

Kinetics of Cyclophosphamide and Ifosfamide Degradation from Aqueous System via TiO₂ Assisted Photocatalysis

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Cyclophosphamide (CP) - N,N-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide and ifosfamide (IF) - N,3-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide degradation under UV-Vis/TiO₂ photocatalysis was studied in the following experimental conditions: photocatalyst dose = 100 – 800 mg/L; irradiation time = 30-360 min; initial pollutant concentration = 1 – 50 mg/L. CP and IF degradation via TiO₂ photocatalysis was found to obey Langmuir – Hinshelwood model and pollutants degradation rate constants: $5.89 \times 10^{-6} \text{ M min}^{-1}$ (CP); $4.86 \times 10^{-6} \text{ M min}^{-1}$ (IF) and pollutants adsorption – desorption on TiO₂ particles equilibrium constants: 5637 M^{-1} (CP); 4930 M^{-1} (IF) were calculated.

Keywords: cyclophosphamide, ifosfamide, photocatalysis, TiO₂ anatase

Cyclophosphamide (CP) – N, N-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide and ifosfamide (IF) N,3-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide are cyclic amides commonly used in cancer treatment and present genotoxic, carcinogenic, mutagenic and teratogenic potential. In the context of prognosis of doubling those drugs consumption during the next ten years and taking into account the persistent character of cyclic amides, there is a need for new CP and IF degradation methods in order to answer to one of the targets or European water strategy, namely conservation of natural resources quality.

The main pollution sources with these pollutants are represented by wastewater discharges from alkylating agents anticancer drugs synthesis industry which contains as active principles CP and IF and effluents discharged by oncological clinics due to the fact that 20% of the administered drug is eliminated by patients through urine without transformation or as human metabolites [1].

Although water related legislation does not impose until now concentration limits for amides, these compounds are known as toxic micro-pollutants that will be strictly normed in the future [2].

Moreover, performed studies proved that long time exposure to CP, IF and their metabolites is leading to negative effects upon aquatic ecosystems and human health [3-8].

Until their introduction within hazardous compounds list, cyclic amides pollution level was determined by global indicator COD, which cannot measure the exact evolution of specific pollutants. Consequently, treatment of those wastewater categories monitored removal of global organic loading and not specific pollutants.

In Romania, industrial wastewater treatment is realised through complex treatment flows which contains physical – chemical (coagulation / flocculation, chemical oxidation, adsorption) and activated sludge biological treatment steps [9, 10]. Appliance of those treatment flows is usually asking for high operating costs, due to the large amounts of reagents used especially within physical – chemical steps. Treatment efficiency, even if can reach 98% of global

organic loading does not assure reduction of specific pollutants concentration (such is the case also for cyclic amides) and compliance with extremely low limits imposed for persistent organic micro-pollutants (lower than 1 µg/L).

A similar situation is recorded for municipal wastewater that contains effluents from oncological clinics, for which the efficiency of municipal wastewater treatment plants is monitored by global indicators COD and BOD5.

At international level, classic treatment of municipal wastewater using activated sludge does not assure proper removal of cyclic amides due to their low value of octanol-water partition coefficients ($\text{Log } K_{ow} < 1$), that doesn't allow their advanced adsorption [11].

Among classic oxidants, the ozone is the only that assures cyclic amides degradation with efficiencies higher than 98%, but due to the low degradation rate is asking for high consumption of oxidant reagent, which consequently leads to high costs related to energy for ozone production [12].

In the last years, researches in the AOPs field proved that a promising method for treatment of water with toxic micro-pollutants' content is represented by their degradation via advanced oxidation processes both in homogenous (UV/H₂O₂, UV/H₂O₂/Fe²⁺) and heterogeneous (UV-Vis/TiO₂) systems [13-17].

The working conditions applied for the studies on CP and IF degradation from synthetic aqueous solutions were initial pollutant concentration = 1 – 50 mg/L, photocatalyst dose = 100-800 mg/L, irradiation time = 30-180 min for CP and 30-360 min for IF, air flow ($Q_{air} = 50 \text{ L/h}$).

