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ASSESSMENT OF SUSCEPTIBILITY OF SULFONAMIDE DRUGS TO BIODEGRADATION IN ENVIRONMENTAL SAMPLES

OCENA PODATNOŚCI LEKÓW SULFONAMIDOWYCH NA BIODEGRADACJĘ W PRÓBKACH ŚRODOWISKOWYCH

Abstract: Sulfonamide drugs enter the environment in different ways, *eg* through wastewater, creating the potential risk of the generation of drug resistant strains of microorganisms. This risk increases when drugs are resistant to degradation and remain in the environment for a long time. Therefore, it is particularly important to know biodegradability of sulfa drugs since the literature data on this subject are often contradictory. In our study the estimation of susceptibility of four sulfonamides was carried out under aerobic and anaerobic conditions. The mixture of these compounds was added directly to environmental samples or natural wastewater samples (from different sources) or to synthetic wastewater containing inoculum from natural sources. The following effects were studied: aerobic/anaerobic conditions, the availability of light, initial concentrations, pH of the solution, sampling time of inoculum, earlier 40-days adaptation of inoculum to sulfanilamide. The effective biodegradation of sulfonamides was observed in 3 samples of sulfanilamide, 25 samples of sulfadiazine, 29 samples of sulfathiazole and 1 sample of sulfamethoxazole among the all investigated 86 samples. It was found that the biodegradation of sulfonamides differ significantly from the results presented in the literature. Moreover, the dynamics of this process depends mainly on the type of sulfonamide, the origin of inoculum and the sampling time.

Keywords: sulfonamides, pharmaceuticals, biodegradation

Introduction

Sulfonamide drugs (SNs) can enter the environment in different ways, *eg* through wastewater, creating the potential risk of the generation of drug-resistant strains of microorganisms [1]. This risk increases when drugs are resistant to degradation processes and they remain in the environment for a long time.

Currently, particularly large amounts of SNs with bacteriostatic properties are used in animal husbandry and in aquaculture, often without restrictions. According to the data contained in the DANMAP report, the use of SNs to produce 1 kg of meat was from 0.0033 (broilers) up to 58.5 mg (farmed fish) [2]. Consequently, the concentration of SNs in manure of farmed animals may reach even 400 mg kg^{-1} [1, 3]. However, more disquieting is the fact that in the environment the frequency of SNs in low concentrations is very high. Additionally, according to some researchers, the presence of SNs and other antibiotics was found in almost 100% of the tested surface water samples [1, 4].

According to majority of the researchers, SNs have been recognised as poor or non-biodegradable compounds in the environment (*ie*, in pure water, surface water and in soil) with a half life time > 30 days [1, 4-6]. The results of standardised tests, such as the ISO 11734:1995, OECD 301D, and the assessment of soil microbial activity suggest that

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most of SNs do not undergo natural biodegradation. In almost 40% of articles describing biological degradation of SNs, they are regarded as non-biodegradable compounds (in pure water, seawater, natural water and wastewater or active sludge) [1, 4, 5, 7-9] . The fact that SNs occur so frequently in the tested samples can also be considered as an evidence of their persistence in the environment.

The aim of our study was to evaluate the biodegradability of four SNs (*ie* sulfanilamide, sulfadiazine, sulfathiazole and sulfamethoxazole). The process was carried out directly in environmental samples or natural wastewater samples (orginating from different sources), and in synthetic wastewater samples containing inoculum orginating from natural sources. In particular, the following effects were studied:

- aerobic/anaerobic conditions,
- the availability of light ($\lambda > 400$ nm),
- initial concentrations of SNs
- pH of the solutions,
- sampling time of inoculum,
- earlier, 40-days adaptation of inoculum to sulfanilamide.

Methods

Materials and samples

The appropriate amount of concentrated solution containing an equal-mass mixture of four SNs was added to the tested samples (Table 1).

Table 1

Characteristics of the studied SNs

Sulfonamides	**CAS number**	**Abbr. in text**	**Manufacturer**	t_R[a] **[min]**	**LOD**[b] **[μg dm^{-3}]**
Sulfanilamide	63-74-1	SAD	POCH	5.45	3.0
Sulfamethoxazole	723-46-6	SMX	Sigma	7.49	4.3
Sulfadiazine	68-35-9	SDZ	Sigma	3.48	3.0
Sulfathiazole	72-14-0	STZ	Sigma	12.66	5.4

[a] retention time for mobile phase flow 1.0 cm^3 · min^{-1}
[b] limit of detection for injection samples at volume 50 mm^3

Environmental samples were taken from the six selected sites (Table 2).

