoretically, the increase in the catalyst loading above an optimum value has no effect on the photodegradation rate since all the light available is already utilized. However, higher loading of TiO<sub>2</sub> led to the aggregation of its particles and thus to a decrease in the contact surface between the reactant and photocatalyst particles, which caused a decrease in the number of active sites, resulting in a lower rate of photodegradation. Also, when TiO<sub>2</sub> is overdosed, the intensity of the incident UV light is attenuated because of the decreased light penetration and increased scattering, which attenuates the positive effect coming from the dosage increment, and therefore the overall performance decreases (Wong & Chu, 2003). Optimum catalyst concentration is a complex function of a number of parameters including catalyst agglomeration, the suspension opacity, light scattering, mixing, reactor type, and the pollutant type (Toor et al., 2006; Mendez-Arriaga et al., 2008); hence it is not constant for all photocatalytic systems. Indeed, optimum catalyst concentrations have been reported to vary between as low as 0.1 mg mL<sup>-1</sup> to as high as around 10 mg mL<sup>-1</sup> (Mendez-Arriaga et al., 2008; Alhakimi et al., 2008; Chu et al., 2009a). An optimum catalyst concentration of around 1 mg mL<sup>-1</sup> has been generally reported in many studies (Chen & Ray, 1998; Lu et al., 1999; Mendez-Arriaga et al., 2008; Rajeswari & Kanmani, 2009; Tizaoui et al., 2011). However, by comparing these results with our previously finding that the optimal catalyst loading of

TiO<sub>2</sub> Degussa P25 was 4.0 mg mL<sup>-1</sup> (Šojić et al., 2009), it can be concluded that the effect of catalyst loading on the efficiency of the photocatalytic removal of CLP is influenced by the type of TiO<sub>2</sub> used. If we compare the efficiencies of the two catalysts at their optimal loadings (i.e. 1.0 mg mL<sup>-1</sup> for TiO<sub>2</sub> Wackherr and 4.0 mg mL<sup>-1</sup> for Degussa P25), it comes out that TiO<sub>2</sub> Wackherr, although being present in a lower amount, is more efficient in the removal of CLP.

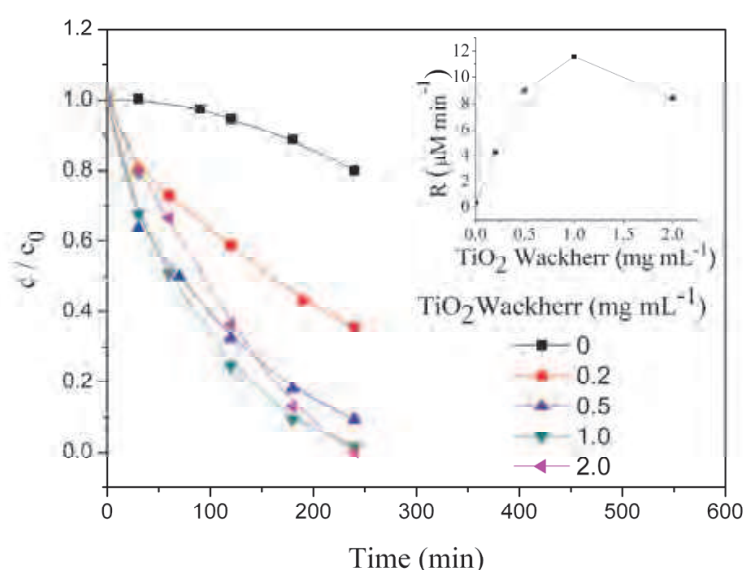


Fig. 3. Effect of the TiO<sub>2</sub> Wackherr catalyst loading on the kinetics of CLP photodegradation. The inset shows the effect of TiO<sub>2</sub> Wackherr loading on the degradation rate ( $R$ ) determined after 120 min of irradiation. Operation conditions:  $c(\text{CLP})_0 = 1.0$  mM,  $t = 40$  °C at pH  $\sim 3.5$ .

### 3.3 Effect of the initial CLP concentration

Many studies have shown that the initial pollutant concentration has a significant effect on the rate of its photocatalytic removal. In this work, the effect of the initial concentration of CLP on the photodegradation rate was studied under UV light using TiO<sub>2</sub> Wackherr in the loading range from 0.25 to 1.0 mM (Figure 4). As can be seen from the inset of Figure 4, the degradation rate decreased with increase in the CLP concentration above to 0.5 mM. Such behaviour may be explained by the fact that at an increased concentration of CLP more of its molecules can be adsorbed on the photocatalyst surface, needing thus a larger catalyst area for their degradation. However, as the intensity of light, irradiation time and amount of catalyst are constant, the relative amounts of O<sub>2</sub><sup>-</sup> and •OH radicals on the catalyst surface do not increase (Atiqur Rahman & Muneer, 2005; Qamar et al., 2006).

An alternative explanation for the effect of the substrate concentration is the competition for reactive species between the substrate and the transformation intermediates, the concentration of which increase with increasing substrate concentration, or the poisoning of the photocatalyst surface by the intermediates themselves (Abramović et al., 2011).