Experimental part

Experimental installation

Heterogeneous photocatalytic experiments were performed using a Heraeus type UV reactor equipped with an immersed TQ-150-Z1 medium pressure mercury lamp. An incident photon flow of $1.12 \times 10^{-6} \text{ einstein}^{-1}$ was determined via ferrioxalat actinometrical method for UV lamp. Prior to irradiation, the samples were bubbled with air (50 L/h) in the dark for half hour in order to avoid holes-

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electrons recombination process. All photocatalytic experiments were at least duplicated in order to assure results' reproducibility.

Reagents

The following reagents were used within photocatalytic degradation experiments: cyclophosphamide (Sigma - Aldrich), ifosfamide (Sigma - Aldrich), titanium dioxide anatase form (Merck).

Analytical methods

For gas chromatographic evaluation of cyclophosphamide (CP) concentration an Agilent 7890A gas chromatograph coupled with an Agilent 240 Ion Trap Mass Detector was used. Volumes of 10 to 500 mL of aqueous samples were saturated with sodium chloride and then where extracted with ethyl acetate using ifosfamide (IF) as surrogate.

The extract was dried on anhydrous sodium sulphate and then dried under nitrogen stream in 2mL GC vial. A volume of 0.1mL of trifluoroacetic anhydride solution in ethyl acetate (volume ratio 1:1) was added to vials for derivatization. After sealing and vigorously shaking, vials where placed for 20 min in water bath (at 70°C).

After derivatization, the solvent, excess trifluoroacetic anhydride and formed trifluoroacetic acid where removed with gentle nitrogen stream. 1 mL of toluene was added in each vial and 1µL of toluene solution was analysed by GC-MS.

Low polarity phase column (5% diphenyl/95% dimethyl polysiloxane, 30 m, 0.25 mm ID, 0.25 µm film) was used for separation of derivatives (RT: 9.88 min for IF derivative and RT:10.38 min for CP derivative).

GC conditions: split less injection with injector temperature 250°C, flow rate: 1mL/min; carrier gas: helium 6.0; temperature ramp employed: 70°C (1 min), 20°C/min to 280, hold 2.5 min.

Mass detector conditions: Ion Trap: 120°C; Manifold: 50°C; Transfer line: 250°C; Ion source: 220°C. Scan mode: single ion monitoring (quantified ion: m/z= 307 and qualifier ion: m/z=309).

Same procedure was used for IF analysis by using CP as surrogate.

Formed ionic inorganic nitrogen compounds and chloride where analysed by a Dionex ICS-3000 Dual Pump Ion Chromatograph, with separate anions and cations columns (Dionex AS23 and CS16 respectively) and suppressed conductivity detectors. As eluents, carbonate/bicarbonate solution and methanesulphonic acid solutions where used at constant flow, 1 mL/min each.

Sample's pH was measured using a Consort C532 pH-meter.

Results and discussions

Photocatalyst concentration

In order to investigate this parameter, photo-oxidation experiments were performed using a solution with initial concentration $[CP]_0 = 20 \text{ mg/L} = 7.66 \times 10^{-5} \text{ M}$ and photocatalyst dose was varied within the domain $[TiO_2] = 100 - 800 \text{ mg/L}$. Concentrations of CP and its main mineralization products: Cl, N ($N-NH_4^+$, $N-NO_2^-$, $N-NO_3^-$), P ($P-PO_4^{3-}$) were monitored for each photocatalyst dose for an irradiation time of 180 min.

Obtained data showed that both CP degradation and its mineralization products formation efficiencies are presenting an ascendant trend until a photocatalyst dose of 400 mg/L. This behaviour represents a consequence of interactions between TiO_2 particles (disperse phase) and incident radiation as well of surface processes.

At low photocatalyst concentrations (lower than 400 mg/L) the surface processes are prevailing and the increase of photocatalyst concentration leads to the increase of active sites available for pollutant adsorption.

At high photocatalyst concentrations (more than 400 mg/L) both CP degradation and organic chlorine, nitrogen and phosphorus mineralization efficiencies are slightly descending due to the light scattering which negatively affects catalyst photo-excitation efficiency and thus on having a negative effect upon pollutant's photocatalytic degradation.