Table 2

The locations of the sampling sites

Samples	**Locations** (latitude;longitude)
Influent, municipal wastewater	50,304897;19,204884
Effluent from WWTP	50,301577;19,202357
Water from a pond	50,342982;19,186119
Landfil leachate	50,375125;19,058443
Septic tank	50,371016;19,038153
Manure from catle	50,294891;19,908038

Only in one experiment, the pH of sample was corrected by addition of HCl solution.

Additionally, synthetic wastewaters were used as the reaction medium [10] and the inoculum from environmental samples were also added to these wastes (1 ml inoculum was added to 100 ml of wastewater). Environmental samples were used within 2 days after collection. Only in one experiment, wastewater from septic tank was stored frozen at –18°C for 40 days.

In order to adapt of microorganisms to SNs, 50 g of SAD was added to dual-chamber septic tank with a capacity of 10 m^3. The samples were taken before SDA addition and after 40 days.

Apparatus

After intensive stirring, the samples were put in the reaction set (for minimum period of 28 days) as shown in Figure 1.

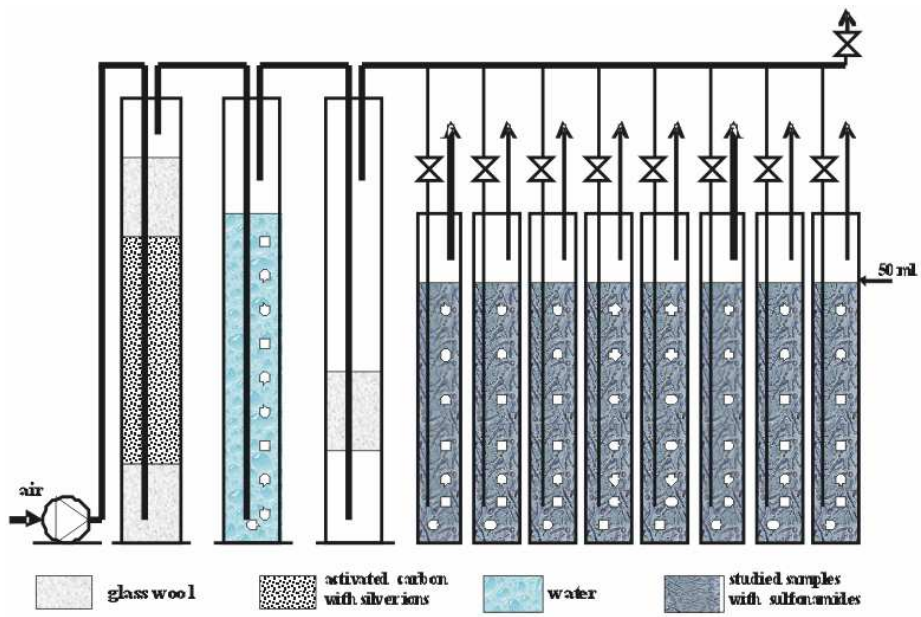

Fig. 1. Scheme of the reactors for aerobic biodegradation

Under aerobic conditions, the air flow rate in the reactors was in the range of ~5 cm^3 min^{-1} (oxygen saturation > 90%), the temperature 20.4 ±0.5ºC, the light intensity < 10 W m^{-2} (< 1% of UV irradiation). Additionally, the experiments in the dark and under anaerobic conditions were carried out.

Analysis

The degree of degradation was determined as the relative decrease in the concentration of each SNs in samples after a definite reaction time. A possible sorption of SNs on

suspended sediments particles of samples was omitted (it was regarded as insignificant based on an analysis of the literature data [9]). The SNs concentrations were determined by HPLC method (detector - Waters TAD 486, λ = 254 nm; pump - Knauer 64, flow - 1.0 $cm^3 \cdot min^{-1}$; column - Supelcosil LC-18, 5 μm; 250 x 4.6 mm; mobile phase - buffer containing 20 mmol dm^{-3} K_2HPO_4 at pH = 8.2 : acetonitrile, 95:5). The time required to reduce the SNs concentrations by 25% ($T_{0.25}$) was determined by the graphical method.

Results and discussion

The decrease the SNs concentration was observed in the test samples. Figure 2 shows the dynamics of this process.

