HHS Public Access

Author manuscript

J Microbiol Methods. Author manuscript; available in PMC 2018 April 30.

Published in final edited form as:

J Microbiol Methods. 2012 September ; 90(3): 145–151. doi:10.1016/j.mimet.2012.04.016.

A Carbon Free Filter for Collection of Large Volume Samples of Cellular Biomass from Oligotrophic Waters

Brian J. Mailloux[a,*], **Audra Dochenetz**[b], **Michael Bishop**[c], **Hailiang Dong**[c], **Lori A. Ziolkowski**[d], **K. Eric Wommack**[e], **Eric G. Sakowski**[e], **Tullis C. Onstott**[f], and **Greg F. Slater**[d]

[a]Department of Environmental Science, 76 Claremont Ave, Barnard College, New York, NY 10025-6598, USA, phone-212-854-7956

[b]School of General Studies, Columbia University, New York, NY 10025, USA

[c]Department of Geology, Miami University, Oxford, OH 45056

[d]School of Geography and Earth Sciences, McMaster University, Hamilton, Ontario, Canada

[e]Delaware Biotechnology Institute, 15 Innovation Way, University of Delaware, Newark, DE 19711

[f]Department of Geosciences, Princeton University, Princeton, NJ 08544

Abstract

Isotopic analysis of cellular biomass has greatly improved our understanding of carbon cycling in the environment. Compound specific radiocarbon analysis (CSRA) of cellular biomass is being increasingly applied in a number of fields. However, it is often difficult to collect sufficient cellular biomass for analysis from oligotrophic waters because easy-to-use filtering methods that are free of carbon contaminants do not exist. The goal of this work was to develop a new column based filter to autonomously collect high volume samples of biomass from oligotrophic waters for CSRA using material that can be baked at 450°C to remove potential organic contaminants. A series of filter materials were tested, including uncoated sand, ferrihydrite-coated sand, goethite-coated sand, aluminum-coated sand, uncoated glass wool, ferrihydrite-coated glass wool, and aluminum-coated glass wool, in the lab with 0.1 and 1.0 μm microspheres and *E. coli*. Results indicated that aluminum-coated glass wool was the most efficient filter and that the retention capacity of the filter far exceeded the biomass requirements for CSRA. Results from laboratory tests indicate that for oligotrophic waters with 1×10^5 cells ml^{-1}, 117 L of water would need to be filtered to collect 100 μg of PLFA for bulk PLFA analysis and 2000 L for analysis of individual PLFAs. For field sampling, filtration tests on South African mine water indicated that after filtering 5955 liters, 450 μg of total PLFAs were present, ample biomass for radiocarbon analysis. In summary, we have developed a filter that is easy to use and deploy for collection of biomass for CSRA including total and individual PLFAs.

[*]Corresponding Author: bmaillou@barnard.edu.

Keywords

Filter; PLFA; Radiocarbon

1. INTRODUCTION

Compound specific radiocarbon analysis (CSRA) of cellular biomass has become a powerful method for determining microbial carbon sources in the environment (Brady et al., 2009; Cherrier et al., 1999; Pearson et al., 2001; Petsch et al., 2001; Slater et al., 2005; Wakeham et al., 2006). The power of this approach is in the large isotopic disparity that can exist in ^{14}C contents in certain environmental systems. Fossil carbon, such as found in petroleum carbon and kerogen, is millions of years old and contains no significant ^{14}C due to radioactive decay ($\Delta^{14}C = -1000$ ‰). In contrast, modern natural organic matter contains modern levels of ^{14}C ($\Delta^{14}C = \sim 50$ ‰). This large disparity raises the potential of identifying microbial cycling of modern versus fossil carbon in situations where these two potential carbon sources coexist.

