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OCCURRENCE OF MICROPLASTIC IN MUNICIPAL WASTEWATER SLUDGE

Bachelor’s thesis
Environmental Engineering

2018

South-Eastern Finland
University of Applied Sciences

Author	Degree	Time
Huong Thu Nguyen	Bachelor of Environmental Engineering	October 2018

Thesis title	
Occurrence of Microplastic in Municipal Wastewater Sludge in Mikkeli	25 pages 3 pages of appendices

Commissioned by

South-Eastern Finland University of Applied Sciences

Supervisor

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Abstract

The objectives of this thesis include: following a simple procedure of detecting and identifying microplastic in sludge samples to determine the amount and characteristics of microplastic in Mikkeli municipal wastewater sludge and comparing the results with literature findings. The method implemented would then be concluded if suitable for beginner researchers or not.

Two types of sludge were involved: conventional activated sludge (CAS) and membrane bioreactor (MBR). The wet samples were extracted and dried, then inspected under microscope.

It was found that there were 14 and 18 microplastic particles per gram dry weight in CAS and MBR, respectively. MBR was found to be more efficient in removing microplastic. Most of these microlitter was fibrous and visually identified to be polyester and polyamide. The results are in agreement with previous studies on the topic. The experimental method followed was found to produce some confusing results and was determined to be more suitable for researchers much more experienced with microplastic rather than beginners who are new to the subject.

Keywords

Microplastic, municipal wastewater, sludge

CONTENTS

1 INTRODUCTION

Microplastic (MP) are plastic particles that have a dimension of less than 5 mm (Talvitie, 2017b). In recent years they have been detected everywhere, in lakes, coastal waters and sewage water, raising concern in scientific world as well as general public. Organic contaminants or heavy metals in the environment have been found to tend to adsorb to the surface of these miniscule plastic particles, which in turn can be consumed by aquatic fauna (Rios et al., 2010; Chua et al., 2004; Rochman et al., 2014). Therefore, microplastic can act as a transporter of these contaminants in the environment and in the food chain, posing possible health hazards to aquatic animals and subsequently, humans (Browne et al., 2013; Rochman et al., 2015).

Microplastic can be divided into primary and secondary microplastic (Talvitie et al., 2017b). Primary microplastic are plastic products that are intentionally manufactured in small sizes such as microbeads in scrubbing cosmetics, or micro-pellets for industrial purposes. Secondary microplastic are miniscule fragments, either from products while in use (for example synthetic textile fibers from clothes) or from plastic waste when they are disposed of into the environment (GESAMP, 2015). Both of these types can enter the sewage water easily, as they are from common products of human consumption and are washed off to household or industry wastewater.

As a result, microplastic has recently become a major concern of wastewater treatment sector and the number of studies conducted on the topic has increased significantly. Although conventional treatment process is already able to filter most of the microplastic available in municipal wastewater, the volume of treated water released to receiving water bodies is still so large that wastewater plants are considered an important source of releasing microplastic to the environment (Talvitie et al., 2017b).

Kenkäveronniemi is a wastewater treatment plant of Mikkeli municipality in south-eastern Finland, where the samples are taken for this thesis study. The samples consist of conventional activated sludge (CAS) from the main treatment process and the MBR sludge from the pilot membrane bioreactor. The MBR pilot treats water from the primary settling tank of the plant, so the comparison would be of microplastic removal efficiency between CAS and MBR as secondary treatment.

A simple method of experimental work will be followed in this study. This refers to parts of methods of preparing, treating and analyzing samples described in Mirka Lares' study on the same topic with samples from the same wastewater plant (Lares et al., 2017). However, the scope of this study only covers simple procedures, and hence Lares' methods were slightly altered to suit it. The most important difference is this study would not involve the use of FTIR or Raman spectroscopy to identify types of microplastic. At the end of this report, the procedure will be determined whether it is reliable and relevant for beginner researchers and can be standardized for future studies.

This study will focus on three main objectives, first is to follow the simple experiment procedure mentioned of detecting and identifying microplastic fibers in sludge samples, second is to find out the amount and characteristics of microplastic in the sludge of municipal wastewater from Kenkäveronniemi plant; third is to compare the MP amount between conventional activated sludge (CAS) and membrane bioreactor sludge (MBR); and last is to compare the results of this study to previous relevant researches.

2 LITERATURE REVIEW

2.1 Conventional Activated Sludge and Membrane Bio-reactor

While conventional activated sludge has been a reliable method to deal with high levels of organic contaminant in wastewater for decades, membrane bioreactor has recently emerged as a relatively new method to replace CAS as secondary treatment for municipal wastewater. Because the latter is remarkably more efficient in terms of almost all of the removal parameters, it has becoming considered more and more as a suitable improvement for wastewater plants to match increasingly stringent regulations (Talvitie et al., 2017b).

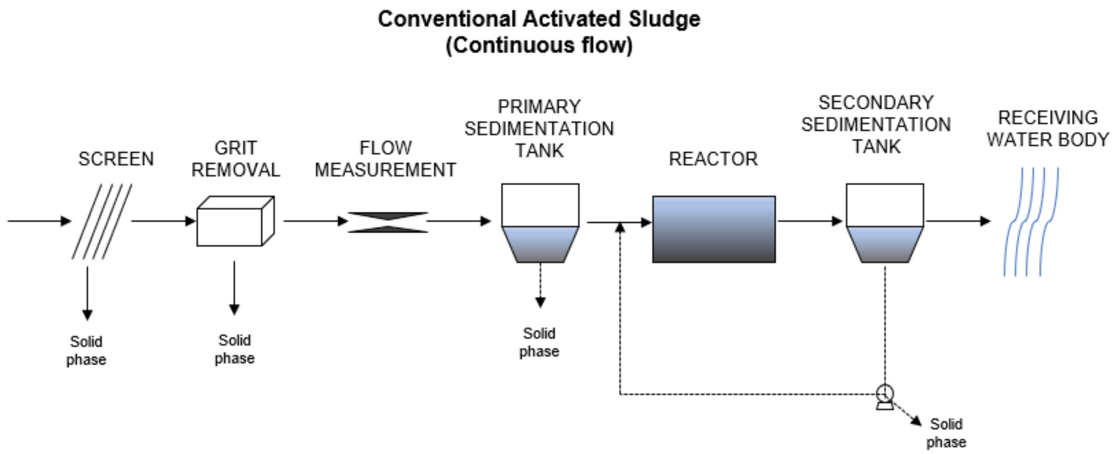

Figure 1. Flow chart of conventional activated sludge treatment process (von Sperling, 2007)

The main feature of CAS is the biological process in aeration tank (reactor) and secondary settling tank. CAS relies mainly on the recirculation of solids (the sludge) from the secondary tank, which ensure sufficient amount of healthy and viable bacteria in the process to consume the high organic load (feed) in the influent water. In other words, CAS relies on sludge to treat the water and since too much sludge can’t be in the system, this would inevitably lead to constant removal of excess sludge (equal to amount produced) (von Sperling, 2007).