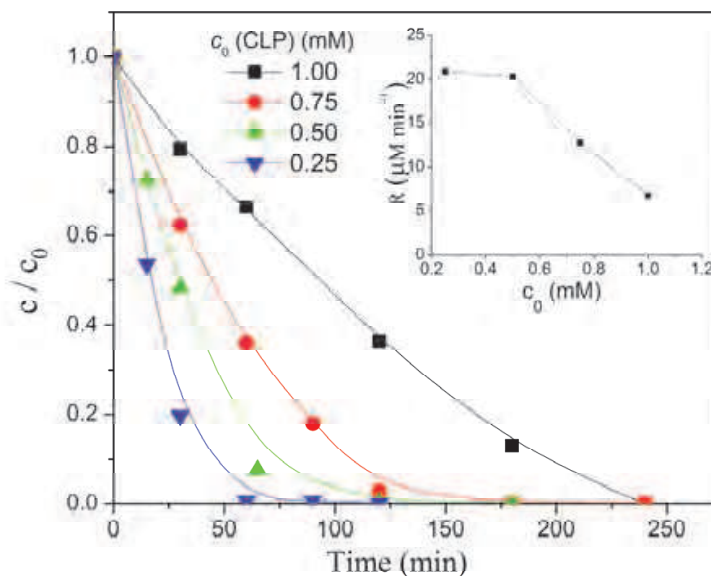


Fig. 4. Effect of the initial CLP concentration on the kinetics of photodegradation. The inset shows the effect of initial CLP concentration on the CLP degradation rate ( $R$ ) calculated for 60 min of irradiation. Operation conditions: TiO<sub>2</sub> Wackherr = 2.0 mg mL<sup>-1</sup>,  $t$  = 40 °C at pH ~ 3.5.

### 3.4 Effect of temperature

The photocatalytic degradation of CLP was studied in a temperatures range from 25 to 40 °C (Figure 5) and the rate constant  $k'$  was determined from the pseudo-first order plots. In general, the rate constant is expected to increase at higher temperatures, but it appeared that CLP more easily degraded at lower temperatures in the TiO<sub>2</sub> Wackherr suspension. Thus, the decrease in the rate constant observed in this temperature range may be attributed to the physisorption between the TiO<sub>2</sub> surface and the CLP molecules (Ishiki et al., 2005). Namely, the temperature of fastest CLP removal was, surprisingly, 25 °C, and hence all the further measurements were carried out at this temperature. The energy of activation,  $E_a$ , was calculated from the Arrhenius plot of  $\ln k$  versus  $1/T$  (K<sup>-1</sup>), and it amounted to 37.9 kJ mol<sup>-1</sup>. Obviously, this value is somewhat higher than that obtained for the photocatalytic degradation of CLP in the presence TiO<sub>2</sub> Degussa P25 (Šojić et al., 2009), but it is acceptable since for TiO<sub>2</sub> photocatalyst, irradiation is the primary source of the electron-hole pair generation at ambient temperature, as the band gap energy is too high to be overcome by thermal activation (Topalov et al., 2004).

### 3.5 Effect of the initial pH

The effect of pH is very important in the heterogeneous photocatalytic removal of organic molecules since it influences both the surface charge of TiO<sub>2</sub> and the ionic form of the reactant, influencing thus the electrostatic interactions between the reactant species and the catalyst surface. Moreover, the pH influences the sizes of TiO<sub>2</sub> aggregates, interaction of the

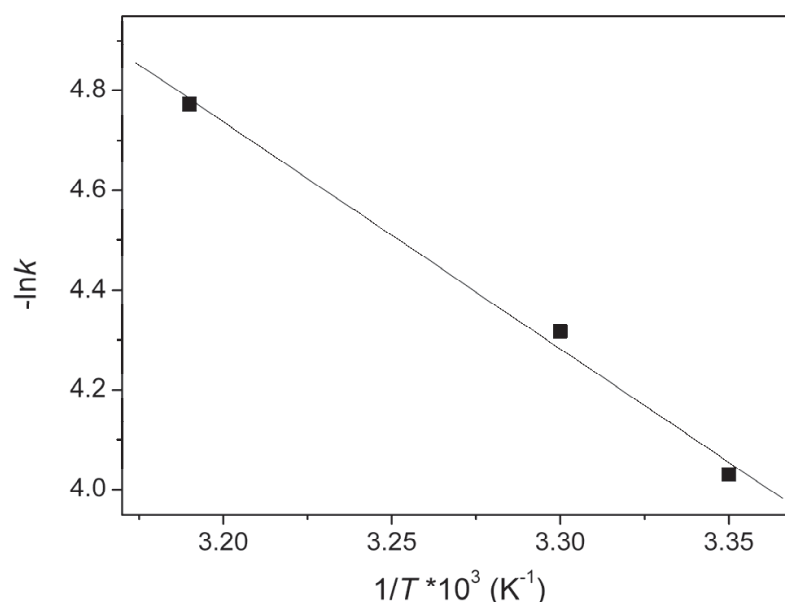


Fig. 5. Arrhenius plot of  $\ln k$  versus  $1/T$  for the photocatalytic degradation of CLP for the first 120 min of irradiation. Operation conditions:  $c(\text{CLP})_0 = 1.0 \text{ mM}$ ,  $\text{TiO}_2 \text{ Wackherr} = 2.0 \text{ mg mL}^{-1}$  at  $\text{pH} \sim 3.5$ .

