Project Report
28244

Method development microplastics

2020-04-07

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Contents

Summary

This project focuses on further development of a screening method for detecting and quantifying fibershaped and non-fiber shaped particles of synthetic origin (microplastics) in wastewaster effluents. Two different chemical pretreatments used in textile testing for calculating percentages of binary fabric blends were studied. Light conversion of a light microscope was also investigated.

The most efficient pretreament to dissolve the cellulosic fractions in wastewater was sulfuric acid combined with ultrasound for better separation of various clogs and bundles of fibers. Pretreament with copper diethylamide did not dissolve the cellulosic fractions completely and needs further development for wastewater samples.

The software of the light microscope was improved to measure elongated length and a light conversion module was installed. Light conversion of the microscope itself was successful, but the filters on the market does not provide enough contrast or have other technical drawbacks for this application. Development of a black filter with sufficient contrast is required. It is also of interest to develop a SEM-analysis (Scanning Electron Microscopy) mapping program especially for fibershaped particles.

The real-life wastewater samples were provided by the wastewater treatment plant in Rimbo. One sample (from the nearby laundry) was sent to Germany to be analysed with a new method called "Dynamic Image Analysis" (DIA). The equipment analyses the wastewater samples directly without prior filtering or pretreatment, which can be an advantage. Thus, light and transparent fibers are still a challenge. Without any pretreatment all particles/fibers, regardless of origin, will be counted, and experience tells us that there is a lot of cellulose in wastewater from laundries. This can also explain why RISE gets lower numbers of fibershaped particles than the DIA-analysis.

There are still many uncertainties and challenges with assessing and analyse microplastics, regardless of shape, from textiles. At present should all the described methods in this project be regarded as screening methods under development with a relatively high uncertainty of measurement.

The experiences in this project will contribute to the standardisation work in the new working group CEN/TC248/WG37 "Microplastics from textile sources" where one of RISE researchers will participate.

Sammanfattning

Detta projekt fokuserar på vidareutveckling av en screeningmetod för analys och kvantifiering av fiberformade samt icke-fiberformade partiklar av syntetiskt ursprung (s.k. mikroplaster) från avloppsvatten. Två olika kemiska förbehandlingar som används inom textil provning för analys av procentandelar för binära fiberblandningar studerades. Ljuskonvertering av ett ljusmikroskop utvärderades också.

Den mest effektiva förbehandlingen för att lösa upp cellulosafraktioner i proverna med avloppsvatten var svavelsyra i kombination med ultraljud för bättre separering av diverse klumpar och fibernystan. Förbehandling med koppar dietylamid visade sig inte lösa upp cellulosan helt och hållet och behöver utvecklas vidare för att tillförlitligt kunna användas för analys av prover från avloppsvatten.

Ljusmikroskopets mjukvara uppgraderades för att kunna mäta fibrer i utsträckt längd och en ljuskonverteringsmodul installerades. Konverteringsmodulen fungerar utmärkt, men de filter som finns på marknaden ger inte tillräcklig kontrast eller har andra tekniska brister som gör att de inte är lämpliga för denna applikationen. Utveckling av ett svart filter som ger tillräcklig kontrast behövs. Det är också av intresse att utveckla ett "mapping" program speciellt för fiberformade partiklar för analys med SEM (Svepelektronmikroskopi).

Vattenverket i Rimbo samlade in proverna och skickade till RISE i Mölndal. Ett av proverna (från det närliggande tvätteriet) skickades också till Hohenstein i Tyskland för analys med den nya metoden "Dynamic Image Analysis"(DIA). Utrustningen analyserar proverna direkt utan förbehandling eller filtrering vilket kan vara en fördel. Dock är ljusa och transparenta fiber fortfarande en utmaning. Utan förbehandling kommer alla partiklar och fibrer med i analysen och erfarenhetsmässigt finns det mycket cellulosa i avloppsvatten från tvätterier. Detta kan förklara varför RISE detekterar lägre antal fiberformade partiklar än DIA-analysen.

Det finns fortfarande många osäkerheter och utmaningar vid utvärdering av mikroplaster (oavsett form) från textiler. Än så länge ska alla de beskrivna metoderna i projektet betraktas som screeningmetoder under utveckling med relativt stor mätosäkerhet.

Erfarenheter från projektet kommer bidra till standardiseringsarbetet i den nya arbetsgruppen CEN/TC248/WG37 "Microplastics from textile sources" där en av RISE forskare kommer delta.

1 Introduction

The Swedish Environmental Agency has identified further development of analytical methods to detect and analyse microplastics as one important step to fill the current knowledge gaps concerning microplastics [1].

The aim of the project is to further develop a screening method for assessing microplastic particles (fiber shaped and non-fiber shaped) in waste water using light microscopy with automatic counting of particles/fibers, FT-IR/SEM including, if possible, additional weighing.
Earlier studies have shown the importance of pretreatment of the samples and there are several ways of dissolving organic substances and other matter; oxidation - for example hydrogen peroxide, acid treatment, alkaline treatment [2]. This project focus on optimisation with sulfuric acid treatment and Copper diethylamide. For better separation of fibers the addition of ultrasound as part of the pretreatment was also investigated.
It is challenging to detect white and/or transparent particles with light microscopy due to lack of contrast. Therefore, one of the tasks was to investigate light conversion. Instead of using a white filter a black filter was used and the microscope was converted to detection of light and semi-transparent particles/ fibers on a black background.
The real-life samples were provided by the wastewater treatment plant in Rimbo, a relatively small treatment plant which has a large laundry (Textila Tvätt och Textilservice in Rimbo) as one the main contributors of industrial wastewater. During early 2020, two sampling points were installed where flow proportional week samples were collected during weekdays and sent to RISE for analysis. One sample was sent to Germany to be analysed with a new method called "Dynamic Image Analysis"(DIA) where the waste water is analysed directly without prior filtering or pretreatment.

2 Pre treatment

2.1 Sulfuric acid

2.1.1 Introduction

Acid treatment is used for analysing the percentage of polyester in polyester/ cotton blends where the cotton fraction is dissolved with sulfuric acid during controlled circumstances (ISO 11827:2016). If there is polyamide present one needs to use copper diethylamide instead since sulfuric acid dissolves polyamide.

2.1.2 Experimental

5 ml of 75% sulfuric acid was poured over the filtrated mass in the funnel of the filtration unit. It was kept at ambient temperature (approx. 20 °C) for 60 minutes before the remaining chemical was removed using air suction and the filter was

rinsed with distilled water several times [3]. This procedure worked well and appears to be robust even if the room temperature varied a little. There was however discoloration, blackening, of the filtrate on the filters pretreated with sulfuric acid that occurred within a week of the sample preparation.

2.1.3 Conclusions

Sulfuric acid can be recommended for dissolving cellulosic fibers from wastewater samples. However, it is not advisable to store the filters for more than two days to be sure to avoid any discoloration.

