

Aquatic Aerobic Biodegradation of Commonly Flushed Materials in Aerobic Wastewater Treatment Plant Solids, Seawater, and Lakewater

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Microfibers and microplastics originating from wastewater treatment plant (WWTP) effluents are significant pollutants in freshwater sources and marine environments. This research investigated the biodegradation of cotton microfibers generated from bleached cotton jersey knit fabric and commercially available flushable wipes, polypropylene-based (PP) nonwoven wipes containing a cellulose component, and tissue paper. Biodegradation was tested in wastewater treatment plants (WWTP) solids, seawater, and lakewater according to the ISO 14852 and ASTM D6691 standard methods in an ECHO respirometer. Degradation experiments continued until a plateau in CO₂ emissions was reached, and the final biodegradation extent was calculated relative to the theoretical CO₂ produced based on elemental analysis. The results showed that the cotton and other cellulosic materials/components biodegrade to a great extent, as expected for all conditions, whereas the PP did not degrade. In general, for the cellulose polypropylene composite wipes, the cellulose biodegraded readily; the presence of the PP did not hinder the cellulose biodegradation.

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INTRODUCTION

Fibers from textile products have been observed in significant quantities in wastewater treatment plant (WWTP) effluents (Talvitie *et al.* 2017). It is believed that these microfibers enter the wastewater treatment plants (WWTPs) with the effluents of the washing machines and also from airborne microfibers that deposit on the ground and then are transferred through the sewer system (Browne *et al.* 2010; Dris *et al.* 2017). Even though WWTPs have relatively high microplastics and microfibers removal capacity (>98%), they continually release large volumes of effluents containing low concentrations of microplastics to freshwater sources and marine environments (Talvitie *et al.* 2017).

Polyethylene terephthalate (PET) and cellulose-based fibers, such as those found in tissue paper, nonwovens, and other textiles, have been detected as well (Ziajahromi *et al.* 2017). These fibers are typically called microfibers due to their small size (< 5mm); however, the textile industry prefers a definition of microfibers as fine fibers with a denier less than one but greater than 0.3 deniers (Clarke 2021). Therefore, The Microfiber Consortium has defined these particles as fiber fragments, short pieces of textile fiber

broken from the main textile construction (The Microfibre Consortium 2020). These fiber fragments could be made of synthetic or natural polymers (Zambrano *et al.* 2019). For this study, the term microfibers will be used to describe pulp fibers and fibers, either natural or synthetic in origin, smaller than 5 mm in size (length). Synthetic microfibers can be classified as a subset of microplastics, but microfibers of natural origin (wool, silk, cotton) are not considered microplastics.

Textile microfibers are contaminants of emerging concern because they have been observed in oceans and coasts, lakes, estuaries, and rivers from the surface to the sediments (Vermaire *et al.* 2017; Fernandez Severini *et al.* 2019; Henry *et al.* 2019; Peller *et al.* 2019). They have also been reported in air, the food web, and humans (Cox *et al.* 2019). The fate and role of natural fibers in aquatic environments is not well-known, although more focus has shifted to this research topic. The environmental impacts of textile finishes on synthetic and natural microfibers have not yet been investigated and are relatively unknown.

Recent literature shows the presence of cellulosic fibers in different ecosystems. For example, Suaria *et al.* (2020) observed fibers, mostly dyed, in 99.7% of all samples collected in six oceanic basins (0.02 to 25.8 fibers liter⁻¹, ~10⁸ fibers km⁻²). These fibers were typical of natural and synthetic textile goods and suspected to be effluents from laundering (Suaria *et al.* 2020). Athey *et al.* (2020) also reported microfibers in 87 to 90% of the anthropogenic particles collected from lake sediments, with around 41% of these microfibers identified as modified cellulose. Studies also propose that cellulose microfibers found in aquatic environments are the result of laundering cotton textiles (De Falco *et al.* 2020). However, the reliability of identifying the source of these cellulosic fibers is currently under debate, which could potentially lead to errors or false positives when distinguishing different sources of cellulosic fibers. Other studies propose that textiles made from synthetic fibers such as nylon and polyester make up the largest sector of the microplastic problem, as they release substantial microfibers during washing and in landfills (Fernandez Severini *et al.* 2019). One garment is estimated to shed greater than 1900 microfibers in one wash (Browne *et al.* 2011). Along with microfibers and fiber fragments generated during laundering, cellulosic fibers are also released into aquatic environments by flushing paper and nonwoven materials in toilets and the like. The USA is estimated to have the highest per capita tissue paper consumption at 141 rolls or 12.69 kg annually (Armstrong and Richter 2018). Germany follows at 134 rolls per capita and the UK comes in third at 127 rolls per capita (Armstrong and Richter 2018).

Along with flushable materials meant to biodegrade in aquatic environments, a “throw-away” culture also exists, wherein unflushable products such as nonwoven wet wipes and sanitary items are disposed of improperly by flushing, causing both environmental and infrastructural issues (Alda-Vidal *et al.* 2020). Being observed not only in oceans and coasts but also in lakes, estuaries, rivers, and the food web, microfibers can be ingested by organisms and cause damage to their digestive tracts, affect reproduction rates, and enzyme activity. The spread of invasive species and pathogenic microorganisms can also occur due to the attachment of microorganisms and other organisms to the surface of microfibers, which are similar in size to low-end organisms in the food chain. As the scope of microfiber pollution continues to expand, it is crucial to address its potential hazards and reduce its environmental impact (Xi *et al.* 2022).

Microfibers from flushed hygiene products are not consistently retained by wastewater treatment plants and make their way to marine environments. Alternatively, whole nonwoven materials, primarily non-biodegradable wet wipes, directly enter waterways and create pipe clogs known as fatbergs, resulting in sewage overflow (Michael

2020). There is an average of 300,000 sewer blockages every year in the UK, costing roughly £100 million (Curran *et al.* 2019). Polypropylene wipes are notoriously challenging to degrade, partly due to the polymers' highly crystalline structure. Biodegradation experiments have shown biodegradation extents of MCC > cotton > rayon > polyester/cotton >> polyester in simulated freshwater environments (Zambrano *et al.* 2019).