solvent molecules with the catalyst and the type of radicals or intermediates formed during the photocatalytic reaction (Muneer & Bahnemann, 2002; Šojić et al., 2009; Tizaoui et al., 2011). All of these factors have a significant effect on the adsorption of solutes on  $\text{TiO}_2$  surfaces and, as a result, on the observed photodegradation rates. Because the real effluent stuff of pesticide can be discharged at a different pH, the pH effect on the photocatalytic rate degradation of CLP was studied in the pH range from 2.4 to 9.8 (Figure 6). The point of zero charge ( $\text{pH}_{\text{pzc}}$ ) of anatase is 5.8 (Karunakaran & Dhanalakshmi, 2009). Thus, the  $\text{TiO}_2$  surface will be positively charged ( $\text{TiOH}_2^+$ ) in acidic media ( $\text{pH} < \text{pH}_{\text{pzc}}$ ) and negatively charged ( $\text{TiO}^-$ ) in alkaline media ( $\text{pH} > \text{pH}_{\text{pzc}}$ ). On the other hand, the  $\text{pK}_a$  values for CLP are  $1.4 \pm 0.1$  and  $4.4 \pm 0.1$  (Corredor et al., 2006), so that at  $\text{pH} < 1.4$  the herbicide is mainly present in its protonated form, and at  $\text{pH} > 4.4$  in the anionic form. In the pH interval from 2.4 to 3.5, one can expect a great increase in the photodegradation rate, arising as a consequence of the dissociation of the carboxylic group and deprotonation of the pyridine nitrogen (to a significantly smaller extent). In this way, favourable electrical forces are generated that are manifested as the attraction between the positively charged surface of the catalyst and CLP anion. As can be seen in Figure 6 (inset), in the pH interval from 3.5 to 4.8, a distinct decrease of the photodegradation rate is observed, arising probably as a consequence of the decrease in the number of positive sites on the catalyst surface. A further increase in the pH up to 9.8 caused a decrease in the photodegradation rate, which was probably a consequence of the influence of several factors. Namely, at  $\text{pH} > \text{pH}_{\text{pzc}}$ , the  $\text{TiO}_2$  surface is negatively charged, causing the repulsion of the CLP anion. Besides, unfavourable electrical forces are generated, i.e., the repulsion between the negatively charged surface of the catalyst and  $\text{OH}^-$ .



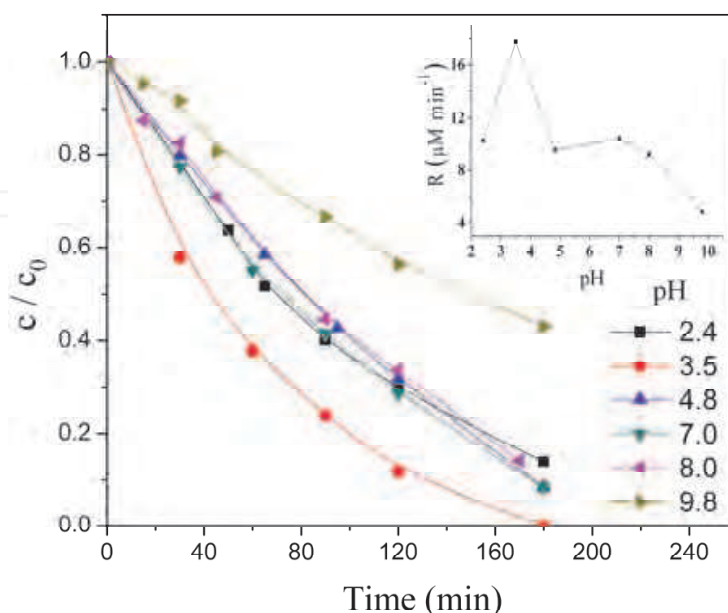


Fig. 6. Effect of the pH on the kinetics of CLP photocatalytic degradation. The inset shows the effect of pH on the degradation rate ( $R$ ) calculated for 120 min of irradiation. Operation conditions:  $c(\text{CLP})_0 = 1.0 \text{ mM}$ ,  $\text{TiO}_2 \text{ Wackherr} = 2.0 \text{ mg mL}^{-1}$ ,  $t = 25^\circ\text{C}$ .

### 3.6 Effect of electron acceptors

A practical problem arising in the use of TiO<sub>2</sub> as a photocatalyst is the undesired  $e^- - h^+$  pair recombination. One strategy to inhibit  $e^- - h^+$  pair recombination is to add other (irreversible) electron acceptors to the reaction mixture. They may have several different effects such as (1) to increase the number of trapped electrons and, consequently, avoid recombination, (2) to generate more radicals and other oxidising species, (3) to increase the oxidation rate of intermediate compounds, and (4) to avoid problems caused by low oxygen concentration. In highly toxic wastewater, where the degradation of organic pollutants is the major concern, the addition of electron acceptors to enhance the degradation rate may often be justified (Singh et al., 2007). The rates of photocatalytic degradation and mineralisation of CLP in the presence of various electron acceptors such as KBrO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, and (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> in addition to the molecular oxygen are shown in Figure 7.

As can be seen, the mentioned electron acceptors showed different effects. Namely, only the addition of KBrO<sub>3</sub> enhanced the rate of photocatalytic degradation of the parent compound (by a factor of 1.4), indicating that this compound is a more effective electron acceptor compared with other oxidants employed in this study. A possible explanation might be the change in the reaction mechanism of the photocatalytic degradation, since the reduction of BrO<sub>3</sub><sup>-</sup> by electrons does not lead directly to the formation of  $\cdot\text{OH}$ , but rather to the formation of other reactive radicals or oxidising reagents e.g. BrO<sub>2</sub><sup>-</sup> and HOBr. Furthermore, BrO<sub>3</sub><sup>-</sup> by themselves can act as oxidising agents (Singh et al., 2007). However, the mineralisation rate is slightly lower (by a factor of 1.1).



However, the presence of  $\text{H}_2\text{O}_2$  caused a decrease in both the rate of removal of CLP (by a factor of 1.7) and its mineralisation (by a factor of 1.3). Such a negative effect of  $\text{H}_2\text{O}_2$  is probably a consequence of the fact that it can also act as an  $\cdot\text{OH}$  scavenger, generating much less reactive hydroperoxyl radicals ( $\text{HO}_2\cdot$ ). The  $\text{HO}_2\cdot$  can further react with the remaining strong  $\cdot\text{OH}$  to form ineffective oxygen and water. Besides, at a higher dose,  $\text{H}_2\text{O}_2$  might absorb and thus attenuate the incident UV light available for the photocatalysis process (Chu & Wong, 2004; Muruganandham & Swaminathan, 2006).