2.2 Copper diethylamide

2.2.1 Introduction

Copper diethylamide is used in textile testing to dissolve cellulose and spare the polyamide when investigating fabric blends of polyamide and cotton under controlled circumstances (ISO 11827:2016). The method was also used in a previous study at industrial laundries with an acceptable result [3].

2.2.2 Experimental

5 ml of Copper diethylamide was poured over the filtrated mass in the funnel of the filtration unit. It was kept at ambient temperature (approx. 20 °C) for 20 minutes before the remaining chemical was removed using air suction and the filter was rinsed with distilled water several times.

However, in the following microscopic analysis, fibers with the characteristic twist of cotton were detected, example see Figure 1.

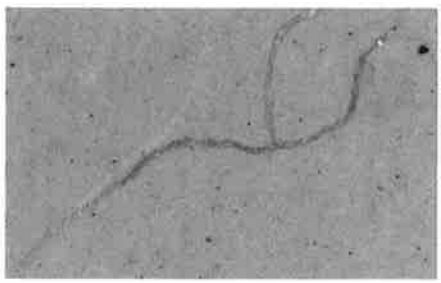

Figure 1. Possible cotton fiber

Several tests were carried out to improve the solubility of cotton using copper diethylamide.

- Pressing a metal weight during approximately 10 minutes after the filtration. However, the fibers moved towards the edges causing more overlapping and consequently more difficult for the automatic counting.
- Stirring during the alkaline treatment. 10 ml of copper diethylamide was added directly to 10 ml of wastewater sample and stirring approximately 5 seconds two times during 10 minutes. This started a chemical reaction that clogged the pores of the filter and left a mat of unidentified substance on the filter.

The results show that during these conditions cotton is not fully dissolved using copper diethylamide. If there are much cellulosic residues left the microscopic analysis becomes especially difficult. This is somewhat surprising since the method is well established for analysing blended fabrics. But since copper diethylamide did work in the previous study at industrial laundries, further investigation is needed to find the cause of why the dissolving was no success in this case and to further develop the pretreatment.

One way could be to dye the cotton fibers with Neocarmine or another specific dyestuff and let the microscope analyse the other fibers. For this to be achievable one needs to develop a program for the microscope, in addition to analyse specific shapes, to also analyse specific colours.

2.3 Dyeing with Neocarmine

Neocarmine is used for fiber identification purposes as it dyes different fiber types in different colors, see figure 2. There has been attempts to dye transparent fibers for better contrast, which will work on cellulosic fibers. But since polyester, which is of much interest in this project, needs both high temperature and pressure to be dyed this was not pursued. Instead Neocarmine was used to see if all the cellulose on the filter had been properly dissolved.

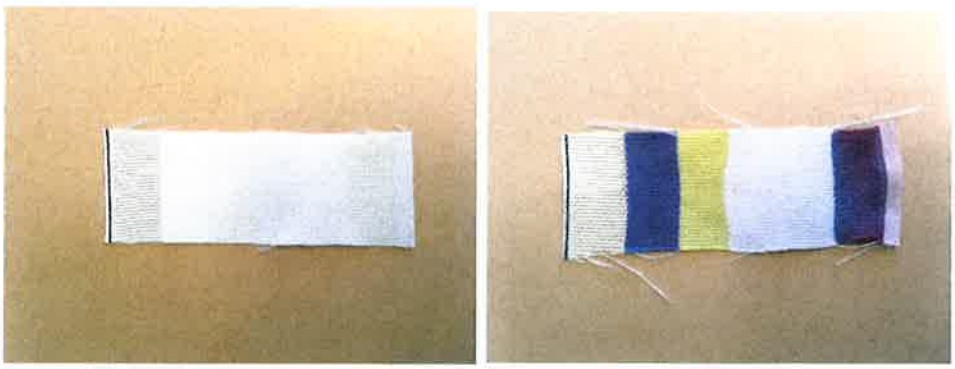

Figure 2: Undyed (left) and dyed (right) standardized multifiber fabric.

Material content from left to right is: Triacetate, cotton, polyamide, polyester, acrylic and viscose.

2.3.1 Experimental

It was found that many of the remaining fibers, after the alkaline treatment, had the spiraling shape typical of cotton. Therefore, the Neocarmine was added and it did indeed dye a lot of the fibers blue. It also dyed many fibers (and particles) not previously identified, also these were mainly blue, i.e. of cellulosic origin, see Figure 3.

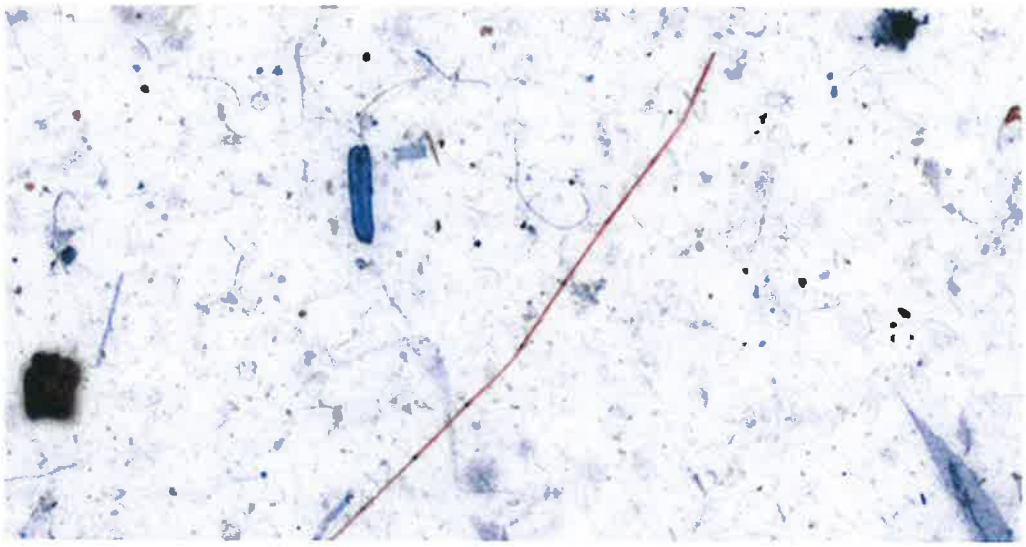

Figure 3. Filter with Neocarmine dyed blue fibershaped and non-fibershaped particles.

2.4 Ultrasound

2.4.1 Introduction

After filtering, the filters often have a lot of particles and fibers to be analysed, sometimes thousands of fibershaped as well as non-fiber shaped particles. This is a challenge for the microscope to detect. This is especially difficult when the fibers are crossed or in bundles. Tests with ultrasound as part of the pretreatment was conducted to see if this could increase the separation the fibers.

2.4.2 Experimental

Different periods of time of ultrasound treatment were evaluated and 30 minutes was decided to be an adequate duration for increasing the fiber separation. In this project a Bandelin Sonorex super RK500 ultrasonic equipment was used.

