

Wojciech BARAN¹, Jolanta SOCHACKA¹,
Ewa ADAMEK¹, Andrzej SOBCZAK¹
and Andrzej MAKOWSKI¹

CHANGES OF TOXICITY AND BIODEGRABILITY OF SULFONAMIDES SOLUTIONS DURING THEIR PHOTOCATALYTIC DEGRADATION

ZMIANY TOKSYCZNOŚCI I PODATNOŚCI NA BIODEGRADACJĘ ROZTWORÓW ZAWIERAJĄCYCH SULFONAMIDY PODCZAS ICH DEGRADACJI METODĄ FOTOKATALITYCZNĄ

Abstract: The aim of this study was to estimate the applicability of the photocatalytic process to: (1) sulfa-drugs degradation in aqueous solutions, (2) a decrease in their toxicity and (3) an increase in their biodegradability. Photocatalytic process initiated by UV-A radiation was carried out in open reactors containing a catalyst (TiO₂ suspension) and solutions of five sulfonamides. After a definite irradiation time, the change of sulfa-drugs concentration in solutions was estimated using HPLC method. Moreover, changes of total organic carbon (TOC), chemical oxygen demand (COD), biochemical oxygen demand (BOD₅) as well as changes of sulfonamides toxicity relative to *Chlorella vulgaris* were examined. The biodegradability of investigated sulfonamides and their photocatalytic degradation products was determined based on BOD₅/COD ratio. It was found that under experimental conditions all investigated sulfonamides underwent the photocatalytic degradation and the decrease in TOC and COD of sulfa-drugs solutions was observed. Simultaneously, products of this process were less toxic and more biodegradable than initial sulfonamides.

Keywords: sulfonamides, photocatalytic process, TiO₂, biodegradation, ecotoxicity, *Chlorella vulgaris*

Sulfonamides have been commonly used for decades in medicine and veterinary as antibacterial agents. After excretion, they may be introduced into the environment as the unchanged products or as metabolites [1–3]. For these reasons their traces are present in almost all kinds of biotopes [3]. Taking into account their toxicity, the sulfa-drugs can induce changes in ecosystems exposed to their influence and, first of all, can cause generation of drug-resistant in microorganisms, including pathogenic ones.

¹ Department of General and Inorganic Chemistry, The Medical University of Silesia: The School of Pharmacy and Division of Laboratory Medicine in Sosnowiec, ul. Jagiellońska 4, 41–200 Sosnowiec, Poland, tel. 032 364 15 63, email: bw-xxl@wp.pl

In the last decade, the problems concerning the occurrence of sulfa-drugs in the environment are subjects of high interest to researchers. This is evidenced by a very large number of scientific publications and conference communications published each year on this topic but available data often give discrepancies in information and results. These differences concern *i.a.* the estimation of susceptibility of sulfonamides to biodegradability. Published data showed that the values of degradation half-life for these compounds varied from about 2.5 days [4] to even one year [5]. Moreover, results of estimation of the environmental risk caused by these drugs are different [6–8]. These discrepancies form *i.a.* as result of differences in the estimation of sulfonamides ecotoxicity (the results depended on tests duration, type of tested organisms as well as sulfonamide type). For example in the case of sulfadimethoxime the values of $L(E)C_{50}$, determined in test with *Artemia solina* varied from $19,5_{96h}$ to 1866_{24h} mg dm^{-3} [2]. In turn, these values obtained in chronic test toxicity using *Lemna* test, were $10 \mu\text{g dm}^{-3}$ and $81 \mu\text{g dm}^{-3}$ for sulfadiazine [6] and sulfamethoxazole [8], respectively.

Particularly high concentration of sulfonamides (10 mg dm^{-3}) was identified in leachates from municipal landfills [9] and in sewage from stock-farming [2] while directly in liquid manure from animals' farm contained even hundreds mg kg^{-1} [4]. The effective treatment of these wastes may limit, in the considerable level, the propagation of sulfa-drugs in the environment and may eliminate disturbances in local ecosystems caused by their toxicity.

