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Determination of aerobic and anaerobic biological degradability of waste tyres

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Abstract

Environmental contamination of rubber from waste tyres poses a risk to the environment. Rubber particles from tyres enter the environment due to the abrasiveness of the road surface. The aim of the scientific work was to observe the biological degradability of waste tyres in aquatic environment and its ecotoxicity. Biodegradability was observed under aerobic and anaerobic conditions that simulate both aerobic and anaerobic conditions in the aquatic natural environment. Aerobic conditions in the aquatic environment take place in flowing fresh water, sea water, lakes. Leachate was prepared to simulate the behaviour of tyres in an aqueous environment. Aerobic degradability was evaluated through complete biodegradability using a 301 F manometric respirometry test. Anaerobic biodegradability was evaluated by measuring biogas production using OECD 311 Anaerobic Biodegradability of Organic Compounds in Digested Sludge. For a better simulation of the natural environment, the pH of the leachates from the tyres was adjusted to a neutral range. It should be noted that standard degradability tests were extended by 7 days due to low biodegradability. Adjusting the pH during the biodegradability test is also a modification of the original test. This modification was used to better simulate biodegradability when the pH of tyres in the natural environment is reduced by acid rain. An essential part of monitoring the behaviour of waste tyres was the assessment of ecotoxicity using standard tests. The contribution of the scientific article lies in the evaluation of the course of decomposition in aerobic and anaerobic conditions with and without pH adjustment and in the use of modified biodegradability tests. The benefit of the scientific work is in the determination of the biodegradability of waste tyres with and without pH treatment, which simulate a comparison of the degradability of tyres in an acid rain environment. Another benefit of the scientific work is the depiction of biodegradation using 3D modelling with calculations of 100% degradability at different input concentrations of waste tyres. Modelling was used for the time for the absolute decomposition of tyres without pH adjustment (outside the acid rain environment) and with pH adjustment (in the acid rain environment). By monitoring, it is possible to determine whether acid rain as an anthropogenic activity influences the degradability of waste tyres in the natural environment. Biodegradability tests confirmed the low biological degradability of waste tyres. The highest average rate of biological degradability—15% was recorded at the input concentration of waste tyres of 350 mg/L. The aerobic degradability test confirmed the improvement of tyre decomposition when adjusting the pH to the level of 6.5–7.5. On the contrary, the anaerobic degradability test confirmed the improvement of the decomposition in the alkaline region compared to the neutral pH values of the mixture. By mathematical–statistical evaluation of aerobic decomposition with preservation of degradability trends at three input concentrations, the time of absolute decomposition of waste tyre particles at a concentration of 370 mg/L was found to be approximately 336 days. By adjusting the pH to the neutral range during aerobic decomposition, the total decomposition time was reduced to 126 days. The ecotoxicity tests performed confirmed the toxic effect of tyre leachate on selected tested organisms. In the future, the authors propose to focus on a more detailed assessment of the ecotoxicity of the waste

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conditions and to modify the biodegradability tests by changing the conditions (wider range of input pH value, longer biodegradability time, temperature) for a better simulation of different types of environments. Adjusting the pH to a neutral environment increased aerobic degradability but had no significant effect on anaerobic degradability. Therefore, it is important to focus future research on the adjustment of various conditions to support the degradability of tyres, of which pH has clearly been confirmed as an important factor.

Keywords Aerobic decomposition, Anaerobic decomposition, Biodegradability, Waste tyres

Introduction

Sales of passenger car and light commercial vehicle tyres in the European Union reached 315 million units in 2020. That same year, 20 million units of medium and heavy commercial vehicle tyres were sold [1]. Natural rubber is the main component of rubber tyres [2]. The composition of tyres is dominated by rubber (natural rubber 21–42%; synthetic rubber 40–55%), carbon black (30–38%), ash (3–7%) [3] Other studies report the composition of passenger car tyres as rubber (41–48%); carbon black (22–28%); metals (13–16%), textiles (4–6%) and additives (10–12%) [4]. Fořt et al. presented composition of waste tyre 47.3% rubber; 31.6% carbon black; additives 12.7%; acetone extract 6.4% and ash 2.1% [5].

Billions of discarded tyres are currently accumulating around the world. Therefore, illegal tyre dumps are the cause of serious environmental problems [6].

Tyres also pose a risk to the environment during operation on roads, when fine particles are released because of abrasion. The wear rate of waste tyres was confirmed to be from 0.006 to 0.09 g km^{-1} per tyre depending on driving conditions [7]. The highest concentrations of various tyre indicators in road dust correspond to particle concentrations in the range from 0.7 to 210 $g/kg_{dry\ weight}$ [8]. At the same time, the accumulation of tyre particles in road dust was detected in the inner spaces of the tunnels [9]. It is important to highlight that the danger in this issue lies in the transport of tyre particles into individual components of the environment. The presence of tyre particles has been confirmed in significant concentrations in various areas of the environment, such as rivers, sediments [10], air [11] and soil, for example, from 1 kg of tyre particles, approximately 1.76 g of zinc reaches 1 kg of dry soil [12].

Ecotoxicity is an important factor in evaluating the harmfulness of tyres in the environment. The chronic toxicity of extracts prepared from worn tyres on green algae, crustaceans and fish eggs was investigated. The highest sensitivity was observed in *Ceriodaphnia dubia* with chronic toxicity values from 50 to 6000 mg/L for survival and from 10 to 1800 mg/L for reproduction [13]. The highest ecotoxicological effect of waste tyres of all investigated species on mussel development was confirmed. EC50 values reached the level of 0.8% [14]. Fish toxicity was investigated in two fish species: mummichogs (*F. heteroclitus*) and fathead minnow (*P. promelas*), which were exposed to concentrations up to 6.0 g/L [15] with a significantly toxic effect. TWP exposure causes both short-term and long-term toxicity to freshwater organisms [16].

The toxicity of tyres has its essence in the composition. The toxicity of tyre leachate is mainly caused by trace elements (metals), polycyclic aromatic hydrocarbons and various volatile organic substances used in vulcanization and as antioxidants [17]. Considering the production of tyres, the persistence of particles and the toxic potential, a significant negative impact is also expected on terrestrial ecosystems [18].

One of the possible ways of removing rubber microparticles of waste tyres from the natural environment is their degradation by microorganisms—biodegradation [19]. Synthetic rubbers are biologically resistant in contrast to natural rubber (NR) which is sensitive to biodegradation. The presence or absence of additives such as fillers, vulcanizing agents, antioxidants, blowing agents and plasticizers also affect biodegradability [20].

The main goal of this contribution is to observe of biodegradability of waste tyre in the natural environment. Waste tyre leachates are characterized by an alkaline region [21, 22], which is probably caused by the release of additives from the waste tyre material during the leaching process [23, 24]. However, in the natural environment there is a decrease in pH due to acid rain [25]. For this reason, for a better simulation, the samples were adjusted to the neutral area. Since acid rain may not affect tyre degradability under all circumstances, biodegradability was monitored with and without pH adjustment. Aerobic conditions in the aquatic environment take place, for example, in sea water, rivers, lakes [26, 27]. Anaerobic conditions occur in aquatic environments during flooding, at high groundwater levels, or in response to mechanical soil compaction [28]. For this reason, we observed the decomposability of waste tyre particles also under anaerobic conditions. Aerobic degradability results were used in 3D modelling evaluation to determine 100% degradability as a function of time at various input concentrations.

