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A holistic approach to determine fiber release by textile laundry, biodegradation and ecotoxicity (DIN SPEC 4872) – preliminary study with dyed cotton textiles

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ABSTRACT

When textiles are washed, natural and synthetic fibers are released that cannot completely be retained by waste water treatment plants. These microfibers can enter freshwater ecosystems and marine habitats and have unpredictable negative effects on the environment. Until now, there has been no standardized approach for determining the environmental impact of microfibers released during washing. Against this background, a new test procedure to investigate and classify the environmental impact of textiles during laundering was developed (DIN SPEC 4872). In this test procedure, textiles are classified with regard to fiber release during the washing process using a suitable analysis system – the Dynamic Image Analysis. In addition, the microfiber release is classified by testing its biodegradability in waste water using a respirometer system. Finally, a suitable ecotoxicity test with a model aquatic organism (Lemna minor) is carried out, to determine the toxicity of the fiber residues after the biodegradation process. With this holistic test procedure, it is possible to investigate the environmental impact of synthetic microfibers (microplastics), as well as natural microfibers in a standardized way. The consideration of textiles made of natural fibers is just as important as the consideration of synthetic ones, because their fiber release capacity, biodegradability and ecotoxicity can be influenced by textile finishes such as dyes and can therefore also pose a risk to the environment. This paper focuses on the presentation of the new test method using exemplary dyed cotton samples.

Keywords
microplastic,
microfiber,
fiber release,
Dynamic Image Analysis,
textile laundry,
waste water,
biodegradation,
ecotoxicity,
environmental impact

1 Introduction

1.1 Relevance

Microfiber and microplastic pollution is a growing environmental problem due to its potential negative impact on the environment. In many studies, the term “microfiber” is used for fibrous or filamentous microplastics (FMP), which are e.g. released from synthetic textile materials [1-4]. Microplastics and FMP are generally insoluble in water, difficult to biodegrade and can accumulate in organisms [5]. In aquatic ecosystems, FMP can impair the development, reproduction and mortality of marine and freshwater organisms [6]. This can also become a global health risk for humans, as plastic particles enter the human food chain and water supply [7-10]. But natural microfibers are also important to consider, because they can also have environmental impacts, due to finishings used in textile production [11]. Therefore, this study refers to the definition that specifies a size of less than 5 mm for microfibers and that the origin can be natural or synthetic [12]. Clothing and textiles lose natural microfibers and FMP throughout their life cycle, during production, during use, e.g. washing and drying, and after disposal [13]. Studies show that approximately 0.12 - 1,107 MMT of synthetic and natural microfibers are generated alone during the washing process [4,13-14]. The reason for this is the mechanical and chemical stress on the textiles during the washing process, also known as Sinner’s parameters (water, chemistry, temperature, mechanics and washing time) [15]. The microfibers discharged via the washing process cannot be completely retained by waste water treatment plants [6,10,16]. This pathway is a potential source for microfibers to enter the environment and be carried further into rivers, lakes and the depths of the oceans [4,7,9-10]. It is estimated that 80% of the world’s waste water is discharged directly into the environment without any waste water treatment, suggesting that microfiber emissions in these areas are significantly higher than in areas with waste water treatment [13]. FMP, which are released from textiles during the washing process, represent one of the largest microplastic fractions in the environment with a total quantity of around 35% [17]. Numerous studies on microfiber emissions focus only on synthetic fibers, as natural fibers tend to biodegrade faster, and it is therefore concluded that they are less harmful to the environment [5]. Nevertheless, approximately 60%-80% of the identified microfibers in the environment are cellulose-based fibers. However, little is currently known about the impact of natural fibers, especially finished ones [4-5,12,18-19].

Raw natural fibers are able to biodegrade without eroding into persistent particles during the biodegradation process. Normally, the biodegradability of natural fibers is higher than of FMP, as most microbes are able to metabolize natural polymers [5,20]. However, this does not mean that natural fibers which are biodegradable are completely harmless. Concerns have been raised about the potential risks associated with non-synthetic fibers [12,16,18,21]. Natural fibers remain in the environment for a certain period as well until they are completely biodegraded and can also have a negative environmental impact during this time or afterwards. To create specific fiber properties, finishings are used in textile production that can influence the fiber release, the degradation process and the ecotoxicological environmental impact of the fibers [4,12,22]. In addition, synthetic and natural fibers can absorb chemical substances, but in different ways. If fibers degrade quickly, such as raw cellulosic fibers, a toxic substance may be available to the environment more rapidly, because the chemicals can be released after fiber degradation. FMP which degrade slowly or not at all, make the chemicals available to the environment later and therefore keep chemical pollutants inaccessible for longer [6,12,16].

