Investigation of Absorbent Material for Dissolved Natural Organic Matter and Improvement of Biodegradation Studies for Dissolved Natural Organic Matter Characterization

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Abstract

Over the past three decades, increasing concentrations of dissolved natural organic matter (DNOM) have been observed in Nordic surface waters. This increase has an effect on the natural environment because DNOM impacts the water chemistry in surface waters through its chemical characteristics and ecosystems through being a source of food for organisms. Moreover, surface waters are used for drinking water and therefore this increase in DNOM has a negative effect for waterworks. It is important to investigate DNOM in the environment to understand its environmental effects and to predict future impacts from DNOM.

This study had two main objectives: to create an absorbent material that will selectively absorb DNOM from water and to improve methods for studying the biodegradability of DNOM. An already developed absorbent material, swellable-organically modified silica (SOMS), was modified with polyethyleneimine (PEI) so that it was selective toward DNOM. After several absorption experiments, it was determined that the material was able to selectively bind humic substances (high molecular weight part of DNOM with more aromaticity) but did not absorb the fulvic substances (lower molecular weight DNOM with more aliphatic character). This material could be further improved to become an effective absorbent for removing DNOM from water.

The amount of nutrients used in studying the biodegradation of DNOM was examined. Nutrients with a ratio of 2N: 1P were compared with 3.54N: 1P to look at different amounts of nitrogen to phosphorous and how it affects respiration rate. It was determined that using 3.54N: 1P ratio nutrients in the biodegradation experiments significantly lowers the biodegradability in the samples and therefore gives a lower signal. The use of 2N: 1P should be used in future biodegradation experiments to have better signal in the experiments. This method improvement is important for accurately characterizing DNOM.
Introduction

1. Dissolved Natural Organic Matter (DNOM)

Dissolved natural organic matter (DNOM) is a complex heterogeneous mixture of many organic compounds found in natural soils and waters that is formed during a process called humification, or the decomposition of plant and microbial remains. DNOM is composed of mainly carbon, as well as oxygen, nitrogen, hydrogen and sulfur. Many of the specific physicochemical characteristics of DNOM such as size, hydrophobicity, and functional group composition depend on the origin of DNOM.\(^1\) It is important to gain knowledge about the physicochemical characteristics of DNOM because these characteristics govern the impact of DNOM on the environment. This has become even more relevant due to increasing concentrations of dissolved natural organic matter (DNOM) over the past three decades in Nordic lakes as well as surface waters in Northeast America and Scotland.\(^2\) Investigation of the characteristics of DNOM also provides information about the source and fate of DNOM in the environment. This will demonstrate how DNOM is being influenced by recent changes in the climate and how these changes impact the environment. Once this is determined, future DNOM trends and environmental effects can be better predicted.

DNOM in surface waters is mainly allochthonous, meaning that the DNOM is from decomposition terrestrial vegetation that has been washed out with precipitation and entered through run-off from soil. It is hypothesized that the recent significant increase in DNOM (i.e. Browning) is due to several complex factors. First, the reduction of acid rain due to less long-range transported air pollutants increases the solubility of organic matter due to less flocculation of humic molecules from the decreased ionic strength and presence of aluminum.\(^3\) Second, the recent increase in global temperature causes an increase in vegetation and biomass (i.e.}
Greening) and therefore an increase in decomposition of plant and microbial remains. The increase in biomass is the main origin of allochthonous DNOM. Climate change has also led to more frequent and more intense rainfall episodes, causing more water only flowing through the upper organic rich forest floor before being flushed into streams. Finally, anthropogenic changes in land-use may also affect vegetation growth and therefore the amount of DNOM in the environment.\textsuperscript{1}

DNOM has several general defining characteristics that affect the boreal environment. DNOM contains many weak acid functional groups and therefore produces a low pH in water. Moreover, due to these acidic groups DNOM is able to complex with metal ions, especially the trivalent iron and aluminum as well as Type B (or Soft) metals. Its organic moieties allow the DNOM to sorb organic pollutants, and the material itself contains nutrients such as phosphate and reactive nitrogen. The DNOM thereby enhances the solubility and mobility, and thus transport of pollutants and nutrients in the environment. DNOM is also able to absorb light due to its chemical structure, consisting primarily of humus, a brown or black complex mixture of organic compounds that are colloidal and weakly acidic.\textsuperscript{4} Humus can be separated into low molecular weight (LMW) non-humic substances such as carbohydrates, amino acids, peptides, fats, alkanes, aldehydes and organic acids which have distinct chemical formulae, and high molecular weight (HMW) humic substances. Humic substances are large organic compounds in which no specific chemical formula can be defined due to the heterogenic complexity of the material. However, it is defined that humic substances consist primarily of three constituents, humin, humic and fulvic acids.\textsuperscript{5} Humin is the fraction of humic substances that is not soluble in water at any pH. Humic acids are not soluble in water when the pH is below 2 and fulvic acids
are soluble in water at all pH. Although the exact structure of humic and fulvic acids is undefined, there are several proposed generic structure models demonstrated in Figures 1 and 2.

![Figure 1. Proposed structure for Humic Acid by Stevenson (1994).](image1)

![Figure 2. Proposed model of Fulvic Acid by Buffle (1977).](image2)

The humic acid proposed model shows that there are a large number of aromatic rings and carboxylic acid functional groups contributing to the acidity of humic acid. Humic acid molecular size mainly ranges from 10,000 to several 100,000. The fulvic acid proposed model shows a smaller molecule (molecular size is 1,000 to 10,000), has less aromatic groups but has higher oxygen content from carboxyl and hydroxyl groups. DNOM has the ability to absorb radiation in the UV and visible spectrum of light due to the high aromaticity and conjugated double bonds in the humic and fulvic acids. DNOM can therefore be characterized using UV-Vis
spectroscopy. Different DNOM characteristics influence the UV absorption of DNOM. For example, LMW organic compounds absorb at a lower wavelength than HMW compounds. This is because the HMW compounds contain more aromatic rings and longer conjugated double bonds. The specific UV absorbency index (sUVa) can be found by dividing the absorbance at 254nm with the concentration of DOC in the sample, which thus demonstrates the normalized aromaticity of the sample. The absorbance at 254nm is used because a small bump on the decline in the UV spectrum is usually observed at this wavelength related to the conjugated double bonds in the sample. In areas of high concentration of DNOM the absorption of light darkens the color of the water and results in a reduction in the depth of the photosynthetically active radiation (PAR) in the lake, which affects the ecosystem and organisms in the lake. DNOM is also an important source of food for aquatic heterotrophic organisms.

2. DNOM Sampling and Analysis

It is necessary to achieve a better understanding of the characteristics and origins of DNOM in order to obtain a better understanding of its impact on present and future environmental conditions. This study originally focused on creating a passive sampler for DNOM to provide an in-situ time-averaged concentration of the DNOM in water over a long period of time while eliminating complex analyte extraction (see project description for details). However, after several complications that will be discussed in more depth later it was decided to partly revise the aim of this study. This study now aims to develop an absorbent material that can be used to absorb DNOM from water.
2.1 Possible Uses for DNOM Absorbent Material

Because Nordic drinking water treatment plants use surface waters, it is important to remove all of the DNOM in the water during water treatment. The process of DNOM removal usually involves coagulation by alum or ferric chloride. However, other methods of removal, such as using adsorbent and absorbent materials, have been studied in attempt to create a more effective method of DNOM removal. Carbon nanotubes, for example, have been used to adsorb DNOM from water. Single-walled carbon nanotubes as well as multiwalled carbon nanotubes were determined to be more effective at removing DNOM from water than alum or ferric chloride. Other adsorbent particles have also been examined, specifically for use in ultrafiltration systems that treat water. Powdered activated carbon and heated iron oxide particles specifically were investigated. These adsorbents were effective but they also lead to membrane fouling which ultimately affects the removal process.

The following study aims to develop an adsorbent/absorbent material that could also potentially be used for removal of DNOM from water. This material, theoretically, will be able to absorb all of the DNOM in a water sample but will not absorb other impurities such as organic waste products. This material can then be used to treat water in drinking water treatment plants and also could be used to remove DNOM from water samples before analysis of water when studying water quality. If successful, this material could be compared to the other adsorbents that are currently used to remove DNOM to determine which material is most effective at removing DNOM from water without negatively impacting the removal process. In this study, an already developed absorbent material, swellable organically modified silica, will be modified to create an absorbent material that is selective toward DNOM.
2.2 Modified Swellable Organically-Modified Silica for DNOM absorption

Swellable organically-modified silica (SOMS) is a newly developed absorbent material that is modified and tested as a sorbent for DNOM in this study. SOMS is a sol-gel material that has an unusual yet useful ability to extensively swell in organic solvents. Some previously reported sol-gel materials have the ability to swell, but this swelling occurs only in response to an environmental stimulus such as changes in pH or temperature. SOMS is the first sol-gel derived organosilica material with the ability to swell in organic solvents without the presence of an environmental stimulus. SOMS is a preferable material in many applications because it has several unique physical and chemical properties. The material is able to rapidly swell 3-5 times its original dried volume in neat organic liquids (Fig. 3). SOMS does not swell in the presence of water, due to its hydrophobic chemical structure. It is not surprising, therefore, that when the material is placed in a mixture of water and organic liquids, SOMS will uptake the organic liquid entirely, leaving the water purified. The material absorbs all non-polar organic solvents as well as some polar organics, and the swelling is completely reversible. SOMS expands with forces measuring >100 N/g when exposed to organics. Nonpolar organic molecules in the gas phase can also be absorbed. These properties make SOMS an attractive media for use in several applications such as controlled drug release or water purification.