Membrane bioreactor is the more recent method of treating wastewater. This is a combination of organic matter oxidation, separation of solids from liquids and microbial decontamination. MBR is scarcely used directly for filtering untreated water but normally after primary sedimentation to reduce fouling (Hai et al., 2014). Submerged MBR is compatible and can be integrated with activated sludge and hence very popular in treating municipal wastewater (as in Kenkäveronniemi plant MBR pilot in Figure 2). It produces effluent of higher quality, smaller footprint and lower sludge production as opposed to CAS as well as smaller space requirement (Gurung, 2014).

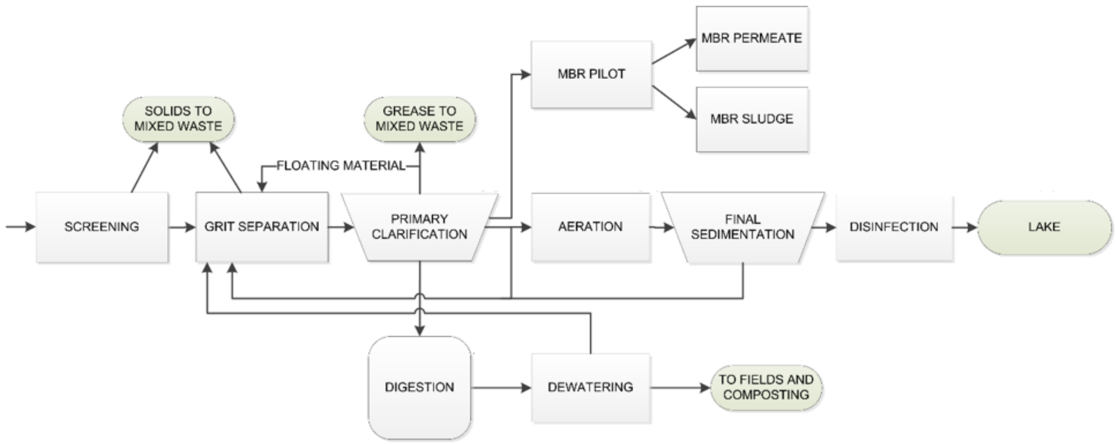

Figure 2. Flow chart of CAS treatment with MBR pilot at Kenkäveronniemi plant (Lares et al., 2017. Permission obtained)

In Kenkäveronniemi plant, the MBR pilot is aerobic (connected with air diffuser), fed with water from primary settling tank. The floating grease (may contain the majority of MP amount, since MP has lower density than water) is removed before the MBR. MBR sludge sample is taken directly from the MBR pilot tank and CAS sample is taken from the aeration tank.

2.2 Previous studies on microplastic in wastewater sludge

Most of available previous studies are on water samples, only a few have measurements on sludge. Therefore, some of the following literature results would be on water samples, not sludge, for an overall picture of MP count in wastewaters.

Some important researches that would be used mainly for comparison of results are listed in the following. Lares took samples from Kenkäveronniemi wastewater treatment plant, which is an important note since the results can be directly compared with present study (Lares et al., 2017). Talvitie also took samples from the same plant, but only did measurements on the liquid fraction, not solids (sludge) (Talvitie et al., 2017b). Murphy had sludge samples, but these were measured after undertaking centrifugal sludge treatment (Murphy et al., 2016). Li’s was the only study that focused solely on sludge (Li et al., 2015). All of these studies conducted MP identification with FTIR spectrometer or Raman spectroscopy after visual identification, so the plastic categorization was more certain and some of it would be used as guidance to sort MP in this study.

Microplastic results generally focuses on their count in the samples, their type (fibers/ particles and chemical composition) and thus implication of their origins (primary or secondary MP, from which product), and finally their physical appearance (size and shape) for visual classification.

2.2.1 Literature results on microplastic count

MP count in sludge was found to be in the order of 15-20 MPs/ g dw (Table 1). Kenkäveronniemi plant was found to have microplastic removal efficiency of 98.3% (Lares et al., 2017). In Talvitie's research, which involved also MBR water from primary settling tank and comparison of different advanced treatments for MP removal efficiency, MBR came out to be the most effective advanced treatment (due to small pore size 0,4 µm) (Talvitie et al., 2017b). She also did testing with water sample taken after secondary settling tank in CAS process to compare between CAS and MBR and MBR was found to be more efficient: CAS effluent had 0.2 (±0.06) MP/L while MBR had 0.005 (±0.004) MP/L. It is said that wastewater is enriched with large numbers of MPs and most of these are retained in the sludge, which showed in the statistics above (Nizzetto et al., 2016).

2.2.2 Literature results on microplastic shape and type

Categorization in previous studies was confirmed with FTIR and/or micro-Raman spectroscopy. Some common types of plastic detected are: polyester (PES), polyethylene (PE), polyamide (PA) and polypropylene (PP). Microscopic images of these different types are shown in Figure 3 (Lares et al., 2017). These images will be used as reference in section 4 to identify the microplastic fibers detected in the samples of this study.