1.2 Objective

The release of fibers from textile products during laundering is actually difficult to generalize and the methods used can hardly be compared, because there are still no standard definitions and internationally uniform, standardized research methods [12-13,16]. So far, no correlation between fiber release during washing and the textile construction or the fiber raw material has been proven. Further research is needed in this area, as well as into the possible negative effects of microfibers out of natural and synthetic origin the environment and health [23-25]. Therefore, new standardized test methods for measuring fiber release from various textile materials and the associated negative environmental impacts must be developed and established [26]. This is the only way to identify and eliminate the negative environmental impacts associated with fiber loss from textiles.

Against this background, Hohenstein and industry partners developed the new test method DIN SPEC 4872, which represents a standardized test procedure to analyze the fiber release from textile materials and their environmental impact during and after the washing process. The fiber release is analyzed with a suitable analysis system, the Dynamic Image Analysis (DIA). The analysis of the environmental impact of the fiber release includes a biodegradability test in aqueous medium and an ecotoxicological screening that evaluates the toxic effects of the textile residues after biodegradation [27].

In this article, the new test method is presented using examples of dyed cotton samples, for a better understanding of the process and also to illustrate the potential environmental impact of dyed natural microfibers.

2 Methods

In order to determine the environmental impact of textiles during washing, the following tests were carried out one after the other. The test procedure is divided into two sections, firstly the analysis of fiber release during washing and secondly the analysis of the environmental impact of the released fibers. In general all textile materials consisting of natural or synthetic raw materials or blends can be tested in this test setup. The textile materials tested can be raw or finished and all textile constructions can be tested, such as woven, knitted and felted piece goods. Identical materials in different colors have to be tested individually [27].

In this study two textile samples in the same construction (single jersey), same raw material (cotton), but differently dyed (grey and pink) are tested exemplarily.

2.1 Analysis of fiber release

For the lab scale washing process, four test specimens had to be cut from each textile material in order to carry out the test in a fourfold approach. The specimens were cut to a size of 150 mm × 290 mm, with two specimens cut lengthwise and two specimens cut crosswise. The edges were folded into a double rolled hem, two on the front and two on the back and were sewn with a lockstitch using 100% polyester thread [27].

2.1.1 Labscale washing process

The determination of the fiber release from textiles during washing was carried out by a simulated washing process on laboratory scale with a Gyrowash washing machine and defined washing conditions, which are relevant for comparable and reproducible results. The washing process is based on the current state of scientific knowledge, the DIN EN ISO 4484-1 [28]. Four Gyrowash containers (1200 ml) were required for the washing process. Each container was loaded with 50 stainless steel balls (⌀ 6 mm), 360 ml ultrapure water and one textile sample, to simulate the mechanical load of the washing process. The washing process took place at (40 ± 3) °C for (45 ± 1) min at a rotation speed of (40 ± 2) min^{-1} without detergent. After the washing process, each test specimen was rinsed three times with ultrapure water and carefully squeezed. The process waste water from each container was decanted through sieves (mesh size < 6 mm) into 4 stationary cylinders. The process water collected in the stationary cylinders was filled up to 500 ml with ultrapure water and was then ready for the measurement of the fiber quantity with the Dynamic Image Analyzer [27].

2.1.2 Dynamic Image Analysis (DIA)

The collected process water was analyzed with a Dynamic Image Analysis device to determine the fiber release. The analysis had to be performed directly after the washing process; otherwise the collected process wastewater has to be homogenized using a magnetic stirrer. The device for dynamic image analysis had to be calibrated before starting the analysis and measured with a control sample with 1000 mg reference fibers in 100 ml ultrapure water. For the actual measurement, 500 ml of the process waste water from each test specimen was passed through the system twice and then cleaned with ultrapure water. Each of the four approaches was measured twice, so that eight measured values were obtained for one textile material, from which an arithmetic mean value was formed. The detection signals from the dynamic image analysis measurements were converted into binary images by the integrated software. Two sets of different evaluation parameters were used to evaluate the results, one for straight and one for bent fibers. Only fibers that correspond to these shape and length properties were considered in the quantitative evaluations. A minimum fiber length of 50 µm and a minimum diameter of 7 µm were defined for the evaluation of the fibers. In addition, the fiber morphology (straightness, bending properties) was also included in the evaluation. The total fiber quantity was calculated by adding the fiber quantities of the straight and bent fibers. The final result was the total amount of fibers per g of textile material, the average length and the length distribution of the detected fibers [29-30].