CSRA analysis can be performed on any cellular component that can be collected and purified in sufficient quantities. This includes cellular membrane lipids, and/or DNA from cellular extracts. Although radiocarbon analysis of lipids has been performed on bacterial, archaeal and eukaryotic biomarker lipids, to this point most environmental studies have focused on phospholipid fatty acids (PLFAs) as these represent viable bacterial cells (White et al., 1979). In addition, most studies have focused on extraction and analysis of easily accessible environmental matrices with high cell densities including sediments or shallow contaminated groundwater (Pearson et al., 2001; Slater et al., 2005). However, many systems of interest exist where low biomass planktonic concentrations dominate, and where solid phase sampling may not be possible, or may inherently contaminate the system. For example, in deep groundwater or mine environments, it is not possible to regularly collect enough pristine sediment for CSRA analysis and therefore filtering of the aqueous phase is the only option.

Normal and tangential flow filters are commonly used to collect biomass from aqueous samples (Venter et al., 2004). Tangential filtration or ultrafiltration has been used successfully in both the lab and field, however the systems can be tedious and utilization in the field with larger volume samples can be difficult (Hill et al., 2007; Knappett et al., 2011). Normal flow filtration or membrane filtration (e.g. filter paper or syringe filters) is the most common method for collecting water samples for molecular analyses (Somerville et al., 1989). For nucleic acid extractions a wide variety of filters have been utilized using traditional extractions or kits. Extractions for nucleic acids are typically not affected by the presence of organic carbon and plastics in the filter material, making this a fast and reliable method. However, the initial extraction step for analysis of lipids is usually an organic extraction (White et al., 1979). Collection of samples onto organic resistant filters (e.g. Durapore) is feasible (Mills et al., 2010) but the filtering progress can become slow to nearly impossible as the filter disks clog and need to be replaced.

Column based filters are commonly used to remove material from water such as pathogens or aqueous phase contaminants such as arsenic (Sobsey et al., 2008). These systems are typically sand based and may contain hydroxide or carbon coatings to improve retention. However, these systems are usually not later extracted to examine microbial properties as the main goal is to reduce concentrations in the water to meet a regulatory or health related criterion. More recently, for virus sampling, a glass wool column filter that uses electrostatic interactions has been utilized to filter large volumes at high flow rates within residential homes while retaining over 60% of viruses (Lambertini et al., 2008). These filters have the potential to be easily deployable at a low cost but these or similarly designed filters have never been tested for CSRA.

The goal of this work was to develop a filter for CSRA of cellular biomass with a focus on PLFAs. The requirements for CSRA are the most stringent, as large sample sizes are required, but the filter could also be used to analyze for PLFA distributions, stable isotopes of cellular biomass, and potentially nucleic acids. For the sampling and analyses the filter needs to meet the following minimum requirements: 1) it must be composed of organic carbon free filter material, 2) the whole apparatus must be bakeable at 450°C to remove all organic contaminants 3) it must be capable of filtering large volumes at high flow rates and high pressures without clogging (>1,000 liters in about a day), 4) it must be autonomous and capable being left unattended for multiple days, 5) it must possess high filtering efficiency in order to capture all the cells in oligotrophic water, 6) it must be capable of filtering small organisms (<1.0 μm and hopefully <0.1 μm), 7) it must operate without power, 8) it must be able to function at high temperatures, up to at least 70°C, and 9) it must be easy to solvent extract with no background contaminants for subsequent analyses. Initial tests examined sand and glass wool based filters with a variety of metal oxide coatings to determine an optimal filter material. Aluminum-coated glass wool proved to be the most promising and was further tested in the lab under varying conditions with microspheres and *E. coli*. The filter was then utilized to collect enough biomass for CSRA of total PLFAs from 1.3 km below land surface in a South African Gold Mine. Initial results indicate this may be a robust filter design to collect cells for CSRA of cellular biomass.