![Figure 3. Swelling of SOMS by drop-wise addition of acetone, reproduced from reference 11.](image)
Currently, SOMS is applied in stormwater and drinking water treatment, oil and gas treatment and personal care.\textsuperscript{17,18} Stormwater run-off, water effluent, and drinking water contain contaminants such as oil, solvents or toxins. SOMS purifies the water by removing organic contaminants (e.g. volatile organic compounds, pesticides, chlorinated solvents, and oils), thus reducing both environmental and health hazards.\textsuperscript{17} In oil and gas treatment, SOMS purifies oilfield water and gas streams by selectively absorbing free, dispersed and water-soluble hydrocarbons from the water.\textsuperscript{17} Recent studies have also established that SOMS technology can be used in personal care products, specifically for controlled fragrance release because SOMS is able to encapsulate and slowly disperse large amounts of fragrances.\textsuperscript{16} Furthermore, current studies have determined that SOMS can also be functionalized to make SOMS-metal composites, such as Iron-Osorb\textsuperscript{®}, which can be used in stormwater treatment to remove runoff pollutants.\textsuperscript{19,20} As a result of SOMS’s non-toxic, regenerable and hydrophobic properties, the material is highly economical, environmentally friendly and valuable in these applications. Researchers continue to investigate the applicability of this unique nanostructured material. A detailed description of how SOMS is prepared through basic sol-gel techniques is described in Appendix A.

The specific conditions and reagents for SOMS production provide an optimally balanced interconnectivity and flexibility of the particles to produce a swellable material.\textsuperscript{11} Molecularly, the final structure of SOMS consists of a partially disorganized network comprised of a hydrophobic exterior that is composed of aromatic groups enclosing a hydrophilic inner layer (Figure 4).\textsuperscript{11}
Morphologically, as determined by scanning electron microscopy (SEM), SOMS is composed of individual nanoparticles. These individual nanoparticles are aggregated together by the interparticle cross-linking of the molecules, to form mass clusters of nanoparticles. Once exposed to organics, the clusters, but not the individual nanoparticles, expand greatly because the noncovalent interactions are broken through solvent disruption. As a result, the surface area increases and porous space is generated for the absorbed liquid, creating ‘nanocavities’ (Figure 5).
To explicitly show sorption by SOMS, the overall swelling mechanism can be explained in a model proposed by Edmiston et al. (Figure 6). The gray boxes in Edmiston’s model represent the polymeric matrix of SOMS, which can be illustrated as collapsed (when the boxes are connected, as in step 1) or expanded in the swollen state (when the boxes are separated, as in step 4). The yellow circles in the blue box depict organic liquid molecules. SOMS has the ability to both adsorb and absorb. Organic molecules are able to adsorb to the hydrophobic surface of the material in its collapsed state (step 1, illustrated by the molecules adsorbing to the connected horizontal gray boxes, demonstrating the hydrophobic barrier). After a sufficient amount of adsorption to the outer surface, the noncovalent interactions between molecules that hold the matrix in a collapsed state are disrupted. This results in expansion and opening up of the SOMS, creating new surface area wherein more of the organic liquid can be absorbed (step 2, depicted by the separation of the horizontal box, creating new space for the molecules). More organic molecules are absorbed further (step 3, the molecules are shown to occupy the new space created) until additional expansion of the matrix is needed to create more surface area for even
further absorption (step 4, the vertical boxes are separated demonstrating additional expansion of the collapsed matrix). Sorption of the liquid ceases when all of the noncovalent interactions have been disrupted and no more surface area can be created.

Figure 6. Proposed model of sorption of organics by SOMS, reproduced from reference 11. (1) Initial adsorption of organic species to surface of SOMS. (2) Triggered matrix expansion leading to absorption of organic species. (3) Further absorption of species. (4) Additional matrix expansion and absorption.

Edmiston’s model demonstrates that the expansion of the polymeric matrix does not occur simultaneously, but rather in steps. SOMS high capacity for absorbing organic liquids is due to its ability to both adsorb and absorb. As a result of this ability, new surface area and volume can be generated within the material, as demonstrated through the proposed model.12

The swellable nature of SOMS, high absorption capacity, and hydrophobicity of the material potentially makes it an ideal material to modify for absorbing DNOM because it will not absorb water and it will be able to uptake a large amount of DNOM, selectively. In order to
exclusively absorb DNOM, SOMS is modified with polyethyleneimine (PEI). PEI is a polyamminated polymer that has been used in water treatment as a cationic polyelectrolyte to remove humic acid from water.\textsuperscript{21} It contains many amine functional groups, which are easily protonated over a wide range of pH. This is significant in coagulation of humic acid with PEI.\textsuperscript{22} Humic acid is negatively charged in the natural environment due to the ionization of the carboxyl and phenolic groups.\textsuperscript{23} The protonated PEI is positively charged and therefore strong electrostatic forces occur between the humic acid and PEI which results in charge neutralization and coagulation.\textsuperscript{21} In addition to the strong electrostatic forces, other chemical forces may be involved in the coagulation such as hydrogen bonding. The aggregation mechanism for humic acid and PEI is shown in Figure 7. The cationic PEI and humic acid are first destabilized by the charge neutralization between the humic acid and the cationic polymer. Cross-linking between the PEI and the humic acid then occurs, which Glaser and Edzwald state is charge-initiated and chemically preferred. Therefore, more humic acid and polymers will be attracted to each growing aggregate.\textsuperscript{21}

\textbf{Figure 7.} Aggregation mechanism of PEI and humic acid, adapted from reference
In this study, PEI is combined with SOMS so that the prepared material is able to selectively uptake humic substances from the environment. SOMS is tailored with PEI using a similar method that was used to attach PEI to silica gels described by Zhang et al.\textsuperscript{24} The PEI/silica gel samples were prepared using a wet-impregnation method in which PEI was dissolved in methanol and dry silica gel was added to the solution. The methanol was subsequently evaporated and the sample was further dried so that PEI was loaded onto the silica gel. This method is used because SOMS is porous organosilica material that has the ability to absorb and adsorb PEI. Several absorption experiments are conducted after tailoring the SOMS with PEI to determine if all of the DNOM can be absorbed by the material and removed from the water sample. Initial absorption experiments using DNOM solutions made from freeze-dried DNOM is conducted. Two main methods of absorption have been investigated; adding the modified SOMS to a water sample followed by filtration of the water/SOMS mixture with a syringe and filter and coating a vacuum filtration system with SOMS and subsequently filtering the water through the filter system. Absorption is measured with UV-Vis spectroscopy and TOC analysis. The results from this study demonstrate that HMW, hydrophobic humic DNOM is absorbed by the material but the LMW fulvic DNOM is left in the water. This is likely because humic substances have a higher affinity toward PEI and that SOMS is more effective at absorbing hydrophobic material.

3. Biodegradation of DNOM

The biodegradability of DNOM provides important information about the characteristics of DNOM in the water sample. This is important for waterworks so that fouling can be controlled in water treatments. The biodegradability of DNOM is also important to understand environmental
conditions and how much of DNOM is available as a source of nutrients and energy for heterotrophic organisms in the environment. Biodegradation is the decomposition of material by biological means, in the case of DNOM this is by microorganisms. Certain chemical characteristics of DNOM govern how easily the material can be biodegraded. It is assumed that DNOM can be split into three categories based on the biodegradability of the material; labile DNOM, moderately biodegradable DNOM and recalcitrant DNOM. The characteristics of each category of DNOM are described in Table 1.

Table 1. Characteristics of DNOM types

<table>
<thead>
<tr>
<th>DNOM category</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Labile</strong></td>
<td>• Easily biodegradable</td>
</tr>
<tr>
<td></td>
<td>• LMW DNOM and fulvic substances</td>
</tr>
<tr>
<td></td>
<td>• High aliphatic character</td>
</tr>
<tr>
<td><strong>Moderately Biodegradable</strong></td>
<td>• Moderately easy to biodegrade</td>
</tr>
<tr>
<td></td>
<td>• Polysaccharides and other slowly degraded products</td>
</tr>
<tr>
<td><strong>Recalcitrant</strong></td>
<td>• Difficult to biodegrade</td>
</tr>
<tr>
<td></td>
<td>• HMW DNOM and humic substances</td>
</tr>
<tr>
<td></td>
<td>• High aromatic character</td>
</tr>
</tbody>
</table>

The Monad equation, which is a mathematical model for the growth of microorganisms, model that low substrate concentration results in low growth rate for bacteria, or low biodegradability. However, if there is a high substrate concentration then there is high bacterial growth. Thus, the significant increase in DNOM in Nordic lakes over the past three decades has likely produced increased bacterial growth. The bacterial growth will continue if DNOM continues to increase in Nordic surface waters. Additionally, increased DNOM increases
shielding of bacteria from harmful UV radiation in surface waters, further suggesting that DNOM increase will lead to increased bacterial growth. Aquatic biomass is likely to have a molar ratio of 106:16:1 of Carbon:Nitrogen:Phosphorus. Alfred Redfield, an oceanographer, determined this from his studies of nutrients in phytoplankton in oceans. It was reported by Cotner that the ratio of nutrients in bacteria in freshwater lakes is also similar to the Redfield ratio. Carbon, nitrogen and phosphorus are vital for bacteria growth and according to Redfield, a ratio of 106:16:1 is optimal for bacterial growth.

Bacteria communities usually take time to adapt to the substrate before the bacteria can exponentially grow—this time period is called the lag phase. The lag phase can last anywhere from days to years in the natural environment depending how easily the bacteria community can adapt to the new substrate. The lag phase usually ends and exponential growth occurs when the bacterial culture has reached about $10^6$ bacteria per mL sample. Exponential growth usually depends on the characteristics of the substrate. The labile portions of the substrate are consumed first before the recalcitrant portions. Once the substrate has been consumed and nutrients begin to deplete, there is a stationary phase where there is no increase or decrease of bacteria. After this the bacteria enter the death phase and the amount of bacteria begins to decrease.

Currently, there are several different methods for studying the biodegradability of DNOM. This leads to variation in results between studies because different methods are used, which create non-comparable data. Biodegradation can be measured by either measuring the change in DNOM concentration, the production of CO$_2$, the changes in spectroscopic characteristics or the consumption of O$_2$, because CO$_2$ is evolved and O$_2$ is consumed with the consumption of DNOM as a substrate. The method for measuring the production of CO$_2$ is difficult because the sample is exposed to the environment thus affecting the results. Measuring DNOM change and
changes in spectroscopic character requires long experiment time periods because bioactivity in natural samples is often too low to generate any measureable changes. Therefore, focusing on the consumption of O₂ is optimal for this study. It is important to develop a standard biodegradation method that is fast, easy and accurate so that results can be compared between researchers.