2.1.3 Chemical separation of cellulose and polyester fibers

In order to determine the percentage of cellulose and polyester fibers in the process waste water of washed blended textiles, the waste water was filtered through a cotton filter after the DIA. This was followed by a sulphuric acid treatment, whereby the cotton filter as well as the cellulose fibers dissolved, leaving the polyester fibers behind. Afterwards it followed a further analysis with the DIA, whereby the number of polyester fibers per g textile material as well as the average length and the length distribution were determined. With this result and the total fiber quantity, it was also possible to calculate the cellulose fiber quantity [29-30].

2.2 Analysis of biodegradation

In order to obtain reproducible and comparable results, it was necessary to use a certain amount of fibers for the biodegradation analysis and the subsequent ecotoxicological assessment. For a triple approach, 60 mg of fibers were required. Another 1 g of the fibers was needed for an elementary analysis of the textile material, which in turn was necessary for the calculation of the biodegradation level of each material. The required amount of fibers was produced by milling in a cryogenic planetary ball mill [27]. Afterwards, the aerobic biodegradation test in aqueous medium in a respirometer system was carried out, according to DIN EN ISO 14851. Each approach consisted of 20 mg of textile fibers in 200 ml of aqueous medium with sludge in a concentration of 5% by volume. Additionally, a blank control was needed, where only the aqueous medium with sludge was added, no fiber discharge. As a positive control, a biodegradable reference material consisting of a raw 100% cotton fabric was used. As negative control, a non-biodegradable reference material consisting of 100% polyester was used. The test duration was limited to a maximum of 2 months. The approach could be stopped earlier if a plateau is reached for at least 7 days. During the test run, the respirometer system tracked the biochemical oxygen demand (BOD) of each sample and provided raw data with which the biodegradation level could be calculated according to mathematical formulas described in DIN EN ISO 14851. The biodegradation level of a sample was determined by relating the biochemical oxygen demand of the respirometer system (BOD) to the theoretical oxygen demand (ThOD), which is the amount of oxygen that is theoretically needed to degrade the total sample and could be calculated with the results from the elementary analysis. The biochemical oxygen demand (BOD) was determined from the difference between the oxygen demand of a waste water sample with testing material and a waste water sample without testing material (blank). The result of the biodegradation test was given as degradation level in percent [27,31].

2.3 Analysis of ecotoxicological impact

For the ecotoxicological testing the duckweed growth inhibition assay was used, according to DIN EN ISO 20079. The liquid media with the fiber residues after biodegradation served as test substances. The threefold approach of each biodegradation test was pooled. For cultivation of duckweed (*Lemna minor*), a duckweed growth medium according to DIN EN ISO 20079 was used. At the beginning of the test, the pooled samples were added to duckweed in growth medium in a threefold approach. Duckweed in growth medium without addition of test substances was used as negative control (no influence on the growth of duckweed) and with addition of potassium chloride as positive control (inhibition of the growth of duckweed). The blank control of the biodegradation test with a sludge concentration of 5% by volume, without any fiber residues, was used as the blank control of the ecotoxicological assay. The blank control of the ecotoxicological assay was diluted to such an extent that it shows no influence on the growth behavior of duckweed, according to DIN EN ISO 20079, Annex B. All the test substances were diluted in the same way, before adding to the duckweed growth medium. For the evaluation of the ecotoxicological test, the number of grown duckweed fronds was counted 48 h and 120 h after start of the test and at the end of the test as the main parameter. As a second monitoring parameter, the dry mass of the grown duckweed was determined at the end of the test (seven days after addition of the test and control substances). After evaluating the monitoring parameters, the mean growth rate per day (%) was calculated from the number of grown fronds of the duckweed plants, using a mathematical formula described in DIN EN ISO 20079. The growth rate per day of the blank was equated to 100% and the test samples were related to the blank. An ecotoxic effect existed, if the mean growth rate per day (%) of a sample to be tested was > 10% lower compared to the blank control. The ecotoxicological impact was rated as either ecotoxic or not ecotoxic, depending on the test result [27,32].