2. METHODS

2.1. Filter Materials

The filter materials tested were uncoated sand, ferrihydrite coated sand, goethite coated sand, aluminum-coated sand, uncoated glass wool, ferrihydrite coated glass wool, and aluminum-coated glass wool. All filter materials were baked at 450°C for 24 hours after cleaning or coating. Sand filters were made from washed (Murphy et al., 1997), unground F-60 silica sand (U.S. Silica, Ottawa Il) sieved to 200–250 μm. The oiled sodocalcic glass wool (Bourre 725QN; Saint Gobain, Isover-Orgel, France) was initially cleaned and rinsed according to previous protocols (Lambertini et al., 2008; Vilaginès et al., 1993) but this did not improve retention of *E. coli* and was used unwashed for all later experiments (referred to as uncoated glass wool). The packing materials were coated separately with up to three types of metal oxides. Fe(III) coatings were performed according to standard protocols (Brooks et al., 1996; Schwertmann and Cornell, 2000). To coat the sand and glass wool with aluminum

hydroxide, 1 M $AlCl_3 \cdot 6H_20$ was neutralized with 6M NaOH, mixed with sand or glass wool, baked at 450°C and then rinsed with deionized water (Hall et al., 2005) (referred to as aluminum-coated sand and aluminum-coated glass wool). The mineralogy of the coatings may change with baking but this was not investigated except for the aluminum-coated glass wool.

2.2. Filter Assembly

Laboratory filters were made in 0.64 cm ID by 8 cm long polycarbonate columns (McMaster-Carr, Atlanta, Ga). The first and last 1cm were packed with plain glass wool and the middle 6 cm were packed with the filter material. Sand materials were dry-packed and glass wool materials were wet-packed (glass wool was wetted with deionized water before packing). Laboratory columns were run upward at a flow rate of approximately 5 mL min^{-1}. Materials for laboratory columns were only baked before packing.

Field columns were 4.1cm (1.61”) ID by 7.62 cm (3”) length 316 stainless steel pipe with a 1.5” to 0.5” reducing flange (McMaster-Carr, Atlanta, Ga). The reducing flanges (end caps) were wet-packed with uncoated glass wool. The pipe was wet-packed with aluminum-coated glass wool. The filters were loosely screwed together and baked at 450°C for 24 hours, after which the end caps removed, Teflon tape added to the pipe threads, and the end caps screwed back onto the pipe, wrapped in aluminum foil, and stored in Ziploc bags until use.

2.3. Laboratory Filter testing

Laboratory filter testing was conducted to pick a filter material, determine retention capacity, and constrain filtering conditions. The Laboratory filters were run with artificial groundwater (AGW) consisting of 0.12 g L^{-1} sodium bicarbonate and 0.16 g L^{-1} calcium chloride at pH 7.0. *E. coli* (ATCC#700891) were grown for 24 hours in Luria Bertani broth (LB) at 37°C.

2.3.1. Filter Material Comparison—To determine the filter material performance, low concentration (~1×10^6 cells ml^{-1}) *E. coli* experiments were conducted with each material to examine retention efficiency while simulating oligotrophic conditions. Cell concentrations were determined by Colilert™ (IDEXX). The low concentration *E. coli* experiments were run for 24 hours with cell concentrations determined at approximately 4 time points.

2.3.2. Variable Groundwater Conditions—Filtering conditions were varied for the aluminum-coated glass wool when using low concentrations of *E. coli* to examine retention under potential field conditions. This included the regular AGW as a control, AGW at pH 6, AGW at pH 8, a short 3 cm filter, a long 10cm filter, a low flow rate filter (0.9 ml min^{-1}), and a high flow rate filter (10 ml min^{-1}). The control filter, AGW at pH6 and AGW at pH 8 were extracted for PLFA analysis to compare the quantity of total and individual PLFAs retained in the filter (see section 1.9).

2.3.3. Sorption Capacity Testing—After choosing the aluminum-coated glass wool as a filter material higher concentrations of microspheres and *E. coli* were utilized to determine the retention capacity of the material. Two different size microspheres were utilized, i.e., 0.1

μm (F8803) and 1.0 μm (F8823) yellow-green fluorescent (505/515 nm) carboxylate-modifed FluoSpheres® microspheres (Invitrogen, Carlsbad CA). Microsphere concentrations were determined with an AquaFluor™ Handheld Fluorometer (Turner Designs, Sunnyvale, CA). For high concentration *E. coli* experiments, cell concentrations were determined by absorbance at 620nm on a UV1601 (Shimadzu, Columbia, MD) in a custom made flow through cell. High concentration experiments were run until complete breakthrough occurred or that filter ripening (the filter was becoming more efficient with clogging over time) was observed.