The presence of  $\text{S}_2\text{O}_8^{2-}$  had an insignificant effect on the rate of degradation of CLP, but it decreased the rate of mineralisation more than  $\text{KBrO}_3$  and  $\text{H}_2\text{O}_2$ , which can be explained by an increase in the concentration of  $\text{SO}_4^{2-}$  adsorbed on the  $\text{TiO}_2$  surface, reducing thus the catalytic activity. The excess of adsorbed  $\text{SO}_4^{2-}$  also reacts with the photogenerated holes and with the  $\cdot\text{OH}$  (San et al., 2001; Muruganandham & Swaminathan, 2006).

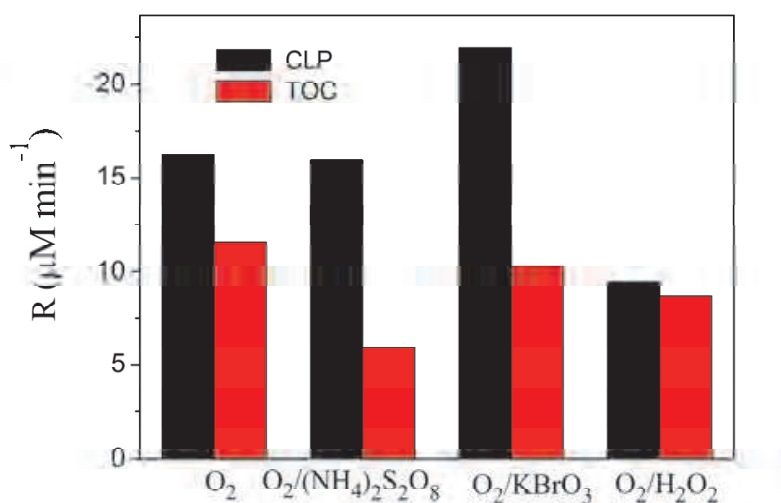


Fig. 7. Comparison of the degradation rate ( $R$ ) of CLP removal and mineralisation in the presence of different electron acceptors (3 mM) calculated for 60 min of irradiation. Operation conditions:  $c(\text{CLP})_0 = 1.0 \text{ mM}$ ,  $\text{TiO}_2 \text{ Wackherr} = 2.0 \text{ mg mL}^{-1}$ ,  $t = 25 \text{ }^\circ\text{C}$ ,  $\text{pH} \sim 3.5$ .

### 3.7 Effect of $\cdot\text{OH}$ scavenger

In order to investigate whether the heterogeneous photocatalysis takes place via  $\cdot\text{OH}$ , ethanol was added to the reaction mixture. Namely, it is known that alcohols, e.g. ethanol, act as  $\cdot\text{OH}$  scavengers (Daneshvar et al., 2004). The results obtained (data not shown) indicate that the degradation rate was significantly slower (by about 100 times) compared to that observed in the absence of ethanol, which proves that the reaction of photocatalytic degradation proceeded via  $\cdot\text{OH}$ .

### 3.8 Intermediates and the mechanism of photodegradation

The LC-MS analysis of the irradiated CLP solutions indicated the formation of six intermediates (labelled 1-7, Table 2), whose kinetic curves are shown in Figure 8. Three of

them, 3,6-dichloro-pyridin-2-ol (compound **3**) and isomeric 3,6-dichloro hydroxypyridine-2-carboxylic acids (compounds **4** and **7**) were previously identified (Šojić et al., 2009) in the presence of TiO<sub>2</sub> Degussa P25. Using the positive and negative ionization MS<sup>2</sup> spectra, it was possible to identify the remaining compounds and propose a photocatalytic degradation scheme (Figure 9).

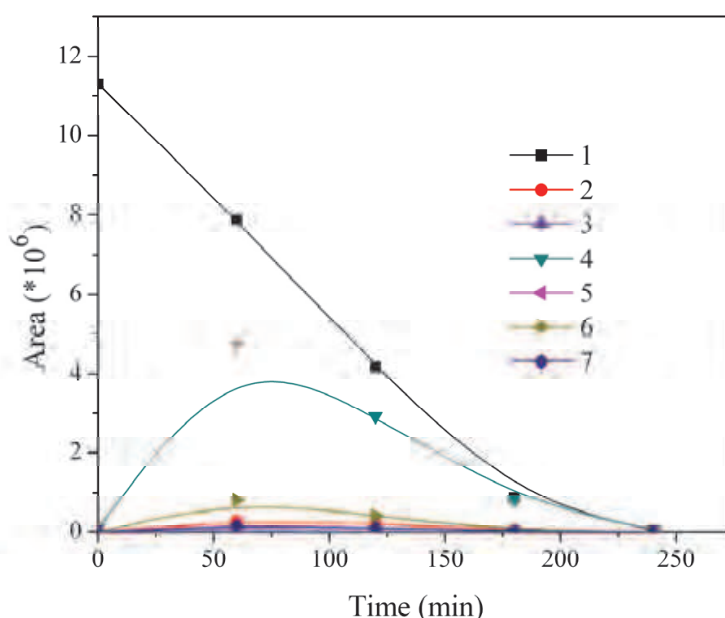


Fig. 8. Kinetics of the appearance/disappearance of CLP and intermediates in the photocatalytic degradation of CLP monitored by LC-ESI-MS/MS. Operation conditions:  $c(\text{CLP})_0 = 1.0 \text{ mM}$ ,  $\text{TiO}_2 \text{ Wackherr} = 2.0 \text{ mg mL}^{-1}$ ,  $t = 25 \text{ }^\circ\text{C}$ ,  $\text{pH} \sim 3.5$ .