3.1 Biodegradation method optimization

Due to the lack of any fast, easy and accurate method for biodegradation measurements, method development for standard biodegradation assays based on O₂ consumption was investigated at UiO by Alexander Håland. SensorDish Reader (SDR) was the analytical tool used by Håland to measure oxygen consumption. The SensorVials (5 mL) contain the samples and at the bottom of the SensorVials there is a sensor spot which is used to measure the oxygen in the sample. Håland focused on the optimizing the following parameters in measuring O₂ consumption as a proxy for measuring DNOM: Inoculum preparation, nutrient solution, reference material, blanks, sample preparation, and temperature control. A brief summary of the optimized parameters follows:

1. Inoculum preparation: Inoculum is defined as a culture of bacteria added to the sterile solutions that are being tested for biodegradability in order to initiate biological activity. Håland tested several different ways to prepare an inoculum. The optimal method of inoculum preparation was determined to be as follows: Filter raw water containing microorganisms and bacteria through a 2.0 μm membrane filter to remove the microorganisms but not bacteria. Transfer approximately 100 mL of the filtrate to a 250 mL Erlenmeyer flask and add Phosphate (PO₄³⁻) and Nitrate (NO₃⁻) to final concentrations of 0.1 mM and cover the Erlenmeyer flask
with aluminum foil to avoid algae growth from light. Incubate the inoculum at room temperature on a shaking table for 2 days to ensure proper oxygen saturation.

2. Nutrient solution: As described above, nutrients such as phosphate and nitrate are essential for bacteria growth. Phosphate and nitrate are added to the samples to stimulate bacterial growth of the bacteria added from the inoculum. It was determined that not only phosphate but also nitrate is needed to initiate bacterial growth and therefore the nutrients solution in Håland’s method was prepared containing 10 mM of phosphate and 10 mM of nitrate.

3. Reference material: It was decided to use glucose as the reference material because it has well documented properties and is easily biodegradable. A concentration of 20 mg C/L was used to match the average concentration of carbon in the samples in Håland’s experiment.

4. Blanks: It was determined that Type 1 water should be used as a blank to account for instrumental drift, temperature fluctuations affecting the oxygen consumption, and leakage of oxygen into or out of the vials. Initially nutrients and inoculum were added to the Type 1 water. Because the raw water that is filtered for the inoculum likely contains biodegradable substances this resulted in a small amount of oxygen consumption in the blanks and therefore the blanks could not account for the parameters previously described.

5. Sample Preparation: 250 μL of nutrients (10 mM nitrate and phosphate) was first added to 25 mL volumetric flasks, followed by a small aliquot of the sample. 250 μL of inoculum was then added- only a small amount of inoculum is added to make sure that there is not an excessive addition of organic carbon in the samples. A surplus volume (5.2 mL) was extracted from the flask and added to the 5 mL SensorVials to ensure that there is no headspace in the vials. The vials were sealed using new screw caps and parafilm to prevent oxygen leakage.
6. Temperature Control: The temperature was set to 25°C because 25°C is the standard temperature for determining chemical equilibrium constants for various compounds. An incubator was used to keep the temperature stable throughout the experiment. The incubator was moved to a cool room so that the temperature inside the incubator was significantly higher than the outside temperature. This helped the incubator to stabilize the temperature at 25°C.

This current study focuses on further improving the standard method of measuring O₂ consumption that was developed by Håland. Specifically, the amount of nutrients added to the samples has been examined. In Håland’s method, a nutrients solution was prepared so that there was an equal amount of nitrate and phosphate (10 mM each). This study investigates adding Håland’s equal amounts of nitrate and phosphate nutrients (2:1 ratio of N:P) to glucose samples with changing carbon concentration as well as adding nutrients so that the ratio of C:N:P in the glucose samples is constant, and the ratio of N:P is higher than 2:1. The ratio of C:N:P in this nutrients addition is 27.5: 3:54: 1. Biodegradability of glucose with different concentrations is studied to determine if there is an effect of concentration on respiration rate. This will determine if respiration rate needs to be normalized by concentration in future biodegradation studies.

Furthermore, nutrients of 2:1 ratio is added (the ratio of C:N:P changes for the glucose samples) as well as nutrients with constant ratio of 27.5:3.54:1 to determine if the ratio of C:N:P in the sample has an effect on biodegradability and if an excess of nitrogen affects the biodegradability. Several reference compounds such as glucose, sucrose, ascorbic acid and maleic acid as well as natural samples from Sognsvann, have been analyzed using both the 2:1 N:P ratio as well as the constant ratio in the nutrients solution for comparison.
3.2 Analysis of Oxygen Consumption

The respiration rate (RR), or the rate of biodegradation, is calculated from the data of the amount of oxygen consumed in the samples. The RR is determined by calculating the amount of oxygen consumed during exponential oxygen consumption and dividing it by the measured time period according to Equation 1, where \( O_{2(i)} \) is the initial amount of oxygen before exponential consumption (\( \mu \text{mol L}^{-1} \)), \( O_{2(f)} \) is the final amount of oxygen at the end of exponential consumption (\( \mu \text{mol L}^{-1} \)) and \( \Delta t \) is the time between \( O_{2(i)} \) and \( O_{2(f)} \) (h) (Equation 1).

\[
RR \left( \mu \text{mol O}_2 \frac{L}{h} \right) = \frac{O_{2(i)} - O_{2(f)}}{\Delta t} \tag{1}
\]

Originally, the start of the exponential consumption and the end of oxygen consumption was determined by visibly inspecting the graph of oxygen consumption vs. time and then calculating manually the RR. However, in order to create a standard methodology of calculation a program in R studio was developed to calculate the RR of the samples. Input to the program are the data from the PreSencse monitoring of \( O_2 \) concentration during the incubation. This program graphs the oxygen consumption vs time and calculates the RR. The start of exponential consumption is defined as the point at which the measured values are more than 3x the standard deviation lower than the initial measurements. This ensures that the initial measurements, which may be skewed from initial temperature fluctuations are not included in the calculation. The R Studio program for graphing the oxygen consumption and calculating the RR is also improved in this study in order to provide a more accurate calculation of the RR.
Experimental

1. DNOM sorption with modified SAMS

1.1 Preparation of modified SAMS

The following procedure was developed from literature. The procedure was repeated, as it was necessary to prepare more modified SAMS for experiments.

Polyethyleneimine (PEI) was loaded into the pores of SAMS to create a PEI/SAMS modified absorbent material. Two samples of the PEI/SAMS were prepared using PEI loadings of 10 and 20 wt %, respectively. This means that 10% and 20% of the final weight of the material is PEI. The theoretical maximum PEI loading amount in the pores of SAMS is approximately 26.8 %, assuming that SAMS pores are mono-modal with a pore volume of 0.366 mL g⁻¹ (Appendix B). Therefore, 10 and 20 wt % were chosen so as to not fully load the pores with PEI. This ensures that there is space within the pores for DNOM absorption. A general procedure for PEI/SAMS preparation is detailed in Appendix C.

The final modified SAMS material appeared very similar to SAMS in its original state- dry, fine, grainy white powder. When preparing the 20 wt % loaded PEI/SAMS the final product was slightly yellow with small clumps of white solid, which could possibly be extra PEI. The surface area, pore volume and pore diameter of regular SAMS, PEI/SAMS-10 (denotes 10 wt % loading) and PEI/SAMS-20 (denotes 20 wt % loading) were measured using the Brunauer-Emmett-Teller (BET) nitrogen absorption/desorption method. N₂ adsorption and desorption on the surface of a porous material gives information about the surface area, pore volume and pore diameter of a material by measuring and plotting the adsorption vs the relative pressure to obtain an adsorption isotherm. The BET method can be employed for microporous, mesoporous and macroporous materials. The pore volume was also determined using the Barrett-Joyner-Halenda nitrogen
adsorption/desorption method. This method only applies to mesopore and small macropore size range and relies on calculating pore size distributions from experimental isotherms.

1.2 Passive sampler absorption experiments

1.2.1 Absorption of DNOM with dry PEI/SOMS-10

The purpose of this experiment was to determine visually if dry PEI/SOMS-10 can absorb DNOM from water. Therefore, an arbitrary amount of DNOM was chosen to dissolve in water and the final concentration was not measured. Svartberget 1999-Fall freeze-dried DNOM (10 mg) was dissolved in 100 mL of type 1 water. Dry PEI/SOMS-10 (250 mg) was transferred to a beaker and 20 mL of the DNOM water was added to the beaker. Visibly, the PEI/SOMS-10 material did not swell or absorb any of the DNOM or water. The material floated on top of the water. The PEI/SOMS-10 was filtered from the water using a 0.2 μm cellulose acetate filter and vacuum filtration. The filter paper was brown after filtration and the PEI/SOMS-10 appeared slightly swollen and slightly brown (Figure 8)

![Figure 8. PEI/SOMS-10 (initially dry) after absorption of DNOM and filtration](image-url)
1.2.2 Absorption of DNOM with PEI/SOMS-10 in stainless steel pouches

Dry PEI/SOMS-10 (~500 mg) was loaded into 3 small stainless steel pouches. There was a bit of leakage of the particles through the pouch. Each of the 3 pouches containing 500 mg of PEI/SOMS-10 was placed in about 100 mL of Svartberget 1999 DNOM water (10 mg of DNOM dissolved in 100 mL Type 1 water). The pouches were fully submerged in the beakers of DNOM water, covered in aluminum foil and shaken on a shaker table for 24 hours. After 24 hours the DNOM in all of the beakers had coagulated in clumps in the beaker. There were also brown clumps adsorbed to the outside of the pouches. When the beakers shaken by hand the DNOM dissolved partially back into the water. This experiment was repeated a second time after the pouches had been rinsed in 500 mL of Type 1 water for 24 hours in attempt to wash out the soluble PEI before it is added to DNOM water. After 48 hours in the second experiment the DNOM had again coagulated in clumps in the water similar to the first experiment.

1.2.3 Control Experiment: Stainless Steel Pouch in DNOM water

Svartberget 1999 DNOM (~ 0.5 mg) was added to 100 mL of Type 1 water. A stainless steel pouch was added to the DNOM water in attempt to see if the DNOM would coagulate from the stainless steel pouch. The pouch was left for 2 days and afterwards the DNOM in the water was still dissolved.

1.3 Absorption of DNOM with pre-wetted PEI/SOMS-10

Two methods were used in attempt to absorb DNOM to the pre-wetted PEI/SOMS-10.