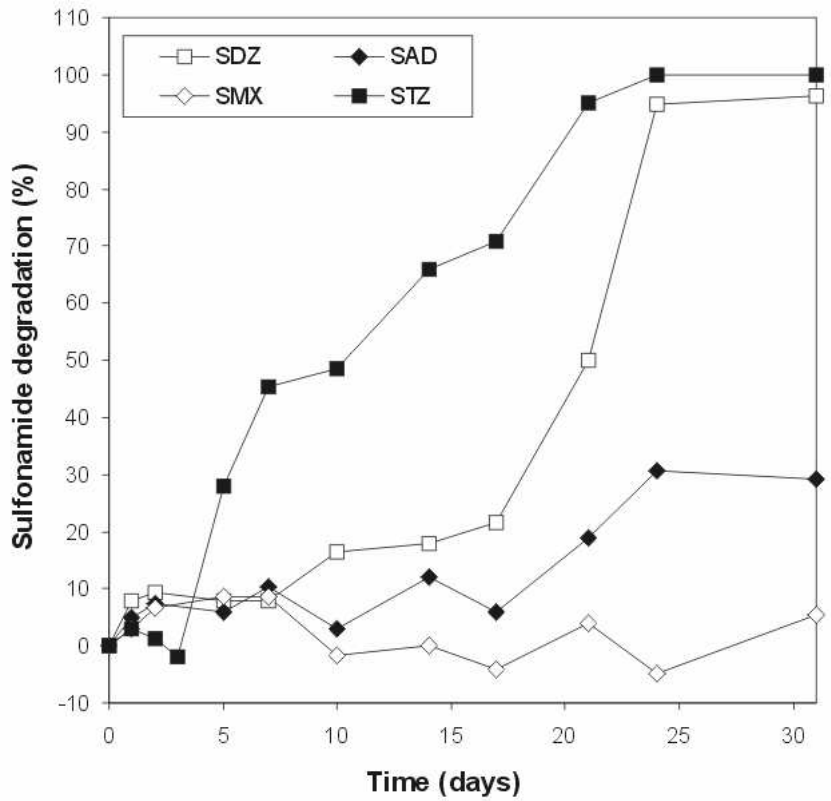

Fig. 2. The relationship between the type of SN and the dynamics of its biodegradation (aerobic conditions; synthetic wastewater inoculated with manure; 08.06.2010; C_o = 10 mg dm^{-3})

It was found that the biodegradation rate was mainly dependent on the type of SN. Moreover, the observed processes did not follow precisely first-order kinetics. In all experimental samples, a decrease in SNs concentrations (probably as a result of sorption) was observed after the first 2-3 days. After that time, their concentration (except STZ) did

not undergo significant changes for a longer time. SMX was the only one among SNs that practically did not undergo the biodegradation processes under these conditions. The effective SNs biodegradation was observed in 3 samples of SAD, 25 samples of SDZ, 29 samples of STZ and only in 1 sample of SMX among all the 86 samples investigated. A factor having a significant effect on the results obtained was also the origin of the inoculum. Figure 3 shows the effect of inoculum type on the $T_{0.25}$ value.