Figure 4. Submerged bottles in the ultrasonic bath

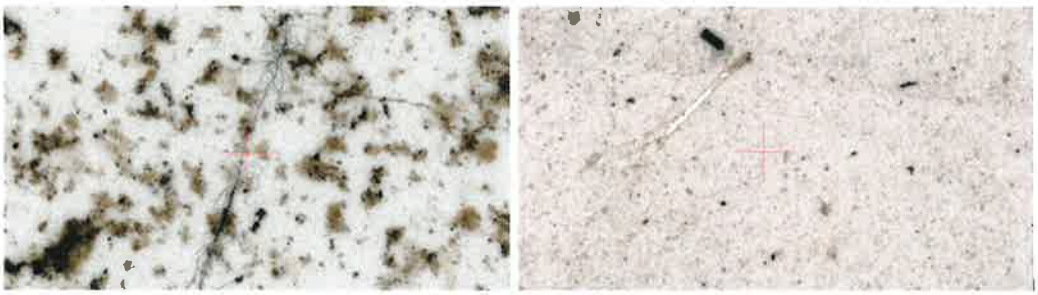

Figure 5. Before (left) and after (right) ultrasonic treatment

After ultrasonic treatment there are much smaller areas of clogging and more evenly distribution of particles/fibers. There are also less bundles and overlapping of fibers.

2.4.3 Final pretreatment of the real-life samples

The weekly water samples were treated with ultrasound during 30 minutes in a Bandelin Sonorex super RK500 ultrasonic equipment. Then mixed thoroughly by stirring, and 10 ml was filtered through a PVDF membrane with a pore size of 0.65µm, using air suction. To dissolve cellulose (mainly paper and cotton fibers) sulfuric acid or copper diethylamide was used. The chemical is poured over the filter in the filtration unit and maintained at a specific time (depending of chemical used), at room temperature before it is also filtered through the membrane using air suction. The remaining mass and filter are rinsed several times with distilled water to remove all residues of the chemical. The latter is also performed in the filtration unit, see figure 6.

Figure 6. Filtration unit with air suction

2.5 Pretreatment conclusions and discussion

Wastewater is contaminated with a lot of different substances, organic as well as inorganic, hence pretreatment is needed to purify the samples before analysing the content of microplastics. The pretreatment with sulfuric acid is time critical, too long time can affect the polyester .The decision was that 60 minutes at ambient temperature (approximately 20 °C) is sufficient for wastewater samples.

Since sulfuric acid dissolves polyamide one can use copper diethylamide instead if there are reasons to believe that there is polyamide present in the sample. In this study the copper diethylamide often reacted and formed salt crystals on the filter. This makes it harder to analyse although it is very clear that the blue spherical crystals are easy to detect visually - but for the microscope it is still a particle that should be counted. Also, the efficiency of dissolving cellulose is not as good as sulfuric acid. This was not the case in the previous study regarding "Microplastics from industrial laundries [3]. Further studies need to be performed to optimize copper diethylamide for wastewater samples with polyamide content. If it is known that polyamide is not present (or a negligible fraction) sulfuric acid is a better choice.

Separating fibers by using ultrasound as part of the pretreament proved to be successful. The fibres are visually more separated after exposure to ultrasound during 30 minutes, see Figure 5.

If one is aiming for a more comprehensive analysis there are other methods to consider: oxidation with hydrogen peroxide for 5 days which is more mild chemical but time consuming, density separation etc. [2] However due to the narrow timeline and budget, these methods were not investigated in this project. There are still many uncertainties in the above described pretreatments, at this point the all mentioned methods is to be regarded as screening methods.

The experiences in this project will contribute to the standardisation work in the new working group CEN/TC248/WG37 "Microplastics from textile sources".

3 Light conversion

3.1 Introduction

One option to be able to detect bright and semi-transparent particles/fibers is to light converse the microscope, i.e. to use a black filter instead of a white filter and shift the detection in the analysis software to detect bright contaminants instead of dark contaminants. To be able to investigate this option we contacted Nyli Metrology AB, a supplier of light microscopes and analysis software, for collaboration.

Nyli Metrology AB is a Swedish measurement technology company accredited for particle analysis [4]. Their analytical system enables automatic ocular counting and the sizing of particles and fibers. Nyli added a function to invert the detection of contaminants to their software. This function makes it possible to count bright particles on black filters instead of the regular counting of dark contaminants on white filters.

3.2 Experimental

Initially an investigation of available dark colored filter types on the market was made. Figure 7 shows the tested filter types and the conclusions of the investigation [5-9].

Filter name	Brand	Pore size	Material	Colour	Conclusion
AABG04700	Millipore	0,8µm	Mixed cellulose ester (MCE) membrane	Grey	Inadequate contrast
AABP04700	Millipore	0,8µm	Mixed cellulose ester (MCE) membrane	Dark grey	Black when wet, grey when dried
HABP04700	Millipore	0,45µm	Mixed cellulose ester (MCE) membrane	Grey	Inadequate contrast
HTBP04700	Isopore	0,4µm	Polycarbonate (PC) membrane	Dark brown	Inadequate contrast and material
CleanDisc5µm	Sefar	5µm	CleanDisc screen fabric (PET) filter	Yellow	Inadequate contrast, material and too large pore size

Figure 7 - Table for tested filter types and outcome

None of the tested filters in this investigation gave enough contrast in the light microscope since the filters were either light grey or light yellow when dried after

filtration. The best contrast was reached with the filter named AABP0047 [6] but in dry condition the contrast was not enough to detect all bright fibers. Figure 8 shows the best detection reached for dry filters.

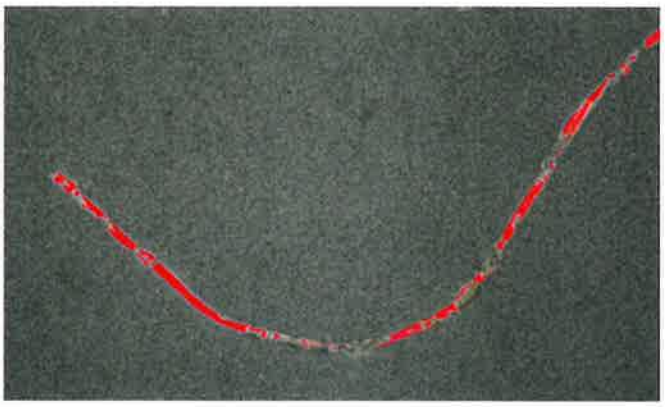

Figure 8 - Best detection reached in dry condition

As an experiment we tried to keep the most suitable filter wet during the microscopic analysis using a soaked absorbent pad under the filter. But since one needs to use an objective glass over the filter during the microscopic analysis, the sharpness turned out to be insufficient due to air bubbles (image in the middle of Figure 9) between the filter and the glass. Figure 9 shows pictures of the wet filter experiment.

