



Surfactant removal efficiency using a multiscale flow laboratory hydrophyte system – a biodegradation experiment

Dobrochna Ginter-Kramarczyk^a, Joanna Zembrzuska^b, Izabela Kruszelnicka^{a,*},
Anna Zajac-Woznialis^c

^aDepartment of Water Supply and Bioeconomy, Faculty of Environmental Engineering and Energy, Poznan University of Technology, Berdychowo 4, 60-965 Poznań, emails: izabela.kruszelnicka@put.poznan.pl (I. Kruszelnicka), dobrochna.ginter-kramarczyk@put.poznan.pl (D. Ginter-Kramarczyk)

^bFaculty of Chemical Technology, Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Berdychowo 4, 60-965 Poznań, email: joanna.zembrzuska@put.poznan.pl

^cDepartment of Biophysics, Poznan University of Medical Science, Grunwaldzka 6, 60-780 Poznań, email: anna.zajac.woznialis@gmail.com

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ABSTRACT

The aim of the study was to evaluate the efficiency of surfactant removal using a laboratory hydrophyte system as well as to determine the impact of the used plant species on the removal of such compounds. The experimental system consisted of seven containers filled with expanded clay pellets planted with vegetation used in hydrophyte treatment plants. Three systems were used with different species of aquatic plants: common reed (*Phragmites australis*), lakeshore bulrush (*Scirpus lacustris*), and great manna grass (*Glyceria maxima*). The systems were loaded with synthetic sewage which contained a polydispersive nonionic surfactant C₁₂E₁₀ (5 mg/L). The experiment lasted for 27 d. The assessment of the biodegradation efficiency of the tested compound was carried out using liquid chromatography combined with tandem mass spectrometry. The reduction of C₁₂E₁₀ and other components of the polydispersive mixture was significant (approximately 90%). Basic relationships regarding the biodegradation of the studied compound as function of time have been established under conditions characteristic for hydrophyte sewage treatment plants. The study also included observations of plants in terms of their response to wastewater containing the C₁₂E₁₀ detergent.

Keywords: Nonionic surfactant; Hydrophyte system; Biodegradation; LC-MS/MS

1. Introduction

The pollution of aquatic environments does not apply to cities and industrial centers since such areas possess sewage treatment plants, however, it is a notable issue in case of small towns and villages, which often lack a proper sewage system and include dispersed buildings.

The use of small, domestic sewage treatment plants, including hydrophyte systems, which are characterized by

high efficiency and the possibility of wide application is a chance to improve the quality of water at the local level [1].

The operation of hydrophyte treatment plants is based on the use of the same physical, chemical, and biological processes which occur in natural wetland ecosystems with the participation of various microorganisms and appropriately selected plants. These plants exist in designed beds (soil filters or ponds) and are periodically or permanently flooded with sewage. They are used to treat not only

* Corresponding author.

domestic and municipal wastewater, but also for remediation of industrial and agricultural wastewater, sewage from gas stations, rainwater, and leachate from landfills or area runoff from arable fields [1–3]. Both water-plant systems with surface sewage flow and soil-plant systems with subsurface sewage flow are often used among such facilities.

Systems with surface sewage flow (SF systems) are ponds and ditches with aquatic macrophyte vegetation. They are characterized by low investment outlays and simple operation. A significant reduction of efficiency outside the growing season can be the main problem of such systems. Weather conditions, especially winter conditions, also determine the effectiveness of treatment in this type of systems.

In case of subsurface flow, two types of systems can be distinguished: systems with horizontal subsurface flow (HSF systems) [4–7] and systems with vertical subsurface flow (VSF systems) – soil-plant deposits [8,9]. In this type of constructions, there is no problem with the freezing of sewage surface and lack of oxygen access to the deposit. In the winter period, after the death of plants, there is a decrease of efficiency of plant bed cleaning. However, the purification process is not stopped, since the microorganisms present in the bed mainly carry out the biodegradation. In winter, the vegetation is left on the bed and it forms an insulating layer for the treatment plant. Additionally, the bed can be further insulated with a layer of straw or other biomass before winter [9].

Soil-plant treatment technology is known in Europe and the world as the use of constructed wetlands. For many years they have been a very popular method of wastewater treatment, as evidenced by numerous scientific reports. These systems were the subject of research, for example, in England [10], Denmark [11], Austria [12], Norway [13], Sweden [14], Poland [9,15], Germany [16], the Czech Republic [17], USA [18] New Zealand [19], Canada [20], Spain [21], and many other countries around the world. In the literature, however, there are not many reports regarding the effectiveness of surfactant biodegradation in such systems.