The opinions concerning the possibility of removal of sulfonamides from wastes during routine, biological treatment processes are also divergent [6, 11]. Moreover, the draining of toxic wastes into wastewater treatment plant using biological processes may disturb their effective functioning and it is unlawful. The physiochemical methods (ultramicrofiltration, reverse osmosis or adsorption [6]) used during wastewater treatment seem well founded and correct for sulfonamides. However, the discussed problem is dissolved only partly because the above-mentioned methods generate large amounts of wastes, still containing sulfa-drugs.

For this reason, the search of methods leading to total destruction of pollutants (mineralization) or their partial decomposition (transformation) to less toxic substances, which are easy biodegradable, seem to be proper and right. The authors of POSEIDON project [6] have proposed the fermentation of wastes and their ozonization as the most suitable methods for removal of sulfonamides. However, results of the latest studies have indicated that part of antibiotics still remains in effluents despite the biological treatment (including after fermentation) [12, 13]. In turn, high operating costs of wastewaters treatment using ozone are also barrier limiting their application. The photocatalytic process may be an alternative method leading to the degradation of organic substances. This process can be effective and cause the decomposition of almost all organic compounds occurring in wastes, irrespective from their toxicity and the resistance to biodegradation [14–16]. Under the influence of UV-A irradiation and in the presence of photocatalysts the organic pollutants can undergo total mineralization even to CO_2 , H_2O and inorganic ions [17]. Unfortunately, the total mineralization of pollutants using photocatalytic methods appeared to be too expensive. The overall cost of this process may be decreased by the shortening of its duration and obtaining only

part of the decomposition of sulfonamides to simpler organic compounds. However, this method may be connected with the risk that the obtained semi-products will be characterized by high toxicity and the resistance to biodegradation.

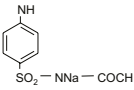
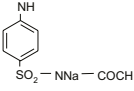
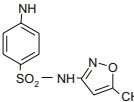
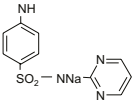
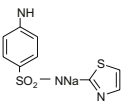
The aim of our work was: (1) the determination of possibility of photocatalytic process application to partial degradation of sulfa-drugs and (2) the estimation of toxicity and susceptibility to biodegradation of the products of this transformation.

Material and methods

All chemicals used throughout this study were analytical grade and their solutions were prepared in distilled water. A commercial TiO_2 from Riedel de Haën was used as catalyst (anatase 100 %, BET surface area of $9\text{--}11 \text{ m}^2 \text{ g}^{-1}$ and residues on filter $> 40 \mu\text{m}$ after dispersion in water $< 0.02 \%$). The photodegradation experiments were carried out using one of five selected sulfonamides (Table 1). TiO_2 powder (250 g dm^{-3}) was added to sulfonamide solutions (100 cm^3 , 0.1 mmol dm^{-3}) and the samples were homogenized for 30 min by means of magnetic stirrers. The applied amount of TiO_2 was established as optimal, based on preliminary experiments.

Table 1

Characteristics of investigated sulfonamides and their determination methods

Sulfonamide ^a	Abbr. in text	Structural formula	pK_{a1}	pK_{a2}	Manufacturer, purity	HPLC ^b mobile phase
Sulfanilamide	SN			10.43	POCH S.A. Gliwice, pure	Buffer: 20 mmol dm^{-3} K_2HPO_4 ; pH 8.9 Buffer: $\text{CH}_3\text{CN} - 92:8$
Sulfacetamide (natrium salt)	SA		1.78	5.38	Polfarma $\approx 98 \%$	Buffer: 20 mmol dm^{-3} K_2HPO_4 ; pH 8.9 Buffer: $\text{CH}_3\text{CN} - 92:8$
Sulfa-methoxazole	SM		1.60	5.81	Sigma $> 98.0 \%$	Buffer: 20 mmol dm^{-3} $\text{HCOONH}_4/\text{HCOOH}$; pH 4.0 Buffer: $\text{CH}_3\text{CN} - 1:1$
Sulfadiazine (natrium salt)	SD		2.00	6.48	Sigma $> 99.0 \%$	Buffer: 20 mmol dm^{-3} $\text{HCOONH}_4/\text{HCOOH}$; pH 4.0 Buffer: $\text{CH}_3\text{CN} - 1:1$
Sulfathiazole (natrium salt)	ST		2.36	7.23	Sigma $> 99.0 \%$	Buffer: 20 mmol dm^{-3} $\text{HCOONH}_4/\text{HCOOH}$; pH 4.0 Buffer: $\text{CH}_3\text{CN} - 1:1$