Compound **2**, eluting at 0.81 min, had  $M_{\text{MI}}$  223, two chlorine atoms (on the basis of the A+2 isotopic peak intensity, as well as two consecutive losses of HCl in the MS<sup>2</sup> spectra:  $178 \rightarrow 142$  and  $142 \rightarrow 106$ ) and an odd number of nitrogen atoms (odd molecular weight), and was visible only in negative mode. In both the first-order and second-order MS spectra, the loss of CO<sub>2</sub> was observed ( $222 \rightarrow 178$ ), pointing out to the presence of carboxylic group. On the basis of the molecular weight (32 units higher than that of CLP) and spectral data, it was concluded that the compound is 3,6-dichloro-4,5-dihydroxypyridine-2-carboxylic acid.

Compound **5** eluted at 1.57 min. On the basis of the A+2 isotopic peak intensity and molecular weight, it could be concluded that it contains one chlorine atom and an odd number of nitrogen atoms. The only fragmentation observable in the NI MS<sup>1</sup> and MS<sup>2</sup> spectra was the loss of the carboxylic group as CO<sub>2</sub> ( $172 \rightarrow 128$ ). The monoisotopic weight of 173 mass units could be explained by the loss of one chlorine atom (which is in agreement with the isotopic profile) from the CLP molecule and introduction of one hydroxyl. Thus, the compound was identified as either 6-chloro-3-hydroxypyridine-2-carboxylic acid or 3-chloro-6-hydroxypyridine-2-carboxylic acid.

Finally, compound **6** was characterized by the odd monoisotopic weight of 179 units (pointing out to the odd number of nitrogen atoms), presence of two chlorine atoms, and the absence of carboxylic group loss both in positive mode (no sequential loss of H<sub>2</sub>O and CO)



concentration of toxic degradation intermediates and to the toxicity of the mixture that is no longer dominated by the parent compound.

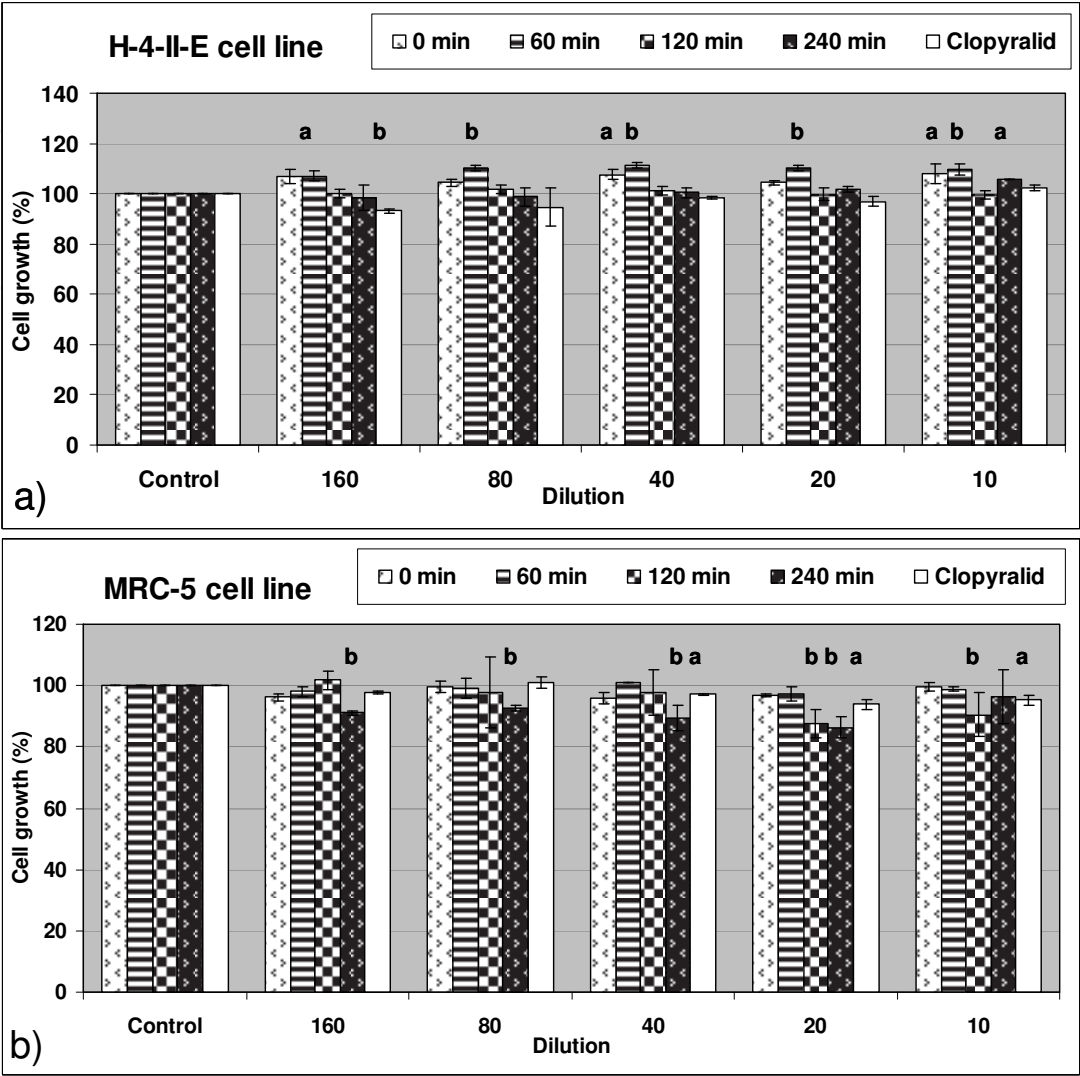


Fig. 10. Cell growth activity of the serial dilutions of CLP and its photocatalytic degradation intermediates obtained after different irradiation times in a) H-4-II-E and b) MRC-5 cell line. (One-way analysis of variance, compared to the control; a:  $p < 0.05$ , b:  $p < 0.01$ ). Results are expressed as mean  $\pm$  SD of two independent experiments, each performed in quadruplicate ( $n = 8$ ).