Studies conducted in the last decade [22,23] have shown that surfactants are the main pollutants in wastewater streams introduced into the natural environment. Therefore, surfactants have become a common source of surface water contamination. Nonionic surfactants are one of the main components of the stream of surfactants introduced to the aquatic environment in the form of washing agents, cleaning agents, emulsifiers, additives, etc. Due to their production scale, ethoxylated alcohols are the main representatives of this group of surfactants [24]. Their production volume is the result of high demand in terms of the broadly understood market. As reported by the European Committee of Organic Surfactants and their Intermediates (CESIO), in 2013 the production of NS in EU alone reached 1,450 thousand tons, 1,320 thousand tons of which were oxyethylates. Among oxyethylates, the highest production is observed for oxyethylated alcohols (AE), which was equal to 962 thousand tons. Both petrochemical and renewable resources are used for the synthesis of AE. In 2013 approximately 574 thousand tons of AE were produced based on renewable resources, whereas 388 thousand tons were produced from petrochemical resources. The advantage of using renewable resources suggest that the majority of

produced oxyethylated alcohols include an aliphatic chain with 10, 12, 14, 16, and 18 carbon atoms [25].

Based on the production volume [25] and described research regarding the pollution of sewage [26–29], and surface waters [29] with surfactants, it was established that currently the nonionic group of surfactants is defiantly dominated by oxyethylated alcohols. The studies allowed to identify 105 individual oxyethylated alcohols comprising an aliphatic chain with 10–18 carbon atoms in their structure (C10, C11, C12, C13, C14, C15, C16, and C18) as well as an oxyethylated chain with 3–23 oxyethylate groups in the structure.

For this reason, oxyethylated polydisperse dodecanol ($C_{12}E_{10}$) was selected as a model representative of the NS group for the purpose of studies [25].

The production volume and common use of oxyethylated surfactants, mainly oxyethylated alcohols, makes them a notable anthropogenic component of the aqueous environment. Based on the literature reports regarding the presence of surface active compounds in surface water and wastewater, it can be observed that nonionic surfactants dominate these systems [26,27]. High AE ratio in total nonionic surfactants was confirmed by an investigation of sewage composition, in which approximately 100 individual AE but no other NS were found [28].

The basic process which determines the concentration of surfactants in surface waters is their biodegradation. Previous studies indicated that the biodegradation of ethoxylated alcohols occurs due to a mixed mechanism, that is, central cleavage, shortening the chain by one EO unit and oxidation of terminal OH groups to carboxyl groups. Such a course of biodegradation has been reported for numerous consortia of microorganisms present in surface waters, sewage, and soil [29–31]. The biological methods based on activated sludge used in wastewater treatment plants [32,33] and methods based on the use of bacterial strains isolated from river waters [30,34,35] are widely used for the removal of surfactants from the aquatic environment. However, the insight regarding the involvement of individual bacterial strains in this processes is still limited and the current state of knowledge on the biodegradation of ethoxylated alcohols does not provide information regarding biodegradation processes carried out with the participation of hydrophyte plants. Therefore, research was undertaken to investigate whether the plants used in hydrophyte sewage treatment plants may impact the efficiency of biodegradation processes. The aim of this study is to demonstrate the basic relationships concerning the biodegradation of a selected nonionic surfactant as a function of time under conditions characteristic for hydrophyte sewage treatment plants by conducting tests in a laboratory environment. The study also includes observations of plants and their response to wastewater containing polydisperse $C_{12}E_{10}$, which is often used in household cleaning agents and in cosmetics.

2. Materials and methods

2.1. Experimental set-up

For the purpose of the research, seven model systems with hydrophyte plants were prepared (three sets of

two containers with three different species of hydrophyte plants). Two systems were prepared for each plant species in order to check the repeatability of the processes occurring in a separate system. After the time needed for plant growth, expanded clay deposits were loaded with model synthetic sewage containing $C_{12}E_{10}$ at a concentration of 5 mg/L. Then, the leachate was collected from each deposit at intervals of several days. Properly prepared samples were subjected to chromatographic analysis in order to examine surfactant biodegradation as a function of time.

2.2. Model hydrophyte system

The hydrophyte systems were prepared in plastic containers with dimensions equal to $0.78 \times 0.56 \times 0.43$ m. The containers were filled to approximately $\frac{3}{4}$ of their volume with single-fraction thick expanded clay characterized by a grain size range of 8–16 mm and density of 400–800 kg/m³.

The plant species used in the experiment included: common reed (*Phragmites australis*), lakeshore bulrush (*Scirpus lacustris*), and great manna grass (*Glyceria maxima*). The selected hydrophytes are vascular plants emerging from monocots.

Before planting, the underground parts of the plants were thoroughly rinsed to remove the soil they originally grew in. Seedlings of a particular plant species were placed in six containers. Two containers were used for each specific plant species. The last, seventh container was filled only with expanded clay pellets (reference system). All deposit models were loaded with synthetic sewage containing 5 mg of $C_{12}E_{10}$ /L after a 20 d adaptation period.