^a common name;

^b the choice of mobile phase was done based on kinetics studies of sulfonamides photodegradation, in the presence of TiO_2 .

The photocatalytic process was carried out in open glass reactors (the exposed surface of samples was 102 cm^2) and was initiated by UV-A light (a four lamps Philips TL-40W/05 with maximum emission at $\lambda = 366 \text{ nm}$; the intensity of UV radiation measured by Parker's actinometer was 2.9 mW cm^{-2}) or with use of the natural sunlight (the total intensity of the natural sunlight measured by luxometer Introl TES-1332 with natural gray filter ($\times 2$) was $\approx 60 \text{ mW cm}^{-2}$). During the whole experiment, the samples had free contact with atmospheric air and were constantly magnetically stirred. The pH value of the irradiated samples were not corrected and ranged from 5.63 (SM) to 7.35 (SA). The temperature of samples was $21 \pm 2 \text{ }^\circ\text{C}$ (during UV irradiation) and $25 \pm 3 \text{ }^\circ\text{C}$ (during natural sunlight irradiation). In order to separate the TiO_2 suspension, after the end of irradiation the samples were centrifuged for 30 min at 4000 RPM.

The concentration determination of sulfonamide remained in samples was performed using HPLC method (HPLC Merck Hitachi, detector UV $\lambda = 254 \text{ nm}$, column Separon SGX C-18, $5 \text{ }\mu\text{m}$, $250 \times 4.6 \text{ mm}$). The conditions of experimental runs are described in details in Table 1. The degree of mineralization of irradiated samples was calculated based on the measurement of Total Organic Carbon (TOC), using a total carbon analyzer (TOC-5000A, Shimadzu). The values of Chemical Oxygen Demand (COD) of the samples were determined without centrifugation of TiO_2 , using chromate titration method according to HACH 8230 procedure. The values of 5-days Biochemical Oxygen Demand (BOD_5) were determined in Closed-Bottle test using mixed biological population of sewage treatment microbes (inoculum) in accordance with the test procedure described in OECD, method 301D [18a]. The measurement of dissolved oxygen concentration was done following the Winkler titration method. A theoretical oxygen demand (TOD) was estimated in accordance with method 301 D [18a].

The toxicity of sulfonamides and their photodegradation products were tested using a method for the determination of the toxic effects of chemical compounds on the growth on unicellular green alga *Chlorella vulgaris* (*C. vulgaris*) Beij., strain 264, Boehm and Borns 1972/1 (Culture Collection of Algal Laboratory – CCALA, Culture Collection of Autotrophic Organisms of the Institute of Botany, Czechoslovak Academy of Science, Trebon). After the end of irradiation, samples were centrifuged (30 min, 4000 RPM) in order to separate TiO_2 and natant was enriched with the liquid medium as a stock solution and an inoculum of alga was added. Besides cultures with photodegradation products (in solution after irradiation) and the cultures with the sulfonamides (individually added), the control cultures were conducted in parallel. The algal cells were cultivated in 250 cm^3 Erlenmeyer flasks with 50 cm^3 of modified Kuehl-Lorenzen [19] liquid medium (pH 6.5) at $24 \pm 2^\circ\text{C}$, under continuous illumination (3000–4000 lx) and at continuous stirring. Biological activity of cultures was determined by a measurement of absorbance at $\lambda = 680 \text{ nm}$. Growth inhibition and the $\text{EC}_{50(0-48)}$ (effective concentration 50 %) values were calculated according to OECD [18b] and ISO 8692 [20].