The research herein aims to model and compare the aquatic biodegradation of commonly flushed materials in wastewater treatment plant solids, lake water, and seawater, which can provide insight into their fate in home sewage environments, sewer systems, and aquatic environments.

EXPERIMENTAL

Materials Preparation and Characterization

Cotton microfibers generated from bleached cotton jersey knit fabric and commercially available flushable wipes (Charmin Freshmates), polypropylene-based (PP) nonwoven wipes (Super Sani-Cloth Wipes), and tissue paper (Charmin Essentials Soft) were evaluated for biodegradability in an inoculum of dilute solids obtained from a wastewater treatment plant (WWTP), lake water, and seawater. The flushable wipes and tissue paper were designed to be flushed down the drain. The polypropylene-based nonwoven is not intended to be flushed; however, the frequent presence of these types of material in sewer systems causes sewage blockage and overflow issues in wastewater infrastructure. Cotton microfibers were used to simulate microfibers from laundering. Each material represented items that are commonly found in water systems.

Flushable wipes and tissue paper were cut into 1 cm x 1 cm pieces. Polypropylene-based wipes were thoroughly rinsed with water to remove any anti-microbial additives, oven-dried at 105 °C for 24 hours, and then cut into 1 cm x 1 cm pieces. Cotton microfibers were generated from a jersey knit, unfinished, bleached cotton fabric using a Wiley Mill (arithmetic mean length of 1.342 ± 0.020 mm, arithmetic mean width of 19.7 ± 0.121 μm). Microfibers were not generated from the other materials (tissue paper, flushable wipes, and polypropylene-based wipes) to keep materials in a more standard state. Microcrystalline cellulose with an average particle size of 50 μm was used as a reference material.

Materials were characterized for composition using elemental analysis (carbon, hydrogen, nitrogen) and Fourier-Transform infrared spectroscopy (FTIR). The morphology of each material was analyzed using scanning electron microscopy (SEM) before and after biodegradation where appropriate. Thermogravimetric analysis (TGA) was completed for the nonwoven polypropylene wipe to quantify the amount of cellulose in the material. Near InfraRed Analysis (NIR) was performed by Cotton, Inc to determine the fiber composition, degree of mercerization, and cellulose type in the materials.

Inoculum Collection

Activated sludge of the aerated zone of a bioreactor from the wastewater treatment plant (WWTP) at the Neuse River Recovery Facility (Raleigh, North Carolina USA) was used as a source of microbes for the biodegradation of the materials studied according to the standard method ISO 14852 in an ECHO respirometer (ECHO Instruments, Slovenske Konjice, Slovenia, EU). Sludge was pumped out of the zone using the recovery facility system and into the collection container. Initially the container was partially filled, swirled, and then emptied. Then the container was filled again, leaving approximately one-third

free volume at the top of the container. After collecting the sludge in November of 2020, the total suspended solids (TSS) were measured. The sludge was added to the media at 1000 ppm of TSS per reaction vessel. The reaction vessels were prepared within 24 hours after the sludge collection. In summary, 12 flasks were prepared for each experiment; two replicates of each sample and two blanks that contained only the test media.

Seawater was collected on May 20, 2021, separately at low tide and high tide from Homer's Point Marina, Salter Path, North Carolina, USA. The container openings were immersed to a depth of approximately six inches. Initially, the container was partially filled, swirled, and then emptied. The container was then filled leaving one-third free volume at the top of the container. The seawater was used as the source of microbes for the biodegradation of the materials studied using the standard method ASTM D6691 in the ECHO respirometer. The low tide and high tide seawater samples were mixed at a 1:1 ratio, and reaction vessels were prepared within 24 hours after the seawater collection. In total, 12 flasks were prepared for each experiment; two replicates of each sample and two blanks that contained only the seawater and the nutrients.

Lakewater was collected on August 31, 2021 from Lake Raleigh, Raleigh, North Carolina, USA. The container opening was immersed to a depth of approximately six inches near the boat ramp. Initially, the container was partially filled, swirled, and then emptied. The container was then filled leaving one-third free volume at the top of the container. The lakewater was used as the source of microbes for the biodegradation of the materials studied using the standard method ISO 14852 in the ECHO respirometer. Reaction vessels were prepared within 24 hours after the lakewater collection. In total, 12 flasks were prepared for each experiment; two replicates of each sample and two blanks that contained only the lakewater and the nutrients.

The optimized nutrient solution recommended by ISO 14852 and the standard test media recommended by ASTM D6691 were used for biodegradation experiments. In each reaction vessel, 800 mL of test media was used to ensure good stirring and enough headspace for oxygen transfer (aerobic). After adding the test medium, each vessel was closed tightly and positioned in the ECHO respirometer. These flasks were incubated in the dark at a constant temperature and under stirring with magnetic bars (300 rpm). After one week of incubation, the material was added to each flask and the respirometer was reinitiated to record the biodegradation data. Material (3.5 grams) was added for WWTP solids and 0.200 grams of material was added for seawater and lakewater. Throughout the experiment, the pH of the system was measured with a probe weekly and adjusted as needed with 1 N sodium hydroxide (Fisher Scientific) and hydrochloric acid (Fisher Scientific) solutions to maintain the pH upon initial sample collection – a pH of 7 for WWTP conditions, a pH of 8.1 for seawater, and a pH of 9.1 for lakewater. In this experiment, the ECHO respirometer was used to track the production and concentration of gases (O₂ and CO₂), using near-infrared (NIR) sensors and flowmeters to monitor the gas concentration and flow. With this system, the biodegradation was calculated by comparing the CO₂ produced with the theoretical CO₂ that should be produced if 100% of the initial materials added were to biodegrade based on their elemental composition.