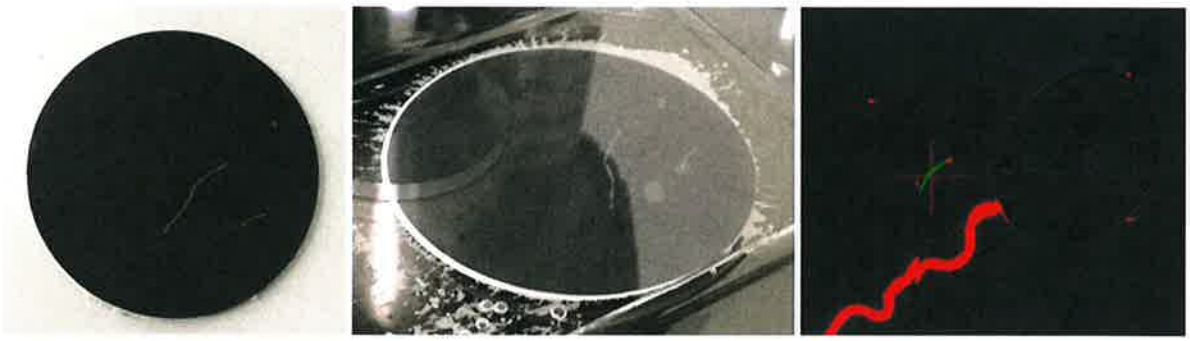

Figure 9 - Wet filter investigation

Another idea was to use a black particle trap [10] and put some bright fibers on to investigate the contrast in the microscope with a completely black background. Figure 10 shows the black particle trap with planted fibers, the contrast for bright fibers and the limitations in detection when the fibers are semi-transparent.

Figure 10 - Black particle trap investigation

3.3 Conclusions

The new function in ParticleView to invert the detection of contaminants from dark to bright is very useful and works well on completely black background. However, there is no suitable hydrophilic filters that is completely black on the market. The most suitable dark colored filter found on the market was a 0.8µm mixed cellulose ester filter [6] but the filter is not completely black when dry. This leads to problems with contrast and detection in the light microscopy.

In addition to testing different filters, there was also a test with a black particle trap. The black particle trap [10] worked well as background and the contrast was better than for all hydrophilic filters tested. The conclusion is that if there would have existed a completely black hydrophilic filter even in dry condition on the market, the analysis system would manage to count the bright fibers. Further development in this area is of very high interest.

4 Analysis of samples from WWTP in Rimbo

4.1 The facility

Rimbo WWTP was built in the 1960's and have had several modifications to its process over the years. The current renovations and constructions consist of a modernization of the wastewater intake and primary treatment as well as adding a nitrogen-removal step and refurbishing the anaerobic sludge digester. At the time of the sampling, the plant process consists of the following steps:

Pre-treatment
A temporary step-screen (as described above) and an aerated sand-trap with a small dose of PIX (iron (III) Chloride) as a flocculent to increase solids removal in the primary sedimentation tank.

Primary sedimentation
In this tank, solid particles are allowed to sink to the bottom of the tank where scrapers gradually transport the sludge to the sludge hopper, from where it is pumped into a sludge-holding tank before dewatering.

Bio-bed
The pre-treated water is dispersed over the bio-bed, allowing the water to trickle down over surface-enhanced bio-carriers that are overgrown with biofilm. The bacteria can then absorb nutrients and dissolved organic compounds from the water.

Secondary sedimentation
Sloughed off biofilm is collected in these sedimentation tanks, scrapers bring the bio-sludge to the sludge hopper and two valves open regularly and the water pressure forces the bio-sludge to flow back to the water intake so that it will pass the primary sedimentation tank and settle there.

Oxidation Pond
Not really used to re-oxygenate water anymore, this pond has a holding time of up to three days and now the mainly function is as a water retention magazine.

Chemical treatment and tertiary sedimentation
In this step, the main dosage of the flocculent (PIX -iron (III) Chloride) is added to the flow. The flocculation chamber consists of intense and slow stirring and the flocs are allowed to settle in the following sedimentation tank. The resulting sludge is scraped to the hoppers and pumped to the sludge-holding tank.

Final polishing
The final polishing step consists of continuous sand filters (Dynasand). These filters help with removing residual flocs or other suspended particles.

4.2 Sampling

Due to extensive renovations at the facility, the incoming water pre-treatment with two step-screens has been substituted with a single step-screen with wider gaps to allow for a higher hydraulic load. Instead of the regular screw pumps, the water has been lifted into the step-screen by two centrifugal pumps. This setup has been constant over the course of the sampling period. It may cause the content of microplastic particles to deviate from normal levels, as the centrifugal pumps excerpt a much greater force on plastics in the water stream. This could lead to a greater than normal disintegration of plastics and non-woven textile (for example wipes) in the water flow. The temporary step-screen may also be less efficient than the normal ones in catching small pieces of plastics.

As in most treatment plants, inflow of ground- and rainwater increase the flow rates during rainy weather. As the weather during the sampling period has been mild and wet, for the most part, inflows to the treatment plant has been higher than the yearly average. Due to the short duration of the project this could not be avoided. Due extremely high inflow rates RISE also decided to exclude the samples from the first two weeks.

Due to the constructions and renovations, it was challenging to set up an optimal sampling point. The point used is affected by recirculated water from the sand filter backwash, bio-sediments removed from the secondary sedimentation and reject water from the sludge dewatering process. The backflow is the major contributor to the recirculated flows, calculated to comprise 10% of the total inflow. The other flows are minor additions, calculated to be less than 1% of the total inflow together.
Nevertheless, the sampling was evaluated to be good enough for the purpose of delivering real-life samples to the project.

In total three different sample points were used, inflow, inflow - night and outflow. Inflow sampling was done with two separate samplers. One did the regular continuous sampling for the daily samples and one was programmed to start at 23 PM and to stop sampling at 5 AM the following morning during weekdays. (Starting Monday night and taking the last sample on Friday morning.) This was done to catch a specific segment of the inflow, as the local laundry (Textilia Tvätt och Textilservice, Rimbo) releases their process water between 11 PM and 4 AM. Outflow samples were taken from the regular continuous sampling. The 24-hour samples are collected from Monday morning to Friday morning

Sampling from week 2 and 3 were not analysed due to heavy inflow and were not considered as suitable samples for this project. The night sample from week 4 (21-24 January) was also sent to Hohenstein Institute in Germany for Dynamic Image Analysis, see chapter 4.6. Samples from the other weeks (week 5-7 plus week 9) were only used at RISE in Mölndal.