Results and discussion

The estimation of the susceptibility of each sulfonamide to photolysis and photocatalytic degradation processes was calculated based on the change in their concentration during

irradiation. Since the photocatalytic degradation processes belong to pseudo first-order reactions, the rate constants values (k) are experimentally determined as the slope of the linear function:

$$\ln(C/C_0) = kt + b \quad (1)$$

where t is reaction time.

The results, calculated from Eq. 1, are presented in Table 2.

Table 2

Reaction rate constants determined during photodegradation of sulfonamides

Sulfonamide	k [min ⁻¹]		
	UV-A without TiO ₂	UV-A with TiO ₂	sunlight with TiO ₂
SN	0.0016 ± 0.0016	0.031 ± 0.003	0.123 ± 0.008
SA	0.0005 ± 0.0022	0.013 ± 0.001	—
SM	0.0004 ± 0.0003	0.030 ± 0.004	—
SD	0.0005 ± 0.0008	0.013 ± 0.001	0.016 ± 0.009
ST	110 ⁻⁵ ± 0.0003	0.020 ± 0.002	—

It was found that the irradiation of solutions of each sulfonamides by UV-A light without TiO₂ did not induce any observable degradation. In this process, the reaction rate constants were basically nearly zero (Table 2). So it was concluded that under the studied conditions practically none of these sulfa-drugs did undergo photolysis. The phenomenon called photolysis was previously described for three from the investigated sulfonamides, namely for SM, ST [21] and SA [22] but this process was proceeded under conditions different than those used in our studies. In the case of ST and SM, it was carried out in the presence of atomic oxygen, free HO[•] radicals and Rose Bengales (a dye with photosensitizing properties), while the SA photolysis proceeded with the use of UV radiation of a greater energy.

During UV-A irradiation and in the presence of TiO₂ suspension all sulfonamides underwent fast degradation (Table 2). The degree of sulfonamide degradation was calculated using the equation:

$$DR = [1 - (C/C_0)] \cdot 100 \% \quad (2)$$

where: DR – degradation rate [%]; C_0 – the initial sulfonamide concentration; C – the determined sulfonamide concentration. The obtained DR values are presented in Fig. 1.

In the case of SN and SM this process proceeded more easily however using appropriately long irradiation time it was obtained high, almost 100 % degradation of all sulfonamides. The significant differences in photocatalytic degradation rate of each sulfa-drugs may be connected with the differences in their affinity to the surface of TiO₂ particles. Since the used TiO₂ had a point of zero charge at pH 3, so in our

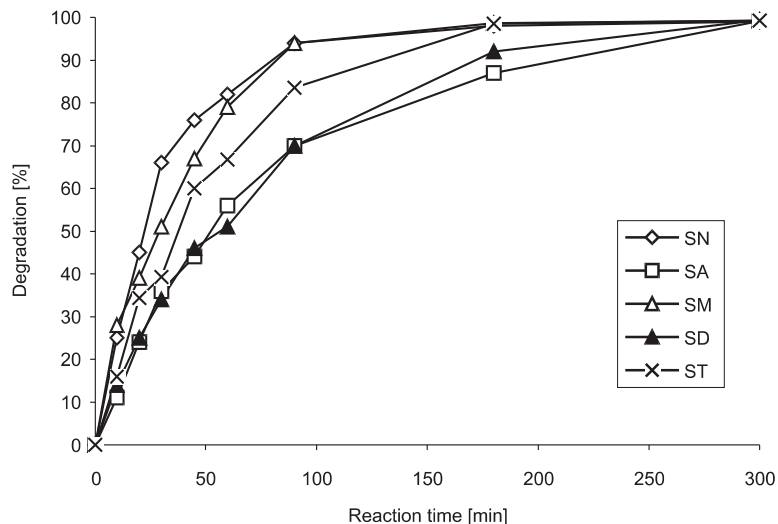


Fig. 1. Dynamics of degradation of sulfonamides during UV-A irradiation in the presence of TiO₂