In these experiments, cotton microfibers produced from cotton fabrics along with cellulosic tissue paper, cellulosic flushable wipes, and polypropylene-based wipes were evaluated. These materials were compared with a positive control of microcrystalline cellulose (MCC, 50 µm particle size, Acros Organics). Duplicates were prepared for all samples, the positive control, and the blanks. Figure 1 summarizes the experimental scheme.

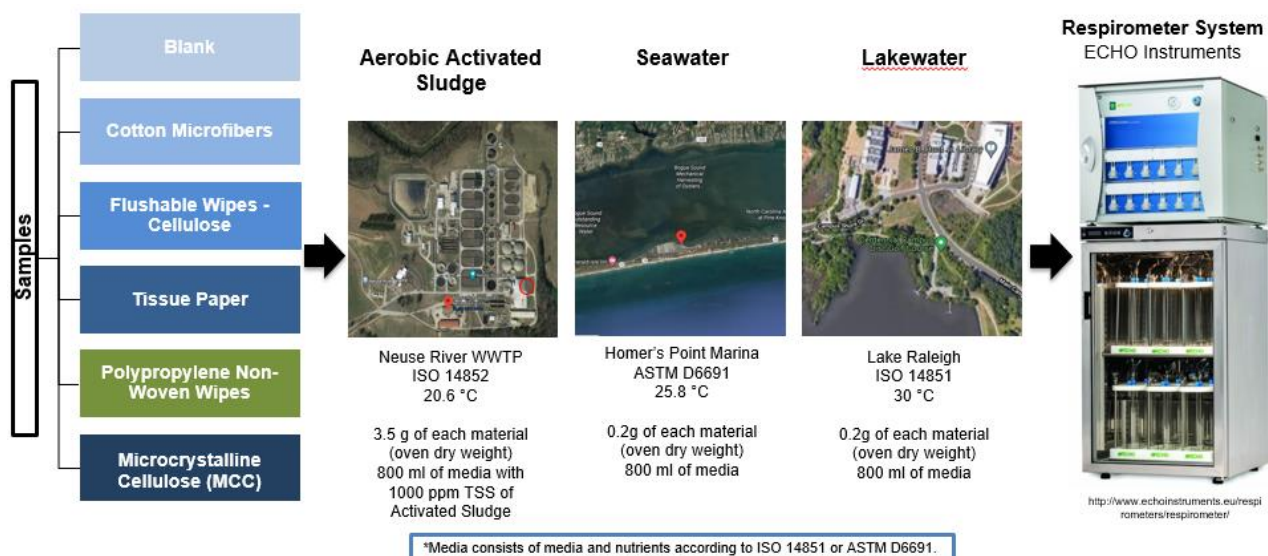


Fig. 1. Experimental plan of biodegradation in WWTP activated sludge and seawater

RESULTS AND DISCUSSION

Material Characterization

Fourier transform infrared (FTIR) spectra were taken of all materials and are shown in Fig. 2. Based on the FTIR spectra, the polypropylene-based wipes also contained some cellulosic fibers, as confirmed by scanning electron microscopy (SEM) imaging. There was a broad peak in the PP-based wipes observed at 3341 cm^{-1} , representing OH stretching consistent with cellulose. Pure polypropylene does not contain hydroxyl groups capable of producing this peak. Additional C-O stretching peaks that are in line with cellulose were observed in the PP based wipe spectra at 1645 cm^{-1} . The flushable wipes sample showed a spectra in accordance with cellulose.

SEM images of all materials before biodegradation are shown in Fig. 3. SEM images were taken on a Variable Pressure Scanning Electron Microscope (Hitachi SU3900) and sputter coated with gold. The images are consistent with the FTIR data and show that cotton microfibers and tissue paper are composed mainly of only one type of fiber material. The PP based wipes showed two different fibers in the SEM image, one smooth as expected in PP and another with a textured surface corresponding to plant-based cellulose. The flushable wipes also showed a smooth fiber from regenerated cellulose and also wood fibers.

NIR Analysis (Cotton, Incorporated) was conducted to determine the fiber composition. NIR identified the composition for samples containing cotton, wood pulp, or regenerated cellulose (Table 1).