- Method 1: PEI/SOMS-10 was added to a water sample followed by filtration with a syringe and a 0.45 μm filter to separate the PEI/SOMS-10 and the water.
• Method 2: A vacuum filtration system was coated with PEI/SOMS-10 and the water was filtered through this system.

The DNOM water that was used for all of the following experiments was made using the Svartberget spring (2000) freeze-dried DNOM sample. A sample of Svartberget freeze-dried DNOM (52.94 mg) was added to 1 L of Type 1 water to make a solution of 18 mg C/L. This was stirred for 1 day on a stir plate and filtered through a 0.2 μm filter to sterilize the sample. The water was stored in brown bottles in a dark cool room when not in use.

1.3.1 Absorption of DNOM via Method 1

3 falcon tubes were each filled with 40 mL of Svartberget DNOM water. PEI/SOMS-10 (500 mg) was pre-wetted with ethanol (so that the material is swollen but not overwhelmed in a puddle of ethanol) and added to the falcon tubes. The PEI/SOMS-10 sinks and collects at the bottom of the falcon tube. The PEI/SOMS-10 sinks because it has been wetted and therefore absorbed some ethanol. This makes the material heavier and able to sink. The falcon tubes were covered with aluminum foil and left on the shaking table for about 24 hours. Visibly, the PEI/SOMS-10 was browner and more swollen than it was originally and the water was still slightly brown in all of the falcon tubes. The PEI/SOMS-10 was filtered from the water using a syringe and 0.45 μm polyethersulfone filter. The PEI/SOMS-10 that was collected on the filter appeared brown. The absorption spectra and the DOC concentration of filtered water was measured. This experiment was repeated, except in dram vials instead of falcon tubes because the PEI/SOMS-10 was stuck in the bottom of the falcon tubes, which may hinder the absorption of DNOM. The absorption spectra and the DOC concentration of the water from the dram vial experiment was also measured.
1.3.2 Absorption of DNOM via Method 2

PEI/SOMS-10 (~ 500 mg) was pre-wetted with ethanol until the material was fully swollen, visibly. A filtration system was set up so that a filter with a 0.45 μm HAWP filter paper was placed over a filtration flask and connected to vacuum. The filter paper was coated with the pre-wetted PEI/SOMS-10. Svartberget 2000 DNOM water (40 mL) was poured onto the filter and was vacuum filtrated. This was repeated three times. The first time the water was filtered through the system the water filtered quickly but each time this was repeated the water filtered slower through the system. The PEI/SOMS-10 retained on the filter was visibly brown after filtration. After 3 filtrations the absorption spectra and DOC concentration was measured. This procedure was repeated except the filter paper with PEI/SOMS-10 was changed after 2 filtrations of the water. This was done in case the first PEI/SOMS-10 was fully loaded. New PEI/SOMS-10 was coated on a new filter paper after which the water was filtered twice again through the system. The first PEI/SOMS-10 was visibly very brown and swollen, however, the second PEI/SOMS-10 filter was white and visibly appeared to not have absorbed the DNOM (Figure 9).

Figure 9. Filter with PEI/SOMS-10 after first filtration of water (right) and after second filtration (left)
These two filtrations (with and without changing the filter) initially were performed with PEI/SOMS-10 that had lost much of its swelling ability during preparation and therefore were repeated with fully swellable PEI/SOMS-10 and analyzed with UV-Vis spectroscopy and TOC analysis.

1.3.3 Absorption of DNOM via Methods 1 and 2 with extra filtering

Methods 1 and 2 were repeated in another experiment with an extra filtering step using regular SOMS. This was done in attempt to absorb any ethanol or PEI that had leaked from the PEI/SOMS-10 material. In method 1, after separation of the PEI/SOMS-10 and the water, regular SOMS (~500 mg) was added to the dram vials and left of the shaker for about 2 hours. The regular SOMS was then filtered out of the water using a 0.45 μm polyethersulfone filter and syringe. The final water was analyzed with UV-Vis spectroscopy. In Method 2, after filtration with and without changing the filter a new filter paper coated with regular SOMS was placed on the filter system. The water was filtered through the regular SOMS two times. The final water sample was analyzed with UV-Vis spectroscopy.

1.3.4 Control Experiments

Each of the experiments described above, except Method 1 in the Falcon tubes and Method 2 with the partially swollen PEI/SOMS-10, were conducted with Type 1 water as a control, of which the absorption spectra were recorded.

UV-Vis spectroscopy was used to analyze the 18 mg C/L DNOM water made from the freeze-dried Svartberget 2000 DNOM.

Water and a mixture of ethanol (5-8 mL) and water (10 mL) was analyzed by UV-Vis spectroscopy. No signal appeared in the spectra (200-800 nm) for either the water or the water/ethanol mix.
Approximately 15 mg of PEI was dissolved in a minimal amount of ethanol (~4 mL).

This was added to about 50 mL of Type 1 water and analyzed with UV-Vis spectroscopy.

Table 2 describes each of the absorption experiments (without the extra filtering) done with pre-wetted PEI/SOMS-10:

Table 2. Absorption experiments (without extra filtering) with pre-wetted PEI/SOMS-10

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>• Performed in Flacon Tubes</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>• Used SVA DNOM water</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>• Performed in Dram Vials</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>• Used SVA DNOM water</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>• Performed in dram vial</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>• Used Type 1 water (control)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>• Filtration 3x through system</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>• Used SVA DNOM water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Partially swellable PEI/SOMS-10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>• Filtration 2x through system, change filter, filtration 2x more</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>• Used SVA DNOM water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Partially swellable PEI/SOMS-10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>• Filtration 3x through system</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>• Used SVA DNOM water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Fully swellable PEI/SOMS-10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>• Filtration 2x through system, change filter, filtration 2x more</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>• Used SVA DNOM water</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Fully swellable PEI/SOMS-10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>• Filtration 3x through system</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>• Used Type 1 water (control)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Fully swellable PEI/SOMS-10</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 describes each of the absorption experiments (with extra filtering) done with pre-wetter PEI/SOMS-10

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Replicates</th>
</tr>
</thead>
</table>
| 1      | ● Performed in dram vials  
         | ● Used SVA DNOM water  
         | ● Extra filter with SOMS | 2 |
| 1      | ● Performed in dram vial  
         | ● Used Type 1 water (control)  
         | ● Extra filter with SOMS | 1 |
| 2      | ● Filtration 3x through system  
         | ● Used SVA DNOM water  
         | ● Fully swellable PEI/SOMS-10  
         | ● Extra filter with regular SOMS | 1 |
| 2      | ● Filtration 2x through system,  
         | change filter, filtration 2x more  
         | ● Used SVA DNOM water  
         | ● Fully swellable PEI/SOMS-10  
         | ● Extra filter with regular SOMS | 1 |
| 2      | ● Filtration 2x through system,  
         | change filter, filtration 2x more  
         | ● Used Type 1 water (control)  
         | ● Fully swellable PEI/SOMS-10  
         | ● Extra filter with regular SOMS | 1 |
2. **Biodegradation**

The following procedures were taken and adapted from Håland’s master thesis at UiO.6,25

2.1 *Stock Solution Preparation*

Stock solutions of glucose, sucrose, ascorbic acid, and maleic acid with 18 mg C/L were prepared. A detailed procedure is given in Appendix D with stock solution calculations. Stock solutions of glucose (10, 30, 45 and 60 mg C/L) were also prepared. All stock solutions were stored in a cool room until needed.

2.2 *Constant Ratio (3.54N: 1P) Nutrients Preparation*

Constant ratio (27.4C: 3.54N: 1P) nutrients were prepared for 10, 18, 30, 45 and 60 mg C/L solutions. The following is the procedure for preparing nutrients for an 18 mg C/L glucose solution, however the amounts were adapted based on the necessary carbon amount:

Ammonium nitrate (NH₄NO₃) and dipotassium hydrogen phosphate (K₂HPO₄) were dried in the oven for approximately 24 hours at 105°C to evaporate the water from the compounds. Ammonium nitrate (0.777 g, 9.7 mM) was weighed using an Ohaus Discovery semi-micro and analytical balance and dissolved in 1 L of Type 1 water in a volumetric flask. The flask was then filled with Type 1 water. Dipotassium hydrogen phosphate (0.955, 5.48 mM) was weighed using an Ohaus Discovery semi-micro and analytical balance and dissolved in 1 L of Type 1 water in a volumetric flask. 100 mL of the dipotassium hydrogen phosphate solution was transferred to a new 1 L volumetric flask. The flask was then filled to the line with the ammonium nitrate solution. This gives a final ratio of 27.4: 3.54: 1 in molar of C:N:P in the solution.

Calculations for constant ratio nutrients are shown in Appendix E.
2.3 Inoculum Preparation

A water sample from Sognsvann was collected on the 10\textsuperscript{th} of January 2018 and used for preparation of the inoculum in all experiments. Sognsvann water was collected because this lake is close to UiO and therefore allowed for a short collection time period. Approximately 100 mL of the Sognsvann water was filtered through a 2.0 μm filter to remove higher trophic layer microorganisms but allow bacteria to pass through. The water was transferred to a 250 mL Erlenmeyer flask to allow for extra headspace oxygen. Nutrients (1 mL), either the constant ratio nutrients (9.7 mM nitrate and 5.48 mM phosphate) or nutrients with equal amounts of nitrate and phosphate (10 mM, prepared by Stevenson Ong) depending on the experiment, were added to the flask. The flask was then wrapped in aluminum foil and shaken on the shaking table for a minimum of 2 days but no longer than 5 days.

2.4 Sample Preparation

The samples for each experiment were prepared in 25 mL volumetric flasks. 250 μL of constant ratio nutrients or nutrients with equal amounts of nitrate and phosphate (10 mM, referred to as 10 mM nutrients) is first added to the flask, depending on the experiment. 12 mL of the sample is then added to the flask followed by 250 μL of the inoculum. The flasks were then topped to the mark with sample and shaken to ensure proper mixing. The final concentration of nitrate and phosphate in the solution is 0.1 mM. The 5 mL sensor vials were filled with 5.2 mL of the sample to ensure no headspace. The vials were capped with new screw caps and covered with parafilm. Blanks were prepared with only Type 1 water and glucose (18 mg C/L) was used as a reference in all experiments. The experiment was placed on an SDR plate and let run for a minimum of 4 days at 25°C in an incubator that was placed in a cool room.
Four experiments were performed in order to study the effect nutrients ratios have on the biodegradability of different quality of organic matter, as well as to see concentration effect. The quality effect experiments measured the biodegradation of 18 mg C/L glucose, sucrose, ascorbic acid, maleic acid and DNOM in Sognsvann using both the constant nutrients ratio and 10 mM nutrients. Each compound was measured in 4 replicates. The concentration effect experiments measured the biodegradation of 10, 18, 30, 45 and 60 mg C/L glucose. The first experiment used constant ratio nutrients prepared for each concentration of glucose, and the second the 10 mM nutrients. Each glucose sample was measured in 4 replicates.