Overall, the biodegradability of vulcanized NR is difficult because the interlinked polymer chains reduce water absorption and permeability, and the majority of studies have focused on the cis configuration. Rose and Steinbuchel reported that intensive attempts to demonstrate the degradation of the trans configuration by known rubber degrading microorganisms all failed [29]. According to Linos, several actinomycetes isolated from nature were able to use both natural rubber (NR) and synthetic *cis*-1,4-polyisoprene rubber (IR) as a sole source of carbon [30]. The purpose of the scientific paper is not to introduce procedures for the biological degradability of tyres. It is to observe the behaviour of waste tyres under different conditions—pH, aerobic/anaerobic, activated/digested sludge.

Material and methods

Used inoculum

According to the OECD 301 F standard test, activated sludge was used as an inoculum for aerobic degradation. According to the OECD 311 standard test, digested sludge was used as inoculum for anaerobic degradation.

Adjust samples

Grinding the sample

The fraction before grinding had dimensions of 3 mm × 40 mm. The tyre sample was cryogenically cooled at −200 °C using liquid nitrogen. Subsequently, the sample was ground on a ball mill. The dimensions of the fraction after grinding were approximately 0.1 mm.

Preparation of leachate

For research purposes, a standard leachate was prepared from ground tyres and deionized water—100 g of sample dry matter per 1000 mL of deionized water. The leaching was conducted according to STN EN 12457-4, 2006 [1]. Then we prepared a mixture of leachate and activated sludge with three concentrations of leachate (350, 380 and 430 mg/L) for aerobic degradability testing. For anaerobic degradability testing, they prepared a mixture of leachate and digested sludge with three concentrations of leachate (350, 380 and 430 mg/L). Five samples were prepared for individual leachate concentrations. Individual tests were performed in five repetitions.

Preparation of inoculum for aerobic and anaerobic decomposition

A sample of activated sludge was taken from the biological grade aeration tank of the wastewater treatment plant. If the sludge has actively respired on the external substrate, it will be converted into an endogenous phase (that is, without residual external substrate). The sludge was aerated (at the desired temperature) for 4 h before use to maintain an aerobic state. An age of less than 24 h is recommended for the use of activated sludge. The age of our sludge was 4 h from the time it was taken from the activation tank. After recalculation, the OLR was set at the level of 20 mg_{COD}/m^3 h (tyre leachate with concentration 350 mg/L); 30 mg_{COD}/m^3 h (tyre leachate with concentration 380 mg/L), 45 mg_{COD}/m^3 h (tyre leachate with concentration 430 mg/L). The low levels of OLR are due to the fact that neither the inoculum nor the leachate was replenished continuously so as not to disturb the anaerobic environment. For this reason, the unit mg_{COD}/m^3 h was used instead of the standard unit for OLR kg_{COD}/m^3 h.

Used analytical methods

Determination of pH

Determination of pH of waste materials were conducted in compliance with the Slovak Technical Standard (STN) ISO No. 10390 using potentiometrically combined electrode [31].

Determination of the dry matter content

Dry matter (total solids—TS) was determined according to the STN No. 14346 [32].

Determination of VS

Determination of the loss by annealing in waste is in compliance with the STN No. 15169 [33]. Volatile solids was referred to dry matter (% DM).

Determination of chemical oxygen demand

Determination of chemical oxygen demand, COD_{Cr} was performed according to the standard STN No. 6060 determination by potassium dichromate/by titration [34].

Determination of biological oxygen demand

Biological oxygen demand (BOD_5) was determined as a difference of concentrations of dissolved oxygen in a sample before and after biochemical oxidation of organic substances under considerably standardized conditions of incubation of the sample. Determination was performed in compliance with the STN No. 1899-2 [35].

Determination of electrical conductivity

Direct determination, using an appropriate instrument, of the electrical conductivity of aqueous solutions [36].

Determination of metal content

The metal content in the rubber sample and tyre leachate before and after decomposition was determined by the AES-ICP atomic emission spectrometry method with inductively coupled plasma. Metals were determined according to ISO 11885—determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES) (ISO 11885:2007).

Biodegradability tests

To evaluate the degradability of waste tyres, we used the OECD 301 F test [37] and the OECD 311 test [38].

Manometric respiration tests 301 F with OxiTop® control measuring system under GLP conditions The test determines the complete biodegradability of organic substances in an aqueous medium by determining the oxygen consumption in a closed respirometer. The test specimen is added to mineral dilution water as the single carbon source. The CO_2 developing due to oxygen consumption is absorbed by concentrated NaOH and manometrically measured as a negative pressure [39]. Incubation took place in diffused light in a closed space in which a constant temperature of 20–25 °C was maintained. The environment could not contain toxic gases. Working volume of bottles was 500 cm^3 (600 ± 10 cm^3 total volume). After the addition of samples and inoculum, the bottles were being blown through by inert N_2 to create anaerobic environment. Consequently, the bottles were incubated at the temperature of 27 °C for the period of 24 h. The test was performed by the measurement of pressure difference during 240 h with continual stirring of the compound. The amount of substrate used for the anaerobic digestion process was calculated with respect to the limiting pressure in the OxiTop device. The declared maximum pressure difference was 300 hPa. In every test series, a reference substance (sodium acetate or sodium benzoate) is tested in a parallel series with a concentration of 100 mg/L. Activated sludge was prepared for biodegradation measurement by washing with water and aeration for 24 h without adding substrate. As an easily degradable reference substance, sodium acetate was used in the experiment. In endogenous respirometric measurements, the respiratory rate was calculated from the dependence of the decrease in oxygen concentration over a time interval. The volume velocity was calculated according to the relation:

$$r_{V,\mathrm{ox}} = \Delta c(\mathrm{O_2})/\Delta t,$$

where $r_{V,\mathrm{ox}}$ (mg/L h).

The specific velocity of respirometry was then calculated from the volume velocity according to the relation:

$$r_{X,\mathrm{ox}} = r_{V,\mathrm{ox}}/X_\mathrm{c},$$

where $r_{X,\mathrm{ox}}$ = 2.29 (mg/g h) and $_{X\mathrm{c}}$ is dry matter activated sludge.

The test was performed with six parallel repetitions and the results were presented as average values. The average value was 2.29 (mg/g h) ± 0.816. Confidence interval was 2.29 ± 0.653 (± 28.5%).

To calculate biodegradation percentage, the amount of oxygen taken up by the microbial population in test suspension (corrected for uptake by biotic control, run in parallel) was expressed as percent of the theoretical oxygen demand (ThOD), shown in formula (1):

$$\text{Biodegradation (\%)} = 100 \times \frac{\mathrm{BOD} - \mathrm{BOD_{Blank}}}{\mathrm{ThOD}}, \quad (1)$$

where BOD is biochemical oxygen demand of the test substance (mg/L), BOD_{blank} is biochemical oxygen demand of the biotic control (mg/L), ThOD is theoretical oxygen demand required when the target compound is completely oxidized (mg/L).