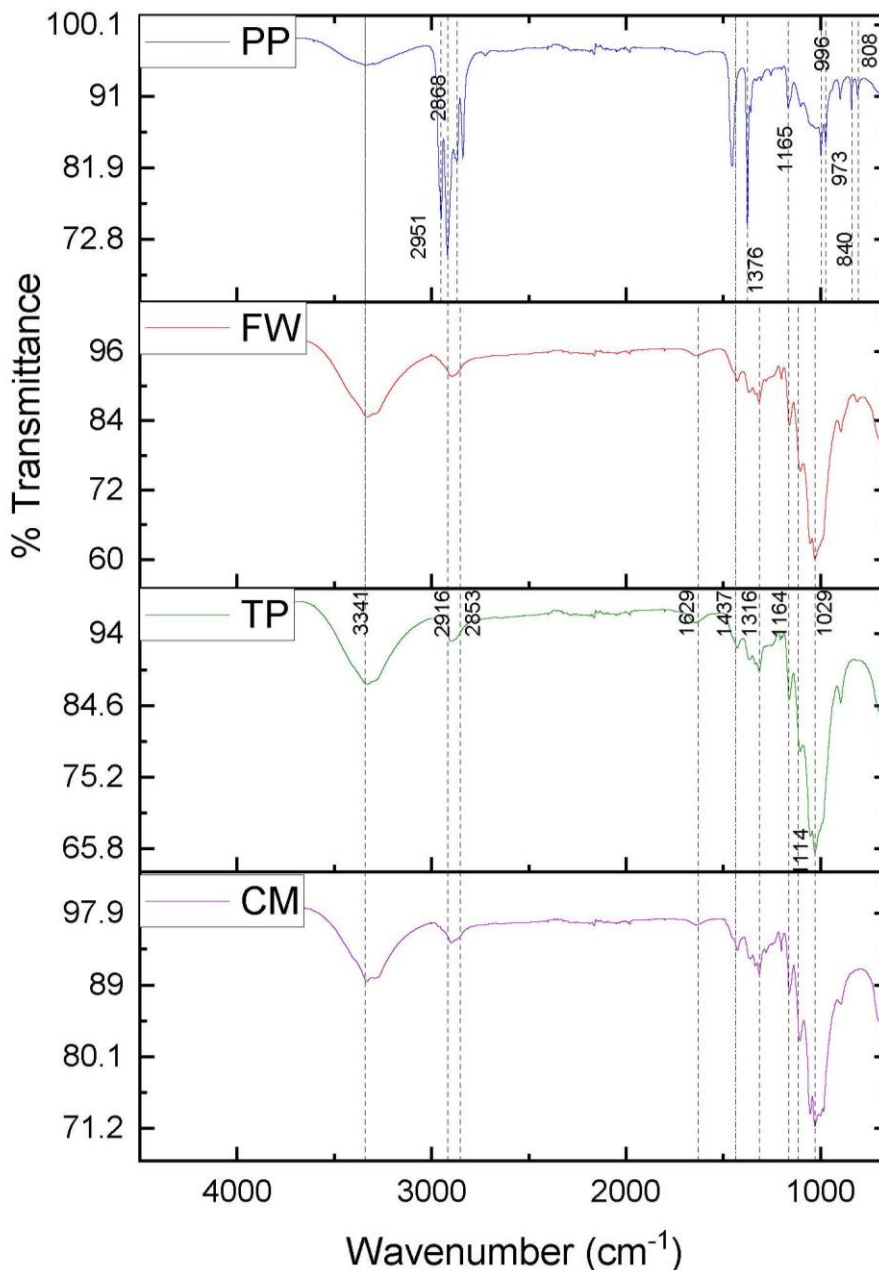


Fig. 2. FTIR spectra of the polypropylene-based nonwoven (PP), flushable wipe (FW), toilet paper (TP), and cotton microfibers (CM)

Table 1. NIR Analysis Identity of Fibers in Commonly Flushed Material

Material	Composition
Cotton Microfibers	Cotton
Flushable Wipes – Cellulose	Cotton Regenerated Cellulose
Tissue Paper	Wood Pulp
Polypropylene-based Wipes	Polypropylene Regenerated Cellulose

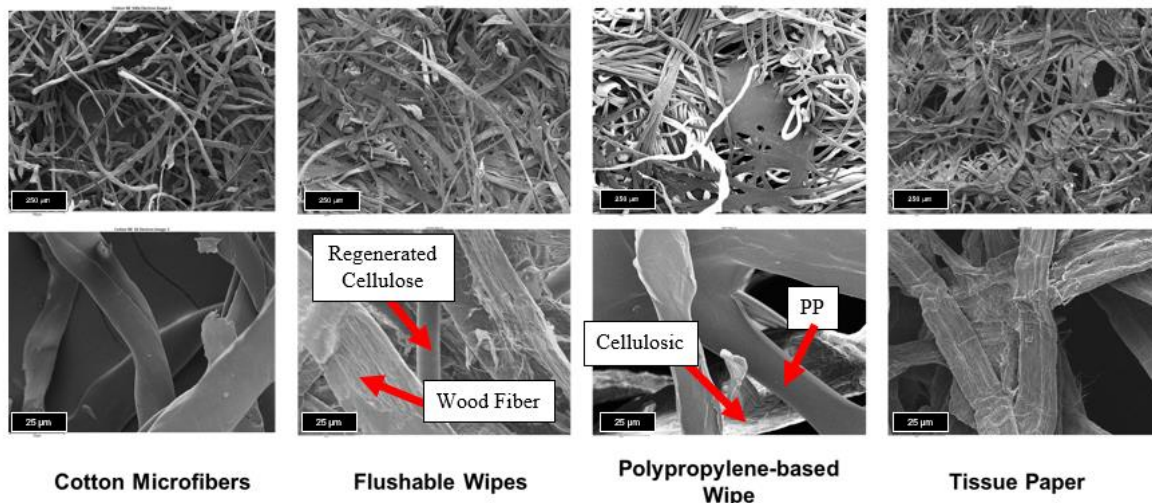
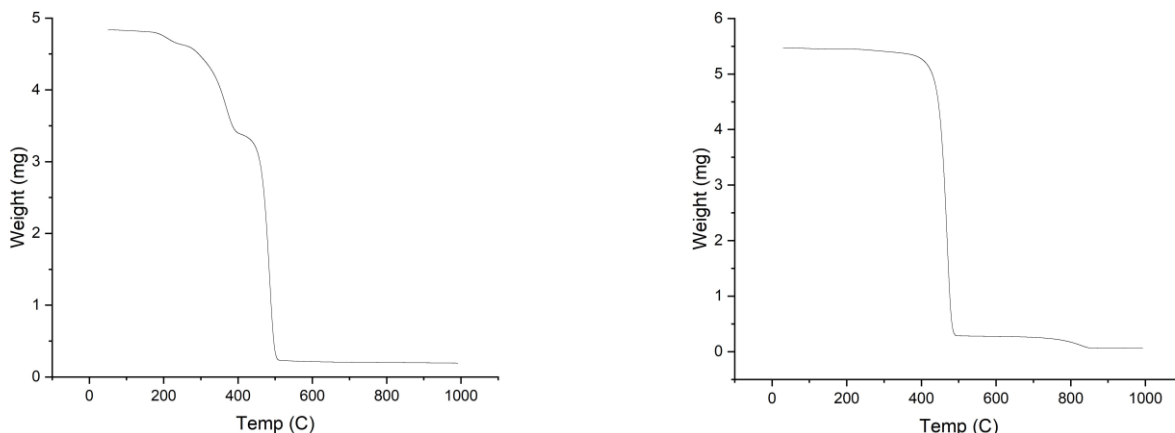


Fig. 3. SEM images of commonly-flushed materials before biodegradation

TGA analysis was completed for the polypropylene-based wipe (see later) and used to determine a cellulose content of 28% by weight. As shown in Fig. 4, the thermal degradation losses of the PP-based wipe are observed beginning at 300 °C corresponding to cellulose and another beginning at 400 °C corresponding to polypropylene (Cichosz and Masek 2020; Ezmizadeh *et al.* 2020).