2.4 Classification Code

Once the test procedure has been completed, a classification code was created as a final result that reflects the results of all three test sections - the amount of fibers released during the washing process (fibers / g textile), the biodegradation rate in waste water (%) and the ecotoxicological potential of the degradation residues, which remain at the end (Table 1) [27].

Table 1. Classification scheme, to reflect the amount of fibers released from a textile material during laundering, the biodegradation rate in waste water and the ecotoxicological potential of the degradation residues.

Fiber release (amount / g textile)	**Biodegradation level (%)**	**Ecotoxicological impact**
A (< 1500)	1 (> 80)	
B (1500-6000)	2 (41-80)	ecotoxic [T]
C (> 6000)	3 (5-40)	not ecotoxic [ ]
	4 (< 5)	

3 Results

In the following, the results of the individual steps of the test method DIN SPEC 4872 for two textile materials made of 100% cotton, with the same construction (single jersey) and fiber treatment, are presented. Only the type of dyeing differs (one material is dyed grey and one material is dyed pink). No specific information is available on the chemical composition of the dyeing. The aim of these results is to show exemplarily the possible influence of finishes on fiber release, biodegradability and ecotoxicity of textile materials, detectable using the new standardized test method.

3.1 Exemplary results of Dynamic Image Analysis (DIA)

Hereafter, typical results recorded and displayed by DIA are shown. For the two tested materials – 100% cotton, single jersey, grey dyed (a) and 100% cotton, single jersey, pink dyed (b) – the total fiber amount per gram textile material, the mean length (µm) of the fibers and the length distribution were obtained as results. Figure 1 (a) shows the fiber length distribution of the fiber release during washing of a 100% cotton single jersey, grey dyed. The total fiber amount per g textile material is 613 fibers. The mean fiber length is 285 µm. Figure 1 (b) shows the fiber length distribution of the fiber release during washing of a 100% cotton single jersey, pink dyed. The total fiber amount per g textile material is 1883 fibers. The mean fiber length is 355 µm.

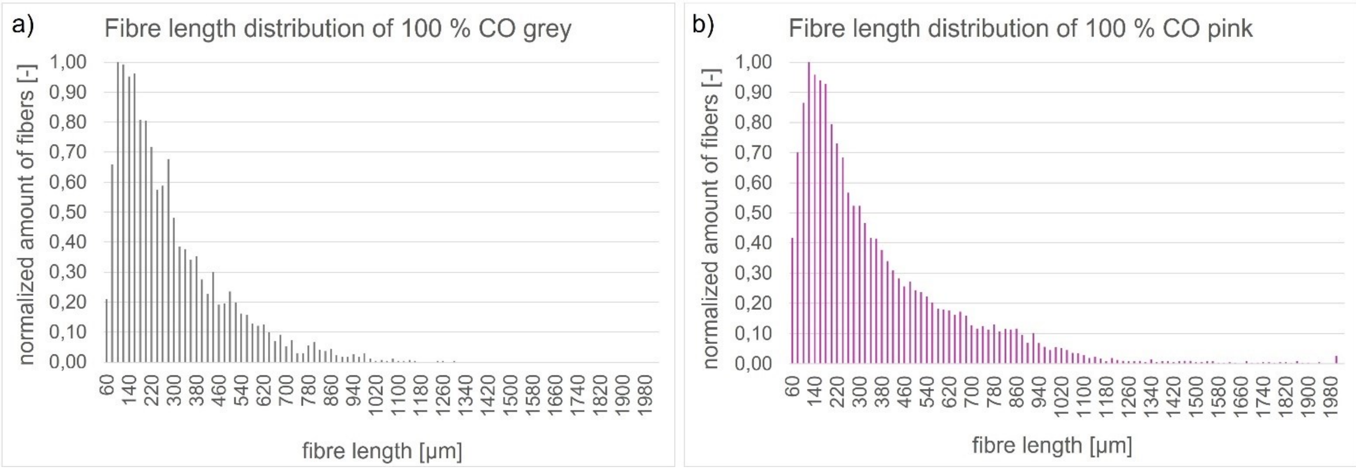

Fig. 1 Length distribution of the fiber release of (a) 100% CO, Single Jersey, grey dyed; (b) 100% CO, Single Jersey, pink dyed. n=8.