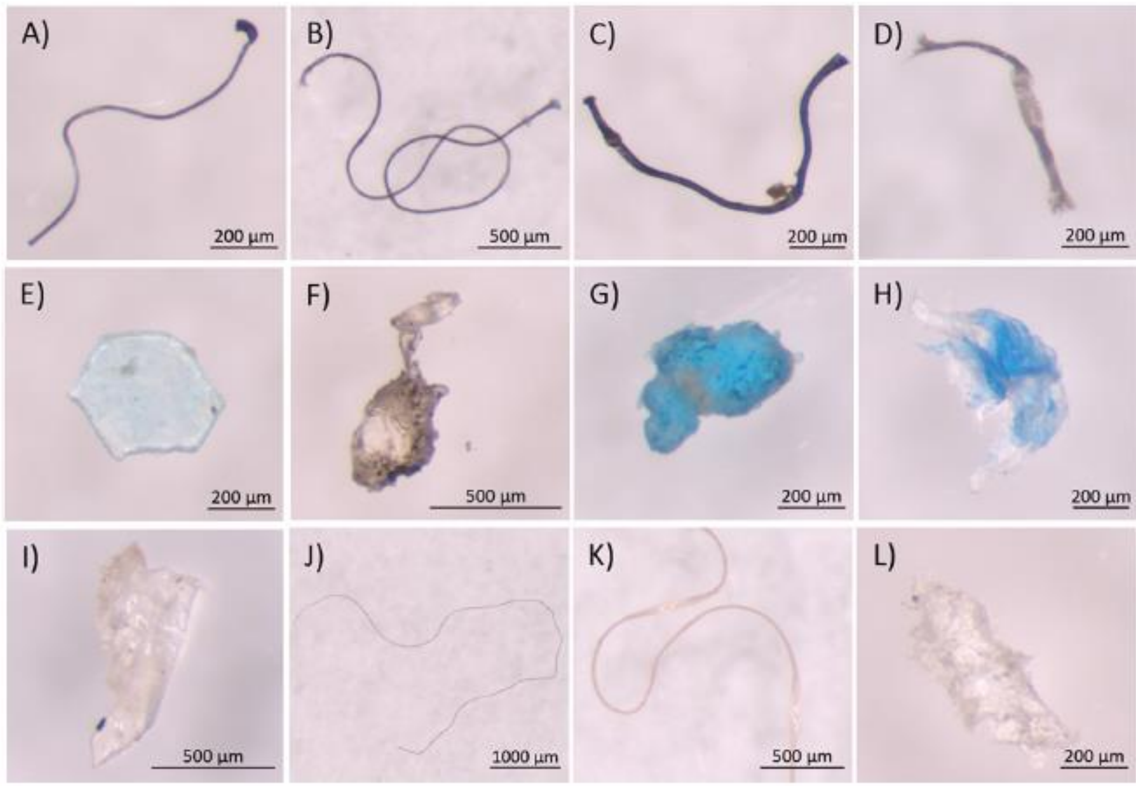

Figure 3. Typical appearances of different polymers identified by micro-FTIR and/or micro-Raman. (A-E) Polyester, (F-I) polyethylene, (J-K) polyamide and (L) polypropylene (Lares et al., 2017. Permission obtained)

Most particles have colors, although some seem to have been faded. Fiber is the most common (probably from textile products). Fibers compose the most fraction: 94% in CA sludge and 92% in initial influent (Lares et al., 2017). There is no plastic industry in the Mikkeli area, so all of these MPs in the discharge wastewater to the plant comes from households and municipal services (Lares et al., 2017).

Of the detected microplastic fibers, polyester accounts for 96.3%, which is 79.1% of total MPs counted. Figure 4 shows results taken from the article, showing percentages of different polymers in different types of sludge samples.

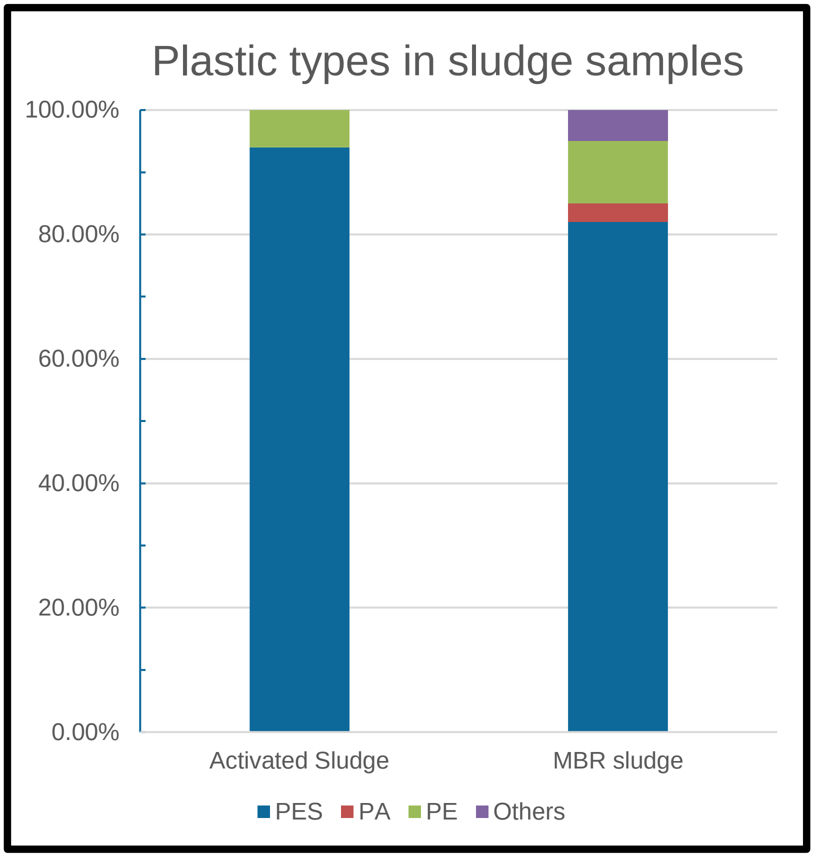

Figure 4. Proportion of different polymers in sludge samples. Various polymers include eg. PE, PP and PES fragments with similar kind of appearance (Lares et al., 2017)

There were only two types of plastic in activated sludge: PES and PE, meaning that most other types escape to the final effluent and eventually to the receiving water body. Meanwhile, MBR was able to filter all types of plastic, which were then found in the MBR sludge. Therefore, it seems that MBR is more effective at removing MP from wastewater than CAS.

In Talvitie's study, it was also reported that most of the PES was from textile fibers and most of the PE seemed to be from personal care products (such as scrubs), based on their distinct appearance (Talvitie et al., 2017b). It was also stated that the visual identification method (as done in the experimental work of this study) "contains the risk of MP over/underestimation due to the misidentification of particles" (Talvitie et al., 2017b). It is true that most researches on this topic conduct FTIR analysis to confirm plastic identification, but the prior visual identification process included may lead to erroneous results. More discussion on this matter will be included in section 4.

The results from Talvitie's study shows that after CAS process, primary MPs amount to 19% and secondary MPs 81% of total MPs found. After advanced treatment, the numbers are 9% and 91%, respectively. The primary MPs were mainly microbeads from personal scrub cosmetics and secondary MPs were mostly fragments of textile fibers (Talvitie et al., 2017b).

MP fibers were reported by Li to be of predominant portion of all MPs found (63%), while the rest shapes are shafts, films, flakes and spheres (Li et al., 2018). The abundance of fibers suggests that most MP in wastewater comes from the washing of clothes and discharge of synthetic fiber manufacturing industry, while flakes are supposedly related to the plastic production process (Mahon et al., 2017; Lechner and Ramler, 2015). Some common plastic types found in Li's study that are found also in other studies mentioned in this thesis are: polyethylene, polyamide, alkyd, polystyrene and acrylic.