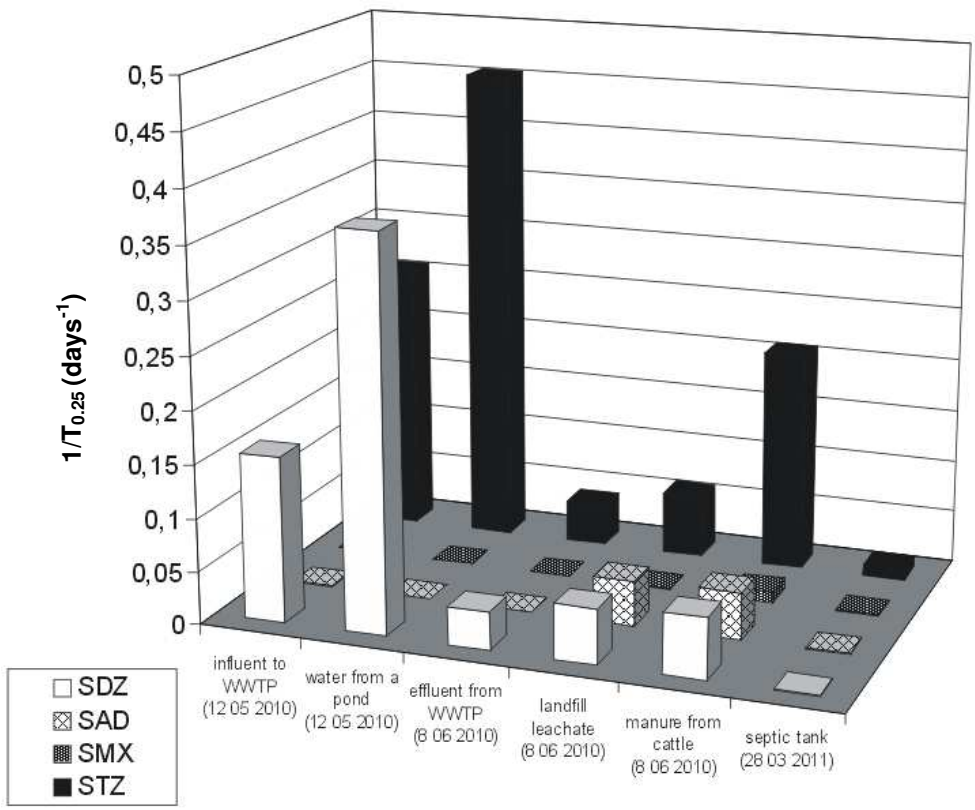

Fig. 3. The relationship between the inoculum type and the biodegradation rate of SNs (aerobic conditions; inoculated synthetic wastewater, C_o = 10 mg dm^{-3})

These results are qualitatively consistent with those obtained during the direct biodegradation in environmental samples. The biodegradation rate of STZ and SDZ in water from a pond turned out to be unexpectedly high. Contrary, these drugs were very slow biodegraded in wastewater from septic tanks. In the first case, the effect may be associated with the possible earlier illegal use of antibiotics by anglers. Such action could theoretically lead to the adaptation of microorganisms. In order to confirm this assumption, we conducted an experiment with wastewater adapted to SNs (*materials and samples*). The obtained results did not differ from those shown in Figure 4. It is possible that the low biodegradation rate in samples from septic tank is due to their prior freezing.

In the case of samples collected from one source, a significant effect on the biodegradation rate may also have the sampling time (Fig. 4).

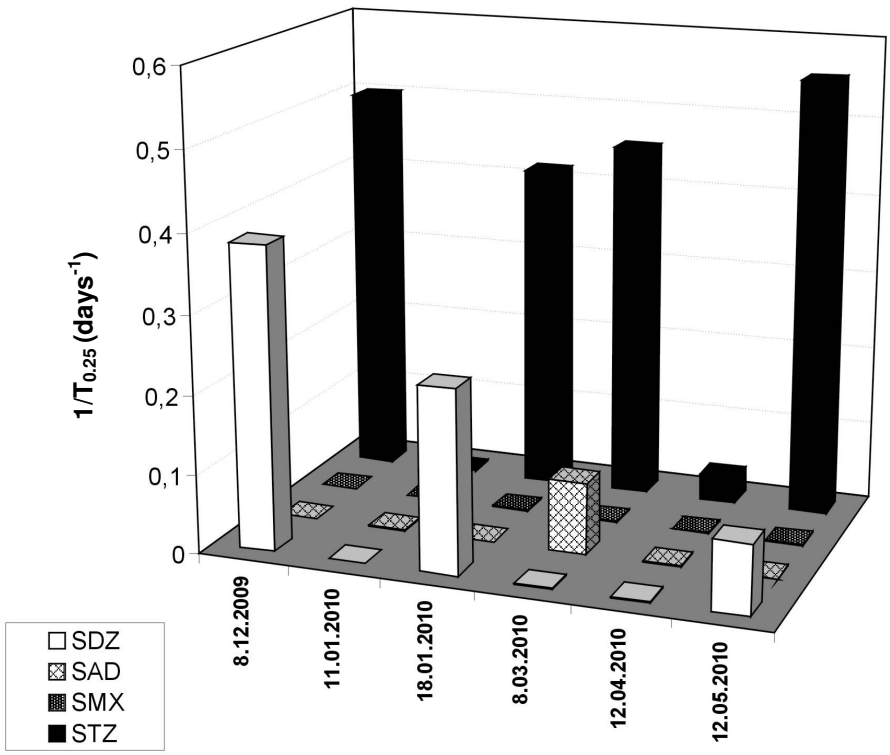

Fig. 4. The relationship between day of sampling and the biodegradation rate of SNs (aerobic conditions; natural influent, natural pH, C_o = 10 mg · dm^{-3})

In our opinion, the weather conditions during the collection of influent may be essential to explain the obtained results. For example, just before 11.01.2010 the temperature was about 0ºC, it was freezing rain and melting snow, the streets were intensively sprinkled with a salt-ice mixture. As a result, municipal wastewater containing runoff from the streets may be poor in microorganisms. These weather conditions were not repeated at any other sampling time (a minimal rainfall was observed only on 04.11.2010) [11].