3.2 Exemplary results of biodegradation

Figure 2 shows the degradation level (%) of the fibers of the two tested textile materials – 100% cotton, grey dyed, and 100% cotton, pink dyed – over a period of 40 days. Additionally, the degradation levels of the positive control fibers 100% cotton, raw and 100% polyester are presented. Moreover, the degree of degradation of 50% cotton / 50% polyester blended fibers is shown, to clarify the difference in degradation of natural and synthetic fibers.

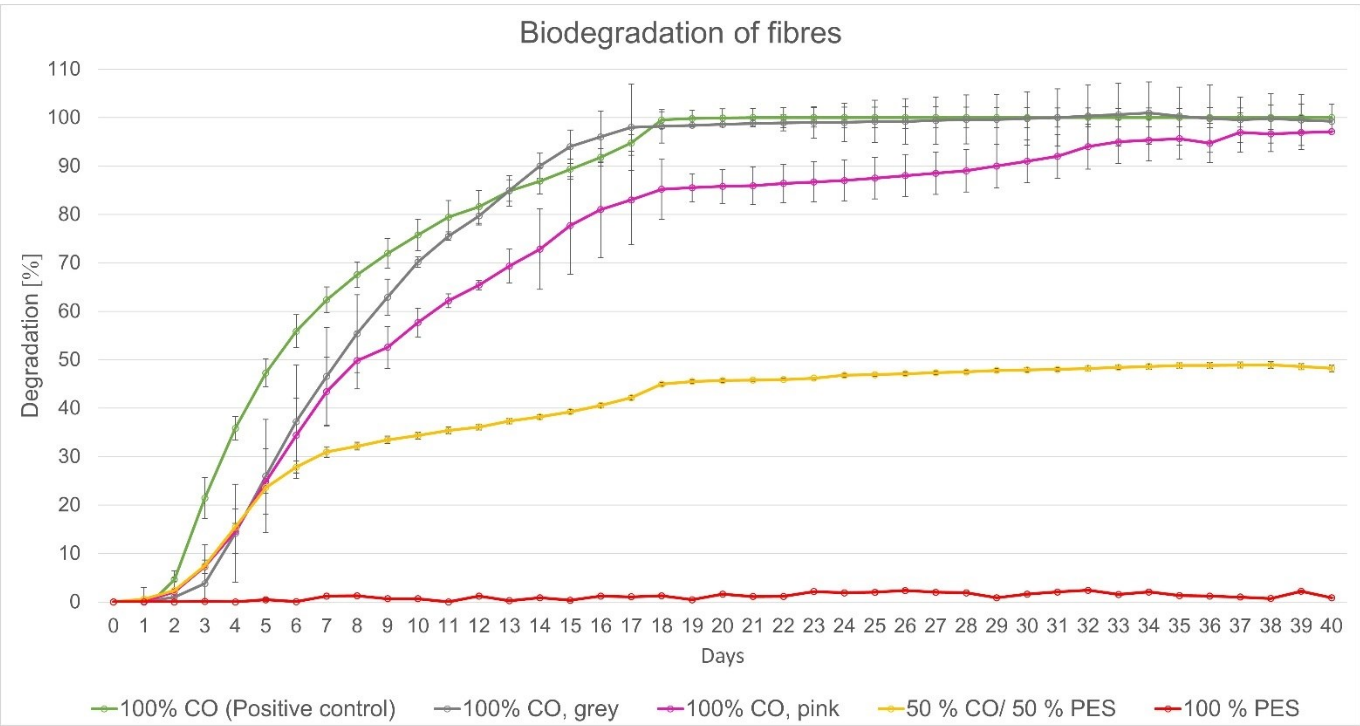

Fig. 2 Degradation level (%) of fibers in waste water over a period of 40 days (n=3). 100% CO, raw (green line), 100% CO, grey dyed (grey line), 100% CO, pink dyed (pink line), 50% CO / 50% PES (yellow line), 100% PES (red line).

3.3 Exemplary results of ecotoxicological screening

Figure 3 shows the results of the duckweed growth inhibition assay (DIN EN ISO 20079) after 40 days of biodegradation of the two tested textile materials – 100% cotton, grey dyed and 100% cotton, pink dyed. The degradation residues of 100% cotton, grey dyed show no negative influence on the growth behavior of duckweed. The degradation residues of 100% cotton, pink dyed, on the other hand, lead to a more than 10% inhibited growth rate compared to the blank, what means that a toxic effect exists.