This fact is sustained by the linearized profiles of degradation rates equations for all five tested photocatalyst concentrations.

Photocatalyst dose $[TiO_2] = 400 \text{ mg/L}$ was selected as optimum, that assures substrate $[CP]_0 = 20 \text{ mg/L} = 7.66 \times 10^{-5} \text{ M}$ degradation after 30 min of irradiation with the highest degradation rate $v_{30 \text{ min}} = 2.08 \times 10^{-6} \text{ M min}^{-1}$.

Optimum photocatalyst dose assures degradation of CP and its mineralization products formation efficiencies of 81.45%, (CP), 20.22% (Cl), 4.66% (N inorganic) and 4.35% (P inorganic) after 30 min of irradiation.

Irradiation time

Irradiation time was varied within the domains 30-180 min (for CP) and 30-360 min (for IF) for CP and IF initial concentrations of $20 \text{ mg/L} = 7.66 \times 10^{-5} \text{ M}$. CP, IF degradation and formation of their mineralization products were monitored during the irradiation period. The results obtained for optimum $[TiO_2] = 400 \text{ mg/L}$ being presented within tables 2 (CP) and 3 (IF).

Prolonged irradiation time has a positive effect upon CP and IF photocatalytic degradation, reaching CP and IF degradation efficiencies of 99.996% after an irradiation time of 180 min (CP) and respectively 360 min (IF).

Formation of oxidative degradation by-products with chlorine, nitrogen and phosphorus content is demonstrated

Table 1

PHOTOCATALYST DOSE INFLUENCE UPON CP DEGRADATION EFFICIENCY AND ORGANIC CHLORINE, NITROGEN AND PHOSPHORUS MINERALIZATION: $[CP]_0 = 20 \text{ mg/L} = 7.66 \times 10^{-5} \text{ M}$, IRRADIATION TIME = 180 min

[TiO ₂] mg/L	[CP]		Cl mg/L	N mg/L	P mg/L	η _{CP} %	η _{Cl} %	η _N %	η _P %
	mg/L	M x 10 ⁹							
100	0.085	325.67	3.80	0.52	0.28	99.58	69.85	24.24	12.18
200	0.025	95.78	3.94	0.60	0.50	99.88	72.42	27.96	21.75
400	0.00075	2.87	4.01	0.69	0.62	99.996	73.70	32.12	26.97
600	0.0015	5.75	3.99	0.67	0.58	99.993	73.34	31.23	25.23
800	0.0025	9.58	3.98	0.59	0.51	99.988	73.15	27.73	22.19