4.3 Light microscopy

The equipment used was a Nikon Eclipse LV150N microscope and ParticleView 4.2.2.27 software. The software was improved by Nyli Metrology AB for better fiber detection and more effective focus setting during the automatic particle counting. All particles (equal to or larger than 1µm) regardless of shape were counted automatically with light microscope. However, the automatic counting was performed with 10X objective lens, 100X nominal magnification and a digital resolution of 0.6871 µm/pixel hence the results for size range 1-5µm may not be accurate. The fiber definition was set to 10:1 (length must be ten times greater than the width to be categorized as a fiber) according to ISO 4407:2002 [11]. The

particle size was defined by the longest distance of the particle and for fibers the elongated length was calculated. The threshold was set at 75% and grey value at 55%.

In this study fibers for all size ranges were counted, but since the fiber definition was set to 10:1 and particle counting below 5µm is uncertain due to the magnification used, the results from 50µm is the main interest in this project. A manual analysis of particles and fibers larger than 500µm was made to control and correct any detection issues. The size classes in the analysis were set according to other studies found during literature research [12-13)] that refer to ISO/TR 21960:2019 [2] and ISO/TR 21960:2020 [14].

4.4 Experimental

All microscopy numbers are an average of 2 replicas. Figure 11 and 12 show the results for the analyzed filters. All samples were pretreated with sulfuric acid except for "Inflow 24h, w.9" which was analyzed with both sulfuric acid and copper diethylamine as a comparison. Sulfuric acid gets a lower number of fibershaped particles than pretreatment with copper diethylamine. The SEM/ FTIR - analysis revealed that there were cellulosic fibershaped fibers left using the copper diethylamine. This is probably the reason for the higher amount of detected fibers for the copper diethylamine. The results also shows that the night samples from the laundry effluent is approximately 50% compared to the "24h samples" (including households).

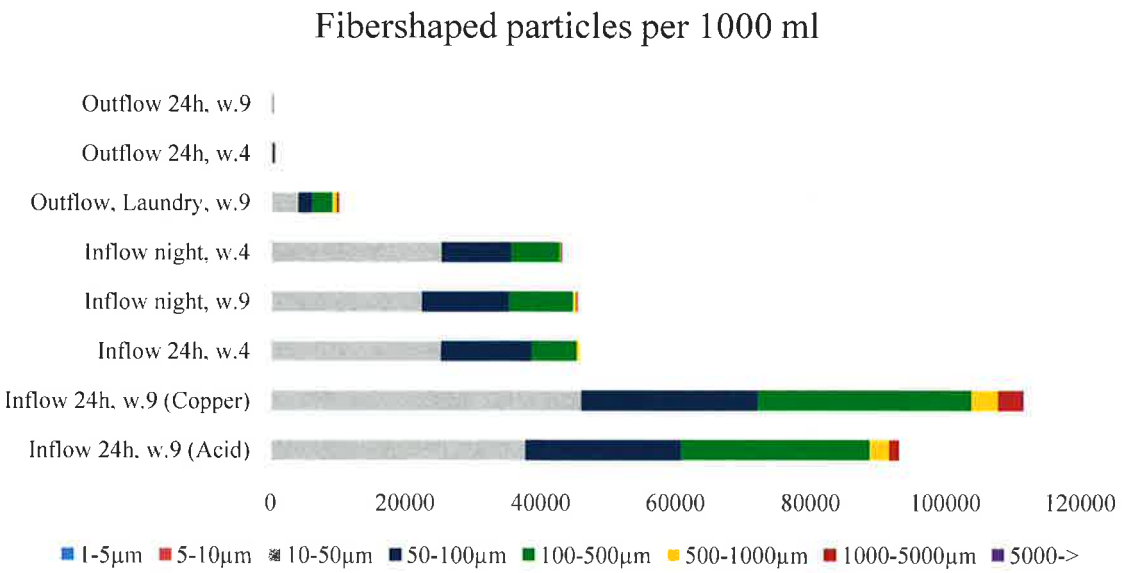

Figure 11. Fibershaped particles per 1 000 ml

The same trend is also shown in Figure 12, including both fibershaped and non-fiber shaped particles. The sulfuric acid gets lower numbers than copper diethylamine also when detecting non-fiber shaped particles. The night samples from the laundry effluent is approximately 50% compared to the "24h samples", in the same range as the same as fibershaped particles. Looking at the numbers one can conclude that a major part of the fibershaped particles is in the size range from 1-10µm. Note that the microscope is not set to count in this size range according to standards (se also chapter 4.3). It is safe though to say that there are a lot of very small particles on the filter.

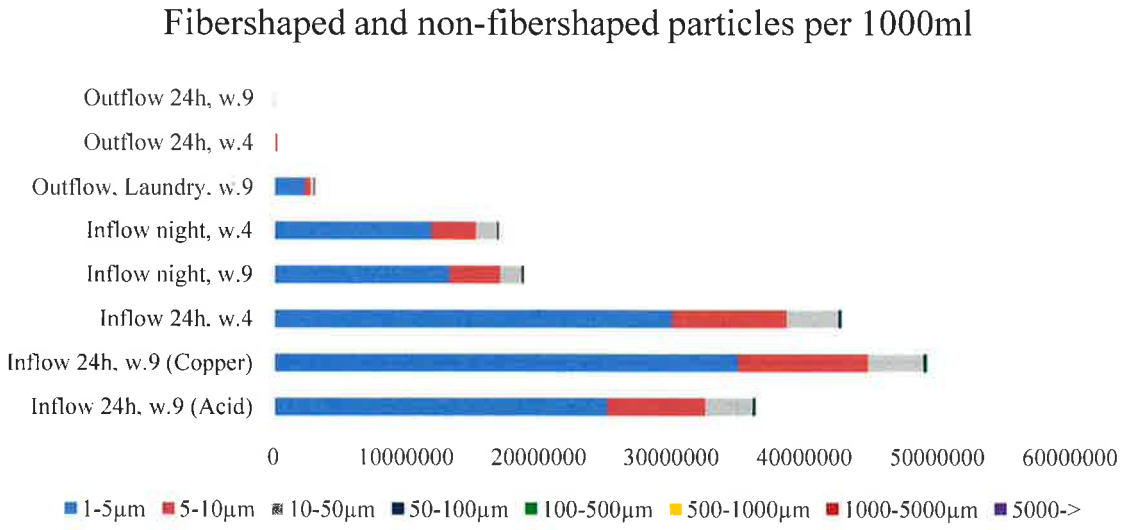

Figure 12. Fibershaped and non-fibershaped particles per 1 000 ml.

A few weighing attempts were carried out in addition to the counting but with inconclusive results. One needs enough matter on the filter to be able to calculate the weight, and with very small weights is it difficult for the scale to show a stable weight. There were also some remaining residues from the pretreatment in some cases which needs further analysing.

Even though the numbers are very low compared to the other samples, surprisingly many particles (including fibershaped) were detected in the outflow samples, see Figure 13. The current renovations and constructions at the WWTP could be a reason for letting particles/fibers accidently coming through.