A - Polypropylene-based wipe before biodegradation

B - Polypropylene-based wipe after biodegradation

Fig. 4. Thermogravimetric analysis (TGA) data for (A) polypropylene-based wipes before (A) and (B) after biodegradation in the WWTP inoculum

Aquatic Biodegradation in Aerobic WWTP, Seawater, and Lakewater

The percent biodegradation of the products (based on the CO₂ produced versus the theoretical CO₂ in the initial material) are shown in Fig. 5 for an experiment with WWTP solids at 1000 ppm to mimic general freshwater conditions in the environment. The reference material (MCC, Microcrystalline Cellulose) reached 64.32 ± 1.90% of biodegradation in the 110 days of the experiment, demonstrating that the microorganisms in the WWTP were active. All of the cellulosic materials (cotton microfibers, tissue paper,

and flushable wipes) biodegraded to a higher extent than even the MCC. The microfibers from cotton degraded the fastest and to the highest extent, presumably due to the high surface area and cellulosic content. The polypropylene-based wipes degraded by $18.23 \pm 0.47\%$ in the WWTP activated sludge inoculum. If all the biodegradation of the polypropylene-based wipe is assumed to be due to the cellulose, and the initial percent of cellulose was determined to be 28%, then the biodegradation of the cellulose component in the PP wipes can be estimated to be 64%, which is similar to the MCC and the other cellulose samples in Fig. 5. This suggests that the PP was not adversely impacting the microorganisms' ability to interact with cellulose component. Another important note is that the remnants of the PP based wipes biodegradation, being predominantly PP, would have very different properties than the initial material. These physical property changes are important to note when trying to determine their fate in an aqueous environment, for example floating versus sinking or adsorption to hydrophobic surfaces.

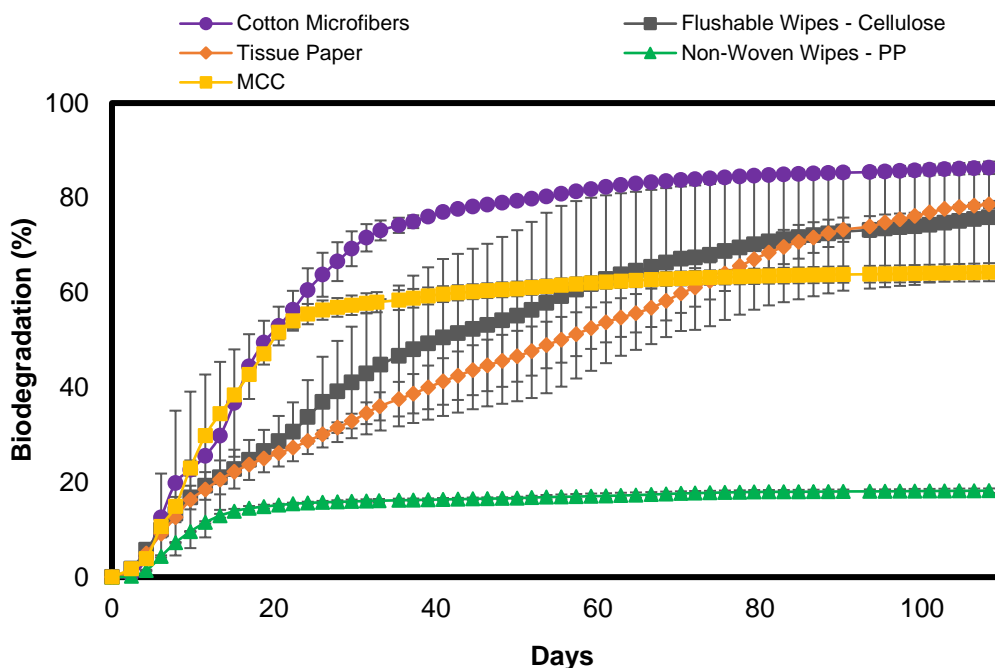


Fig. 5. Biodegradation curves of commonly-flushed materials over 105 days in activated sludge from WWTP using standard method ISO 14852. Error bars represent the standard error of the mean, $n = 2$.

Figure 6 shows the biodegradation results for the same materials in seawater. Note that the time scale is different than Fig. 5. In this case all of the predominantly cellulose samples and the reference MCC biodegraded in the range of 74 to 80%. Again, the PP based wipe degraded to a much lower extent, 22.5%, than the other materials. Assuming that only the cellulose component biodegrades, this translates to an 80% cellulose biodegradation extent. It appears that the activity of the seawater (Fig. 6) was actually higher than the WWTP inoculum (Fig. 5). The use of combining both high and low tide water during the summertime is thought to have assisted in producing an active seawater inoculum.