According to OECD 301 Annex IV formulas, the theoretical oxygen demand (ThOD) is calculated with the empirical formula, which differentiates between the mineralization of nitrogen to ammonia ($ThOD_{NH4}$) and the nitrification of nitrogen to nitrate ($ThOD_{NO3}$). The standard evaluation method uses the ThODNH4; the results for both reference values are quoted if necessary. With wastewater samples, the Chemical Oxygen Demand (COD) of the sample is used as the reference point instead of the calculated ThOD. For the toxicity test solutions with the reference substance, the degradation values relate to the $ThOD_{total}$ (sum of $ThOD_{Reference\ substance} + ThOD_{Test\ specimen}$). With wastewater samples, the chemical oxygen demand (COD) of the sample is used as the reference point instead of the calculated ThOD. We decided to use COD instead of ThOD. We made this decision for two reasons. First, we assume the formation of wastewater when leaching from tyres. Secondly, due to the complexity of the composition of tyres with the addition of various additives and the variable representation of the basic components, ThOD would be difficult to determine unambiguously. On the other hand, the titration determination of COD with subsequent recalculation of biodegradability is a more representative solution for us:

$$\text{Biodegradation (\%)} = 100 \times \frac{\mathrm{BOD} - \mathrm{BOD_{Blank}}}{\mathrm{COD}}. \quad (2)$$

Manometric respiration tests with pH adjustment Due to the nature of the tyres, the tested leachates reached high pH values. To achieve suitable conditions for aerobic decomposition, it was necessary to lower the pH. The pH of the leachates was adjusted using a diluted H_2SO_4 solution to the level 6.5–7.5.

OECD 311 anaerobic biodegradability of organic compounds in digested sludge: method by measurement of gas production This test was conducted according to OECD standard 311: anaerobic biodegradability of organic compounds in fermented sludge by measuring gas production [38]. Measuring principle—anaerobic degradation causes the formation of biological gas (CH_4 and CO_2) that results in an increase in pressure in the headspace of the gas-proof, closed graduated flask. The determination of the anaerobic biological degradability of organic compounds in digested sludge is described in the DIN EN ISO 11734 (1998) or DEV L47 as a process that involves the measurement of the biological gas production. Therefore, we consider the measurement of biogas production as an indirect determination of anaerobic biodegradability. Of course, the term anaerobic biodegradability and biogas production are not identical. Incubation took place in diffused light in a closed space in which a constant temperature of 20–25 °C was maintained. The length of the test was extended from the standard 28 days to 35 days. After recalculation, the OLR was set at the level of 2.314 kg_{COD}/m^3 h (tyre leachate with concentration 350 mg/L); 3.11 kg_{COD}/m^3 h (tyre leachate with concentration 380 mg/L), 4.34 kg_{COD}/m^3 h (tyre leachate with concentration 430 mg/L). The low levels of OLR are due to the fact that neither the inoculum nor the leachate was replenished continuously so as not to disturb the anaerobic environment. The environment could not contain toxic gases. To obtain a uniform temperature in the samples, the test solutions are stirred at a slow speed by an inductive stirring system. CO_2 was absorbed with KOH through a septum in a volumetric flask stopper. The absorption process leads to a decrease in pressure in the space above the head. Since the analysis of the biogas produced was not carried out, it is not possible to consider it as produced biogas without the share of CO_2. The test was performed with six parallel repetitions and the results were presented as average values.

OECD 311 anaerobic biodegradability with pH adjustment Due to the nature of the tyres, the tested leachates reached high pH values. To achieve suitable conditions for anaerobic decomposition, it was necessary to lower the pH. The pH of the leachates was changed using a diluted H_2SO_4 solution. In order to achieve suitable conditions for anaerobic decomposition, tyre leachates were adjusted to a value of 7. After adjusting the pH, the OECD 311 anaerobic degradability test was performed.

Ecotoxicity tests

Ecotoxicological tests (biotests) monitor reactions in which the test organism is exposed to the action of the examined sample. From the reaction of the test organism, it is subsequently possible to draw conclusions about the influence of the tested sample on free-living organisms [40, 41].

Test of inhibition (stimulation) of the growth of Lemna minor (Lemna minor) The principle of the test is the response of the growth rate of *Lemna minor* individuals in the test substance, compared to the control. The aquatic test is one of the tests on producers and was carried out in parallel on four 50-mL samples and one control. In each sample, the test started with 15 leaves of *Lemna minor* individuals. The samples were stored in an incubator with the simulation of day and night.

The determination of growth inhibition is based on the area under the growth curve (A), which is calculated for individual samples and the control, from which the inhibition (or stimulation) of growth (IAi) for individual samples is subsequently calculated. The growth rate (μ) is also calculated for each test sample and control, from which the growth inhibition (stimulation) (Iμi) is finally calculated for each test sample, the results are averaged.

Aquatic plant *Lemna minor*, which belongs to angiosperms (Angiospermophyta), class of monocots (Monocotyledonopsida), family of free-floating plants (Lemnaceae) [41–43].

Acute toxicity test on Daphnia magna The aim of the acute toxicity test is to quantify the effects of harmful substances on *Daphnia magna* organisms. The aquatic test is one of the tests on consumers and was carried out in parallel on four samples and a control. Individuals of guinea fowl are placed in an oxygenated test sample with a volume of 10 mL, in the number of 5 pieces, and in two intervals (24 and 48 h) all immobilized individuals of guinea fowl are counted. From the recorded results, the percentage of immobilization of the individuals in the samples is then determined in comparison with the control [41, 42, 44]. *Daphnia magna* is a crustacean of the Phyllopoda subclass, usually 2 to 5 mm in size, which looks like a human flea (*Pulex irritans*). Test individuals came from laboratory breeding [45].

White mustard (Sinapis alba) root growth inhibition (stimulation) test This terrestrial biotest is one of the tests on producers. The test consists in assessing the effect of the test substance on seed germination and root growth of white mustard *Sinapis alba* in the early stages of development compared to the control. The growth inhibition test takes place at a temperature of 20 ± 1 °C without access to light for a period of 72 h. There are 15 *Sinapis alba* seeds per 10 mL of the tested solution [41, 42, 46].