2.3. Synthetic sewage

The presented $C_{12}E_{10}$ degradation studies were carried out for an artificial sewage, since only such sewage was devoid of both the tested compound and its biological degradation products. The presence of these compounds in the matrix itself would not allow for the assessment of the removal efficiency of the introduced polydisperse analyte from the tested system. The main component of the synthetic sewage used in the experiment was the $C_{12}E_{10}$ nonionic surfactant (Sigma-Aldrich, Germany). It was the only source of organic carbon in the system. The $C_{12}E_{10}$ surfactant is a polydisperse ethoxylated alcohol with a dodecyl chain as its hydrophobic part and a hydrophilic part which includes 10 oxyethylene groups on the average. This compound is a polydisperse mixture – it consists of many dispersed components characterized by different oxyethylene chain length, containing from 2 to 19 ethoxylated groups, which are connected to a hydrocarbon chain with 12 carbon atoms.

2.4. Analytical procedure

2.4.1. Extraction

In order to examine the content of $C_{12}E_{10}$ during the operation of the hydrophyte treatment plant, 50 mL of the leachate was collected each time and subjected to liquid-liquid extraction according to the procedure described earlier [28,29,30]. Then, 15 g of NaCl and 0.1 g of $NaHCO_3$ were

added to the effluent to increase the salinity. After dissolution of the salt, the solution was transferred to a separatory funnel and extracted with two portions of ethyl acetate (2×10 mL). The content of the separating funnel was shaken vigorously and allowed to reach equilibrium for 3 min to separate the phases. The acetate phase was collected into a 25 mL volumetric flask. Finally, the flask was filled with solvent up to the mark. Then, 500 mL of the extract were collected and evaporated under a stream of nitrogen. The dry residue was dissolved in 1 mL of initial phase used for chromatographic separation.

2.4.2. Chromatographic analysis

Qualitative and quantitative analysis of the tested samples was carried out using the combined LC-MS/MS technique. A Dionex Ultimate 3000 RSCL liquid chromatograph was used. A Hypersil Gold C18 RP column (100 mm \times 2.1 mm \times 1.9 μ m) from Thermo Scientific was used for chromatographic separation of the surfactant. A mixture of 5 mM ammonium acetate (A) and methanol (B) was used as the mobile phase. Analytes from the column were eluted using a gradient elution: 0–2 min 30% B, 10 min 100% B, 15 min 100% B. The flow of the mobile phase through the column was at 0.2 mL/min. The separation was carried out at 35°C, the volume of sample loaded into the column was equal to 10 mL. The API 4000 QTRAP tandem mass spectrometer (Biosystem, MDS Sciex, USA) was used as the detector. The analysis was carried out by recording the total ion current chromatograms in the mass range m/z from 100 to 1,200 Da. Ionization was carried out using electrospray (ESI) in positive ion mode. Mass spectrometer operated using the following conditions: ion source temperature at 400°C, shielding gas pressure at 10 psi, atomizer gas pressure at 30 psi, drying gas pressure at 50 psi, voltage applied to the capillary equal to 3,500 V, and fragmentation potential at 36 V.

3. Results and discussion

The result of the chromatographic analysis for each prepared sample were obtained as chromatograms of total ionic current and polydisperse $C_{12}E_{10}$ mass distribution spectra. The TIC chromatogram for the original synthetic sewage containing 5 mg/L $C_{12}E_{10}$ is presented in Fig. 1, while the mass spectrum for a peak with a retention time of 9 min is presented in Fig. 2. This is the $C_{12}E_{10}$ polydisperse peak. Based on the mass spectrum, 19 individual ethoxylated alcohols were identified which contained an aliphatic chain consisting of 12 carbon atoms and an oxyethylene chain containing from 3 to 21 oxyethylene groups in the molecule. The mass spectrum of this compound indicates its polydisperse nature. TIC chromatograms and mass spectra were obtained for each effluent extract collected during a given experiment duration.

Based on the obtained peak area values with a retention time of 9 min, the total concentration of polydisperse $C_{12}E_{10}$ during the experiment was determined for individual extracts. Changes in the concentration of this mixture over time for the tested plants are presented in Fig. 3 and at divided time intervals in Figs. 4–6.