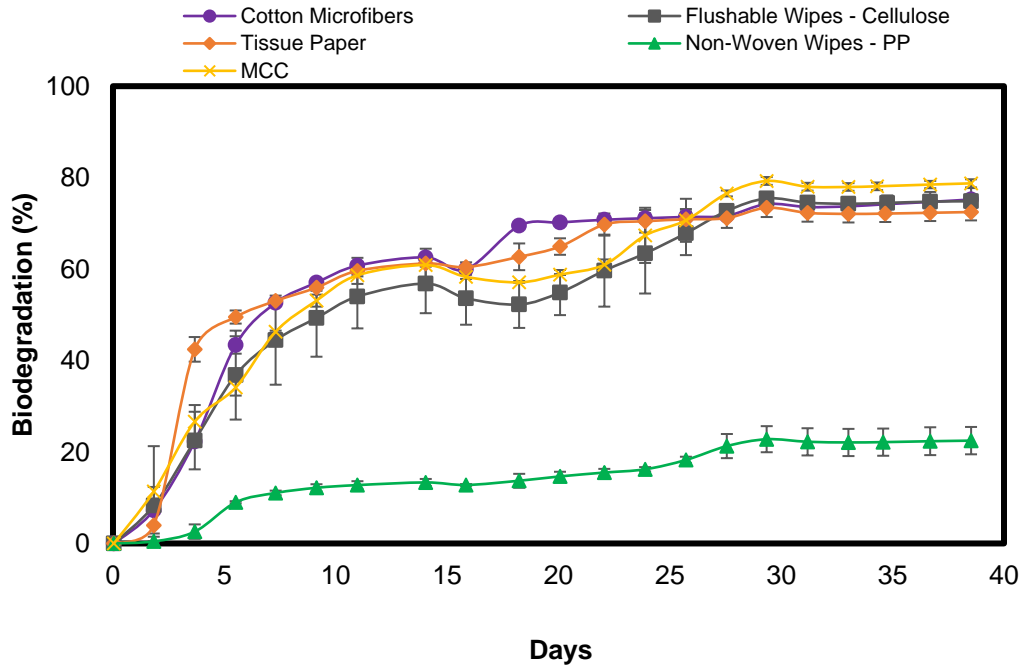


Fig. 6. Biodegradation curves of commonly-flushed materials over 40 days in seawater evaluated using the standard method ASTM D6691. Error bars represent the standard error of the mean, $n = 2$.

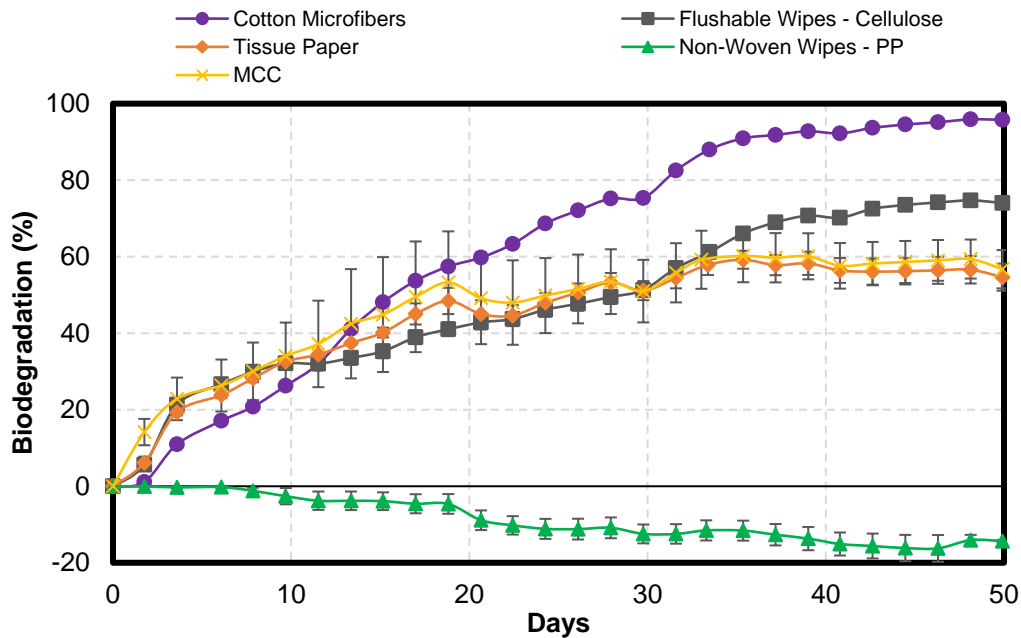


Fig. 7. Biodegradation curves of commonly-flushed materials over 50 days in lakewater evaluated using the standard method ISO 14852. Error bars represent the standard error of the mean, $n = 2$.

Figure 7 shows the biodegradation results for the same materials in lakewater. Again, the cellulose samples and the reference MCC biodegraded to a very significant extent, above 56%. Interestingly, the cotton microfibers biodegraded almost completely

to near 100%. For this experiment the PP based wipes were determined to have a negative biodegradation, indicating that the control experiment showed higher emissions of CO₂ than the sample with the PP based wipes. This may be from the PP component interfering with the biodegradation of the cellulose component or simply from some error/unexpected variation in the experimental results which originate from the sample oxygen consumption minus the control sample (with no substrate) oxygen consumption. When considering the significant biodegradation results from Figs. 5 and 6 for the PP wipe, it is most likely that the results in Fig. 7 is an experimental issue.

SEM images of the PP-based wipes after biodegradation are shown in Fig. 8 and reveal a preponderance of the smooth PP component. Some small particulate type objects are observed attached to the PP, and it is suspected that these are remnants of the cellulose component. These images are in agreement with Fig. 4b, which shows the residual PP-based wipe material after biodegradation only having a mass loss around 400 to 500 °C, which is indicative of the PP alone. There was no mass loss below 300 °C, which would indicate cellulose as shown in Fig. 4a.