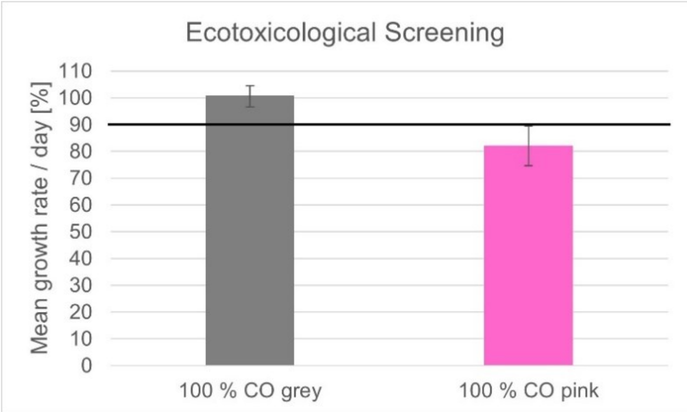

Fig. 3 Ecotoxicological effect of degradation residues from 100% cotton, grey dyed and 100% cotton, pink dyed after 40 days of biodegradation (n=3).

3.4 Exemplary classification codes

In Table 2 the classification codes for the two tested textile materials are shown, resulting of the three test sections (fiber release, biodegradation, ecotoxicity). 100% cotton, single jersey, grey dyed, received the classification code “A1”, based on the results of fiber release with 613 fibers per gram textile material, the biodegradation level of 99.5% and no ecotoxicological impact. 100% cotton, single jersey, pink dyed, received the classification code “B1[T]”, based on the results of fiber release with 1883 fibers per gram textile material, the biodegradation level of 96.9% and an ecotoxicological impact.

Table 2. Exemplary classification codes of three different textile materials.

Textile material	Fiber release (amount / g textile)	Biodegradation level (%)	Ecotoxicological impact	Classification Code
100% CO grey	613	99.5	-	A1
100% CO pink	1883	96.9	[T]	B1[T]

4 Conclusion and outlook

In this study, the new test method according to DIN SPEC 4872 was presented. It is a test method for textiles including the analysis of fiber release from textiles during the washing process, the determination of the biodegradation rate of the fibers in waste water and the potential ecotoxicological impact of the fiber residues after biodegradation. This new standard makes it possible for the first time to test, evaluate and compare textile products regarding fiber release during washing and its environmental impact.

To determine the fiber release of textiles, Dynamic Image Analysis was used in this study. Dynamic Image Analysis is an optical detection system for the analysis of particles in fluids that has the potential to overcome disadvantages of other methods known for the analysis of fiber discharge from textiles, mentioned in the following [29,33]. One method which is often used to analyze the fiber release of textiles is a waste water filtration method with subsequent manual or automated counting of the fibers by light microscopy or scanning electron microscopy [3,33]. This is a highly time-consuming method that risks inaccuracy. One difficulty is that too many fibers on the filter can lead to an overlap of the individual fibers. Furthermore, most studies tended to only count the fibers present within selected areas of the filter and conclude the results as representative for the whole filter [3,25,29,34]. Consequently, the number of fibers often is underestimated. The gravimetric measurement of collected particles after filtration of waste water is another usual method in the field of fiber release analysis [35]. However, no information about particle morphology and dimension is received with this method. But these are important characteristics for the research about fiber release of textiles [36]. If this information is missing, dust, production residues and other contaminants, which also be released from textiles during the washing process, cannot be distinguished from microfibers [30,34]. In this way, the amount of fibers could be overestimated. In general, Dynamic Image Analysis is used in pharmacy, food industry and geology for particle characterization [37]. But it also can be used as a rapid technology with high precision and reliability to characterize and quantify fibers in liquids. A high resolution camera creates physical information about the particle properties and transmits them to a computer. Size and shape information are determined for each individual particle with the help of an evaluation software [30,33]. This makes it possible to quantify only the particles with typical microfiber shape and size and to generate statistically relevant data sets.