experiments its surface had a negative charge. This fact can explain why slightly dissociated molecules of SN and SM (Table 1) undergo the photocatalytic degradation in the presence of TiO₂. On the other hand, others sulfonamides occurring in solutions as anions (coming from well-dissociated sodium salts) practically did not undergo any the degradation. In accordance with this hypothesis, the neutral molecules of SN and SM should be adsorbed onto TiO₂ surface in the highest level. However, before beginning irradiation but after addition of TiO₂ and after homogenization of samples for 30 min, the decrease in sulfonamide concentration (its adsorption) was observed in none of the investigated cases. The importance is that the photocatalytic sulfonamides degradation is also possible in solutions irradiated by solar light. This fact was confirmed by results of reactions with SN and SD (Table 2).

It is known that nearly total degradation of sulfonamides is not evidence of their simultaneous mineralization. On the basis of TOC determination it was found that after 300 min of irradiation the sulfonamides solutions contained from 30 to 75 % of initial concentration of organic carbon. There is the evidence that some of the intermediate organic products of sulfa-drugs degradation were remained in these solutions.

The COD value is an important parameter used as a measure of the oxygen needed for the complete oxidation of all organic compounds content in solution. The percentage decrease in COD values was estimated according to the equation:

$$Y = \left(1 - \frac{\text{COD}}{\text{COD}_0} \right) \cdot 100 \% \quad (3)$$

where: Y – the decrease in COD value [%]; COD₀ – the value determined for the initial sulfonamide solution; COD – the value determined for solution after the end of irradiation.

In irradiated sulfonamide solutions containing TiO_2 the decrease in COD values proceeded considerably slower than the photocatalytic degradation. However, for the majority of the investigated sulfonamides after 300 min of irradiation the degree of COD removal was ranged from 82 to 98 % while for SD was about 40 % (Fig. 2).

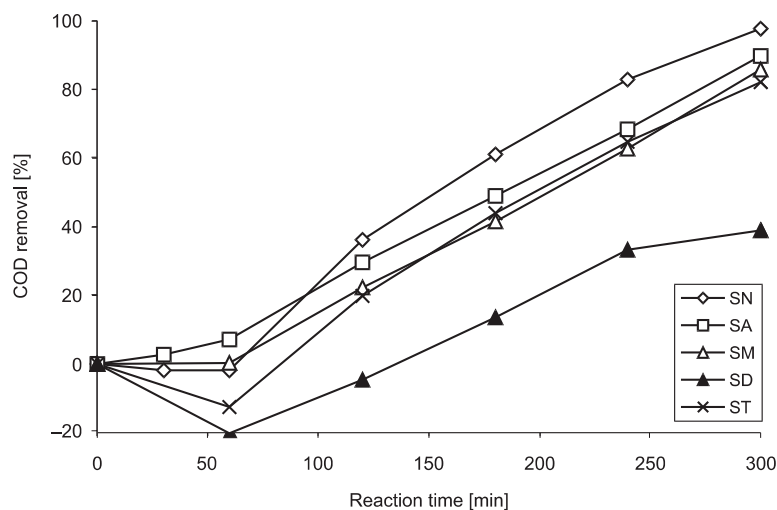


Fig. 2. Changes of COD of sulfonamides during UV-A irradiation in the presence of TiO_2

In our experiment some anomaly was observed. The COD values of SD and ST solutions increased after short-term irradiation (0–60 min). This phenomenon may explain that degradation products which form in the beginning stage of photocatalytic process are oxidized easier than the initial sulfonamides.

The BOD_5/COD ratio can be used to estimate the susceptibility to biodegradation (biodegradability) of organic compounds, including the investigated sulfa-drugs and products of their photocatalytic degradation. The percentage susceptibility to biodegradation of the investigated sulfonamides and products of photocatalytic reaction was calculated using the equation:

$$\text{BD} = \frac{\text{BOD}_5}{\text{COD}} \cdot 100\% \quad (4)$$

where: BD – biodegradability [%]; BOD_5 , COD – the values determined for the same solution after the end of irradiation.