Sinapis alba is an economically important species of higher plant from the cabbage family (Brassicaceae). The

Table 1 Characteristics of substrates and inoculum in testing the aerobic biological degradability of waste tyres

	COD (mg/L)	Dry matter (%)	Volatile solids (% DM)	Conductivity (mS/cm)	pH
Tyre	X	99.90	58	x	x
Activated sludge	11,257.92	0.73	70	0.57	7.83
Leachate from tyres	450	0.07	0.06	2.20	11.74
A[a]	364.84	0.02	0.019	0.20	11.18
B[a]	406.54	0.05	0.04	0.80	11.12
C[a]	427.38	0.06	0.05	0.36	10.82

[a] A mixture of tyre leachate with concentration 350 mg/L and activated sludge; B mixture of tyre leachate with concentration 380 mg/L and activated sludge; C mixture of tyre leachate with concentration 430 mg/L and activated sludge

Table 2 Characteristics of substrates and inoculum in testing the anaerobic biological degradability of waste tyres

	COD (mg/L)	Dry matter (%)	Volatile solids (% DM)	Conductivity (mS/cm)	pH
Tyre	x	93.4	72.83	x	x
Digested sludge	23,607.2	1.17	80.25	6.84	7.51
A[a]	4413.5	0.10	0.09	2.26	7.99
B[a]	9237.6	0.10	0.09	2.05	8.08
C[a]	13,343.2	0.10	0.09	2.28	8.08

[a] A mixture of tyre leachate with concentration 350 mg/L and digested sludge; B mixture of tyre leachate with concentration 380 mg/L and digested sludge; C mixture of tyre leachate with concentration 430 mg/L and digested sludge

Table 3 Elemental analysis of the tyre

	N (%)	C (%)	H (%)
Sample tyre 3 mm × 40 mm	0.485	79,277	7329

Table 4 Metal content in tyre rubber

Heavy metal	Concentrations of heavy metals (mg/kg)	
	Our results	Study
Cadmium	0.186	2 [49]
Lead	2.93	15 [49]; 357 [50]
Chromium	3.86	175 [50]
Iron	307	381 [48]
Zinc	2533	6012 [49]; 5089 [50]

collected seeds of white mustard, of the Mega ochre-yellow colour, with a size of 1.5 to 2.5 mm and a germination rate of 99%, are used for the laboratory test [41, 42, 47].

Results and discussion

Evaluation of physico-chemical analysis and metal content in the sample

Physico-chemical analysis of substrates was an essential part of preliminary results for biodegradability testing. Table 1 presents the characterization of inputs during aerobic biological decomposition. It is important to perform an analysis not only of individual input substances, but also of mixtures.

Table 2 presents the characterization of inputs during anaerobic biological decomposition. Similarly, in the aerobic test, in addition to the substances, it is also necessary to analyse mixtures of leachates with sludge.

Table 3 presents the elemental analysis of tyre crumb. The measurement was carried out in the external laboratory of the LECO company in Prague, using the ASTM D 5373 method. It is an instrumental method that determines the elements nitrogen, carbon, hydrogen in a solid sample.

In the case of biological decomposition, it is also important to monitor metal concentrations. In the case of high concentrations of metals, inhibition or collapse of the entire process may occur. The concentrations of selected metals in tyre rube and their comparison with other studies are presented in Table 4. High concentrations of some of the heavy metals can lead to the assumption of disturbance of the stability of the decomposition process.

The concentrations of selected metals in tyre leachate and their comparison with other studies are presented in Table 5. High concentrations of some of the heavy metals can lead to the assumption of disturbance of the stability of the decomposition process.

A rapid decrease in the concentration of iron and zinc can be observed after leaching the waste in tyre runoff. The COD of waste tyre leachate in our analysis reached an average value of 427 mg/L. This value correlates with

Table 5 Metal content in tyre leachate

Heavy metal	Concentrations of heavy metals (mg/kg)	
	Our results	Study
Cadmium	0.011	0.28–0.96 [48]
Lead	0.134	1.0–160 [48]
Chromium	0.011	0.4–6.73 [48]
Iron	0.012	2.12–533 [48]
Zinc	0.005	463 [49]

[a] The concentrations of heavy metals our results in the leachate were compared with a study aimed at measuring heavy metals in tyre runoff

the results obtained by Sarasa et al. [51], who analysed a COD of 508 mg/L was found. Due to the low COD values, we can assume a low production of methane in the anaerobic biodegradability test.

Evaluation of biodegradability tests

Evaluation of aerobic biodegradability without pH adjustment

When evaluating aerobic decomposition, it was necessary to extend the standard length of the test from 28 to 35 days (Fig. 1).

The highest value of biodegradability (15%) was observed at the input leachate concentration of 350 mg/L. The most pronounced decomposition at the input tyre concentration of 350 mg/L was observed within 7–14 days of the test duration. In the same time interval, a significant course of biodegradability was detected even at the input concentration of 380 mg/L. At an initial concentration of 430 mg/L, a later onset of significant decomposition was observed within 14–21 days and then towards the end of the test 28–35 days. At an input concentration of 380 mg/L, the interval was 28–35 days.

Tsuchii and Tokiwa investigated the microbial degradation of strips or strips was investigated in growth experiments in which a strip of rubber (120 mm × 0.3 to 0.5 mm) was added as the sole substrate for growth. Using *Nocardia* sp. 835A strain Rc achieved 80% tyre reduction over 8 weeks. Using *Nocardia* sp. 835A more than 90% degradability was achieved within 3 weeks [52]. Linos et al. achieved using *Gordonia* sp. VH2 during 4 weeks more than 50% degradation of synthetic and natural rubber [30]. The reasons for the different degradability with our results can be seen in the activity of microorganisms—we used mixed cultures of microorganisms compared to isolated microorganisms. Differences in the implemented tests could also have caused the difference.

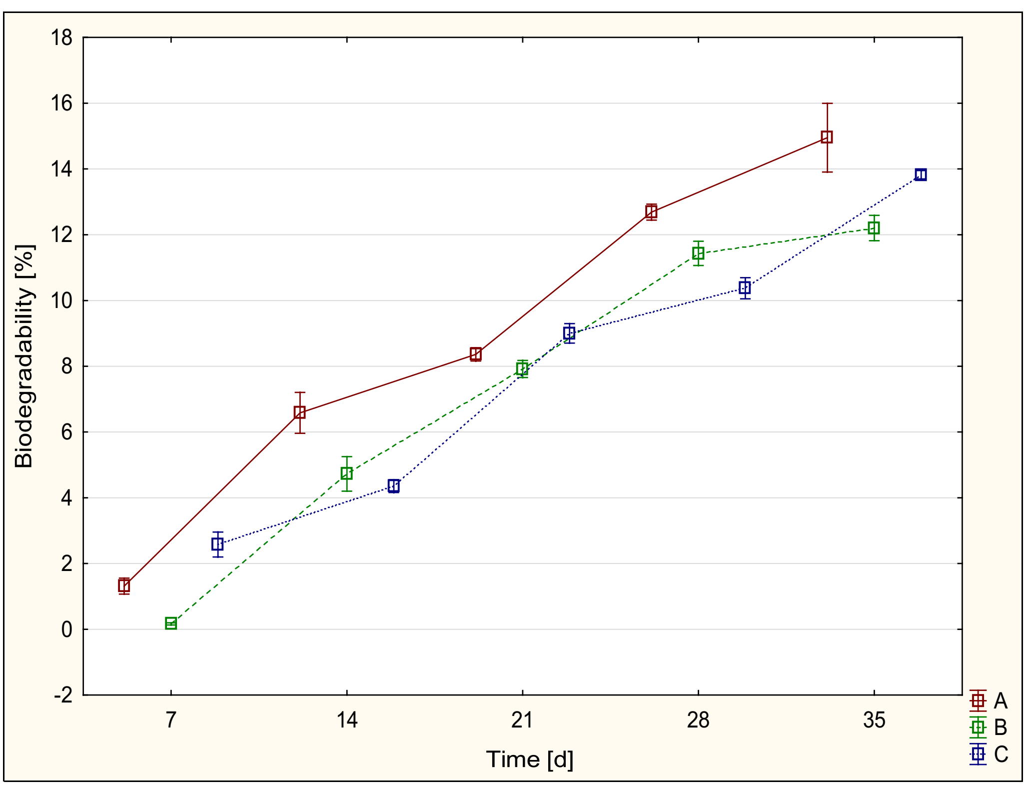

Fig. 1 Course of aerobic decomposition of waste tyres without pH adjustment. *A = 350 mg/L; B = 380 mg/L; C = 430 mg/L