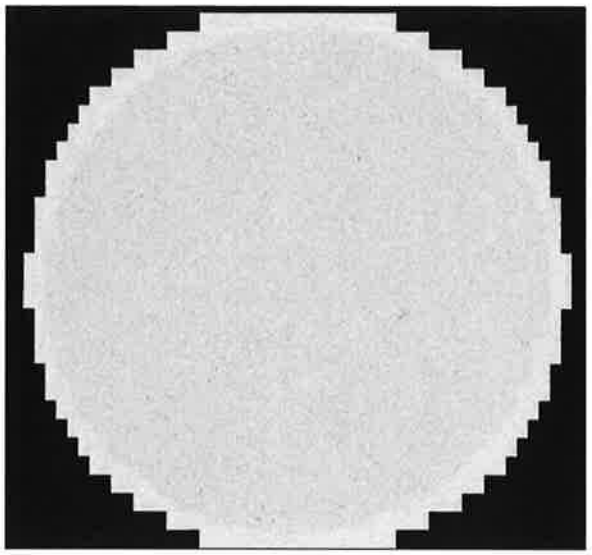

Figure 13. Image of filter from outflow water

4.5 FT-IR and SEM

Additional analysis for identification of fiber content was performed with FT-IR (Fourier Transformed Infrared Spectroscopy) and SEM (Scanning Electron Microscopy).

FT-IR analysis

A Bruker Lumos spectrophotometer instrument equipped with a microscope and an ATR (Attenuated Total Reflection) device was used for the analyses. This allows focusing the IR beam on a specific spot on the sample.
Typical FT-IR spectra of a polyester fiber from one of the samples from week 4 is shown in figure 14.

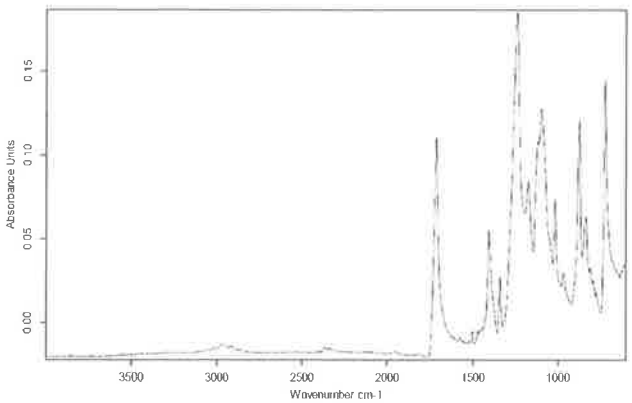

Figure 14. Spectrum with typical absorption bands for polyester at wave number 1715 cm^{-1}, 1243 cm^{-1}, 1098 cm^{-1} (ester) and 720 cm^{-1} (hydrocarbon).

SEM-EDS

Images was taken with a Scanning Electron Microscope from JEOL, model JSM - 6610LV, operated at low vacuum with a chamber pressure of 60 Pa. Elemental analysis is performed with an Energy Dispersive X-ray Spectroscopy (EDS) detector from Bruker (XFlash 5010) and Espirit software version 2.1. SEM images and EDS analysis are performed using an accelerating voltage of 15kW at a working distance of 10 mm.

Figure 15. Typical image of a fibershaped polyester particle.

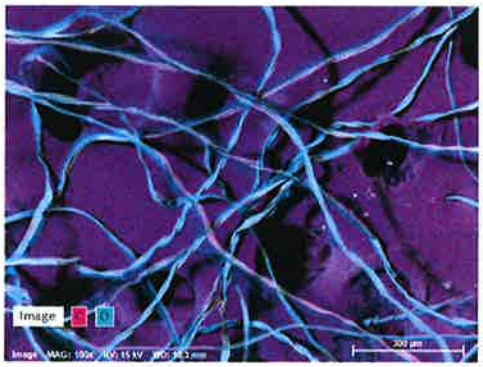

Figure 16. Cellulosic fibers on a filter.
The colors indicate presence of different elements. The more intense colour the higher the concentration. Carbon (C=pink) and oxygen (O=blue).

Figure 17 show cellulosic fibers that are darker compared to the background (the filtermedia) whereas the polyester fibers a more intensely pink.

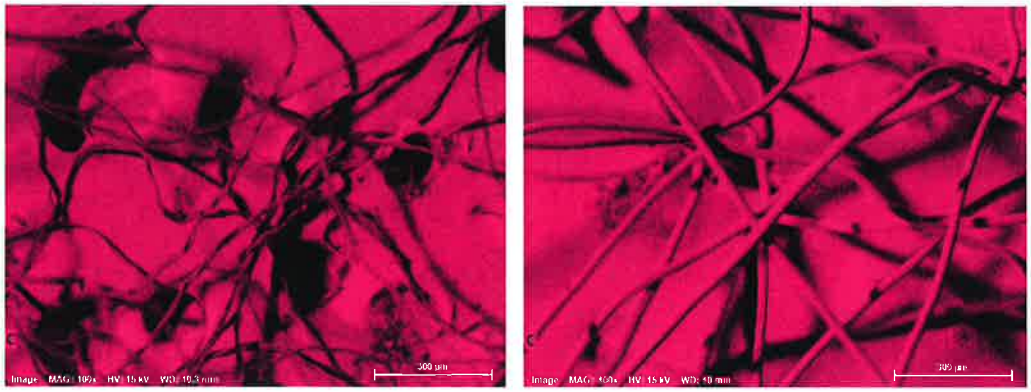

Figure 17. Cotton fibers (left image) and polyester fibers (right image)

4.6 Dynamic Image Analysis (DIA)

One night sample from week 4 was sent to Hohenstein Institute for analysis with the Dynamic Image Analysis [15]. The method requires no filtering and the fluid is photographed through a flow cell at the rate of 85 frames per second. The detected signals are converted into binary images by an integrated software.

According to DIA the sample from week 4 contained 69 250 ± 24 880 fibers per liter in the size range of 50 - 2 000 µm. The setting for defining a particle as a fiber was set to the length ≥ 50 µm and diameter ≥ 7 µm and. (approximately 7:1). The mean fiber length was 288µm and the highest data point was 144µm, see Figure 18.