2.5 Data Analysis

All of the biodegradation data was analyzed using the R-Studio program developed at UiO by Tomas Mikoviny and Alexander Håland. The program was improved by Tomas Mikoviny by allowing the user to manually select the end point of exponential oxygen consumption. In the previous code oxygen consumption may have been zero because there was no more biodegradable compounds but the program still included the time that oxygen consumption was zero in the final respiration rate. Therefore, manual selection of the end of exponential oxygen consumption is needed so that the program will not include the period when oxygen consumption is zero. The start point for measuring exponential oxygen consumption for all experiments was 3x the standard deviation of the initial measurements.
Results and Discussion

There were two different objectives in this study. The first objective was to develop an absorbent material to selectively absorb DNOM from water. The material was partially successful in absorbing DNOM. It was determined from UV-Vis spectroscopy that the developed material was able to absorb humic substances, with higher molecular weight and more aromaticity, but the fulvic substances, with lower molecular weight and more aliphatic character, remained in the water. The second objective was to improve the standard method for measuring biodegradation through measuring O₂ consumption with a focus on improving the amount of nutrients in the experiment. It was determined that using nutrients with a constant ratio significantly affects the biodegradation of the sample. There is less phosphorous compared to nitrogen when using the constant ratio nutrients which likely results in less oxygen consumption. Having a more equal amount of phosphorous and nitrogen allows for more bacteria growth. It was also determined that respiration rate is not dependent on concentration and therefore respiration rate does not need to be normalized by concentration.

1. Preparation of modified SOMS with PEI

A solution of PEI in methanol was added to regular SOMS in order to load the PEI into the pores of SOMS. The surface area, pore volume and pore diameter of the regular SOMS, PEI/SOMS-10 and PEI/SOMS-20 were analyzed using N₂ absorption and desorption to determine of the materials. It is hypothesized that the surface area and pore volume of the material should decrease with increasing amount of PEI because the PEI will be absorbed into the SOMS pores. Table 4 gives the results from the N₂ absorption/desorption experiment.
As expected, the BET surface area, total pore volume and BJH total pore volume decreases as the amount of PEI loaded into the pores increases. The BET and BJH methods for total pore volume give slightly different results because the BJH method only measures mesopores whereas the BET method measures both mesopores and micropores. This decrease in surface area and pore volume demonstrates that the PEI is being absorbed into the pores and not just adsorbed to the surface of the material. The PEI/SOMS-20 material has a very small surface area and almost no total pore volume, which suggests that the pores of the material area almost completely filled. Therefore, it was decided to use PEI/SOMS-10 for future experiments because there is extra space for DNOM to be absorbed in the material. However, due to the swelling ability of the material, it is likely that the increased pore volume and surface area is created during absorption so it is possible that PEI/SOMS-20 would still theoretically be able to absorb DNOM.

<table>
<thead>
<tr>
<th>Sample</th>
<th>BET surface area (m² g⁻¹)</th>
<th>BET total pore volume (cm³ g⁻¹)</th>
<th>BET mean pore diameter (nm)</th>
<th>BJH total pore volume (cm³ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOMS</td>
<td>426</td>
<td>0,597</td>
<td>5,59</td>
<td>0,529</td>
</tr>
<tr>
<td>PEI/SOMS-10</td>
<td>264</td>
<td>0,321</td>
<td>4,87</td>
<td>0,289</td>
</tr>
<tr>
<td>PEI/SOMS-20</td>
<td>57,4</td>
<td>0,064</td>
<td>4,45</td>
<td>0,058</td>
</tr>
</tbody>
</table>

Table 4. Summary of surface area, pore volume and pore diameter for each SOMS sample.

2. Passive sampler absorption experiments

2.1 Absorption of DNOM with dry PEI/SOMS-10

Dry PEI/SOMS-10 was added to DNOM water in beakers and shaken. The material was subsequently separated from the water through filtering with a 0.2 μm filter. The PEI/SOMS-10 did not swell when added to the DNOM water. The material floated on top of the water during
addition. After filtering, the PEI/SOMS-10 appeared slightly clumpy and slightly brown suggesting that a small amount of DNOM was absorbed to the material. However, the water did not appear to be lighter in color than it was originally and therefore it was determined that the addition of dry PEI/SOMS-10 to DNOM water is not able to successfully absorb DNOM from the water.

2.2 Absorption of DNOM with dry PEI/SOMS-10 in pouches

Dry PEI/SOMS-10 was added to 3 stainless steel pouches, which were placed in DNOM water and left on a shaker for 24 hours. After 24 hours the DNOM appeared to have coagulated in the water and on the outside of the stainless steel pouch. The PEI/SOMS-10 was taken out of the pouch and it appeared slightly brown and swollen. However, it appears that most of the DNOM was coagulated in the water. It is hypothesized that PEI is leaking from the PEI/SOMS-10 material out of the passive sampler and into the water. The PEI is then coagulating with the DNOM, which causes the DNOM to precipitate from the water. The experiment was repeated except first adding the pouch loaded with PEI/SOMS-10 to a beaker of 500 mL of Type 1 water. It was predicted that the soluble PEI might leak into the Type 1 water and not leak when placed in the DNOM water. However, after leaving the pouches in Type 1 water for 24 hours and then submerging them in DNOM water for 48 hours there was still coagulation of the DNOM in the water and on the outside of the pouch. It was determined that this method of absorbing DNOM is not effective and therefore it was decided to attempt to open the PEI/SOMS-10 pores before addition to DNOM water. This will open more sites to allow for DNOM absorption.
3. Absorption of DNOM with pre-wetted PEI/SOMS-10

Due to the unsuccessful attempt of absorbing DNOM with dry PEI/SOMS-10, it was predicted that swelling the material first with ethanol might improve the absorption of DNOM from the water because there may be more space in the pores for DNOM when the pores have expanded. First, three Falcon tubes were loaded with pre-wetted PEI/SOMS-10 and Svartherget 2000 DNOM water (Method 1). After 24 hours of being shaken on the shaking table and separation of the water from the PEI/SOMS-10, the water in each falcon tube was analyzed with UV-Vis spectroscopy. The UV-Vis spectrum of the water from each Flacon tube and the original Svartherget water is shown in Figure 10.

![Method 1- Falcon Tubes](image)

Figure 10. UV-Vis spectrum of Svartherget 2000 water and the three replicates of Method 1 in the Falcon tubes.

It is apparent from the spectrum that the DNOM in the water after absorption by PEI/SOMS-10 using Method 1 is different than the original Svartherget (SVA) DNOM water.
Additionally, visibly, the PEI/SOMS-10 was brown after the 24 hours on the shaking table suggesting that DNOM was in fact absorbed on the material. The DNOM in the SVA water absorbs radiation from about 200-400 nm, with a notable shoulder in the curve around 260 nm. The replicates also absorb in this range and there is still a shoulder in the curve around 250 nm. The replicates, however, absorb much stronger from 200-220 nm. It is possible that ethanol or PEI is also in the water from leaking from the SOMS. Therefore, the strong peak from 200-220 nm could be the absorbance of ethanol or PEI. The specific absorbance ratio (SAR) of a UV-Vis spectrum can be calculated to determine characteristics about the organic compounds. It is expected that a high absorbance in the low wavelengths of the spectrum indicates a higher amount of LMW organics compared to HMW. The SAR is obtained by calculating the ratio of absorption at 254nm/400nm. Low SAR would suggest that there is a lot of HMW compounds in the sample whereas a higher SAR would suggest LMW compounds in the sample. The SAR of SVA water was calculated and determined to be 7.63. The three replicates had SAR values of 6.45, 8.33, and 7.09, respectively. These values are similar to the SAR value of SVA so no conclusions can be drawn from the SAR values.

Method 1 was repeated in dram vials instead of Falcon tubes because the PEI/SOMS-10 appeared stuck in the hole at the bottom of the Falcon tube and therefore not as exposed to the water. Pre-wetted PEI/SOMS-10 was added to DNOM water, left on a shaker for 24 hours and afterwards separated by filtration. This was done in 3 replicates and each replicate was analyzed with UV-Vis spectroscopy. Pre-wetted PEI/SOMS-10 was also placed in a dram vial with type 1 water, instead of SVA water to act as a control. After being left of the shaker and filtered this water was also analyzed via UV-Vis spectroscopy. The UV-Vis spectrum of SVA, the three replicates from this trial, and the control in Type 1 water can be seen in Figure 11.
The spectra of the three replicates in this experiment have a lower absorbance from 200-400 nm than in the Method 1 in the falcon tubes experiment. Whereas the curves from the replicates in Met-1 Falcon appeared more similar to the SVA water curve, the curves in this experiment appear more similar to the control curve. There is still a shoulder in the curves for the replicates around 250 nm, which could be from some DNOM absorption, however the absorbance is not as strong. The control, which was performed in Type 1 water, should theoretically not have any absorbance because it should be only Type 1 water. However, there is absorbance from 200-300 nm with very strong absorbance from 200-220 nm. The only other possibly compounds in the water could be ethanol or PEI and therefore it is hypothesized that the
strong peak at around 200 is from one of these compounds. The PEI/SOMS-10 material also appeared swollen and brown, visibly conveying that it did absorb DNOM from the water. The SAR values for these replicates and the control were also calculated for this experiment. The SAR value for SVA is 7.63, the three replicates are 15, 14.14, and 15.93, respectively, and the SAR for the control is 5.85. These three replicates have a much higher SAR than the control or the SVA water, suggesting more LMW compounds. It is notable that the three replicates have much higher SAR than the SVA because this suggests that the PEI/SOMS-10 absorbed the HMW DNOM compounds and not the LMW compounds.