Bode et al. using *Pseudomonas citronellolis* and *Acinetobacter calcoaceticus* achieved biodegradability of vulcanized natural rubber and synthetic high-molecular-weight poly(*cis*-1,4-isoprene) at the level of 13 and 13 during 10 weeks of incubation, respectively, 12% [53]. Bode et al. confirmed 12% biodegradation using *Xanthomonas* sp. and 18% biodegradation of natural and synthetic rubber using *Streptomyces griseus* 1D [54]. Rose and Steinbuchel achieved 18% biodegradation of natural and synthetic rubber using *Streptomyces griseus* 1D and *Streptomyces coelicolor* 1A [29]. This rate of biodegradation was closer to our results. It can be stated that the results of biological degradability are in some cases similar to our results, sometimes very different—we understand the test conditions (temperature, length of incubation), the method of carrying out the biodegradability test, or the choice of microorganisms used for decomposition as decisive factors.

Modelling the course of degradability without pH adjustment using a quadratic 3D graph

Mathematical analysis is one of the applicable tools for modelling the degradability of waste tyres in the natural environment. The final indicator was the achievement of 100% decomposition level (Fig. 2).

The course of biodegradability itself refers more to a quadratic course as a function of time and concentration compared to a linear course of degradability. Based on the model calculation, the highest levels of biodegradability would be achieved at an input concentration of waste tyres of approximately 400 mg/L. By modelling the quadratic dependence of degradability on time and input concentration:

$$\begin{aligned}\text{Biodegradability} = {} & 115.3298 + 0.9861x \\ & - 0.6196y - 0.0052x^2 \\ & - 0.0008xy + 0.0008y^2,\end{aligned} \quad (3)$$

X is time (d); y is the concentration (mg/L); z is the biodegradability (%).

After entering the values, the correlation coefficient reached 0.9495.

Based on the obtained equation, the duration of tyre decomposition can be determined, for example, at the input concentration of waste tyre 370 mg/L. Based on

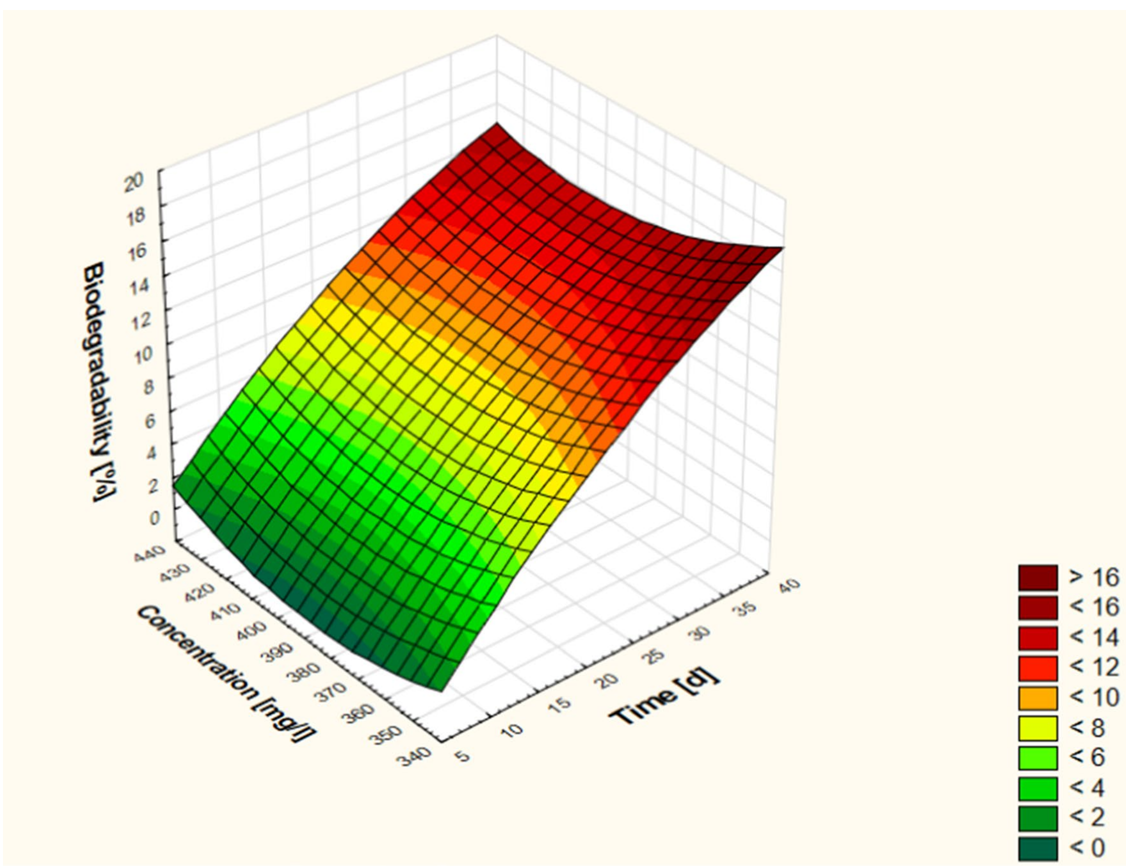

Fig. 2 Evaluation of the course of decomposition by 3D modelling

the calculation, waste tyres completely decompose in 336 days under our test conditions according to Eq. 3.

Evaluation of aerobic biodegradability with pH adjustment

In the aerobic test with pH adjustment, it was again necessary to extend the standard length of the test from 28 to 35 days (Fig. 3).

The highest values of biodegradability were found at the average concentration of tyre leachate (380 mg/L). The highest concentration of tyre leachate was defined by the lowest value of biodegradability. The highest biodegradability value of waste tyre leachate reached an average value of 23%. The lowest value of biodegradability of leachate of waste tyres reached an average value of 18.5%. Compared to the aerobic degradability test without pH adjustment, no significant deviations between parallel samples were observed. The aerobic degradability test confirmed the improvement of tyre decomposition when the pH was adjusted. Tyre biodegradability studies have not yet focused on adjusting the pH to neutral levels by acidification. Therefore, we understand this experiment as filling an empty space for future discussions.

Modelling the course of degradability with pH adjustment using a quadratic 3D graph

Modelling through a 3D graph is represented in Fig. 4.