In Murphy's research, it was found in solid fraction that: the highest count is in grease (7.87 MP/g), next is grit (1.8 MP/g) and the least is sludge cake (0.7 MP/g), in line with other studies that most MP is efficiently removed from wastewater in the primary skimming and settling step (Murphy et al., 2016; Carr et al., 2016; Talvitie et al., 2015). Found polymers in this study were polyester, acrylic, polypropylene, alkyd and polystyrene.

2.2.3 Literature results on microplastic size

The particles (E-I, L) are around 0.5-1 mm in length, and 0.2-0.5 mm in width (Figure 3). The fibers are long, about more than 1 mm long. However, 64% of the MPs found in Lares' study are smaller than 1 mm, and half of which smaller than 0.5 mm (Lares et al., 2017). The fibers shown in Figure 3 must fall in the remaining 36%, since most of them are more than 1 mm long. Comparison among samples showed that MBR permeate had the highest proportion of smallest-sized microliter (<0.25 mm at 20% of MPs found in sample), though MBR is still much more efficient at removing MPs than CAS process (Lares et al., 2017). Explanation for this may be because since MBR removes more MPs, the bigger-sized MPs are retained more, leading to their reduced proportion, making the smallest-sized portion (which is the hardest to catch) highlighted in the result.

In Murphy's research, MPs from the liquid fraction (water/permeate samples) were on average around 0.6 mm in size, remarkably smaller than those found in the solid fraction (grease, grit, sludge), which was 1.3 mm (Murphy et al., 2016). In Li's, the range of MPs found from all sludge samples was reported to be 37 µm – 5 mm (Li et al., 2018). However, looking at the picture taken directly from their article (Figure 5), which shows visuals of representatives of MPs identified, most MPs are 1-1.3 mm in size, except for the white sphere (which was said to be primary MPs from cosmetic scrub products) and shafts (which may be fragments from larger plastic particles) (Li et al., 2018).

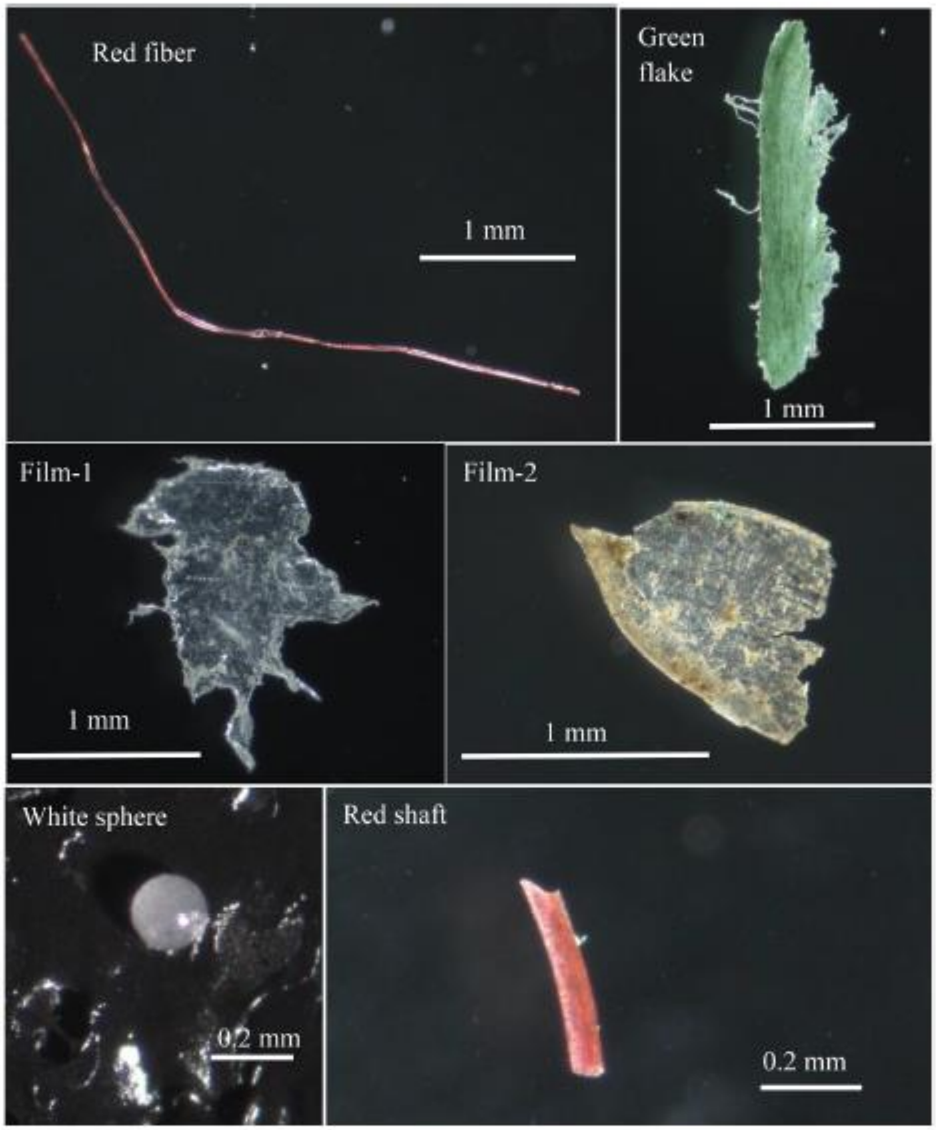

Figure 5. Stereomicrograph of representative MP particles from sewage sludge in China (Li et al., 2018. Permission obtained)

Pretreatment efficiently removes larger-sized MPs (>0.3 mm) and the smallest size fraction (0.02-0.1 mm) grew to be the most abundant toward the later stages in the treatment process (Talvitie et al., 2017a and 2017b). Before MBR treatment, count of each size fraction in the water are: >0.3 mm MPs: 0.1/L, 0.1-0.3 mm MPs: 1.9/L and 0.02-0.1 mm MPs: 5/L (Talvitie et al., 2017b). All of this microlitter was removed after MBR treatment. Since Talvitie only did experiments on water samples, the size of the MPs found in their study is significantly smaller than those of the other studies listed here, which did sampling on sludge samples (or solid fraction in Murphy's study) as well. In other words, MPs found in sludge are larger than in liquids and the smaller the MPs, the higher its count.

In summary, in sludge there are usually 15-20 MPs/ g dw. The plastic types are typically PES, PE, PP and PA, orginating from personal care products or textile products. MPs detected in sludge are larger than those in liquids and commonly falls between 0.2-3 mm.