The absorbance from the control was subtracted from each of the replicates and graphed to see the curves (Figure 12). The strong absorbance around 200 nm disappears from the curves and the curves appear similar to the SVA curve except with lower absorbance. This suggests that the PEI/SOMS-10 material absorbed much of the DNOM but there is some DNOM still left in the water. This agrees with the visual appearance of the water, which appears less brown than it was originally but still slightly colored.
Several controls were performed in order to determine what is strongly absorbing around 200-220 nm in the three replicates. PEI was dissolved in a small amount of ethanol and added to Type 1 water. This was analyzed by UV-Vis spectroscopy. A small amount of ethanol was also added to 10 mL of Type 1 water, which was analyzed by UV-Vis to determine if ethanol has any absorbance in the UV range. Type 1 water was also analyzed with UV-Vis to make sure that there is no absorbance from water molecules. The UV-Vis spectra of these controls are shown in Figure 13.

Figure 12. UV-Vis spectrum of SVA water, the three replicates Met-1 in dram vials minus the control

![Method 1-Dram Vials (minus control)]
The spectra of water and ethanol show that there is no absorbance from either of these compounds. This is expected because neither of these compounds have conjugated double bonds and therefore are not expected to absorb in the UV-Vis range. The PEI has a strong absorbance around 200-220 nm. This demonstrates that the controls and the replicates from the previous experiments with strong absorbance around 200-220 nm is from PEI. Therefore, PEI is likely being leaked from the PEI/SOMS-10 material. The PEI may be absorbed in the pores of the SOMS, however, when the pores are opened it is likely that since the PEI is not chemically bound to the SOMS some PEI molecules leak from the material into the water, creating a strong absorbance around 200-220 nm when analyzed by UV-Vis spectroscopy.

Figure 13. UV-Vis spectrum of PEI, water and ethanol
A second method was attempted to absorb the DNOM with PEI/SOMS-10. This method (Method 2) involved setting up a vacuum filtration system and coating a 0.45 μm filter with pre-wetter PEI/SOMS-10. SVA water (40 mL) was then filtered through the system 3 times. This is referred to as FIL1 because the filter was only coated with PEI/SOMS-10 once. SVA water was also filtered through the system with changing the PEI/SOMS-10 coated filter once in case the first PEI/SOMS-10 coated filter reached its capacity for absorbing DNOM. Filtering through new PEI/SOMS-10 coated filter would, in theory, be able to absorb the remaining DNOM that could not be absorbed by the PEI/SOMS-10 material added to the sample. This filtration is referred to as FIL2 because the water was filtered through 2 sets of PEI/SOMS-10. These two filtrations were each performed twice. First, they were performed with only partially swollen PEI/SOMS-10 and therefore the experiment was repeated with fully swollen PEI/SOMS-10 in order to maximize the amount of DNOM that could be absorbed. For a control, Type 1 water was also filtered through one set of PEI/SOMS-10 three times. The UV-VIS absorbency in all the filtered samples were measured (Figure 14).
The filtration curves appear to absorb at an absorbance between the SVA water and the control. This suggests that there is more than just PEI and water in the sample but some of the DNOM was absorbed during filtration. There is not much difference in the curves between the filtrations that used only the partially swollen PEI/SOMS-10 and the completely swollen PEI/SOMS-10. The water from the FIL2 experiments has the highest absorbance at 200 nm. This may be because more PEI is collected in the water since it is filtered through two sets of PEI/SOMS-10. Visibly, the PEI/SOMS-10 on the filter becomes brown during filtration. For the FIL2 experiments, the first PEI/SOMS-10 is very brown suggesting that DNOM is absorbed during this filtration. However, the second PEI/SOMS-10 is mostly white, suggesting that not much DNOM is absorbed during this second filtration. The SAR for SVA, FIL1-swellable, FIL2-swellable, FIL1-partially swellable and FIL2-partially swellable was calculated to be 7.63, 6.78, 10, 9.87 and 13.09, respectively. Three of the filtrations have a higher SAR than the original.

Figure 14. UV-Vis spectrum of SVA, all filtrations of the SVA water, and a control filtration
SVA water demonstrating that more LMW DNOM is left in the water and the HMW compounds are absorbed by the material. HMW compounds are usually less hydrophilic than LMW compounds. Since SOMS absorbs hydrophobic compounds easier than hydrophilic compounds it makes sense that the HMW compounds are absorbed by the material.

The control, which was only filtered with one set of PEI/SOMS-10, was subtracted from each of the filtrations that were performed (Figure 15). The FIL1 samples have a lower absorbance than the SVA water but are similar in curve-shape to the SVA, demonstrating that there is still DNOM in the samples. The FIL2 samples still have a high absorbance around 200 nm even after subtraction of the control. This is probably because there is a higher amount of PEI in these samples from filtering with two sets of PEI/SOMS-10 and therefore having a higher amount of leakage of PEI in to the samples. Since the control was only filtered with 1 set of PEI/SOMS-10 it does not account for all of the PEI in these samples.
It can be concluded that both Method 1 and Method 2 partially absorb DNOM from the sample. This is concluded based on visible data (visual absorption of DNOM with PEI/SOMS-10) and from the UV-Vis spectra obtained from Methods 1 and 2 (different curves with lower absorbance but similar curve-shape to the SVA). Also, when analyzing the SAR values, most of the SAR values of the water samples after absorption by PEI/SOMS-10 are higher than the original SVA SAR value. This demonstrates that LMW DNOM compounds are left in the water and higher molecular weight compounds are absorbed by the PEI/SOMS-10. This could be because PEI has a high affinity for humic substances but DNOM is composed of both humic and fulvic substances. The humic substances have a higher molecular weight and more aromaticity.
than fulvic substances and because PEI is selective towards humic substances, the humic part of DNOM is absorbed leaving the fulvic, LMW compounds.

In attempt to absorb the PEI that leaks from the SOMS material from the water samples, each of the experiments described above were repeated with an extra filtration using regular SOMS. In theory, the regular SOMS will absorb the PEI from the water leaving only the non-absorbed DNOM in the sample. This was done for both Methods 1 and 2. Each sample was analyzed with UV-Vis Spectroscopy.

![Method 1-extra filter](image)

**Figure 16. UV-Vis of SVA, two replicates of method 1 with extra filtration and a control**

Based on the UV-Vis of the replicates and control in Method 1 with extra filtering, there is still PEI in the water samples. This is concluded because there is strong absorbance from 200-220 nm that reflects the absorbance of PEI. When the control is subtracted from the replicates the
spectra appear similar to Method 1 without extra filtering. There is still absorbance from 200-400 nm demonstrating that there is still DNOM in the sample but the absorbance is not as strong and the curve is not the same as the SVA curve demonstrating that DNOM has been absorbed to a certain extent. The SAR values for the SVA water, replicate 1 and replicate 2 in this experiment are 6.36, 11.43, and 14.44, respectively. This agrees with what was previously concluded- the higher SAR values after absorption demonstrate HMW DNOM was absorbed and there is more leftover LMW compounds in the water.

Method 2 was also performed with extra filtering through regular SOMS. The UV-Vis spectra of the filtrations are similar to Method 2 without the extra filtering demonstrating that PEI is still present in the water samples (Figure 18).

Figure 17. UV-Vis of SVA and replicates 1 and 2 from method 1 minus the control.
The Filtration curves are in-between the SVA curve and the control curve showing that there is still DNOM in the samples. After subtraction of the control from the samples the filtrations still have some absorption in the 200-400 nm range and therefore DNOM is still present in the samples (Figure 19). Visibly, in both methods 1 and 2 the PEI/SOMS-10 was brown and swollen showing that DNOM was absorbed. The SAR values for the SVA, FIL1 and FIL2 are 6.36, 7.14, and 7.51, respectively. The SAR of the filtrations are not as high as the other experiments but it is still larger than the SVA value suggesting that LMW compounds are in the water filtration samples.
In conclusion from this absorption experiment, both methods 1 and 2 absorbed some DNOM from the SVA water samples. However, there was still DNOM left in the water noted visibly by the brown color and from UV-Vis spectroscopy because there were still DNOM absorbance signals in each of the samples. From the higher SAR values of the samples for Methods 1 and 2 after absorption by PEI/SOMS-10 compared to the original SVA, it was determined that the material likely absorbs the more HMW DNOM compounds in the water. As stated above, this is likely because humic substances have high affinity for PEI, which may leave the fulvic, LMW substances in the water. Overall, the material was partly successful in absorbing DNOM from water. However, future work should focus on chemically adsorbing the PEI to the SOMS so that there is no leakage of PEI into the water and absorbing fulvic substances as well.
as humic substances. It is possible to create a material with a different compound that is selective to both humic and fulvic substances that will then be able to absorb all of the DNOM in a water sample.

4. Nutrients Quantity in Biodegradation Experiments

Four biodegradation experiments were performed to assess if the quantity of nutrients added to the samples in biodegradation experiments affects how much organic matter is consumed in the samples by the bacteria. In the original biodegradation method, developed by Alexander Håland, a solution of 10 mM of phosphate and 10 mM of nitrate was added as nutrients to the samples. This resulted in a final concentration of 0.1 mM nitrate and phosphate in the samples (2N:1P). This study compares using 0.1 mM nitrate and phosphate to using nutrients that have been prepared so that they have a constant ratio of 27.5:3.54:1 of C:N:P. It was determined that the respiration rate of the samples with 3:54N:1P ratio nutrients is significantly smaller when using this constant than when using the 2:1 ratio.

4.1. Effect of nutrient concentration on Biodegradation

The respiration rate of a set of easy degradable reference materials (glucose, sucrose, ascorbic acid and maleic acid (18 mg C/L)), as well as DNOM in a natural water sample that was collected from Sognsvann, was measured according to the standardized method developed by Håland described in the material and method chapter. The analysis were performed using two different nutrient concentrations, one using a nutrients solution of 10 mM phosphate and nitrate (ratio of 15:2:1 of C:N:P) and one using a nutrients solution of 9.7 mM nitrate and 5.48 mM phosphate (27.5:3.54:1 of C:N:P). The respiration rates of the compounds using the nutrients of 10 mM phosphate and nitrate is shown in Figure 20.
Figure 20. Respiration rates of glucose, sucrose, ascorbic acid, maleic acid and DNOM in Sognsvann water using a nutrients solution of 10 mM phosphate and nitrate.