On the other hand, in the H-4-II-E cell line, the reaction mixture obtained after 60 minutes of irradiation produced significant ( $p < 0.01$ ) stimulation of cell growth compared to control (Figure 10a). The effects that are not concentration-dependent may be explained by the well known concept of hormesis (low dose stimulation and high dose inhibition). Since hormetic effects have been reported in a highly diverse array of biological models, for numerous organs and endpoints and chemical/physical stressors, it is evident that no single mechanism can account for these phenomena. In pharmacology, such dose responses have been studied with the aid of synthetic agonists and antagonists of receptors which mediate hormetic biphasic effects (Calabrese & Baldwin, 2001). A single agonist with differential

binding (i.e. high and low receptor affinities) that affects two opposite acting receptors will induce hormetic-like biphasic dose responses in numerous biological systems, as has been shown for dozens of receptor systems. Pollutants, for example, may initiate significant changes in complex receptor systems, and affect biphasic dose responses by inducing changes in the concentrations of endogenous agonists. When such changes occur over a broad dose range, biphasic dose responses typically become manifested (Calabrese, 2005).

The IC<sub>50</sub> values of Aspirin®, two well known cytotoxic drugs (Doxorubicin® and Gemcitabine®) and HgCl<sub>2</sub> (Table 3), as well as cell growth inhibition of TiO<sub>2</sub> Wackherr (Figure 11) were obtained in the same panel of cell lines.

Cell line	IC <sub>50</sub> (μM)			
	ASP <sup>a</sup>	DOX <sup>b</sup>	GEM <sup>c</sup>	HgCl <sub>2</sub>
H-4-II-E	>5551	0.272	0.004	3.189
MRC-5	>5551	0.408	0.384	69.578

<sup>a</sup>Aspirin®; <sup>b</sup>Doxorubicin®; <sup>c</sup>Gemcitabine®

Table 3. IC<sub>50</sub> values (μM) of Aspirin®, Doxorubicin®, Gemcitabine® and HgCl<sub>2</sub> in selected cell lines.

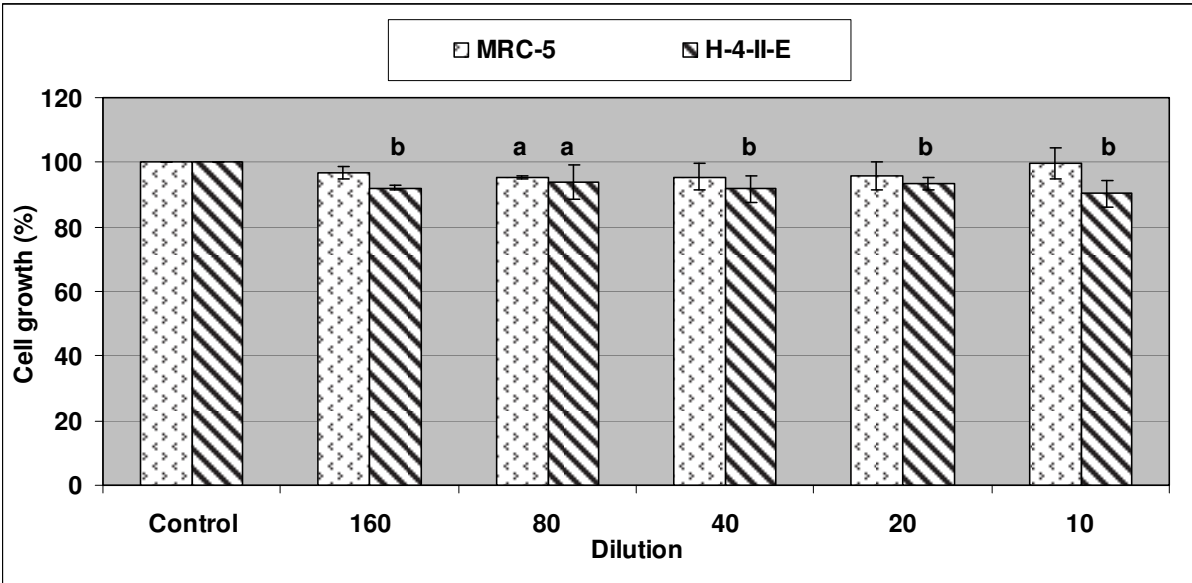


Fig. 11. Cell growth activity of different concentrations of TiO<sub>2</sub> Wackherr catalyst in the MRC-5 and H-4-II-E cell lines. (One-way analysis of variance, compared to the control; **a**:  $p < 0.05$ , **b**:  $p < 0.01$ ). Results are expressed as mean  $\pm$  SD of two independent experiments, each performed in quadruplicate ( $n = 8$ ).

In order to check whether the cell growth activity presented in Figure 10 is a consequence of the presence of CLP and its degradation intermediates only, it was necessary to run a blank test. To this end, aqueous suspension of TiO<sub>2</sub> Wackherr (2 mg cm<sup>-1</sup> without CPL) was sonicated in the dark for 15 min, as in the case of photodegradation of CLP, filtered through Millipore membrane filter, to apply then the same dilutions from 10 to 160. There were no significant effects ( $p < 0.01$ ) on the growth of MRC-5 cell line. On the other hand, the

H-4-II-E cell line appeared to be more sensitive to the presence of catalyst compared to the MRC-5 cell line (Figure 11). In the H-4-II-E cell line, the inhibition of cell growth influenced by TiO<sub>2</sub> Wackherr was significantly different compared to control ( $p < 0.01$ ), even at the 160 dilution (Figure 11), but in all cases was below 10%. Solvent (DDW) was also tested after different irradiation times and it was shown to be nontoxic, i.e. all values were at the level of control that was treated with DDW (data not shown).