After entering the values, the correlation coefficient reached 0.920485.

By modelling the quadratic dependence of degradability on time and input concentration, the following equation was obtained:

$$\begin{aligned}\text{Biodegradability} =& -63.1933 + 2.313x \\ &+ 0.2892y - 0.0162x^2 \\ &- 0.003xy - 0.0003y^2,\end{aligned} \tag{4}$$

x is time (d); y is the input concentration (mg/L); z is the biodegradability (%).

Based on the obtained equation, the duration of tyre decomposition can be determined, for example, at the input concentration of waste tyre 370 mg/L. Based on the calculation, waste tyres completely decompose in 126 days under our test conditions according to Eq. 4. The total length of degradability was reduced by 63% due to adjustment.

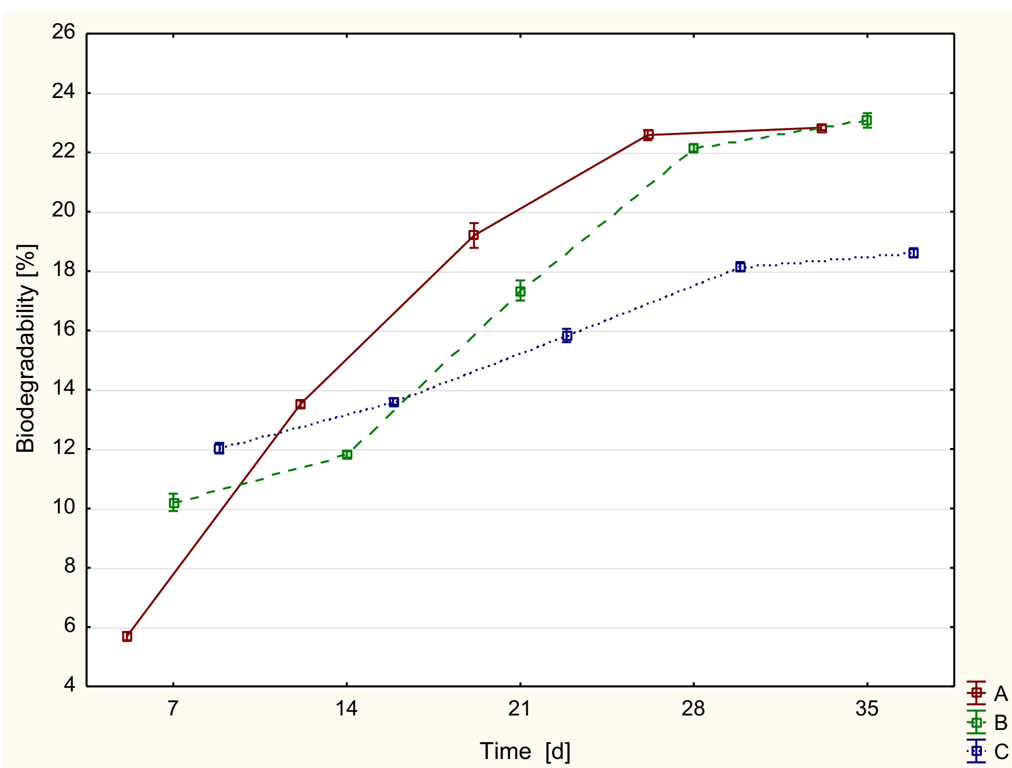

Fig. 3 Progress of aerobic decomposition of waste tyres with pH adjustment. *A = 350 mg/L; B = 380 mg/L; C = 430 mg/L

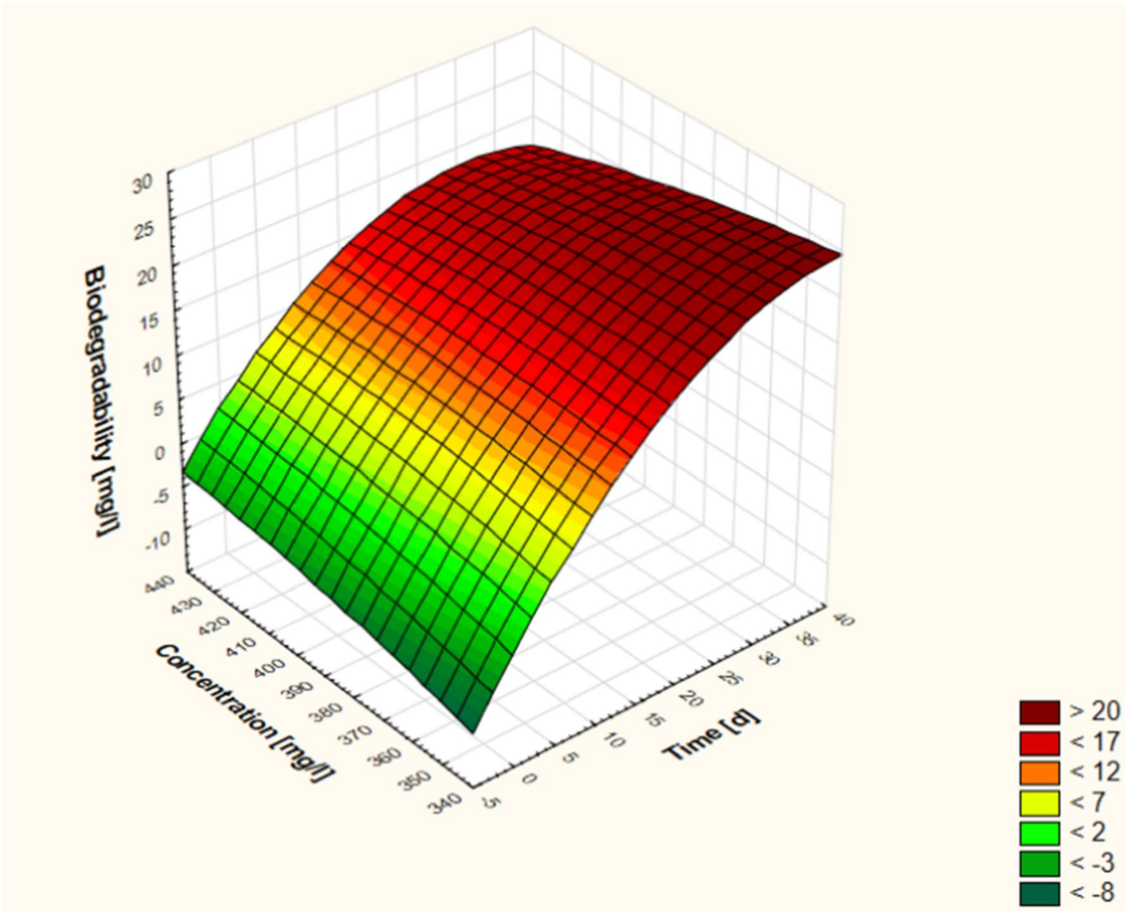

Fig. 4 Evaluation of the course of decomposition by 3D modelling

Evaluation of anaerobic degradability without pH adjustment by measuring biogas production

Anaerobic decomposition without pH adjustment is presented in Fig. 5. Anaerobic decomposition without pH adjustment was carried out at 4 different concentrations. The specific biogas production was selected as a parameter of anaerobic digestion.