3 METHOD AND MATERIALS

3.1 Sample preparing and treating

The samples were provided by XAMK's laboratory. The MBR samples were taken on 14th August 2018 and the CAS on 13th September 2018. Each type of sludge sample was given in a 20-liter plastic container. After the container was shaken, about 250 ml was drawn to a beaker to begin preparation.

The sample was thoroughly stirred with a spoon to ensure complete mixing of the solids and the liquid part. Then, it was poured into petri dishes placed on a scale so that the target wet weight of each CAS replicate was 20 g and of MBR was 10 g (Figure 6). These wet weights were recommended by Lares to ensure the approximately same amount of dry weight (0.1g) between two types of sludge later (Lares et al., 2017). Each type of sludge had 5 replicates, so in total 10 petri dishes were examined and analyzed.

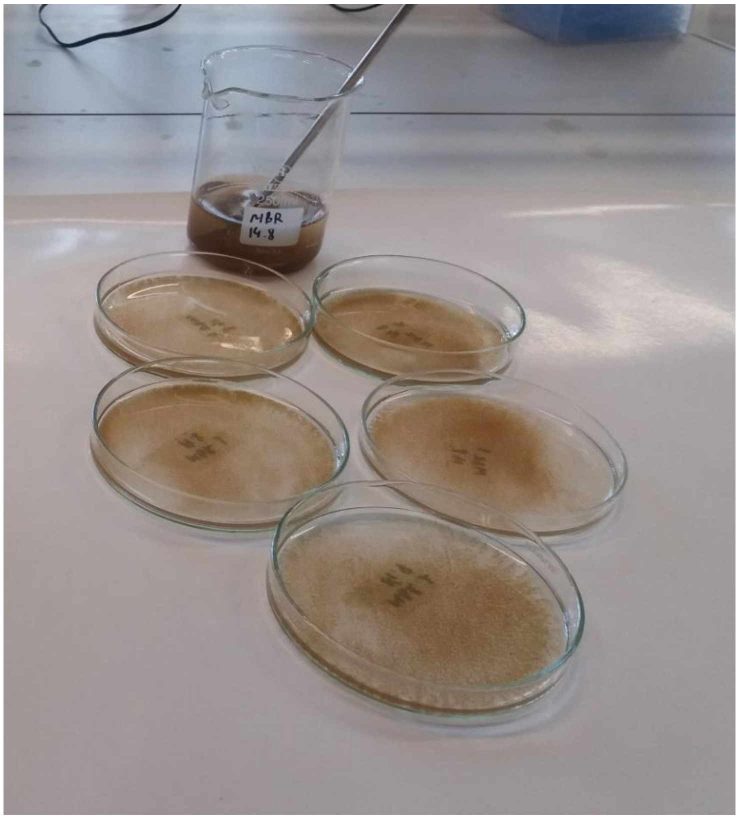

Figure 6. Membrane bioreactor sludge extracted wet samples

The petri dishes were then covered with punctured aluminium foil (to minimize contamination while still enable drying) and placed in an oven until completely dried. The MBR samples were dried at 42°C, for 42h and the CAS at 45°C, for 119h.

For analyzing, each petri dish was placed under microscope. Two microscopes were used: VWR with lens E-PLAN 10x/0.25 and OPTIKA 4083.11 with lens Meiji Japan 13065 SMPlan 10x/0.25, the latter was connected to a computer to take monochrome photos of what can be seen through the lens. Eyepiece magnification of the microscopes was 10x, so together with the objective magnification specified above, the magnification was 100x. Microplastic fibers were detected and counted for each replicate. More photos taken during sample treatment and examination can be viewed in Appendix 1.

No contamination control was conducted for this study, due to its scope and level of simplicity. The sample was given to the author in a plastic container. All tool or material used henceforth was either glass or steel. Only vinyl gloves were used during all procedure. No control samples were included and even if they were, they could not have been from the start (extraction of samples from the wastewater plant). Therefore, the risk of contamination was present, but whether it was high or not was not evaluated and was omitted from this study.

3.2 Sample inspection

After following Lares' method of treating sludge samples (no chemical treatment, drying in oven with pierced tinfoil cover), the dried sludge was examined under 100x magnification microscope (Lares et al., 2017). One of the microscopes used had attached camera and some photos of the (suspected) microplastic particles were taken. However, the photos were monochrome, and the dried sludge matter were shown in the images as well, which may have caused difficulties in distinguishing the MP fiber/particle.

Any particle or fiber that obviously appeared to be microplastic were counted and the uncertain particles were not. The results would be compared between CAS and MBR samples and would further be compared with results from previous studies, whenever applicable, to see if the findings are in accordance with what have been found by more detailed, professional researchers.

An attempt was made at visual categorization of the MPs found. They were classified based on their color and shape, taking reference from images of previous studies in literature (specifically, Figure 3 in this report). Obviously, this method of classification relied solely on author's own judgement and thus introduced some uncertainty.

4 RESULTS AND ANALYSIS

4.1 Microplastic count

CAS had 7 MPs in total, on average 1.4 MPs/sample; MBR had 9 MPs in total, on average 1.8 MPs/sample. Each sample was 0.1 g dw, therefore CAS had 14 MP/g dw and MBR had 18 MPs/g dw, Comparison to previous studies were shown in Table 1 whenever applicable.

Table 1. Average values of microplastic counts from various studies (Standard deviation is stated in parentheses)

Studies	Activated sludge		MBR sludge	
	MP particles per gram dw	MP fibers per gram dw	MP particles per gram dw	MP fibers per gram dw
Present study	0	14	1	17
Lares et al., 2017	1.3 (±1.3)	21.7 (±4.6)	3.3 (±2.4)	24.1 (±6.1)
Li et al., 2018	22.7 (±12.1)			
Magnusson et al., 2014	16.72 (±1.96)			
Murphy et al., 2016	1 (±1)			
Mahon et al., 2017	9.8			
Zubris et al., 2005		4 (±0.5)		

The only direct comparison to all categories of samples are with Lares' results, which shows that present study results are agreeable and along the same line, considering the standard deviation, though fewer MP was found (Lares et al., 2017). Results of Li's study were in the range, considering that the count in his study is the combination of both particles and fibers whereas here only fibers were found in CAS samples. The same could be said with Magnusson's study. On the other hand, Murphy's samples were after sludge treatment in centrifuge, so were Mahon's and Zubris' (either with anaerobic digestion, thermal drying, lime stabilization treatment processes), which may explain the large difference with present study and indicate that any method of sludge treatment may reduce MP count considerably (Murphy et al., 2016; Mahon et al., 2017; Zubris et al., 2005).