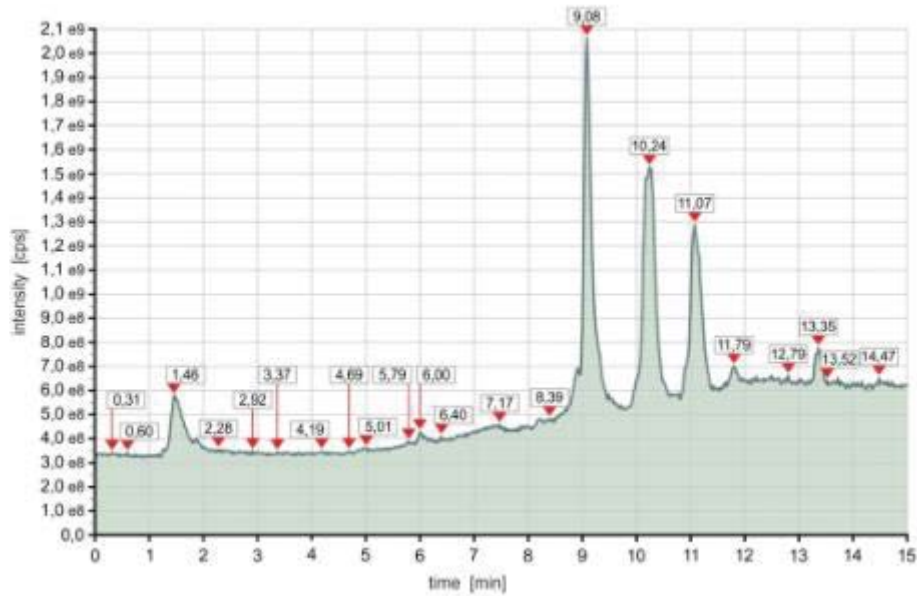


Fig. 1. Total ionic current chromatogram for the extract from a stock solution including 5 mg/L of the standard ([cps] = counts per second).

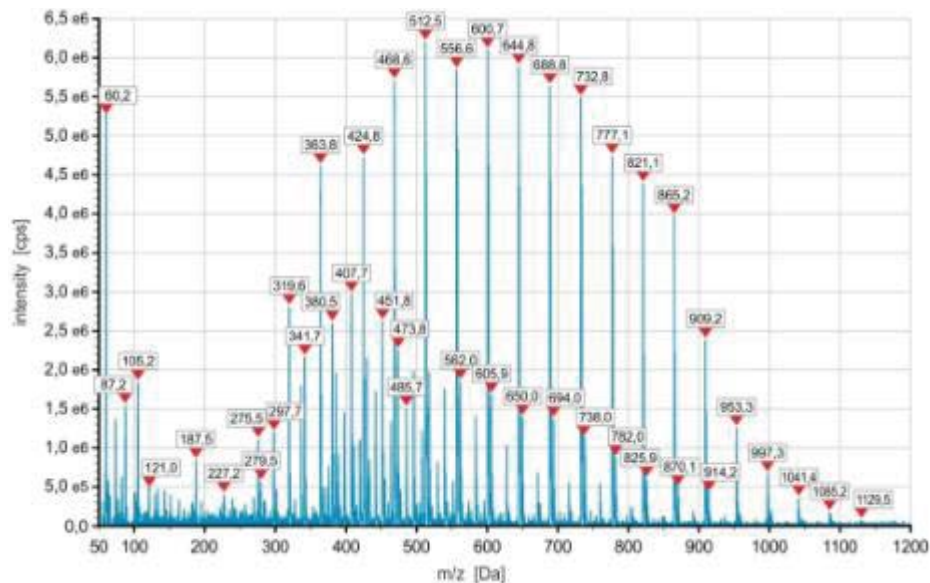


Fig. 2. Peak mass spectrum with a retention time of 9 min – distribution of polydisperse.

$C_{12}E_{10}$ ($[C_{12}E_{10}+NH_4]^+$, for example m/z for $[C_{12}E_3+NH_4]^+ = 336$)

In the case of leachate samples collected from the beds immediately after dosing, a decrease in the concentration of $C_{12}E_{10}$ by half was observed, hence, the initial concentration of the dosed surfactant is equal to 2.5 mg/L in Figs. 3 and 4. The half reduction is due to the adsorption of this polydisperse compound on expanded clay pellets. On the first day of the experiment, a significant decrease in the concentration of the $C_{12}E_{10}$ mixture occurred in each deposit. The highest concentration was recorded for the leachate obtained from a container filled with only expanded clay pellets – 970 ng/L. Degradation

in deposits with the same plant species did not occur in a similar manner. For example, for common reed, 934 ng/L was measured in one system and 532 ng/L in the other, the values were equal to 664 and 480 ng/L in case of great manna grass, and 829 and 648 ng/L for lakeshore bulrush. The lowest surfactant concentration on the 8th day of the experiment was observed in case of container 5 with great manna grass and was equal to 79 ng/L. Comparable results were obtained in deposits with common reed and lakeshore bulrush, which were equal to 100 and 89 ng/L, respectively. In the container with expanded clay pellets, the surfactant concentration was lower (169 ng/L) than in systems 1 (251 ng/L), 2 (274 ng/L), and 3 (185 ng/L).