Anaerobic decomposition of waste tyres was confirmed the highest production of biogas at a concentration of 380 mg/L. At this concentration, the maximum biogas production of 5.3 m^3/t_{VS} was found. The lowest average biogas production was observed at the waste tyres concentration of 430 mg/L. At this concentration, an excessive biogas production of 3.1 m^3/t_{VS} was found.

Evaluation of anaerobic degradability with pH adjustment by measuring biogas production

Anaerobic decomposition with treatment is shown in Fig. 6. Anaerobic decomposition with pH adjustment was carried out at 4 different concentrations. The specific biogas production was selected as a parameter of anaerobic digestion.

The highest production of biogas was found at the level of 4.4 m^3/t_{VS} at a concentration of 350 mg/L. Ward et al. [55], reported that the pH range of 6.8–7.2 is ideal for anaerobic digestion. They also reported that the optimal pH of methanogenesis is around 7.0 and the optimum pH of hydrolysis and acidogenesis is between 5.5 and 6.5 [55]. The improvement of the anaerobic degradability of tyres was not confirmed by changing the pH.

Evaluation of ecotoxicity tests

Testing of the leachate was conducted by a preliminary acute toxicity test. The test subjects used were younger than 24 h, at least third generation. The conditions of the test indicate that the result of the preliminary test is negative if < 50% of the beads died or immobilized during the test compared to the control group.

The result of the preliminary test was positive, as after 24 h immobilization occurred in 60% and after 42 h in

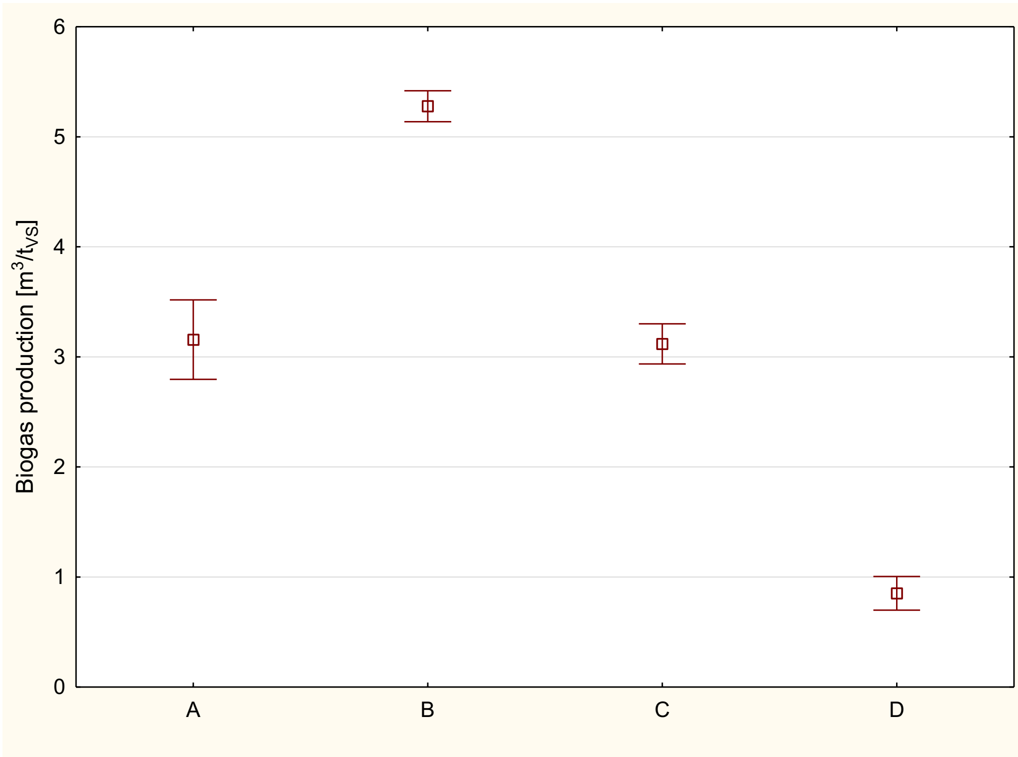

Fig. 5 Biogas production during anaerobic digestion without pH adjustment. *A = 350 mg/L; B = 380 mg/L; C = 430 mg/L; D = blank sample

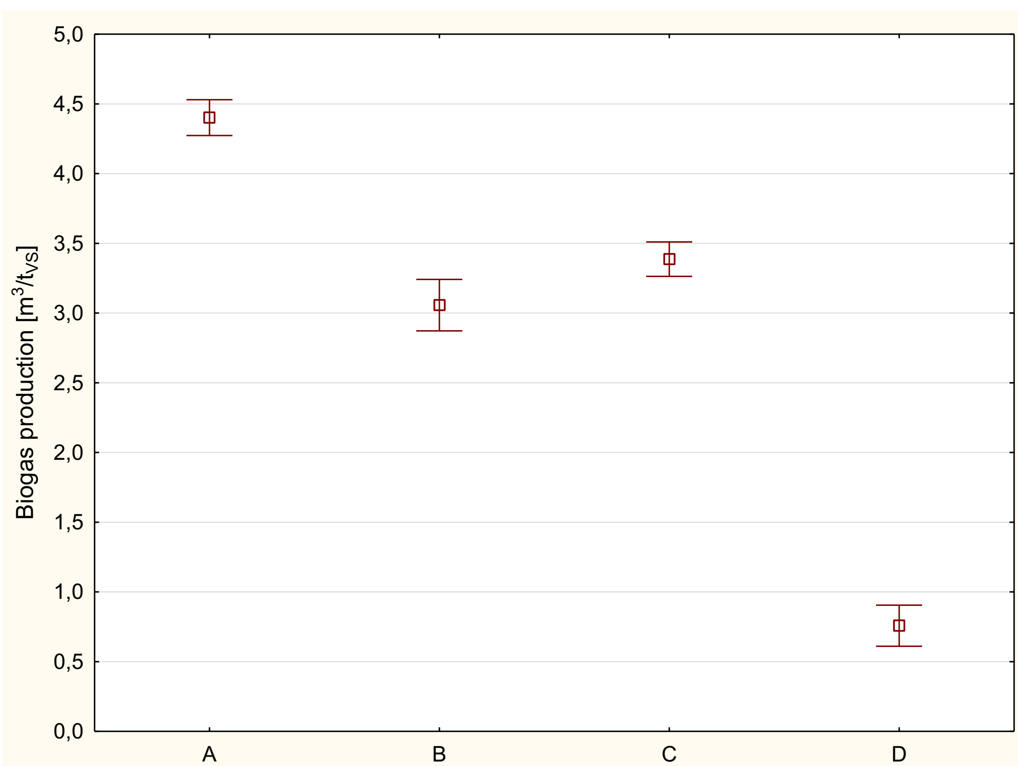

Fig. 6 Biogas production during anaerobic digestion with pH adjustment. *A = 350 mg/L; B = 380 mg/L; C = 430 mg/L; D = blank sample; E = reference sample

75% of the test individuals. The test results showed that the leachate from waste tyres contains substances that were toxic to this type of aquatic organisms.