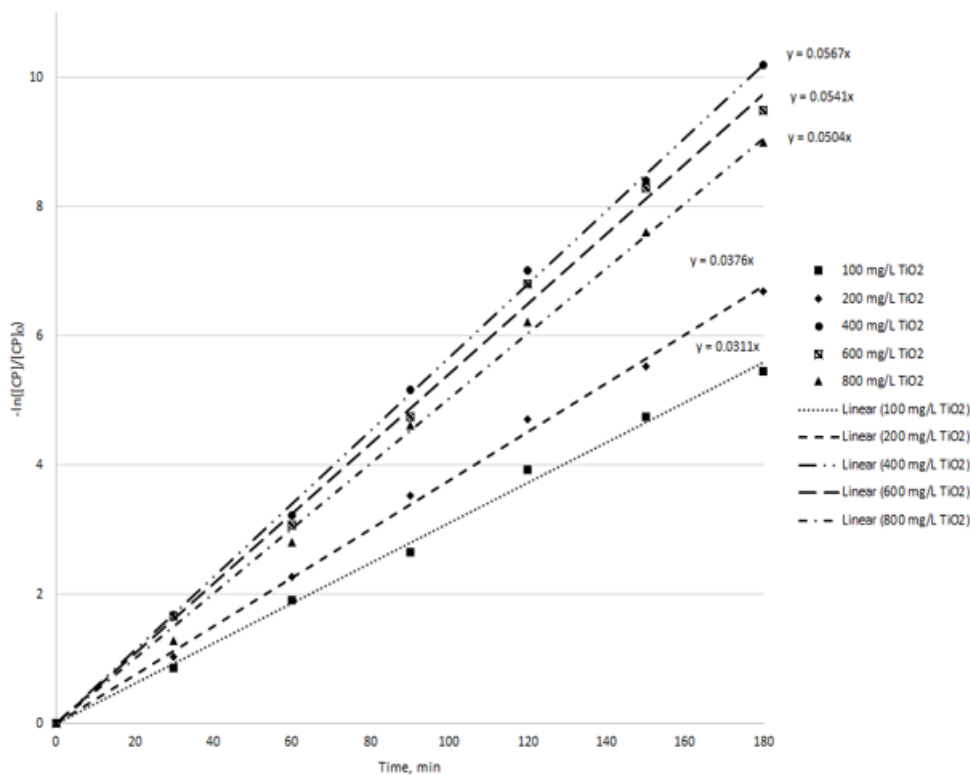


Fig. 1. Photocatalyst dose influence upon CP photocatalytic degradation rate $[CP]_0 = 20 \text{ mg/L} = 7.66 \times 10^{-5} \text{ M}$, irradiation time = 180 min

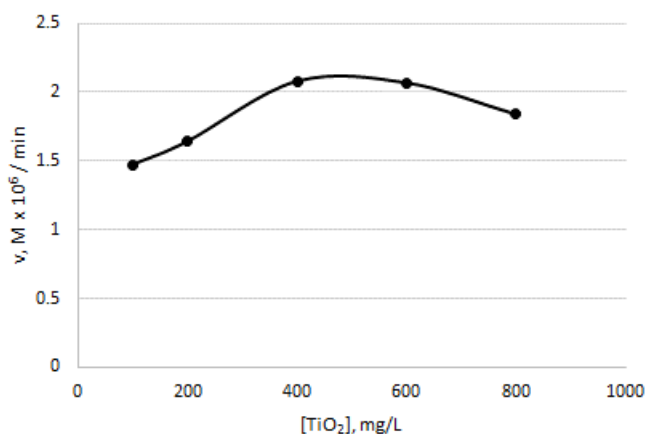


Fig. 2. CP degradation rate vs. photocatalyst dose $[CP]_0 = 20 \text{ mg/L} = 7.66 \times 10^{-5} \text{ M}$, irradiation time = 30 min

by mineralisation efficiencies that are permanently lower compared with pollutant degradation efficiency: 73.70% (CP) and 83.63% (IF) for organic chlorine mineralization, 32.12% (CP) and 50.80% (IF) for organic nitrogen mineralization, 26.97% (CP) and 45.59% (IF) for an irradiation time of 180 min (CP) and respectively 360 min (IF).

Kinetic curves for CP and IF degradation and their mineralization products formation were linearized using a

1st order kinetic and rate constants for CP and IF degradation and mineralisation of organic chlorine, nitrogen and phosphorus were calculated for an initial pollutant concentration of $20 \text{ mg/L} = 7.66 \times 10^{-5} \text{ M}$

Taking into account pollutants remanent concentration ($< \mu\text{g/L}$) optimum irradiation times were selected: 180 min for CP and respectively 360 min for IF photocatalytic degradation.

Initial pollutant concentration

Increase of initial pollutant concentration presents a limitative character for TiO_2 assisted photocatalysis. The influence of pollutant initial concentration was investigated within the domain $1 - 50 \text{ mg/L} = 3.83 \times 10^{-6} - 1.91 \times 10^{-4} \text{ M}$. Degradation experiments were performed using optimum photocatalyst dose $[\text{TiO}_2] = 400 \text{ mg/L}$ for an irradiation time of 30 min.

Increase of initial pollutant concentration determines the decrease of pollutant degradation rate asking for prolonging irradiation time in order to obtain good efficiencies for substrate degradation. This behaviour is explained by internal competition between pollutant and its degradation by-products for consumption of radical species involved in photo-oxidation reaction, which consequently leads to a decrease of pollutant degradation efficiency.