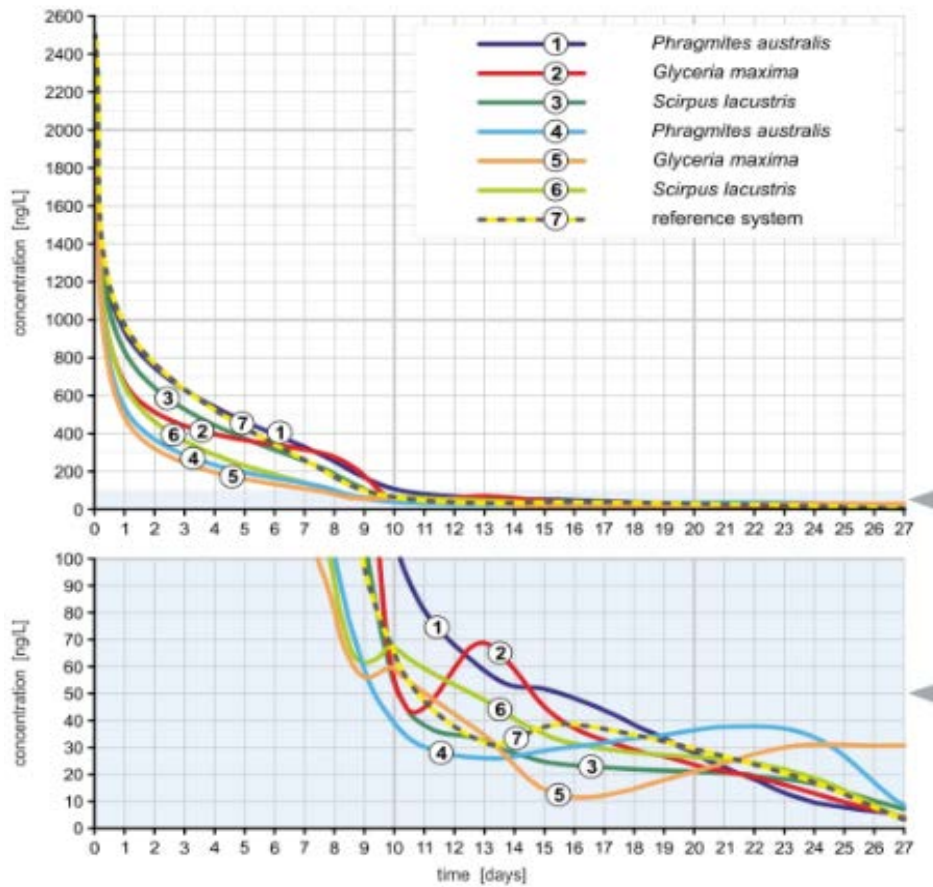


Fig. 3. Biodegradation of $C_{12}E_{10}$ as a function of time.

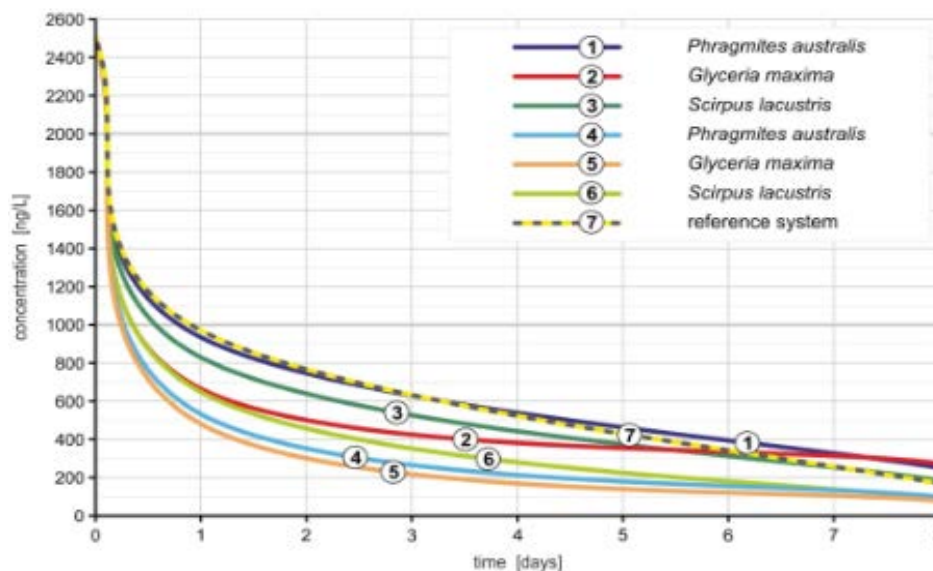


Fig. 4. Biodegradation of $C_{12}E_{10}$ – from the 1st to the 8th day of the experiment.

On the 10th day of the test, the highest surfactant concentration was observed in container 1 with common reed and was equal to 106 ng/L. In container number 4 (with the same plant) the concentration was equal to 48 ng/L, and

for other systems the surfactant concentration ranged from 53 up to 65 ng/L. After 13 d, the concentration in container 2 with great manna grass was equal to 68 ng/L. The lowest concentration both on the 10th and the 13th day was