The effects of examined samples on the growth of selected cell lines were dependent on the type of cell line, concentration and time of irradiation. It can be concluded that CLP and reaction mixture of CLP and its photocatalytic degradation intermediates effected mildly the cell growth of both cell lines. In the examined concentration range, none of the treatments produced cell growth inhibition higher than 50%, i.e. the IC<sub>50</sub> values were not reached, either by the parent compound nor by samples obtained after different irradiation times. All examined samples exhibited lower toxicity in selected cell lines compared to the cytotoxicity of controls and HgCl<sub>2</sub>.

A low level of free oxygen species is necessary for the promotion of cell proliferation (Burdon & Gill, 1993; Wei & Lee, 2002). The redox alterations play a significant role in a signal transduction pathway important for cell growth regulation. It is reasonable to propose that the examined samples obtained using UV irradiation in the presence of O<sub>2</sub> and TiO<sub>2</sub> Wackherr catalyst might influence the cell redox state, altering the cell proliferation.

Multi-endpoint bioassays that are based on whole cell response in human cell lines are a powerful indicator of metabolic, biochemical and genetic alterations that arise under the influence of evaluated compounds. This study presents an example of a systematic and simple first tier method to assess the toxicity of degradation products.

### 3.10 Effect of water type

Since natural aquatic systems contain dissolved organic matter (DOM) and different ionic species, it can be expected that they may complicate the photodegradation process. It has been reported that higher contents of inorganic and organic matter in tap and river water affect the efficiency of removal by UV/TiO<sub>2</sub> process (Buxton et al., 1988). To functionalise a TiO<sub>2</sub> water treatment process, the basic understanding of the effect of these inorganic ions on the photocatalytic performance is essential (Crittenden et al., 1996). Due to the zwitter ionic nature of the TiO<sub>2</sub> particles, it is also possible that the pH might have a profound effect on the selective inhibition of inorganic ions on the surface of the TiO<sub>2</sub> particles (Guillard et al., 2003). In this study, the effect of the matrix on the photocatalytic degradation of CLP was studied on the example of drinking and Danube water. As can be seen from Table 4, the rate of CLP removal from the samples of tap and river water was by about two/three times slower than that from DDW. The observed decrease in the degradation rate can be a consequence of the presence of HCO<sub>3</sub><sup>-</sup> and HUM in the examined water samples (Neppolian et al., 2002). Namely, the addition of HCO<sub>3</sub><sup>-</sup> and HUM to DDW in the amounts present in Danube water (Table 1), caused a decrease in the rate of photocatalytic degradation compared to that observed in DDW. As can be seen in Table 4, the concentration of HCO<sub>3</sub><sup>-</sup> in the examined tap water was somewhat higher, whereas the river water contained more HUM.



	$\text{HCO}_3^-$ (mg L <sup>-1</sup> )	HUM (mg L <sup>-1</sup> )	$R$ (μM min <sup>-1</sup> )
DDW			10.44
DDW	285	5	4.98
Tap water			5.41
DDW	182	15	4.51
Danube water			3.82

Table 4. The influence of water type on the degradation rate ( $R$ ) of CLP determined after 120 min of irradiation. Operation conditions:  $c(\text{CLP})_0 = 1.0 \text{ mM}$ ,  $\text{TiO}_2 \text{ Wackherr} = 2.0 \text{ mg mL}^{-1}$ ,  $t = 25 \text{ }^\circ\text{C}$ ,  $\text{pH} \sim 7.0$ .

In the literature, the inhibition of photocatalytic properties in the presence of ions is often explained by the scavenging of  $\bullet\text{OH}$  radical by ions. Of ionic species,  $\text{HCO}_3^-$  can especially, inhibit the degradation rate due to the high rate constant  $k'$  of its reaction with  $\bullet\text{OH}$  ( $8.5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ) (Buxton et al., 1988). Because of that we focused our attention on the influence of different concentrations of this ion on the photocatalytic degradation (Figure 12). Expectedly, an inhibition of CLP degradation was observed after adding  $\text{HCO}_3^-$  to DDW up to about  $285 \text{ mg L}^{-1}$ .

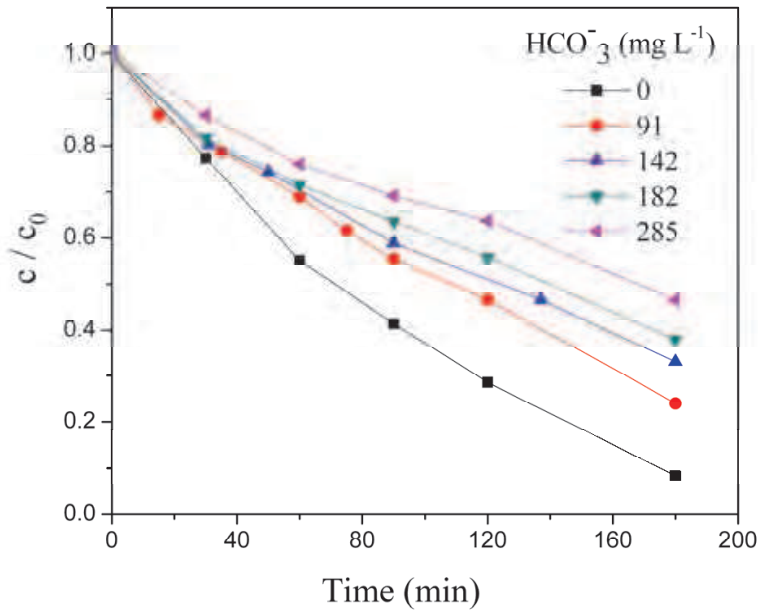


Fig. 12. Effect of the concentration of  $\text{HCO}_3^-$  on photodegradation of CLP in DDW. Operation conditions:  $c(\text{CLP})_0 = 1.0 \text{ mM}$ ,  $\text{TiO}_2 \text{ Wackherr} = 2.0 \text{ mg mL}^{-1}$ ,  $t = 25 \text{ }^\circ\text{C}$ ,  $\text{pH} \sim 7.0$ .