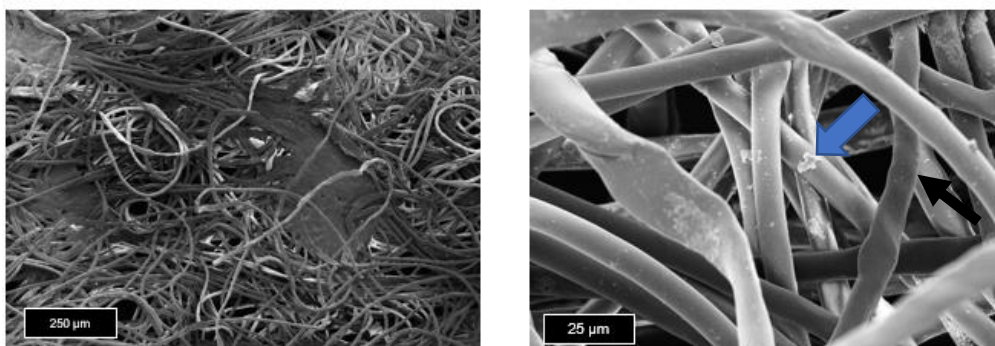


Fig. 8. SEM images of polypropylene-based wipe after biodegradation in WWTP activated sludge. The blue arrow points to a suspected remnant of cellulosic material.

Statistical Analysis of Extent of Biodegradation

For the statistical analysis, SAS 9.4 and Excel 2016 software versions were used to assess the differences in biodegradation between the materials using the data from the plateau phase in the biodegradation curve. Multiple comparisons were made using a non-parametric model for One-Way ANOVA (proc NPAR1WAY, Wilcoxon Model, Median One-Way Analysis) at a 95% significant level ($\alpha=0.05$). In addition, the kinetic models were fitted using a non-linear function (proc NLIN) in SAS 9.4 for the biodegradation process.

Tables 2 through 4 show the average and standard error of the percent biodegradation, and the maximum observed biodegradation of an individual experiment for a given sample. Additionally, samples that could not be judged as statistically different are denoted by common letters. In all three inocula, the PP-based wipes percent biodegradation were statistically different than the cellulosic other samples. The three cellulose-based products and the MCC percent biodegradation results were clustered and were often but not always statistically the same.

Table 2. Summary – Biodegradation Extent of Materials after 110 days of Incubation in WWTP

Samples	Biodegradation (%) Mean; N = 2	Statistically the Same	Maximum Observed Biodegradation (%) in an individual sample
Cotton Microfibers ^a	88.16 ± 1.65 %	A	89.81 %
Flushable Wipes – Cellulose ^{a,b,c}	75.88 ± 11.22 %	A, B, C	87.10 %
Tissue Paper ^b	78.61 ± 0.74 %	B	79.35 %
Polypropylene-based Wipes ^d	18.23 ± 0.47 %	D	18.70 %
MCC ^c	64.32 ± 1.90 %	C	66.22 %

Note: The inoculum was 1000 ppm TSS of activated sludge from Neuse River WWTP. The ISO 14852 standard method was followed. The percentage of biodegradation was based on the carbon dioxide production of the system versus the theoretical carbon dioxide calculated for each material. Samples sharing the same letter superscript are not statistically different. Standard error, $n=2$.

Table 3. Summary – Biodegradation Extent of Materials after 38 Days of Incubation in Seawater

Samples	Biodegradation (%) Mean of Two Samples	Statistically the Same	Maximum Observed Biodegradation (%) in an individual sample
Cotton Microfibers ^{a,b}	75.80 ± 2.82 %	A,B	78.62 %
Flushable Wipes – Cellulose ^c	75.10 ± 1.57 %	C	76.67 %
Tissue Paper ^{a,c}	72.69 ± 1.85 %	A,C	74.54 %
Polypropylene-based Wipes ^d	22.66 ± 3.03 %	D	25.69 %
MCC ^b	78.98 ± 0.88 %	B	79.86 %

Note: The inoculum was seawater from Salter Path, NC and the standard method ASTM D6691 was followed. The percentage of biodegradation was based on the carbon dioxide production of the system versus the theoretical carbon dioxide calculated for each material. Samples sharing the same letter superscript are not statistically different. Standard error, $n=2$.

Table 4. Biodegradation Extent of Materials after 50 Days of Incubation in Lakewater

Samples	Biodegradation (%) Mean of Two Samples	Statistically the Same	Maximum Observed Biodegradation (%) in an individual sample
Cotton Microfibers	96.79 %	B	96.79 %
Flushable Wipes – Cellulose	74.04 ± 1.29 %	C	75.33 %
Tissue Paper	54.63 ± 3.53 %	A	58.16 %
Polypropylene-based Wipes	-14.33 ± 0.33 %	D	-14.00 %
MCC	56.73 ± 4.99 %	A	61.72 %

Note: The inoculum was lakewater and the standard method ISO 14852 was followed. The percentage of biodegradation was based on the carbon dioxide production of the system versus the theoretical carbon dioxide calculated for each material. Samples sharing the same letter are not statistically different. Standard error, $n=2$.

Comparison of the Results for Different Inoculum

Figures 9 and 10 show the final extent of biodegradation of all materials in all three environments.