A large dependence on the sampling time was found in samples collected from the pond. In synthetic wastewater inoculed by a sample collected in spring, the $T_{0.25}$ values for STZ and SDZ were 2.2 and 2.7 days, respectively. In the sample taken from under ice in winter (20.12.2010), there was no degradation of SNs within 28 days.

Additionally, the relationships between pH (Fig. 5) and the initial concentration of SNs in wastewater (Fig. 6) on the biodegradation rate were determined.

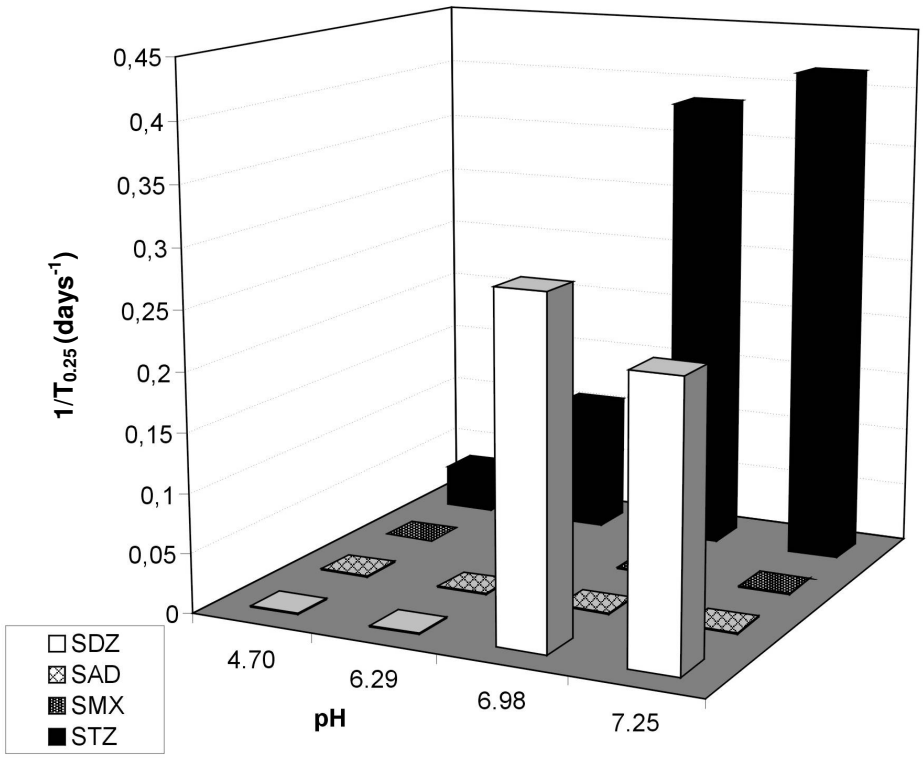

Fig. 5. Effect of pH on the biodegradation rate of SNs (aerobic conditions, natural influent; 18.01.2010; C_o = 10 mg dm^{-3})

It was found that the decrease in pH values below ~7 caused an inhibition of SNs biodegradation and in acidic samples (pH < 4.7) the process practically did not occur.

However, it can not be excluded that the results in naturally acidic environmental samples might be different from these obtained in our experiments.

From the viewpoint of biodegradation rate of SNs, their optimal concentrations in the used wastewater were in the range of 2-4 mg dm^{-3}. It is not excluded that these values will be different for other samples. Therefore, the optimal concentrations of SNs during their biodegradation in wastewater samples may also depend on the sensitivity of microorganisms occurring in the reaction medium.

The results of experiments suggest also that aeration of samples has a beneficial effect on the SNs biodegradation. In influent (sampling time: 08.12.2009) the $T_{0.25}$ values for SDZ and STZ were 2.2 and 2.7 days under aerobic conditions and > 28 and 3.3 days under anaerobic conditions, respectively.

Additionally, there was no significant effect of:

- short-term adaptation of microorganisms to SNs and,
- exposure to low light intensity.