There was also an added comment in the report:" *The sample was quite difficult to measure, we had issues with the large/coarse particles which tended to clog the cuvette / flow cell. Additionally, the fibers were white/transparent, so that the contour of the fibers was not captured 100% due to the low contrast between the liquid and the fibers. This is also very likely the explanation for the high STD".*

(STD=Standard deviation)

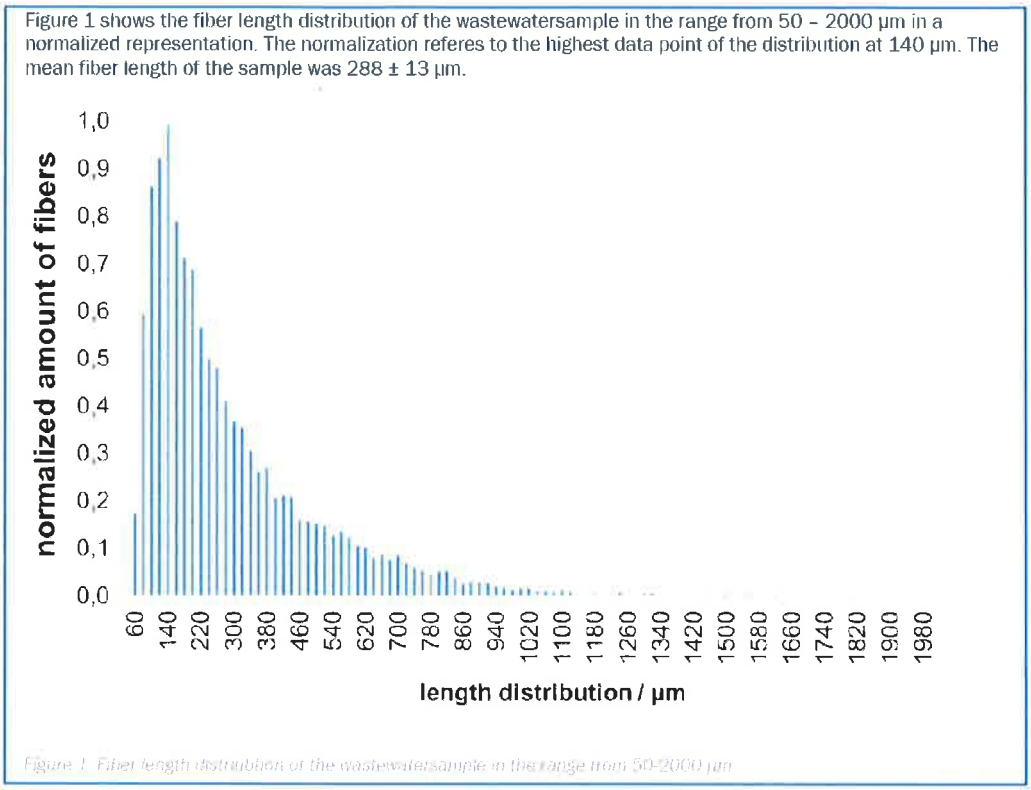

Figure 18. Length distribution, excerpt from the DIA-report

The full DIA-report is attached in Appendix A.

As Hohenstein reports "fibers per liter" and the used volume is 300 ml it is assumed that the values are recalculated to "fibers per liter". Note that RISE diluted the original sample from 360 ml up to 1 000 ml before sending it to Hohenstein according to the requirements. Recalculated estimated value per 1 000 ml is 69 250 x (1000/360) ± 24 880 x (1000/360) = 192 391 ± 69 111 fibers per liter.

4.7 Conclusions and discussion

Discussion

The result from the real-life samples show that there are a lot of small particles, roughly 40-50% of the total detected amounts of particles. An estimation from an earlier study is that approximately 95% of the fibershaped particles in the effluent coming directly from industrial laundries are of synthetic origin and 95% of the non-fibershaped particles are of cellulosic origin [3]. In this study the focus was on fibershaped particles ≥50 µm, and both polyester and polyamide fibers were detected. We also found examples of "large" cellulosic fibers surviving the copper dietylamide especially from the "25h samples" which includes wastewater from households. In this minor project there was no possibility for in-dept analysis of a large number of particles to be able to state any exact percentage of fiber content.

Both FT-IR and SEM are well established and known to be suitable for particle identification. Analyzing fibershaped particles from textile sources almost always includes many particles on the same filter, hence the analysis is very time consuming. To streamline the analysis, it is of interest to develop a SEM mapping program especially for fibershaped particles. This enables the SEM equipment to identify all, by definition, selected particles as a first step before the in-depth analysis starts.

There are still many uncertainties in the different analyses. The challenge of light and transparent fibers, the ability to identify the origin of a large number of particles within a reasonable timeline etc. At present, the all mentioned methods are to be regarded as screening methods under development.

The new DIA-analysis is very effective in the way that no pretreatment is needed. There are though still challenges with light and transparent fibers as one can read in the added comment in the report.

An accurate comparison between the night sample from week 4 analysed both at RISE and Hohenstein cannot really be performed. The DIA-analysis appears to detect substantially more fibershaped particles than RISE, more than double the amount. However, the median length seems to be in the same range. Approximately 140-150 µm looking at the raw data from the microscopic analysis compared to DIA stating 144 µm as the highest data point.

One big difference is that the DIA-analysis is performed without any pretreatment, which means that all particles regardless of origin is counted. RISE on the other hand dissolved the cellulosic fractions before counting. This probably has a major impact of the detected amount of fibers since the sample in question is a wastewaster sample directly from an industrial laundry with a presumed large fraction of cellulose.

There is also a difference in settings, RISE having 10:1 ratio [11] compared to DIA 7:1 - which means that the DIA-method detects more fibers per definition. Assuming that the size distribution of the fibers is linear that would mean that RISE can add 30% to the amount of detected fibers.

The DIA-analysis is however an interesting method, especially if it in the future will be combined with an on-line technique to identify the origin of the detected particles.

5 References

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6 Appendix A

Full report from the Dynamic Image Analysis (DIA)

APPENDIX A

HOHENSTEIN

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Contact person	Our ref.	Date
Dr. Jan Beringer	———	19.02.2020

Report No. 20.8.6.0003

Client:	see address
Test sample:	see page 2
Date of order:	05.02.2020
Receipt of test samples:	11.02.2020
Period of testing:	12.02.2020 to 14.02.2020
Sampling:	The test sample has been delivered to us by the client.

The report comprises 6 pages.

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AIM OF TEST

The wastewater sample was analyzed regarding the content of fibers and their morphology.

TEST SAMPLES

Table 1 shows the image of the wastewater sample and the sample description. The sample was used like handed over by the customer.

Table 1: Sample description

Sample No.	Test sample
20.8.6.0003-1	1 l wastewater sample "RISE IVF AB 1" 

The sample was used like handed over by the customer.

METHODS

PRE-TREATMENT

No sample pre-treatment was applied.

DYNAMIC IMAGE ANALYSIS

The measurement was performed on the dynamic image analysis device QICPIC (Sympatec GmbH) which allows the analysis of particle and fibers in liquids. The device was equipped with a 0.2 mm flow cell, a reservoir (drainage beaker) and a magnetic stirrer to disperse the liquid. A peristaltic pump (MFC Process with 6 rollers, Ismatec) was used for liquid transportation. The measurements were conducted with the following parameters:

Volume flow:	172 ml/min
Frame rate:	85 frames/second
Sample volume:	300 ml
Sample homogenization:	magnetically stirred repeatedly for 5 s with intervals of 30 s

The measurement was stopped, after the cuvette was drained with liquid.