Since the present study found that MBR filtered more MPs than CAS process (there were more MPs in MBR sludge than in the latter), this may indicate that

MBR was more effective at removing MPs from wastewater than CAS. If the regulations on MPs become even more strict and/or the application of the excess sludge is very MP-sensitive, it'd be reasonable for the concerned wastewater plant to consider the use of MBR instead of or in addition to CAS.

4.2 Physical appearance and visual categorization of microplastic found

All MPs found was from the sludge, which was the part filtered from the water, and since influents to both the aeration tank (CAS sampling origin) and MBR pilot were from the same primary settling tank, this explains why there is no apparent difference in the type and appearance of the MP found in the sludge between the two processes. Indeed, both sludge had very long and very short fibers and of the same chemical types. However, at this point, the results from present study contradicts results of Lares', where there is difference between MPs dimensions and type between the two types of sludge (as shown in Section 2.2.2 and 2.2.3) (Lares et al., 2017). No other explanation has been found except for the smaller sample pool and numerous uncertainties in present study, which would be shown in section 4.3.

MBR had fibers of averagely 1.1 mm, ranging from 0.3-3 mm; CAS had fibers of averagely 1.52 mm, ranging from 0.2-3.2 mm. Average length in this study is slightly longer than that reported by Lares, possibly because in present study these large MPs were the only one apparent enough to be sure of. Nonetheless, MPs found in this study are still in the range of microplastic (<5 mm) (Talvitie et al., 2017).

There was only one round particle detected. However, due to its unclear appearance, it will be discussed more in Section 4.3 Uncertainties. All of the other detected MPs were fibers, some microscopic images of which are provided in Figure 7.

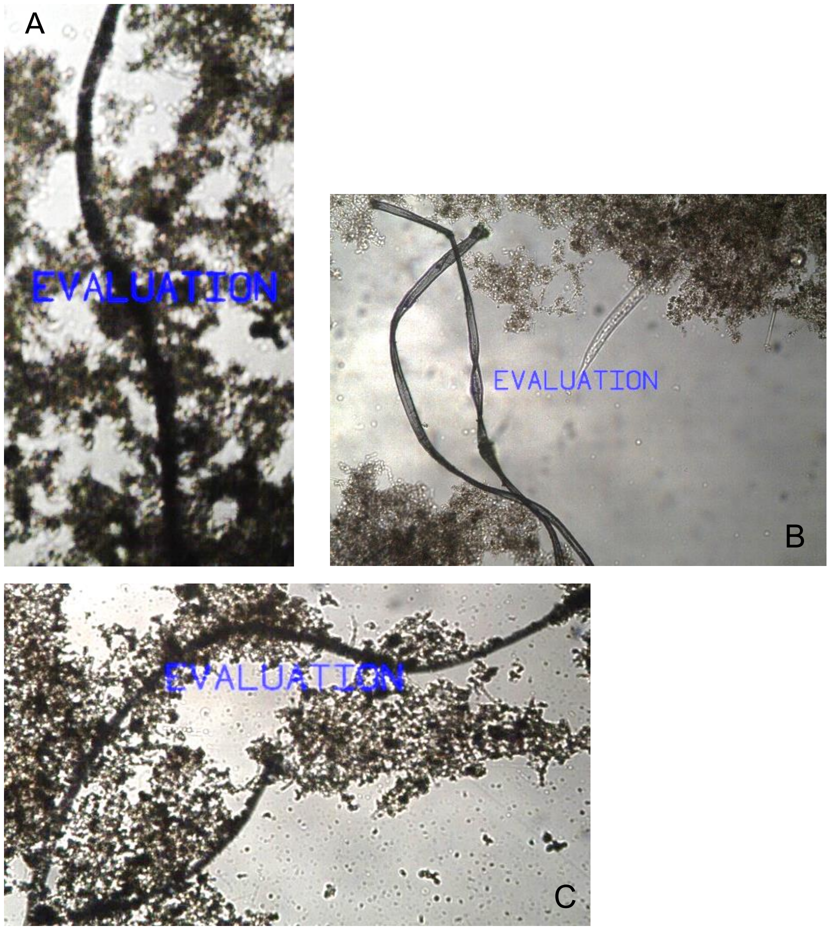

Figure 7. Microscopic images of representative microplastic fibers found in present study: A, B: blue fiber; C: black fiber

Figure 7 shows that the fibers seem considerably distinguished from their surroundings and hence, should be quite easy to detect under the microscope. However, there were other fibrous particles, which did not appear as distinct, and caused difficulty for author trying to determine if they were MP or not (hence, were not counted in the results). They will be discussed in the next section.

Blue was the most common color, then green and black. Some fibers appeared to be clear but it’s not sure whether they were clear originally or faded from any color (probably the latter, since there were some appearing to be faded from original green or blue). According to Lares, most would be polyester (A-D) and some clear ones are polyamide (J) (Figure 3), which are two types often found of MPs in wastewater (Lares et al., 2017; Carr et al., 2016; Talvitie et al., 2017).

Both polyester and polyamide are common textile materials and would be easily fragmented and washed off from clothes during laundry (Carr et al., 2016).

4.3 Uncertainties, limitations of present study and further recommendations

First, sludge samples treating could use chemicals to help dissolve organic matter, as in (Li et al., 2018). Sludge was poured into a flask with deionized water (which already had sodium chloride added to saturation). Then it was stirred for 15 minutes and left to settle for 2 hours. After that, the top water layer was filtered in a vacuum filtration unit with a 37 µm-pore-sized sieve, which was subsequently washed with distilled water to remove salt residues. This extract was then treated overnight with hydrogen peroxide solution (H_2O_2, 30%) to remove the soft and easily disintegrating organic materials. This mixture was then poured into distilled water, vacuum-filtrated through glass fiber filters, rinsed several times with deionized water and dried in a desiccator for 3 days.

Such method of treating sludge samples is deemed superior to method adopted in present study in which the former minimizes the presence of organic matter upon inspecting through the microscope, further aiding the visual identification of MPs.

Second, working with sample in a petri dish was tricky using the wrong kind of microscope. In this study, the dried sludge samples were inspected with normal light microscope, which had a staging area that is much better suited for specimens on a glass plate, rather than in a petri dish. This caused much difficulties during identification of MP. The different lenses attached on such a microscope obstructed the petri dish and hindered its movement in the process of inspection, which may have caused errors in counting the MP fibers.