The obtained results of biodegradability are presented in Fig. 3. Before irradiation, all investigated sulfonamides solutions had the BOD_5 nearly zero, (lower than 1.5 % of TOD) and very low BOD_5/COD ratio. This is the evidence that under experimental conditions these sulfa-drugs are completely biodegradation-resistant. However, even short-term irradiation (0–180 min) caused an increase of BOD_5/COD ratio. It may indicate that the organic products of degradation, forming under these conditions, are

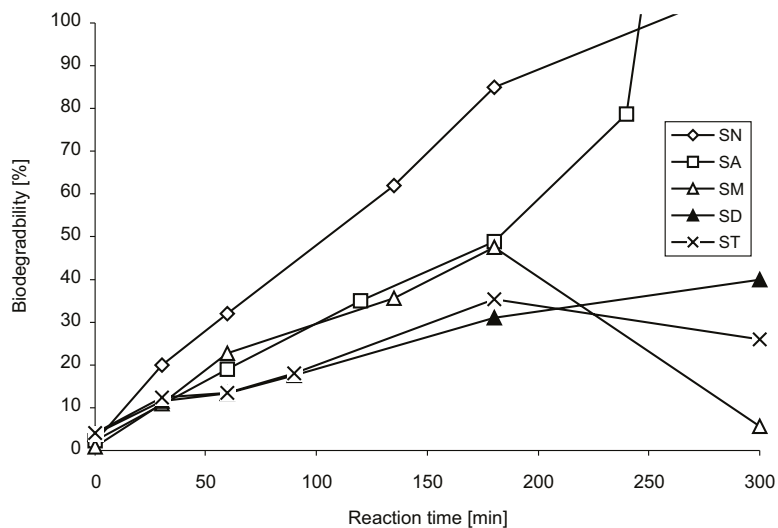


Fig. 3. Changes of biodegradability of sulfonamides solutions during UV-A irradiation in the presence of TiO_2

biodegradable, contrary to initial sulfonamides. The results obtained during long-term irradiation of samples (300 min) cause some problems with their interpretation. This fact may be caused by a high mineralization degree and a relatively low precision of COD determination that is obtainable at so low values of this parameter.

In order to estimate the ecotoxicity of sulfa-drugs and products their photocatalytic degradation, the test with *C. vulgaris* was carried out. The obtained $\text{EC}_{50(0-48)}$ values are presented in Fig. 4. In experiments, the toxicity of initial solutions containing one

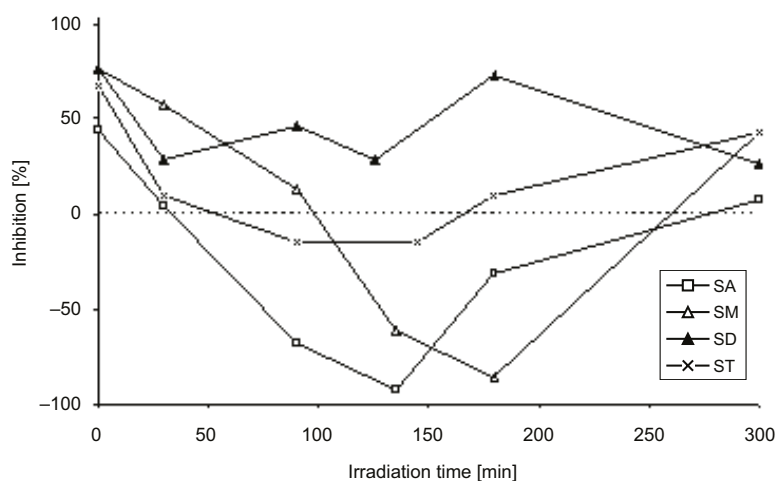


Fig. 4. Changes of toxicity of sulfonamides (changes of inhibition in relation to *C. vulgaris*) during UV-A irradiation in the presence of TiO_2

from four sulfonamides (SA, SM, SD or ST) and the toxicity of these solutions irradiated by UV-A light in the presence of TiO_2 were studied.