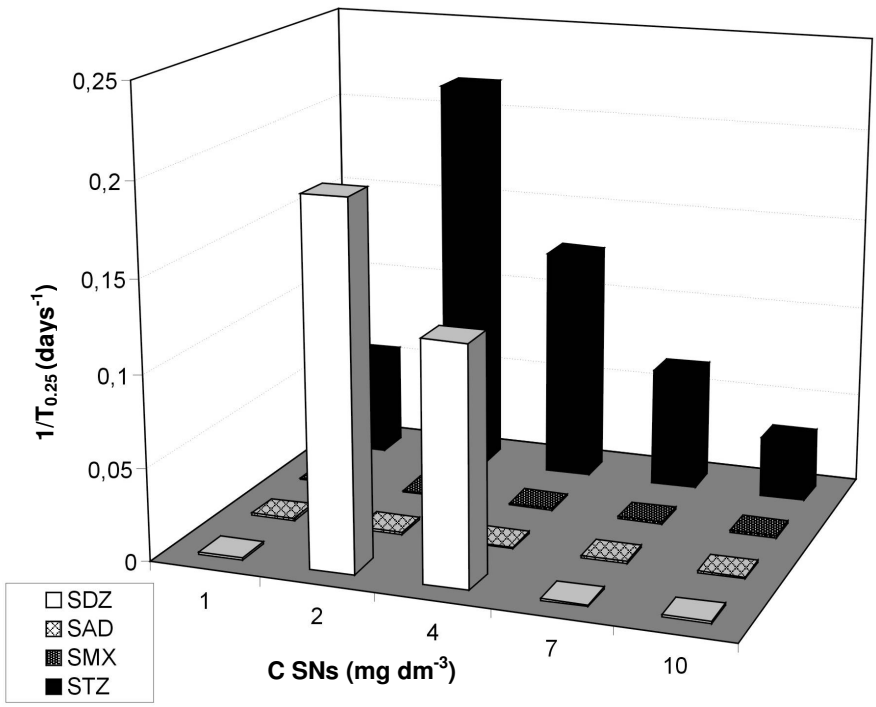

Fig. 6. The relationship between the SNs concentration and their biodegradation rate (aerobic conditions; natural influent, 12.04.2010, natural pH)

Conclusions

- The individual SNs may differ significantly from each other.
- Anaerobic, poor in microorganisms or acidic conditions do not favor the rapid biodegradation of SNs.

Acknowledgements

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Abstrakt: Leki sulfonamidowe, trafiając do środowiska, powodują ryzyko polegające na generowaniu lekooporności drobnoustrojów. Ryzyko to wzrasta w przypadku odporności tych leków na rozkład i pozostawania w środowisku przez dłuższy czas. Z tego powodu szczególnie ważne jest poznanie ich podatności na biodegradację, a dane zawarte w literaturze są rozbieżne. W trakcie badań dokonano oceny podatności na biodegradację 4 sulfonamidów (sulfanilamidu, sulfadiazyny, sulfatiazolu i sulfametoksazolu). Mieszaninę tych sulfonamidów wprowadzano bezpośrednio do próbek środowiskowych lub naturalnych ścieków pochodzących ze zróżnicowanych źródeł i do ścieków syntetycznych zawierających inokulum przygotowane z naturalnych źródeł. Określono, jaki wpływ na przebieg biodegradacji mają warunki tlenowe prowadzenia procesu, dostępność światła (λ > 400 nm), stężenie degradowanej substancji, pH roztworu, pora roku, w jakiej pobierano inokulum, oraz wcześniejsza 40-dniowa adaptacja inokulum w obecności sulfanilamidu. Stopień degradacji sulfonamidów oceniano na podstawie obniżenia ich stężenia oznaczanego metodą HPLC w ciągu 28 dni. Stwierdzono, że na 86 badanych próbek efektywną biodegradację sulfonamidów ($T_{1/2}$ < 21 dni) obserwowano odpowiednio: sulfanilamid - 3 próbki, sulfadiazyna - 25 próbek, sulfatiazol - 29 próbek i sulfametoksazol - 1 próbka. Stwierdzono również, że przebieg biodegradacji sulfonamidów znacznie różni się od wyników opisywanych w literaturze, a jego dynamika zależy głównie od rodzaju sulfonamidu, pochodzenia inokulum i pory roku jego pozyskania.

Słowa kluczowe: sulfonamidy, farmaceutyki, biodegradacja