Lemna minor growth inhibition (stimulation) test In the growth inhibition test, the growth rate (μ) was calculated based on the number of leaves recorded in the time periods, and the growth rate inhibition (Iμ) was then calculated from it. The test is negative if the inhibition of the growth of the plant culture is < 30% or the stimulation is < 75% compared to the control. The calculated inhibition of the growth rate (Iμ) reached a value of 81.24%, which means that the test was positive, and the aqueous leachate contained substances that were toxic to this type of aquatic organism. No necrotic leaflets were observed in the test organisms during the test.

According to Rozman and Kalčíková, a significant reduction in specific growth rate was observed when *Lemna minor* was exposed to tyre wear particles [56]. It was confirmed that microplastic particles can impact root length of *Lemna minor* due to the irregular shape of particles and rough surface, which is in accordance with the surface analysis [57]. Because biomonitoring and the use of bioindicators are an important way to monitor pollutants as complex as waste tyres, both a systematic survey and a field study under environmentally relevant conditions are recommended. Root length was significantly reduced when duckweed was exposed to microplastics of old tyre. Old tyre reduced root length by 37 ± 6%, respectively [58]. Fořt et al. in an ecotoxicological test, when *Lemna minor* was exposed to a waste tyre, it achieved 48% growth inhibition [5]. These results agree with our results.

White mustard (Sinapis alba) root growth inhibition (stimulation) test Testing of the aqueous tyre leach sample was conducted with a preliminary test of inhibition (stimulation) of the growth of the root of white mustard in the initial stages of development. In the inhibition test, white mustard was a representative of higher plants and cultivated crops. In the test, based on the recorded length of the roots, the average root length was calculated for each of the parallel samples, including the control, and growth inhibition (Iμi) was subsequently calculated from them.

The test conditions indicate that the test result is positive if root growth inhibition ≥ 30% or stimulation ≥ 75% compared to the control. The calculated growth inhibition (Iμi) reached a value of 65.1%, which means that the test was positive, and the water leachate contained substances that were toxic to this type of higher plant.

It was demonstrated that growth of *Sinapis alba* was significantly limited due to of microplastics from ground tyres [59]. Rozman et al. studied effects of microplastics and microplastic leachates on a common duckweed (*Lemna minor*). Microplastic of waste tyre most significantly affected the root length of duckweed [58]. Fořt et al. deals with the exposure of freshwater and soil organisms to rubber crumb. *Sinapis alba* reached 31% inhibition of growth root *Lemna minor* [5]. Our results agree with the positive growth inhibition results of these ecotoxicological tests.

Leachate of waste tyre not significantly affected the root length of duckweed [58]. Differences in the toxicity of waste tyre leachates can be caused by the different concentration of tyre leachates.

Conclusion

The aim of the scientific work was to observe the biological degradability of waste tyres in aquatic environment and its ecotoxicity. The benefit of the scientific work is in the determination of the biodegradability of waste tyres with and without pH treatment, which simulate a comparison of the degradability of tyres in an acid rain environment. By monitoring, it is possible to determine whether acid rain as an anthropogenic activity influences the degradability of waste tyres in the natural environment.

Biodegradability tests confirmed the low biological degradability of waste tyres.

Biodegradability tests confirmed the low biological degradability of waste tyres. The highest value of biodegradability (15%) was observed at the input leachate concentration of 350 mg/L. The most pronounced decomposition at the input tyre concentration of 350 mg/L was observed within 7–14 days of the test duration. The highest values of aerobic biodegradability with pH adjustment were found at the average concentration of tyre leachate (380 mg/L). The highest concentration of tyre leachate was defined by the lowest value of biodegradability. The highest biodegradability value of waste tyre leachate reached an average value of 23%. Adjusting the pH to a neutral environment increased aerobic degradability, but had no significant effect on anaerobic degradability. Based on the calculation, waste tyres completely decompose in 126 days under our test conditions. The total length of degradability was reduced by 63% due to adjustment—at the input concentration of waste tyre 370 mg/L. Therefore, it is important to focus future research on the adjustment of various conditions to support the degradability of tyres, of which pH has clearly been confirmed as an important factor.

The low rate of biogas production was confirmed by our study. Anaerobic decomposition of waste tyres without pH adjustment was confirmed the highest production of biogas at a concentration of 380 mg/L. At this concentration, the maximum biogas production of 5.3 m^3/t_{VS} was found. The highest production of biogas with pH adjustment was found at the level of 4.4 m^3/t_{VS} at a concentration of 350 mg/L.

The result of the preliminary ecotoxic test was positive, as after 24 h immobilization occurred in 60% and after 42 h in 75% of the test individuals. The test results showed that the leachate from waste tyres contains substances that were toxic to this type of aquatic organisms.

The calculated inhibition of the growth rate (Iμ) reached a value of 81.24%, which means that the test was positive, and the aqueous leachate contained substances that were toxic to this type of aquatic organism. No necrotic leaflets were observed in the test organisms during the test. The calculated growth inhibition (Iμi) reached a value of 65.1%, which means that the test was positive, and the water leachate contained substances that were toxic to this type of higher plant.

The ecotoxicity tests performed confirmed the toxic effect of tyre leachate on selected tested organisms. The toxic effect was probably caused by the presence of metals in waste tyres.

In our study, the toxicity of waste tyres was evaluated via preliminary toxicity tests. For a closer specification of toxicity (low, medium, high), it is necessary to carry out basic tests, which can be the subject of future research. In the future, the authors propose to focus on modifying the biodegradability tests by changing the conditions (wider range of input pH value, longer biodegradability time, temperature) for a better simulation of different types of environments.

Abbreviations

AES-ICP	Inductively coupled plasma atomic emission spectroscopy
AD	Anaerobic digestion
BMP	Biochemical methane potential
BR	Butadiene rubber
BOD	Biochemical oxygen consumption
BT	Benzothiazoles
COD	Chemical oxygen demand
EC_{50}	Effective concentration EC_{50} (effective concentration), the concentration of a chemical substance that causes a toxic effect on 50% of test organisms
GPC	Gel permeation chromatography
MBT	2-Mercaptobenzthiazole
NH	Non-hazardous
NR	Natural rubber
OLR	Organic load rate
PECs	Expected concentrations with effect
SBR	Styrene butadiene rubber
VS	Volatile solids
TCR	Tyre crumb rubber
TWP	Tyre waste particles

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Author contributions

DS and HH wrote the main manuscript text. AP and JP performed the analysis and biodegradability tests. MS prepared Tables 1, 2, 3 and 4. JS prepared Figs. 1, 2, 3, 4, 5, and 6. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article (and/or) its additional files.

Declarations

Ethics approval and consent to participate

Informed consent/consent to participate was obtained from all individual participants included in the study.

Consent for publication

Consent to publish was obtained from all individual participants included in the study.

Competing interests

The authors have no relevant competing interests to disclose.

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