Table 2

CP DEGRADATION EFFICIENCY IN UV-Vis/ TiO_2 SYSTEM $[CP]_0 = 20 \text{ mg/L} = 7.66 \times 10^{-5} \text{ M}$, $[\text{TiO}_2] = 400 \text{ mg/L}$

Time min	[CP] mg/L	Cl ⁻ mg/L	N mg/L	P mg/L	$-\ln([CP]_t/[CP]_0)$	$-\ln(1 - \text{Cl}_t/\text{Cl}_\infty)$	$-\ln(1 - \text{N}_t/\text{N}_\infty)$	$-\ln(1 - \text{P}_t/\text{P}_\infty)$
0	20	0	0	0	0	0	0	0
30	3.711	1.10	0.10	0.10	1.684	0.225	0.048	0.044
60	0.802	2.17	0.27	0.23	3.216	0.508	0.134	0.105
90	0.114	2.67	0.35	0.35	5.167	0.674	0.178	0.165
120	0.018	3.44	0.45	0.46	7.013	1.000	0.234	0.223
150	0.0045	3.82	0.56	0.52	8.399	1.211	0.304	0.256
180	0.00075	4.01	0.69	0.62	10.191	1.335	0.387	0.314

Table 3

IF DEGRADATION EFFICIENCY IN UV-Vis/TiO₂ SYSTEM [IF]₀ = 20 mg/L = 7.66 x 10⁻⁵ M, [TiO₂] = 400 mg/L

Time min	[IF] mg/L	Cl ⁻ mg/L	N mg/L	P mg/L	-ln ([IF] _t /[IF] ₀)	-ln (1-Cl _t /Cl _∞)	-ln (1-N _t /N _∞)	-ln (1-P _t /P _∞)
0	20	0	0	0	1	0	0	0
30	8.12	0.98	0.09	0.08	0.901	0.198	0.042	0.042
60	1.98	1.51	0.23	0.17	2.312	0.325	0.113	0.074
120	1.04	2.28	0.44	0.33	2.956	0.543	0.229	0.153
180	0.10	3.28	0.61	0.57	5.298	0.923	0.334	0.287
240	0.01	3.70	0.77	0.76	7.600	1.139	0.444	0.398
300	0.0048	4.16	0.97	0.96	8.334	1.446	0.597	0.537
360	0.0009	4.55	1.09	1.05	10.008	1.809	0.709	0.608

Table 4

RATE CONSTANTS FOR INITIAL POLLUTANT CONCENTRATION OF 20 mg/L = 7.66 x 10⁻⁵ M

Rate constant	Cyclophosphamide (CP)	Ifosfamide (IF)
Photocatalytic degradation	5.67 x 10 ⁻² min ⁻¹	2.86 x 10 ⁻² min ⁻¹
Chlorine mineralization	7.8 x 10 ⁻³ min ⁻¹	4.9 x 10 ⁻³ min ⁻¹
Nitrogen mineralization	2.1 x 10 ⁻³ min ⁻¹	1.9 x 10 ⁻³ min ⁻¹
Phosphorus mineralization	1.8 x 10 ⁻³ min ⁻¹	1.7 x 10 ⁻³ min ⁻¹

Table 5

INFLUENCE OF POLLUTANT INITIAL CONCENTRATION UPON DEGRADATION EFFICIENCY [TiO₂] = 400 mg/L, IRRADIATION TIME = 30 min

Initial concentration		Cyclophosphamide			Ifosfamide		
		Concentration after 30 minutes of irradiation		η _{CP}	Concentration after 30 minutes of irradiation		η _{IF}
mg/L	M	mg/L	M	%	mg/L	M	%
1	3.83 x 10 ⁻⁶	0.05	1.91 x 10 ⁻⁷	95.02	0.29	1.11 x 10 ⁻⁶	71.00
2	7.66 x 10 ⁻⁶	0.23	9.00 x 10 ⁻⁷	88.25	0.64	2.45 x 10 ⁻⁷	68.00
5	1.91 x 10 ⁻⁵	0.61	2.34 x 10 ⁻⁶	87.78	1.67	6.39 x 10 ⁻⁶	66.60
10	3.83 x 10 ⁻⁵	1.24	4.77 x 10 ⁻⁶	87.55	3.98	1.52 x 10 ⁻⁵	60.20
20	7.66 x 10 ⁻⁵	3.71	1.42 x 10 ⁻⁵	81.45	8.12	3.11 x 10 ⁻⁵	59.40
50	1.91 x 10 ⁻⁴	19.09	7.31 x 10 ⁻⁵	61.81	33.22	1.27 x 10 ⁻⁴	33.56