The respiration rates of the compounds using the 3:54N:1P ratio nutrients is shown in Figure 21.

Figure 21. Respiration rate of glucose, sucrose, ascorbic acid, maleic acid and DNOM in Sognsvann water using a nutrients solution with the redfield ratio.
Glucose and sucrose have slightly lower respiration rates when using 3.54N:1P ratio nutrients. Sognsvann water has a slightly higher respiration rate in the 3.54N:1P experiment. Because the carbon concentration in the water was not measured, no conclusions can be drawn from the Sognsvann water samples. The respiration rates of ascorbic acid and maleic acid are significantly different in the two experiments. The graphs of the ascorbic acid (Figure 22) suggest that ascorbic acid reacts with oxygen. This affects the respiration rate because not only bacteria is consuming the oxygen but the ascorbic acid is also reacting with the oxygen. Figure 22 shows an example of one of the ascorbic acid respiration rate graphs from the experiment using 10 mM phosphate and nitrate nutrients. The red part shows the part of the graph that was used to calculate respiration rate. There is initial oxygen consumption followed by a lag time where it appears that oxygen is not being consumed. This is unusual if bacteria were the only source of consumption. It was hypothesized that ascorbic acid may react with $O_2$ and this was confirmed online. Therefore, the results from ascorbic acid biodegradation are inconclusive.

![Figure 22](image)

Figure 22. Example graph of the biodegradation of ascorbic acid from the experiment using nutrients of 2N:1P.
The results from the biodegradation of maleic acid are also inconclusive although it is unknown what is happening during oxygen consumption. There is an extreme difference in respiration rates for maleic acid in the two experiments (3.6 for the nutrients of 2N:1P and 0.11 for 3.54N:1P nutrients). This large variance is unexpected and the graphs in the two experiments are also very different. The graphs are shown in Figure 23.

![Graphs of biodegradation of maleic acid from the experiment using 3:54N:1P ratio nutrients (left) and the experiment using nutrients of 2N:1P ratio (right).](image)

Figure 23. Graphs of biodegradation of maleic acid from the experiment using 3:54N:1P ratio nutrients (left) and the experiment using nutrients of 2N:1P ratio (right).

It is unlikely that this large difference in graphs is due only to a difference in the quantity of nutrients. Therefore, the results from the biodegradation of maleic acid are inconclusive. The only compounds that can be assessed from these experiments are glucose and sucrose. These compounds show a slight decrease in oxygen consumption when 3:54N:1P ratio nutrients is used. Therefore, it is predicted that the use of this ratio lowers the respiration rate because an excess of nitrate compared to phosphate is added. Due to the lack of phosphorous compared to nitrogen there is less oxygen consumption. The biodegradation graphs from these two experiments can be found in Appendices F and G.
4.1. Biodegradation of glucose with different concentrations

Due to the inconclusive results from the biodegradation of ascorbic acid, maleic acid and DNOM in Sognsvann water, it was decided to use only glucose, with different concentrations, using nutrients of 2N:1P and 3:54N:1P. Glucose with concentrations of 10, 18, 30, 45 and 60 mg C/L were prepared. Based on the preliminary findings in the previous biodegradation experiments it was hypothesized that the experiment using 3:54N:1P ratio nutrients would have lower respiration rates than the experiment using 2N:1P nutrients. The concentration of nutrients and nutrients ratios are shown for each experiment in Table 5. The respiration rates of the glucose are shown in Figures 24 and 25.

Table 5. Concentration of nutrients and nutrients ratios for both glucose experiments.

<table>
<thead>
<tr>
<th>Sample (mg C L⁻¹)</th>
<th>2N:1P nutrients concentration (mM)*</th>
<th>2N:1P nutrients ratio (C:N:P)</th>
<th>3.54N:1P nutrients concentration (mM)*</th>
<th>3:54N:1P nutrients ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-10</td>
<td>0.1 nitrate 0.1 phosphate</td>
<td>8.33: 2: 1</td>
<td>0.054 nitrate 0.0304 phosphate</td>
<td>27.5: 3.54: 1</td>
</tr>
<tr>
<td>Glucose-15</td>
<td>0.1 nitrate 0.1 phosphate</td>
<td>15: 2: 1</td>
<td>0.097 nitrate 0.0548 phosphate</td>
<td>27.5: 3.54: 1</td>
</tr>
<tr>
<td>Glucose-30</td>
<td>0.1 nitrate 0.1 phosphate</td>
<td>25: 2: 1</td>
<td>0.1616 nitrate 0.0914 phosphate</td>
<td>27.5: 3.54: 1</td>
</tr>
<tr>
<td>Glucose-45</td>
<td>0.1 nitrate 0.1 phosphate</td>
<td>37.5: 2: 1</td>
<td>0.242 nitrate 0.137 phosphate</td>
<td>27.5: 3.54: 1</td>
</tr>
<tr>
<td>Glucose-60</td>
<td>0.1 nitrate 0.1 phosphate</td>
<td>50: 2: 1</td>
<td>0.323 nitrate 0.1795 phosphate</td>
<td>27.5: 3.54: 1</td>
</tr>
</tbody>
</table>

*This is the final concentration in the sample
It is apparent that the respiration rates for the glucose in the experiment with 3:54N: 1P ratio nutrients is much lower than the respiration rates with nutrients of 2N:1P. This demonstrates that using the constant ratio nutrients of 27.5C: 3.54N: 1P significantly affects the
respiration rate. The use of nutrients with a 2:1 ratio of N:P provides more phosphorous compared to nitrogen than the use of nutrients with 3.54N:1P. Due to the lack of phosphorous compared to nitrogen in the latter experiment the respiration rate is probably lower because of a lack of phosphorous. It can be concluded from these experiments that having more phosphorous with respect to nitrogen is optimal because it provides a better signal for measuring oxygen consumption. There is no clear pattern in respiration rates when comparing the different concentrations of glucose. In the experiment with nutrients 2N: 1P, glucose of 18 mg C/L has the highest respiration rate, however in the experiment with 3.54N: 1P nutrients, glucose of 45 mg C/L has the highest respiration rate. There is likely no pattern for glucose of different concentrations because the different concentrations might not make a significant difference in respiration rate. Glucose is easily biodegradable and therefore it is possible that the different concentrations do not significantly affect oxygen consumption. However, the lower respiration rates when 3.54N: 1P ratio nutrients were used compared to 2N: 1P ratio nutrients demonstrates that the amount of phosphorous with respect to nitrogen significantly affects the respiration rate and a larger amount of phosphorous should be used to improve the signal in biodegradation experiments. The biodegradation graphs of these two experiments can be found in Appendices H and I.
Summary

The first objective of this study was to create an absorbent material that is able to absorb DNOM selectively. The results indicate that the developed absorbent material is able to partially absorb DNOM. Swellable-organically modified silica was modified with polyethyleneimine because of its high affinity toward DNOM. This material was found to be able to absorb humic substances (higher molecular weight DNOM) but was unable to absorb lower molecular weight fulvic substances. This is likely due to the fact that PEI has a high affinity toward humic substances and less so toward fulvic substances. It is possible that with another chemical which has a high affinity toward both humic and fulvic substances the material would be able to absorb all of the DNOM from the water.

The second objective of this experiment was to improve biodegradation study methods through the investigation of the nutrients quantity, specifically the amount of nutrients, in biodegradation experiments. In previous biodegradation studies, the amount of nutrients that was added to the samples was 10 mM nitrate and phosphate (2N: 1P). A constant ratio of 27.5C: 3.54N: 1P nutrients was investigated during a glucose biodegradation experiment. The results indicate that using 3.54N: 1P ratio nutrients in the biodegradation experiments significantly lowers the biodegradability of the samples. This is likely due to the lack of phosphorous in comparison to nitrogen. The ratio of 2N: 1P gives a better signal in biodegradation studies and therefore should be used in future biodegradation experiments. This study should be followed up by more biodegradation experiments comparing different amounts of nutrients. Only one experiment was conducted successfully in this study and therefore more experiments comparing different amounts of nutrients will be important to confirm the results of this study. Experiments
using water samples from the environment should also be performed because the only compound that was studied in this experiment was glucose, which is easily biodegradable.

The results from this study show that a new absorbent material for selectively absorbing DNOM is in process of being developed with preliminary results demonstrating that this absorbent material is possible to develop. The results also show that using 2N: 1P ratio nutrients in biodegradation experiments is likely necessary to accurately characterize DNOM in water samples.
Appendices

A  Description of SOMS production
B  SOMS pore data and calculation of maximum theoretical loading of SOMS with PEI
C  Detailed procedure of PEI/SOMS preparation
D  Stock Solution Sample Calculation
E  3.54N: 1P Nutrients Sample Calculation
F  Biodegradation graphs of glucose, sucrose, ascorbic acid, maleic acid (all 18 mg C/L) and Sognsvann water using nutrients of 2N: 1P
G  Biodegradation graphs of glucose, sucrose, ascorbic acid, maleic acid (all 18 mg C/L) and Sognsvann water using nutrients of 3.54N: 1P
H  Biodegradation graphs of glucose (10, 18, 30, 45 and 60 mg C/L) using nutrients of 2N: 1P
I  Biodegradation graphs of glucose (10, 18, 30, 45 and 60 mg C/L) using nutrients of 3.54N: 1P
Appendix A: Description of SOMS Production

SOMS media is prepared through basic sol-gel techniques by carefully controlling the precursors and conditions during synthesis to obtain the swellable optically transparent sol-gel material. The sol-gel process is a chemical method used for producing solid materials from small molecules. Sol-gel materials are formed through the following steps: mixing, gelation, aging, and drying. Mixing, for SOMS synthesis, is accomplished by combining a precursor, an alkoxy silane such as bis(trimethoxysilyl)benzene (BTEB, Figure 1), with organic solvent such as acetone, water, and tetra-n-butylammonium fluoride (TBAF) as a catalyst. The alkoxy silane subsequently undergoes hydrolysis and polycondensation to create a cross-linked three-dimensional solid structure.

Scheme 1 demonstrates an example condensation reaction that occurs after hydrolysis of the alkoxy silane. Many condensations of the hydrolyzed molecules occur, thereby creating a polymeric cross-linked network.