After the quantitative determination of the fiber release, it follows a biodegradation analysis to check the ability of the released fibers to degrade. In general, a material is biodegradable, if under defined conditions like temperature, oxygen, moisture concentration, the pH value and by the action of microorganisms, it degrades to water, carbon dioxide, minerals and biomass within a certain time [5,38]. The rate of biodegradation is determined by various site-specific factors, such as environmental properties, the presence of microorganisms and the type of microorganisms. However, the properties of the textile material to be biodegraded also have an important influence on the rate of biodegradation or even the ability to be biodegradable, such as the raw material and the associated chemical structure, crystallinity and finishing, like for example dyeing [5,12,21]. In this study, a method according to an existing standard (DIN EN ISO 14851) is chosen, for the analysis of biodegradation. This method is based on the determination of the ultimate aerobic biodegradability of plastics in an aqueous medium. For this purpose, the oxygen demand is measured in a closed respirometer. As a novelty, instead of plastic materials, textile materials are used as test object in the test setup, to determine their aerobic biodegradability. To create perfect conditions that imitate the biodegradation process of textile fibers in waste water, sludge is added to the aqueous medium used in this respirometer approach. In this way, the natural aquatic waste water environment in waste water treatment plants could be simulated in a closed respirometer system.

After the biodegradation analysis, an ecotoxicological assessment of the fiber residues after biodegradation follows. The ecotoxicological screening is performed according to the standardized duckweed growth inhibition test (DIN EN ISO 20079). This test is applied to textile products for the first time and is chosen because duckweed is an easy handling and sensitive representative for water organisms. In the test setup it indicates if a substance has negative toxicological impacts on water organisms or not. Other common ecotoxicity tests, such as the *Daphnia* test, did not prove to be optimal in this research. This test is very time-consuming, and the results are sometimes inaccurate, as only the hard-to-recognize female *Daphnia* are indicators. Other possible ecotoxicity tests for water organisms are the *algae* and luminescent bacteria test. But all these tests are measured photometrically. However, a colorless solution is required for an accurate assessment. Suspended solids from sewage sludge or substances that discolor the waste water can falsify the ecotoxicological result.

At the end of the test, a classification code serves as an orientation how textile materials can be improved to make a better contribution to the environment. After testing and classification, more targeted material and process development can take place, in order to optimize textile materials with regard to their environmental impact. A ranking of the test results is only possible within each individual classification category, i.e. either for fiber release or biodegradation. An overall ranking is not possible, because it cannot be said that a large amount of released fibers with a high biodegradation rate is less harmful to the environment than a small amount of fiber release with a low biodegradation rate. It is not known whether the fibers released from the textiles during washing are biodegraded before they become an environmental risk. There is still no scientific basis for making this kind of statement.

It is necessary to understand the potential environmental impact of all types of fibers, not limiting investigations to synthetic ones (FMP), also known as microplastics. As the exemplary results indicate, natural fiber release can also have environmental impacts. Textile finishings like dyes, commonly used in textile production, can influence fiber release, biodegradation and the ecotoxicity of natural fibers, simply due to their presence [4,11,12]. The varying ecotoxicological effects of degradation products shown in this study are likely attributed to the type of dye used in the samples. Specifically, the grey and pink dyes have differing influences on the ecotoxicity of fibers from the tested samples. In this instance, the pink dye leads to an ecotoxic effect, whereas the grey dye does not. It also seems that the dyeing also caused a slightly difference in fiber release, length distribution and biodegradation rate. However, this observation cannot be generalized to all dyeings. Therefore, differently colored materials must be considered separately, even when the raw material is the same.

Definitely, further research is needed to prove specific effects of different textile materials and the specific influence of textile characteristics like construction, treatments and finishings on fiber release, biodegradability in waste water and ecotoxicity. To derive correlations here, much more data is required. These investigations will be the subject of future research using the newly developed standardized test method (DIN SPEC 4872). To date, most studies have focused on biodegradation under composting conditions. Therefore, there is a particular need for research into the biodegradability of textile fragments in waste water [6]. The biodegradation of fibers under near-natural environmental conditions must also be given greater focus. In order to show the actual path of the microfibers, the biodegradability of the already partially biodegraded fiber fragments in freshwater ecosystems should also be considered, because this is where the fiber residues end up after their path through the wastewater treatment plants. The possible effects of microfibers and FMP on living organisms must also be intensively researched. The test method presented in this study can be improved and expanded once more scientific findings are available. Knowledge about the negative effects of fiber release from textiles is only possible if the effects can be measured and assessed using objective test methods. Ultimately, the overarching goal should be to reduce fiber release and environmental impact throughout the whole life cycle of a textile material.

Author Contributions

J. Alberts, E. Glink: conceptualization, methodology, visualization, formal analysis, investigation, writing - original draft preparation; E. Classen: review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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