Kinetics

Degradation of organic compound via TiO₂ assisted photocatalysis can be described by a Langmuir - Hinshelwood type kinetic. Its application on photocatalytic degradation of CP and IF was investigated, starting from the following reaction rate equation:

$$v_0 = \frac{k_r K_{ad} [P]_0}{1 + K_{ad} [P]_0} \quad (1)$$

where:

- v₀ = initial pollutant degradation rate (M min⁻¹)
- [P]₀ = initial pollutant concentration (M)
- k_r = rate constant for pollutant photocatalytic degradation (min⁻¹)

K_{ad} = equilibrium constant of adsorption - desorption of pollutant on photocatalyst surface (M⁻¹)

Rearranging the reaction rate equation (1) a linear dependence can be obtained:

$$\frac{1}{v_0} = \frac{1}{k_r K_{ad} [P]_0} + \frac{1}{k_r} \quad (2)$$

Plotting of equation (2) can be used to calculate the values for rate constant k_r and adsorption - desorption equilibrium constant K_{ad}.

The intercept is 1/k_r and the slope is 1/k_rK_{ad} for 1/v₀ = f(1/[P]₀) plotted function.

Verification of Langmuir - Hinshelwood equation attests that degradation of adsorbed pollutant takes place at TiO₂

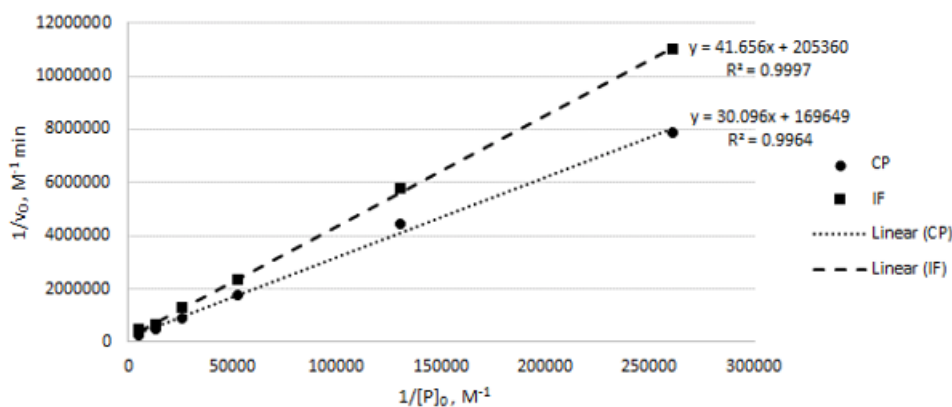


Fig. 3. Linearization of Langmuir - Hinshelwood equation for CP and IF degradation

particles surface, where the radical species generated by photocatalyst irradiation are also adsorbed. Adsorption – desorption equilibrium constants K_d , 5637 M⁻¹ (CP) and 4930 M⁻¹ (IF) and k_d rate constants 5.89 x 10⁻⁶ M min⁻¹ (CP) and 4.86 x 10⁻⁶ M min⁻¹ (IF) were calculated from equations slopes (K_{ad}) and intercepts (k_r).

Conclusions

Experimental results proved that TiO₂ assisted photocatalysis applied in optimum working parameters for the advanced degradation of CP and IF can assure pollutant removal efficiencies of 99.996% (remnant concentration < 1µg/L) for an initial pollutant concentration of 20 mg/L.

CP and IF photocatalytic degradation can be described by Langmuir – Hinshelwood model and rate constants and adsorption – desorption equilibrium constants were calculated using experimental values from the first 30 min of irradiation.

CP degradation occurred at higher rate compared with IF a possible explanation being the chemical structure of IF that presents steric hindrance in comparison with CP structure.

Obtained kinetic data confirms that TiO₂ assisted photocatalysis represent a promising method for the advanced degradation of CP and IF.

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