Scheme 1
Aging of the gel involves even further polycondensation reactions over a period of hours to days, increasing the cross-linking of the network. For SOMS production, after aging but prior to drying, derivitization of the silanol groups that were produced from hydrolysis occurs by reacting the condensed molecules with hexamethyldisilazane (Figure 2). The silanol groups specifically react with hexamethyldisilazane, thus preventing further condensation reactions or cross-linking to occur during drying. Derivitization of the silanol groups is an extremely important step to obtain a swellable material. During the drying process of sol-gels, the solvent is evaporated. Due to the evaporation of solvent, further cross-linking polycondensations between molecules are able to occur in most sol-gels and as a result, the area of the polymeric network that the solvent previously occupied is able to collapse. However, during SOMS production, polycondensations are prevented during drying by derivitization and thus, when the material is dried, noncovalent interactions between molecules are generated to create a temporarily collapsed polymeric network. These non-covalent interparticle interactions can be disrupted by an organic solvent, which results in swelling of the material. Swelling is lost when the material is too highly cross-linked because the strong covalent bonds generated from cross-linking polycondensations of the molecules cannot be interrupted by solvent. In addition to derivitization, the alkoxy silane precursor must have enough flexibility to collapse within the polymeric matrix from the noncovalent interactions. BTEB is used in SOMS production because the aromatic group is flexibly tethered with an ethylene group to the silicon centers and thus there is enough flexibility for the material to collapse by noncovalent interactions to create a swellable material.

A more detailed description of SOMS synthesis is described by Edmiston et al. elsewhere.
Appendix B: SOMS pore data and calculation of maximum theoretical loading of SOMS with PEI

1. SOMS pore data from Dr. Paul Bonvallet and ABS materials in Wooster, Ohio

<table>
<thead>
<tr>
<th>Pore diameter range (nm)</th>
<th>Pore volume (mL per gram of SOMS)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 6</td>
<td>0.36583</td>
<td>55.19</td>
</tr>
<tr>
<td>6 - 8</td>
<td>0.23861</td>
<td>36.00</td>
</tr>
<tr>
<td>8 - 10</td>
<td>0.02475</td>
<td>3.73</td>
</tr>
<tr>
<td>10 - 12</td>
<td>0.01065</td>
<td>1.61</td>
</tr>
<tr>
<td>12 - 16</td>
<td>0.00774</td>
<td>1.17</td>
</tr>
<tr>
<td>16 - 20</td>
<td>0.00472</td>
<td>0.71</td>
</tr>
<tr>
<td>20 - 80</td>
<td>0.00903</td>
<td>1.36</td>
</tr>
<tr>
<td>Over 80</td>
<td>0.00150</td>
<td>0.23</td>
</tr>
<tr>
<td>Total</td>
<td>0.66284</td>
<td>100.00</td>
</tr>
</tbody>
</table>

2. Maximum theoretical loading of SOMS with PEI calculation

SOMS pore volume: 0.366 mL/g (biggest percentage of pores in SOMS according to data above)
PEI Density: 1.00 cm$^3$/g

Maximum Loading: $\frac{0.366}{0.366+1} \times 100\% = 26.8\%$
Appendix C: Detailed procedure of PEI/SOMS preparation

The following general procedure assumes 10% weight loading of PEI into 500 mg of SOMS, however these amounts changed based on the necessary wt % and amount of SOMS used during each PEI/SOMS preparation:

PEI (55.55 mg) was dissolved in methanol (~ 2 mL) in a round bottom flask. The solution was stirred for approximately 1 hour so that all of the PEI was dissolved. SOMS (500 mg) was added to the round bottom flask. The SOMS swelled in the organic solution. Additional methanol was added to the flask so that visibly the SOMS was completely swollen but not overwhelmed with methanol (i.e. the SOMS is wet but not floating in excess methanol). The solution was stirred and left in a fume hood for approximately 12 hours or until the methanol had evaporated entirely. During one experiment, the SOMS was stirred for longer than 48 hours. This resulted in loss of some swelling ability. Visibly, the 500 mg SOMS was able to uptake less than 1 mL methanol whereas unmodified SOMS (500 mg) is able to uptake approximately 2 mL of methanol. It was determined that the optimal stirring time for the solution was 12-48 hours.
Appendix D: Stock Solution Sample Calculation

Preparation of a glucose solution with 18 mg C/L:

Carbon = 12.01 g/mol
Glucose = 180.1559 g/mol (6 carbons in glucose)

Calculation:

18 mg C/L = 0.018 g C/L

0.018 g C/L * 1 mol/12.01 C = 0.000149 mol/L C

180.1559 g/mol * 0.000149 mol/L/ 6 carbons = 0.045 g glucose/L

This calculation was performed for 18 mg C/L glucose, sucrose, ascorbic acid and maleic acid as well as glucose solutions of 10, 30, 45 and 60 mg C/L

The following procedure is for preparation of 18 mg C/L glucose and the amounts were modified for each compound based on the stock solution calculations: glucose (0.09 g) was weighed using an Ohaus Discovery semi-micro and analytical balance and transferred to a 2 L volumetric flask. The 2 L volumetric flask was then filled with Type 1 water.
Appendix E: 3.54N: 1P Ratio Nutrients Sample Calculation

This sample calculation is for preparing 3.54N: 1P ratio nutrients with potassium phosphate and ammonium nitrate assuming a carbon concentration of 18 mg C/L.

Ammonium Nitrate: 80.043 g/mol (2Nitrogens)
Nitrogen: 14.01 g/mol

Potassium Phosphate: 174.2 g/mol
Phosphorous: 30.97 g/mol

Calculation (Nitrate):

1. Change nitrogen grams to moles
   \[ 2.72\times10^{-3} \text{ g/L Nitrogen} \times \frac{1 \text{ mol}}{14.01 \text{ g}} = 1.94\times10^{-4} \text{ mol/L N} \]

2. Calculate how many grams of nitrate you need to get \(1.94\times10^{-4} \text{ mol/L N} \) in solution:
   \[
   \frac{1.94 \times 10^{-4} \text{ mol N}}{2 \text{ Nitrogens}} \times 80.052 \frac{\text{g}}{\text{mol}} \text{NH}_4\text{NO}_3 = 7.77 \times 10^{-3} \frac{\text{g}}{\text{L}} \times (100)
   \]
   You need: 0.777 g nitrate/L water (multiplied by 100 because need to weigh out at least 200 mg on the analytical balance)

3. In a 25 mL sample, how much of the solution do you need to add to get \(7.77 \times 10^{-3} \text{ g nitrate} \) in the solution?
   \[ C_1V_1 = C_2V_2 \]
   \( (X) \text{ L} \times 777 \text{ mg/L nitrate} = 0.025 \text{ L} \times 7.77 \text{ mg/L nitrate} \)
   \[ X = 2.5\times10^{-4} \text{ L} = 250 \mu\text{L} \text{NH}_4\text{NO}_3 \text{ of the solution in the 25 mL sample} \]

Calculation (Phosphate):

1. Change phosphorous grams to moles
   \[ 0.1698 \times 10^{-3} \text{ g/L Phosphorous} \times \frac{1 \text{ mol}}{30.97 \text{ g}} = 5.48\times10^{-6} \text{ mol/L P} \]

2. Calculate how many grams of phosphate you need to get \(5.48\times10^{-6} \text{ mol/L P} \) in solution
\[
\frac{5.48 \times 10^{-6} \text{ mol} \text{ P}}{1 \text{ Phosphorous}} \times 174.2 \frac{g}{\text{mol}} K_2HPO_4 = 9.55 \times 10^{-4} \frac{g}{L} \times (1000)
\]

You need: 0.955 g phosphate/L water (multiplied by 1000 because need to weight over 200 mg on the analytical balance)

3. In a 25 mL sample, how much of the solution do you need to add to get $9.55 \times 10^{-4}$ g phosphate in the solution?

\[
C_1 V_1 = C_2 V_2
\]

\[
(X) \text{L} \times 955 \text{ mg/L phosphate} = 0.025 \text{L} \times 0.955 \text{ mg/L}
\]

\[X = 2.5 \times 10^{-5} \text{L} = 25 \mu \text{L} \text{ K}_2\text{HPO}_4 \text{ of the solution in the 25 mL sample}
\]

4. Need to dilute the sample because cannot measure 25 \mu L.

\[100 \text{mL} \times 955 \text{mg/L} \text{ K}_2\text{PO}_4 = 1000 \text{mL} \times X \text{mg}
\]

\[X = 95.5 \text{ mg K}_2\text{PO}_4
\]

Take 100 mL of the K_2PO_4 and dilute with the NH_4NO_3 solution to 1 L. Use 250 \mu L of this solution when preparing the 25 mL biodegradation solutions.
Appendix F: Biodegradation graphs of glucose, sucrose, ascorbic acid, maleic acid (all 18 mg C/L) and Sognsvann water using nutrients of 2N: 1P.

Blanks:
Glucose 18 mg C/L:
Sucrose (18 mg C/L):
Ascorbic Acid (18 mg C/L):
Maleic Acid (18 mg C/L):
Sognsvann Water:

A5

B5

C5

D5
Appendix G: Biodegradation graphs of glucose, sucrose, ascorbic acid, maleic acid (all 18 mg C/L) and Sognsvann water using nutrients of 3.54N: 1P.

Blanks:
Glucose (18 mg C/L):
Sucrose (18 mg C/L):
Ascorbic Acid (18 mg C/L):
Maleic Acid (18 mg C/L):
Sognsvann Water:

A6

B6

C6

D6
Appendix H: Biodegradation graphs of glucose (10, 18, 30, 45, and 60 mg C/L) using nutrients of 2N: 1P.

Blanks:
Glucose (10 mg C/L):
Glucose (18 mg C/L):
Glucose (30 mg C/L):
Glucose (45 mg C/L):
Glucose (60 mg C/L):
Appendix I: Biodegradation graphs of glucose (10, 18, 30, 45, and 60 mg C/L) using nutrients of 3.54N: 1P.

Blanks:
Glucose (10 mg C/L):
Glucose (18 mg C/L):

A3

B3

C3

D3
Glucose (30 mg C/L):
Glucose (45 mg C/L):

A5

B5

C5

D5
Glucose (60 mg C/L):
References


(3) Vogt, R. Natural Organic Matter in Nordic Drinking Waters (NOMiNOR).