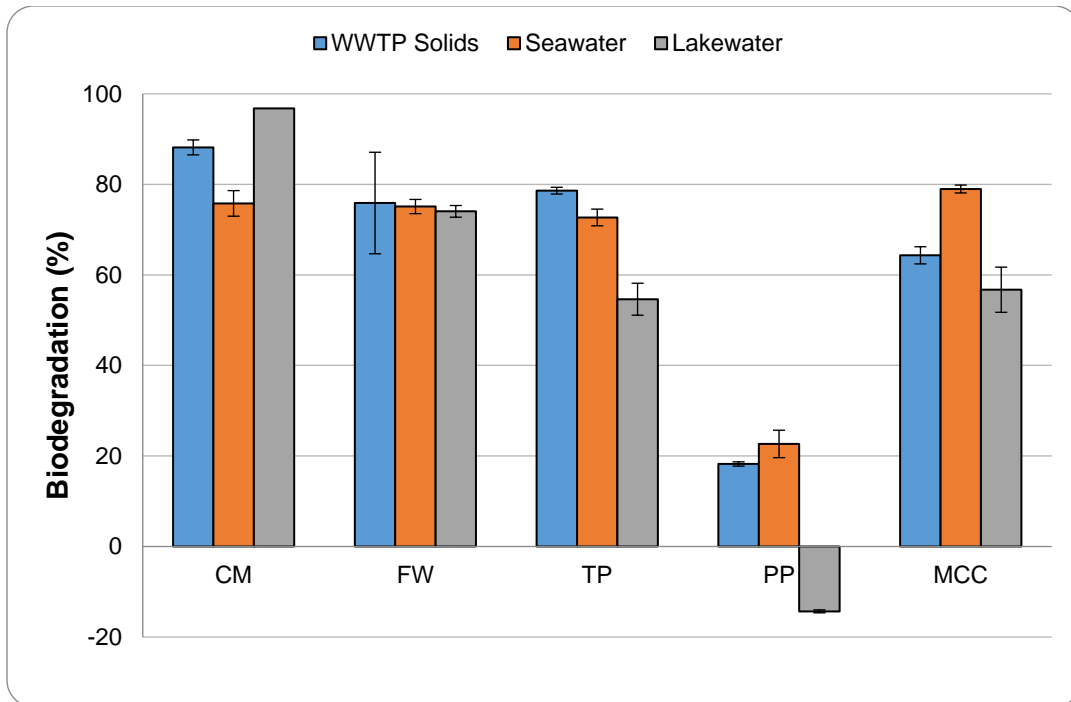


Fig. 9. Biodegradation of commonly-flushed materials in WWTP activated solids, seawater, and lakewater. Error bars represent the standard error, $n = 2$.

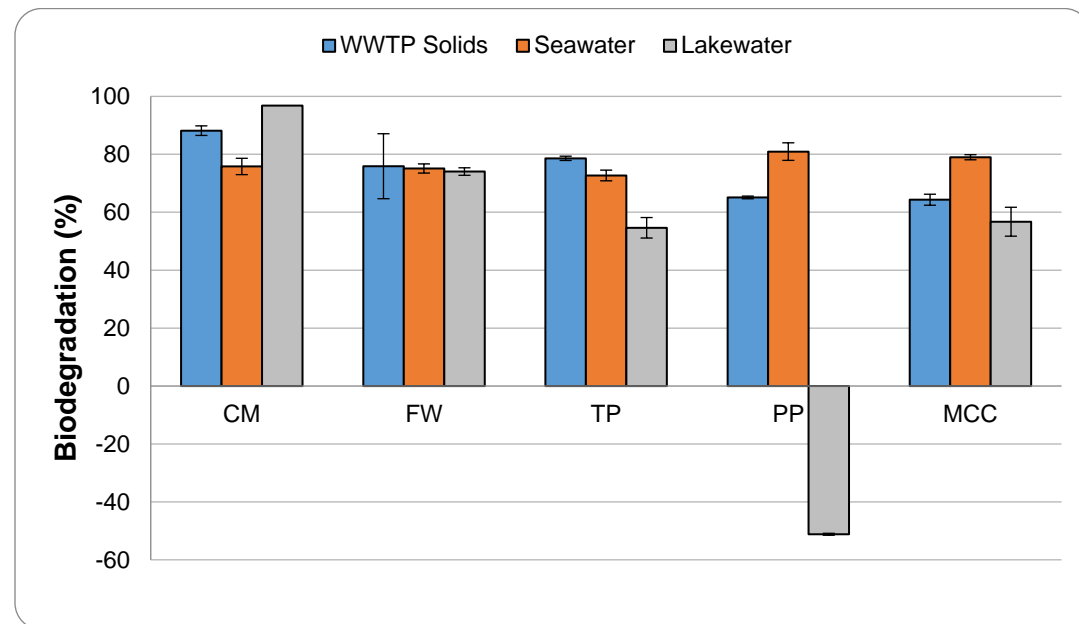


Fig. 10. Biodegradation (based only on the measured cellulosic content) of commonly-flushed cellulosic materials in seawater and WWTP activated solids. Error bars represent the standard error, $n = 2$.

Figure 9 includes the polypropylene and cellulosic contents of the polypropylene-based wipe in the calculations, whereas in Fig. 10, the final extent of biodegradation was recalculated assuming the cellulose is the only degrading component in the polypropylene-based material. All cellulosic materials degraded more than 50% whether cellulose was the only component of the material or just a small portion. The data shown in Fig. 10 supports the notion that in a blended wipe, each fiber degrades independently of each other, and a non-biodegradable fiber will not hinder or limit the biodegradation of a biodegradable fiber in either WWTP or seawater conditions. Due to the low cellulosic content, the polypropylene-based wipe exhibited the lowest percent biodegradation.

CONCLUSIONS

1. All of the cellulosic materials and the cellulosic components in the polypropylene (PP)-containing wipe readily biodegraded in all inoculums including wastewater treatment plant (WWTP) solids, seawater, and lakewater.
2. For these experiments, the type of inoculum did not significantly affect the final extent of biodegradation.
3. Cotton biodegraded significantly faster than tissue paper in wastewater inoculum and lakewater, but at a similar rate to tissue paper in seawater. The polypropylene-based wipe's biodegradation was lower due to its 72% PP and 28% cellulosic content; however, the polypropylene did not impede the cellulosic component's biodegradation.
4. The research reaffirmed that fibers found in these cellulosic cotton-based or wood-based products can biodegrade easily in various environments. By choosing natural fibers over synthetic alternatives, a reduction in the accumulation of non-biodegradable waste in our environment is expected to promote a more sustainable future.

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