All sulfa-drugs were toxic relative to *C. vulgaris*. The determined values of $\text{EC}_{50(0-48)}$ for SA, SM, SD and ST were $6.2 \cdot 10^{-2}$, $6.2 \cdot 10^{-3}$, $4.9 \cdot 10^{-3}$ and $6.4 \cdot 10^{-2}$ mmol dm^{-3} , respectively. It means that two sulfonamides, namely SM and SD, having lower values of $\text{EC}_{50(0-48)}$ were more toxic to *C. vulgaris*. The influence of products obtained during photocatalytic degradation (0–300 min) of sulfonamides on the growth of cultures is shown in Fig. 4.

After short-term irradiation the inhibition of growth of algal culture caused by degradation products was, in each case, lower than the initial sulfonamides. The prolonged irradiation times (30–180 min) led to the formation of less-toxic products and were revealed as a stimulation of cultures growth (the inhibition values lower than zero were established as a stimulation of growth of culture). The long-term irradiation of sulfonamides (> 180 min) caused the inhibition of growth of alga cultures. For this reason the irradiation time (ranging from 135 to 180 min) is optimal to receive less-toxic products of photocatalytic degradation of sulfonamides.

Conclusions

The investigated sulfonamides do not undergo biodegradation. However, they undergo the photocatalytic degradation and the slow mineralization during irradiation by UV-A or sunlight, in the presence of TiO_2 . Contrary to investigated sulfonamides their degradation products, forming in these solutions, are biodegradable. Moreover, it was found that sulfonamides are toxic to *C. vulgaris*. The important is the fact that toxicity of products of photocatalytic degradation was significantly lower than the toxicity of initial sulfonamides. The above-mentioned facts indicate that the removal of sulfonamides from wastewaters using the photocatalytic method may be limited only to their partial degradation. Further steps of treatment leading to total mineralization of pollutants can continue using cheaper biodegradation methods.

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ZMIANY TOKSYCZNOŚCI I PODATNOŚCI NA BIODEGRADACJĘ ROZTWORÓW ZAWIERAJĄCYCH SULFONAMIDY PODCZAS ICH DEGRADACJI METODĄ FOTOKATALITYCZNĄ

Zakład Chemii Ogólnej i Nieorganicznej,
Wydział Farmaceutyczny z Oddziałem Medycyny Laboratoryjnej w Sosnowcu
Śląski Uniwersytet Medyczny

Abstrakt: Celem badań było określenie możliwości wykorzystania procesu fotokatalitycznego do degradacji leków sulfonamidowych w roztworach wodnych oraz do zmniejszenia ich toksyczności i zwiększenia podatności na biodegradację. Proces fotokatalityczny inicjowany za pomocą promieniowania UV-A oraz światła słonecznego prowadzono w otwartych reaktorach zawierających katalizator (zawiesinę TiO_2) oraz roztwory pięciu wybranych sulfonamidów. W roztworach uzyskanych po fotokatalitycznej degradacji oznaczano: stężenie sulfonamidów (metodą HPLC), stężenie ogólnego węgla organicznego (OWO) oraz zmiany chemicznego (ChZT) i 5-dobowego biochemicznego (BZT₅) zapotrzebowania na tlen. Ponadto, sulfonamidy i produkty ich fotokatalitycznej degradacji oceniano pod względem ich toksyczności wobec *Chlorella vulgaris* oraz podatności na biodegradację (BZT₅/ChZT). Stwierdzono, że w stosowanych warunkach wszystkie badane sulfonamidy ulegały fotokatalitycznej degradacji ze zmniejszeniem OWO i ChZT roztworów. Jednocześnie produkty tego procesu okazały się mniej toksyczne i bardziej podatne na biodegradację niż wyjściowe sulfonamidy.

Słowa kluczowe: sulfonamidy, proces fotokatalityczny, TiO_2 , biodegradacja, toksyczność, *Chlorella vulgaris*