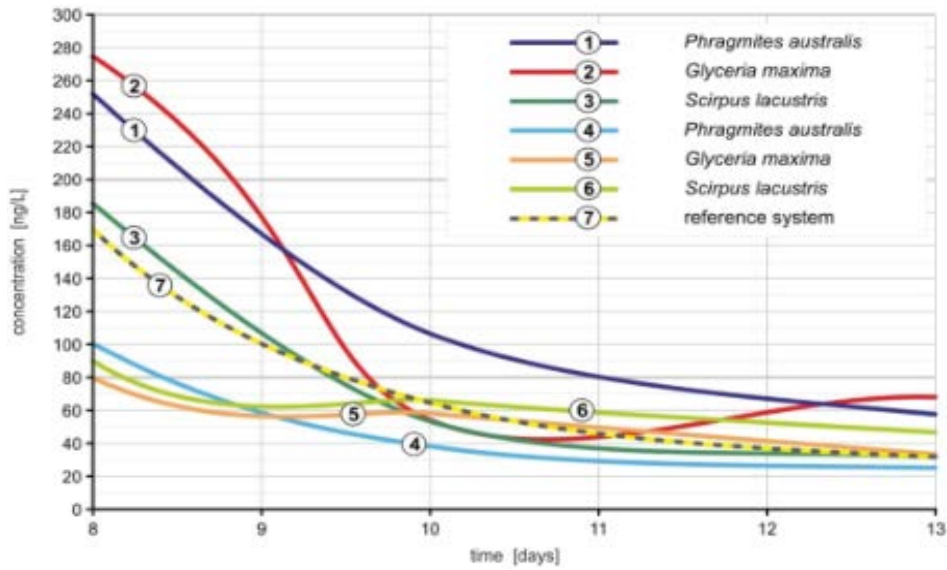


Fig. 5. Biodegradation of $C_{12}E_{10}$ – from the 8th to the 13th day of the experiment.

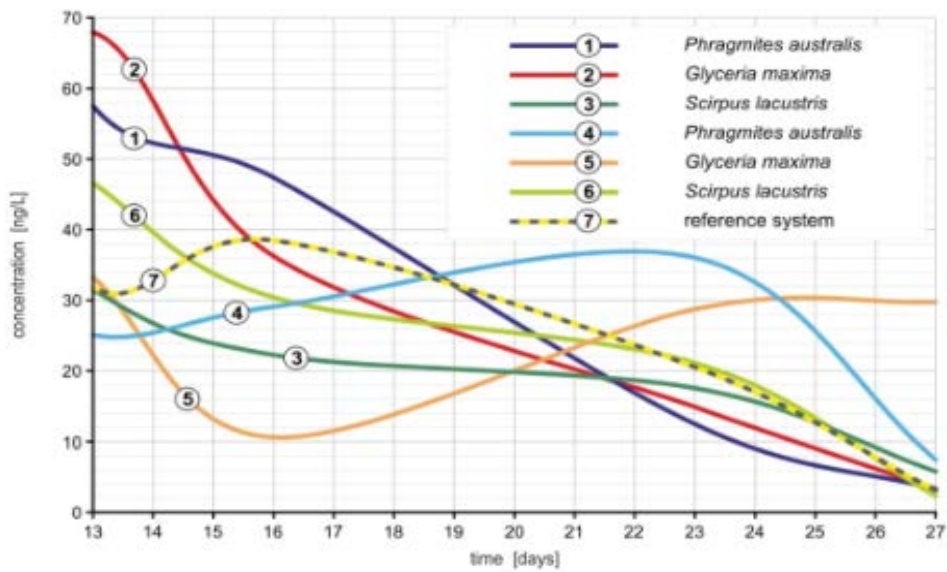


Fig. 6. Biodegradation of $C_{12}E_{10}$ – from the 13th to the 27th day of the experiment.

observed for common reed in container 4. Degradation in the bed filled with expanded clay pellets was similar to that in beds 3, 4, and 5. This indicates that the degradation of the dosed surfactant is more likely caused by microorganisms which developed on the bed, while plants only slightly affect its distribution.

On the 16th day, an increase in the surfactant concentration in expanded clay pellets (38 ng/L) was observed relative to the value recorded on the 13th day of the test (31 ng/L). These differences in $C_{12}E_{20}$ concentration on day 13 and 16 may result from evaporation of the synthetic sewage and/or measurement error. In containers with plants, the highest concentration recorded at that time was in container 1 with common reed – 47 ng/L. In other systems, the concentration of the surfactant was determined at a level ranging from 10 to 36 ng/L.

On the 23rd day, an increase in the concentration was observed in beds with common reed – container 4 – and great manna grass – container 5, compared to the previous readings. After the 27th day, the concentration in most deposits was lower than 10 ng/L, only the surfactant concentration in the container 5 with great manna grass was equal to approximately 30 ng/L.

The biodegradation of $C_{12}E_{10}$ in systems with a selected plant species and for the deposit with expanded clay only is presented in Figs. 7–9.