The effect of HUM can be explained by the reaction with  $\bullet\text{OH}$ , which lowers the availability of the latter for the reaction with CLP. Moreover, the actually available UV radiation reduces because some organic matters (especially aromatic compounds) absorb strongly UV

irradiation (Chu et al., 2009b). Expectedly, the degradation rate decreased after the addition of HUM up to 20 mg L<sup>-1</sup> (Figure 13). The behaviour observed in the presence of HUM suggests a predominant effect of the  $\cdot\text{OH}$  radical inhibition, due to the complex structure of HUM and their high reactivity with  $\cdot\text{OH}$  radicals (Basfar et al., 2005; Prados-Joya et al., 2011).

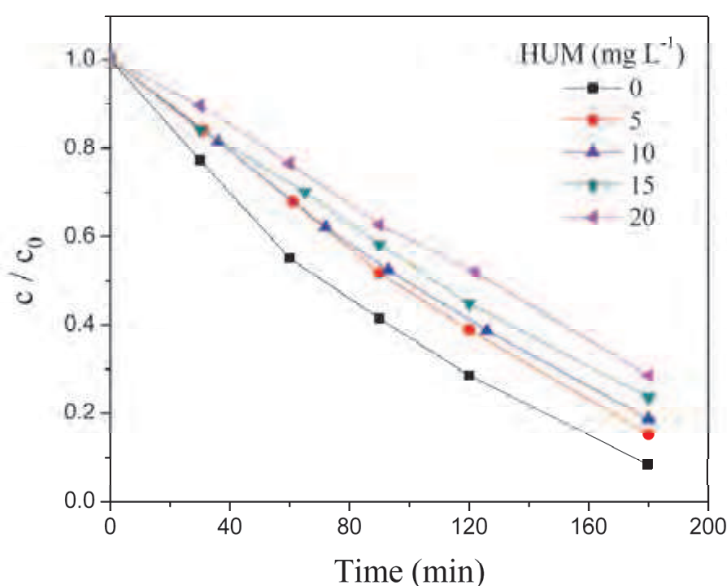


Fig. 13. Effect of the concentration of HUM on photodegradation of CLP in DDW. Operation conditions:  $c(\text{CLP})_0 = 1.0 \text{ mM}$ ,  $\text{TiO}_2 \text{ Wackherr} = 2.0 \text{ mg mL}^{-1}$ ,  $t = 25 \text{ }^\circ\text{C}$ ,  $\text{pH} \sim 7.0$ .

#### 4. Conclusion

The results of this study clearly indicate that under the UV irradiation TiO<sub>2</sub> Wackherr was more efficient than Degussa P25 in both the process of removal of CLP from water and its mineralisation. The reaction followed the pseudo-first order kinetics. The optimum loading of TiO<sub>2</sub> Wackherr was 1.0 mg mL<sup>-1</sup> at pH 3.5. The photodegradation rate was dependent on the temperature, and the apparent activation energy was 37.9 kJ mol<sup>-1</sup>. Along with molecular oxygen, KBrO<sub>3</sub> was the most efficient electron acceptor when concerning the degradation of the parent compound, whereas its mineralisation was most efficient in the presence of O<sub>2</sub> only. It was found that the presence of ethanol as a scavenger of  $\cdot\text{OH}$  inhibited the CLP photodecomposition, suggesting that the reaction mechanism mainly involved free  $\cdot\text{OH}$ . The LC-DAD, and LC-ESI-MS/MS monitoring of the process showed that six intermediates were formed. The analysis of the intermediate product formed during the photocatalytic degradation could be a useful source of information about the degradation pathways. The rate of photodegradation of CLP in DDW was about two/three times higher than in tap and river waters. The photodegradation rate was dominantly influenced by the pH of the medium and the presence of HCO<sub>3</sub><sup>-</sup> and DOM. Our work validates the presented screening methodology of ecotoxicological risk assessment for transformation products, and can be used as a first step in toxicity assessment of degradation products and for prioritisation and planning of more detailed investigations.

## 5. Acknowledgment

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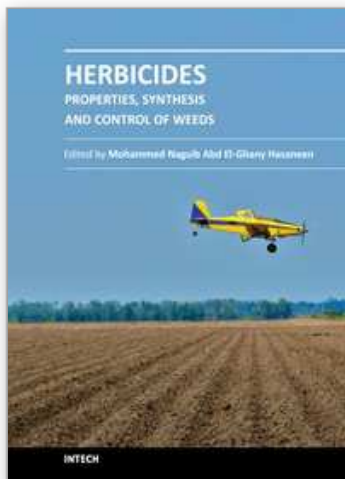
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## **Herbicides - Properties, Synthesis and Control of Weeds**

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This book is divided into two sections namely: synthesis and properties of herbicides and herbicidal control of weeds. Chapters 1 to 11 deal with the study of different synthetic pathways of certain herbicides and the physical and chemical properties of other synthesized herbicides. The other 14 chapters (12-25) discussed the different methods by which each herbicide controls specific weed population. The overall purpose of the book, is to show properties and characterization of herbicides, the physical and chemical properties of selected types of herbicides, and the influence of certain herbicides on soil physical and chemical properties on microflora. In addition, an evaluation of the degree of contamination of either soils and/or crops by herbicides is discussed alongside an investigation into the performance and photochemistry of herbicides and the fate of excess herbicides in soils and field crops.

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