A stereomicroscope, on the other hand, has a staging area that is meant for petri dishes (Figure 6 in Appendix 1) and it is considered easier and less erroneous using one for inspection of samples in petri dishes, which explains its being used in several previous studies (Li et al., 2016; Talvitie et al., 2017)

Third, since visual identification is prone to errors, it'd be more thorough and credible to incorporate the use of FTIR and/or Raman spectroscopy, if possible, either as a mean to confirm all MP existence (rather than the usual process of visual detection, then only some MPs are selected for FTIR/Raman) or to replace visual method altogether (Murphy et al., 2016). These methods would also constitute to higher certainty with plastic categorization than visual identification. Certainly, to use FTIR/Raman with large numbers of MP in a large sample pool would be time-consuming, costly and impractical in most situations. Nonetheless, the likability to produce errors of visual identification is worth noting whenever the purpose is to construct a creditable study with the least uncertainties possible.

Due to the uncertainties above, some examples of unclear particles were produced and are shown in Figure 8 and 9.

Figure 8. Detected round microplastic particle in MBR sludge sample

The image appears to be a sphere MP particle, which is clear-colored and has diameter of about 0.1 mm. The particle could be microbeads from facial scrub products, which are made of polyethylene (Fendall et al., 2009), since it was found in municipal wastewater and there is no plastic industry in Mikkeli. It seems that there was some organic matter attached to the surface of the particle. However, there is a chance that this particle was not really microplastic and could be just a drop of water with some sludge matter adsorbed to it.

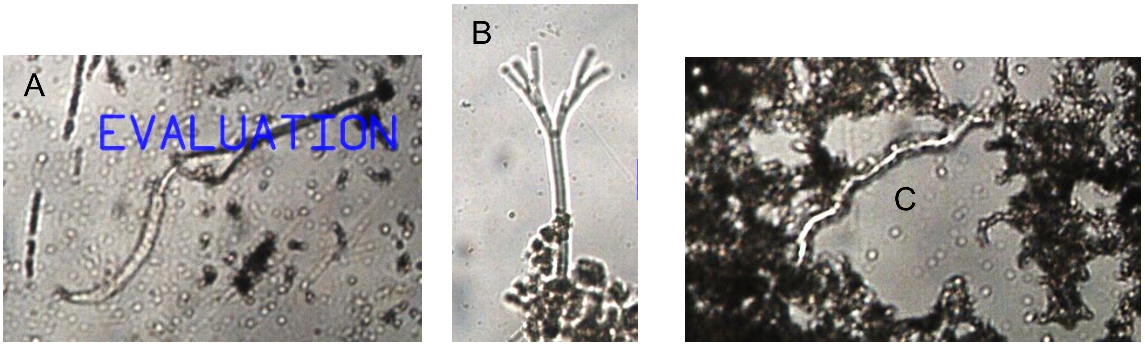

Figure 9. Microscopic images of unidentified fibrous particles detected in present study

It is not apparent if these are particles, or just streaks of liquid that got magnified under the microscope or scratches of the layer of sludge water on the petri dish that was accidentally made by the pierced tinfoil cover during sample treatment. The particles in Figure 9-A may be a short (about 0.2 mm) and faded, clear fiber but the latter two may fall in the case of scratches or liquid streaks. Due to this uncertainty, the author didn’t include these three or any such findings in the final MP results.

Other uncertainties in present study that can be mentioned is small sample pool, which means statistically, the results are not highly credible; determination of plastic type based on author’s own judgement on references from previous studies, which may not be correct, and the surface of MPs are easily caught on by organic matter or other toxic contaminants, which may appears quite confusingly under the microscope, thus making the identification process even more challenging (Holmes et al., 2012; Koelmans et al., 2013, 2016; Rochman et al., 2014; Wright and Kelly, 2017; Ziajahromi et al., 2016).

In short, some recommendations from this study would be: use chemicals to dissolve organic matters in treating samples; use stereomicroscope for samples in petri dishes; use FTIR/Raman spectroscopy if applicable and visual identification should be used by experienced researchers.

5 CONCLUSION

Firstly, this study found that there were 14 MPs and 18 MPs per gram dw in conventional activated sludge and membrane bio-reactor sludge, respectively, implying that MBR is the superior method to CAS in filtering MPs. These MPs were visually identified to be polyester and polyamide, of which possible origins are textile products. Secondly, the results are in accordance with previous studies on microplastic in wastewater sludge. Lastly, the method of treating and inspecting samples implemented in this study, though simple and not costly to follow, is more suitable for experienced researchers, who have done numerous experiments with microplastic and thus would be able to determine on first sight whether the particle detected is microplastic or not. Otherwise, for beginner researchers, this method could produce some unclear and unapparent images, causing confusion and uncertainties, leading to erroneous results. Therefore, in future studies, it is suggested to use chemicals (such as hydrogen peroxide, H_2O_2 30%) in treating sludge samples to dissolve organic matter and to use stereomicroscope in examining samples, both of which would considerably facilitate the inspection process and hence, improve result certainty.