Significant differences were observed after considering the dynamics of the biodegradation process in containers with the same plant species compared to sole expanded clay pellets. The degradation dynamics for common reed in both containers was different. In container 4, the $C_{12}E_{10}$ degradation was faster and more effective during 16 d than

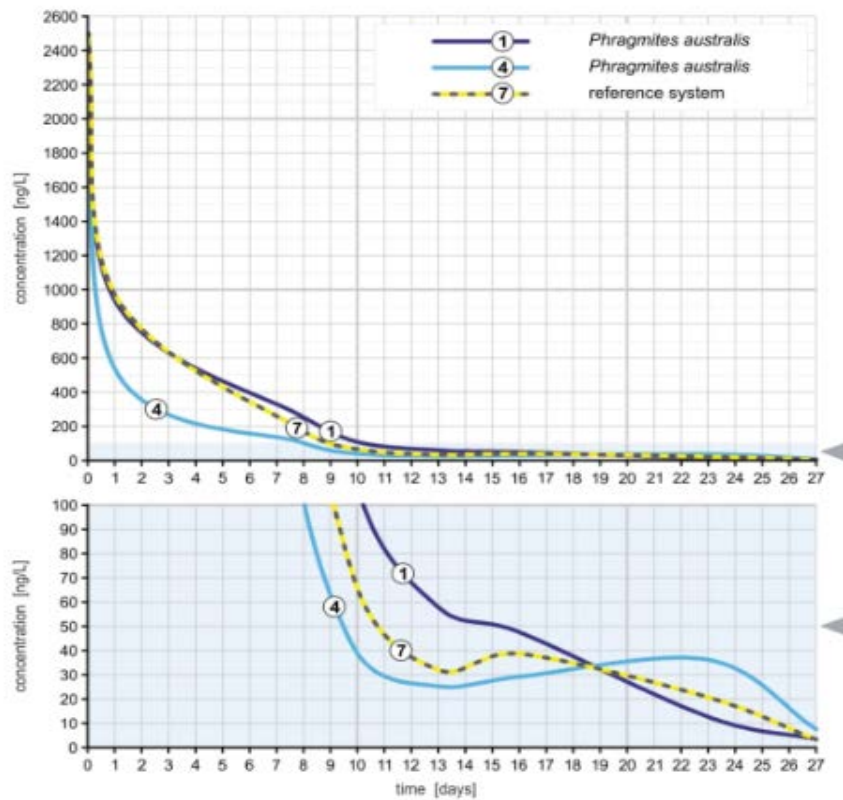


Fig. 7. Biodegradation of $C_{12}E_{10}$ as a function of time – common reed and expanded clay pellets.

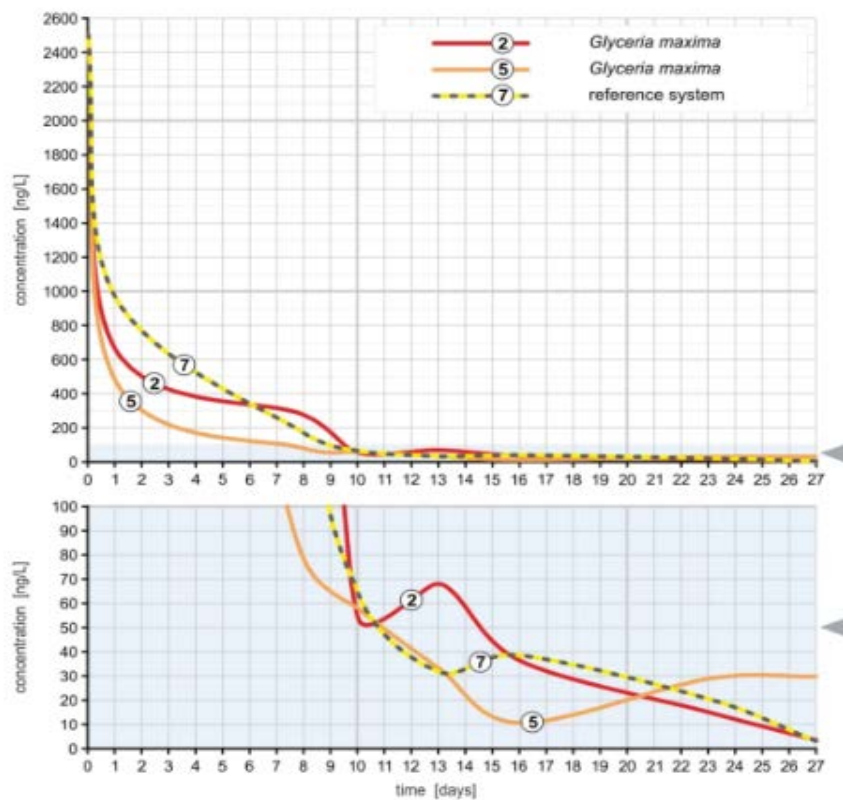


Fig. 8. Biodegradation of $C_{12}E_{10}$ as a function of time – great manna grass and expanded clay pellets.