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DATA ANALYSIS

The detection signals from the dynamic image analysis measurements were converted into binary images by the integrated software Windox 5.10.0.3. For the quantitative evaluation two different assessment parameters were applied. Table 2 shows the filter criteria for straight fibers, in Table 3 the filter criteria for bended fibers are summarized. Only fibers matching these shape and length properties were considered in the quantitative evaluations.

Table 2: Assessment parameters for the quantitative evaluation straight fibers by dynamic image analysis.

Assessment parameter	Parameter Limit
Length of fiber	≥ 50 µm
Diameter of fiber	≥ 7 µm
Sphericity	≤ 0.85
Straigthness	≥ 0.92
Aspect ratio	x ≥ 0.05; x ≤ 0.2

Table 3: Assessment parameters for the quantitative evaluation of bended fibers by dynamic image analysis.

Assessment parameter	Parameter Limit
Length of fiber	≥ 50 µm
Diameter of fiber	≥ 7 µm
Sphericity	≤ 0.85
Straigthness	≥ 0.5
Aspect ratio	≤ 0.5
Convexity	≤ 0.5

The total fiber count was calculated by adding the fiber counts determined for the evaluation criteria of straight and the fiber counts of bended fibers. Eventually occurring fiber doublings due to inhomogeneous volume flow events during measurement were subtracted manually from the counting results.

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RESULTS

QUANTITATIVE ANALYSIS

The wastewater sample contained 69 250 ± 24 880 fibers / l wastewater.

MORPHOLOGY ANALYSIS

Table 4 shows examples of photos from the measurement and the picture gallery of the dynamic image analysis.

Table 4: Examples of photos from the dynamic image analysis measurement

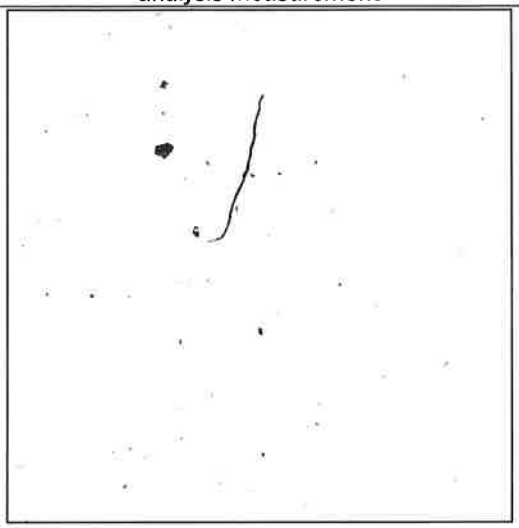
Sample	Example of a photo from the dynamic image analysis measurement	Examples from the picture qallery oft he dynamic image analysis measurement			
20.8.6.0001		 LEFI 511.346µm DIFI 10.713µm Sphärizität 0.222 Seitenverhältnis 0.169 Konvexität 0.238 Geradheit 0.919 Bildnummer 570	 LEFI 88.330µm DIFI 11.279µm Sphärizität 0.274 Seitenverhältnis 0.176 Konvexität 0.468 Geradheit 1.000 Bildnummer 1845	 LEFI 243.800µm DIFI 11.451µm Sphärizität 0.312 Seitenverhältnis 0.221 Konvexität 0.372 Geradheit 0.936 Bildnummer 1858	 LEFI 1022.956µm DIFI 13.940µm Sphärizität 0.184 Seitenverhältnis 0.114 Konvexität 0.275 Geradheit 0.922 Bildnummer 1865

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Figure 1 shows the fiber length distribution of the wastewatersample in the range from 50 – 2000 µm in a normalized representation. The normalization referes to the highest data point of the distribution at 140 µm. The mean fiber length of the sample was 288 ± 13 µm.

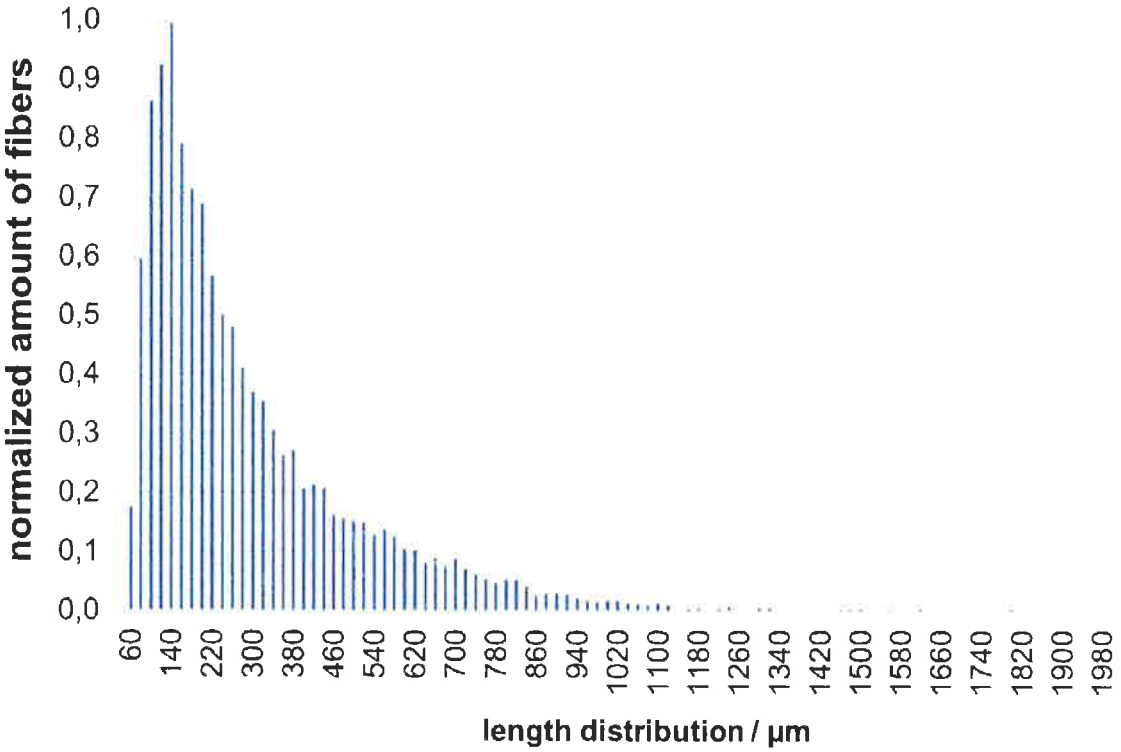

Figure 1: Fiber length distriubtion of the wastewatersample in the range from 50-2000 µm.

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CONCLUSION

The wastewater sample contained 69 250 ± 24 880 fibers / l wastewater. The fibers showed different sizes and shapes.

Schloss Hohenstein, 19.02.2020

Director
Life Science & Care

Dr. Timo Hammer

Hohenstein Institut für Textilinnovation gGmbH · Schloss Hohenstein ·

Scientific Expert

Dr. Jan Beringer