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Appendix 1

OTHER PHOTOS TAKEN DURING SAMPLE TREATING AND INSPECTION

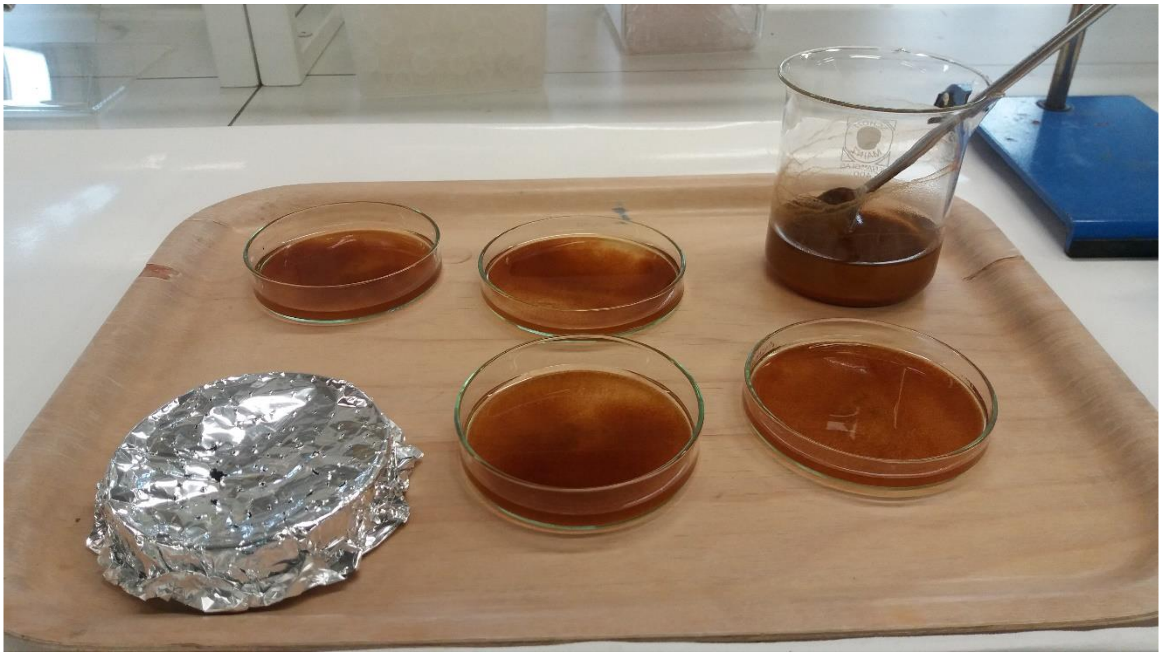

Figure 1. Conventional activated sludge wet samples

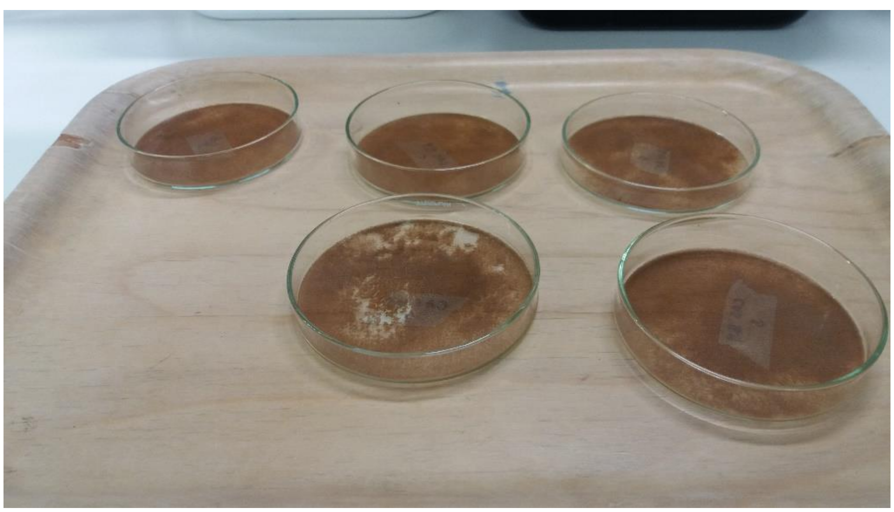

Figure 2. Conventional activated sludge dried samples

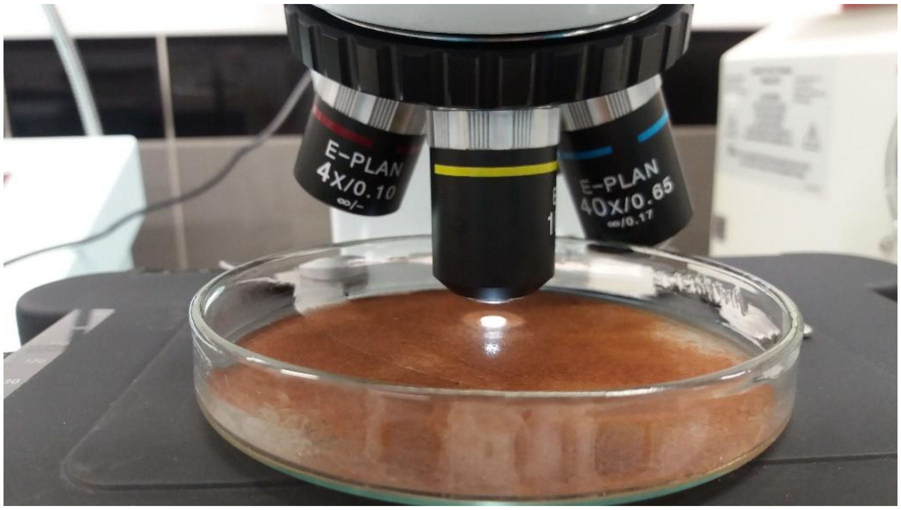

Figure 3. Petri dish placed under microscope VWR for examination

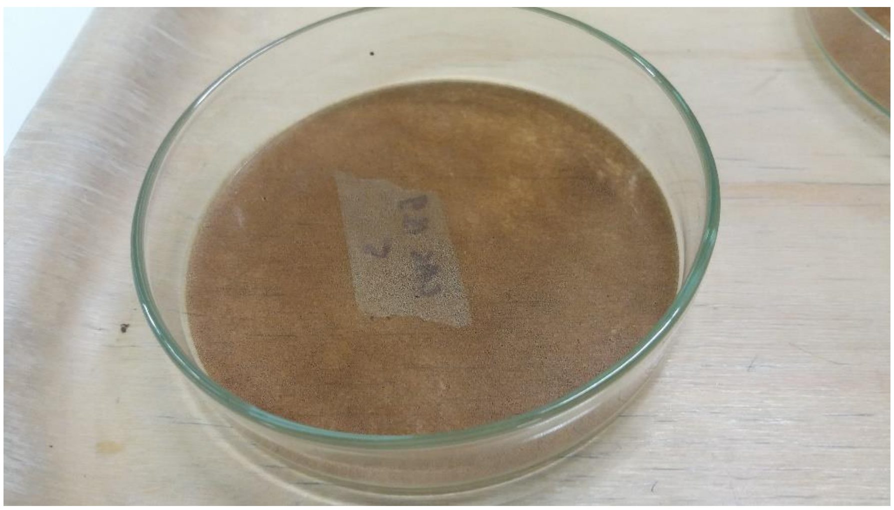

Figure 4. Conventional activated sludge dried sample, replicate 5

Figure 5. Normal light microscope OPTIKA 4083.11

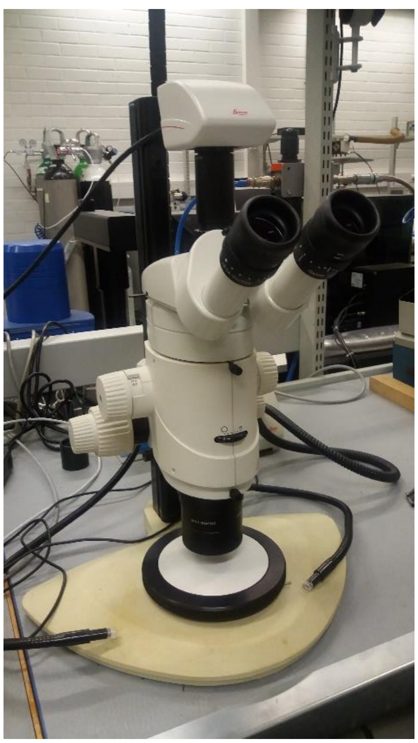

Figure 6. An example of a stereomicroscope