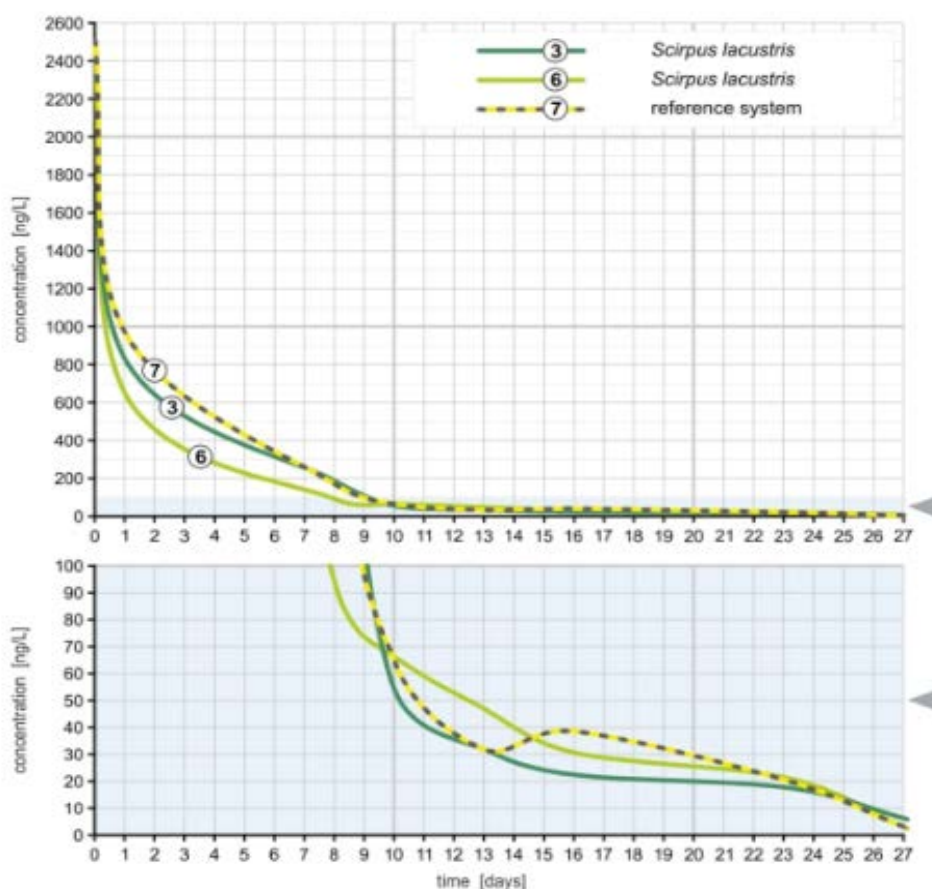


Fig. 9. Biodegradation of $C_{12}E_{10}$ as a function of time – lakeshore bulrush and expanded clay pellets.

for the same plant in container 1. Finally, after 27 d of testing, systems 1, 4, and 7 showed almost the same surfactant degradation efficiency.

The dynamics of the biological degradation process in containers with great manna grass also varied compared to sole expanded clay pellets. By the 10th day in container 5 with great manna grass, faster surfactant degradation occurred compared to biodegradation in container 2 and in the system with expanded clay pellets. Starting from the 10th day, the concentration fluctuated. On the last day of the study, the highest concentration was determined in container 5, while in systems 2 and 7 it reached concentrations below 5 ng/L.

The biodegradation dynamics in containers with lakeshore bulrush were also different. Up to the 8th day in container 6 with lakeshore bulrush, the surfactant was degraded more rapidly compared to container 3 and sole expanded clay pellets. On the last day of the study, the highest concentration was determined in container 3, while in case of bed 6 and 7 the content was lower than 3.5 ng/L.

Analysis of the test results allowed to establish that the tested surfactant was completely biodegradable in each of the studied deposits. The first phenomenon after dosing of the synthetic sewage which included the surfactant was its adsorption on expanded clay pellets, manifested by a decrease of the $C_{12}E_{10}$ concentration in the leachate by 50%. On the first day of the experiment, the concentration of the

test compound in each of the systems decreased by more than 65%. Additionally, biodegradation of $C_{12}E_{10}$ by approximately 60% occurred in the deposit with only expanded clay pellets. After the next 7 d, the concentration in each leachate was lower than 280 ng/L. This corresponds to 89% removal of the surfactant. On the 10th day, $C_{12}E_{10}$ concentration in the systems did not exceed 120 ng/L – which results in 95% biodegradation. Concentrations of this compound in subsequent days continued to decrease regardless of the system. The decrease of the $C_{12}E_{10}$ concentration in all systems indicates that the biological degradation of the surfactant is mainly caused by microorganisms which settled on the bed from the environment, and not the presence of the plants. After an analysis of the research results, it cannot be clearly stated which species of hydrophyte plants promoted the degradation of the studied nonionic surfactant and whether the presence of the plants played a significant role in the removal of this contamination.

4. Conclusion

In our research, we confirmed that the reduction of $C_{12}E_{10}$ and other components of the polydisperse mixture was significant (approximately 90%). Additionally, basic relationships regarding the biodegradation of the selected compound as a function of time have been demonstrated under conditions characteristic for hydrophyte sewage

treatment plants. The fact that various degradation efficiencies occurred for the same plant species and the same number of plant seedlings is an interesting observation.

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