2.3.4. Analyses—For the laboratory testing, the influent and effluent concentration of microspheres and cells were monitored. For statistical purposes, a non-detect was counted as one-half the lowest detection limit. Concentrations were reported as normalized concentrations (C/C_o or effluent/influent). It is possible to determine both the absolute and fraction of cells retained and eluted from the column. Since all filter materials worked well with greater than 90% of the influent cells retained, presenting results as cells retained or fraction retained would mask differences (e.g 99% versus 99.9%), therefore initial results are presented as fraction eluted, average C/C_o. The amount of microspheres or *E. coli* captured on the lab filters were used to predict the amount that could be captured on a field filter. This conversion was done based solely on the volume of the two filters and the field filter contained 39.7 times as much filter material and it was assumed it's filter capacity was 39.7 times that of the laboratory filters.

2.4. Field Filter Testing

The aluminum-coated glass wool filters were tested on two boreholes located in the Beatrix Gold Mine (Gold Fields, Ltd.) in South Africa. The mine is located approximately 240 km southwest of Johannesburg near the southwestern margin of the Witwatersrand Basin and consists of four shafts with operating levels ranging from 600 to 2,155 meters below the surface. The two boreholes, BH1 and BH2, are located on level 26 of shaft #3, 1.3 km below the surface in the Witwatersrand Supergroup quartzite (Borgonie et al., 2011). Sterile methods were utilized for assembling the apparatus and sampling the boreholes. After sampling, the filter material was then transferred into sterile Whirlpaks and frozen at −80°C. The control filter material was white, the same color as the starting material, whereas the filter material from the filters that had seen borehole water was black at the inlet end to very light grey at the outlet end due to the Fe sulfides present in the borehole water. This color variation suggests that the filter material had not been fully saturated after thousands of liters of water had passed through them. The filter material remained frozen until extracted.

2.4.1. PLFA Extractions—Filter samples were extracted twice using the modified Bligh and Dyer process (White et al., 1979). The phospholipid fatty acid (PLFA) fraction was separated from the neutral and glyco-lipids by silica gel chromatography and was subsequently reacted to fatty acid methyl esters (FAMEs) via milk alkaline methanolysis (Guckert et al., 1985). An Agilent 6890 gas chromatograph coupled to an Agilent 5973 quadrapole mass spectrometer outfitted with an HP-88 MS column was used for separation, identification and quantification of FAMEs. FAMEs were identified and quantified using commercially available calibration standards (Supelco, St Louis, MO), mass-fragmentation

patterns, and retention times. The cellular biomass retained on the filter was calculated from the quantified FAMEs (Green and Scow, 2000) and subsequently used to predict sample volumes required in the field assuming the field samples have a similar FAME distribution to *E. coli*. This calculation then accounts for filtering and extraction efficiency when scaling to field samples.

2.5. Lab Methods

BH1 and BH2 total cell count samples were collected in 5mL cryogenic vials, fixed with formalin (final concentration, 1%), filtered onto 13mm 0.02μm-pore-size Anodisc (Whatman) filters, and stained with 2.5X SYBR gold (Invitrogen). Bacteria were visualized by epifluorescence microscopy at 1,000x and counted using the iVision software (BioVision Technologies). Average bacterial counts were calculated based on two replicate filters per sample. Filter material was examined by scanning electron microscopy (SEM) to better understand microbe-filter interactions (Dong et al., 2003). Powder X-ray diffraction (XRD) patterns were collected using a Scintag XDS-2000 diffractometer and mineral phases were determined using the Jade 7 program.

3. RESULTS

3.1. Filter Material Comparison

The goal of the initial testing was to screen materials for their ability to filter cells and potential usage for compound specific radiocarbon analysis (CSRA) of cellular biomass. In the uncoated sand, the fraction eluted was 0.13 and decreased to 4.9×10^{-4}, 3.6×10^{-3}, and 1.5×10^{-4} for the ferrihydrite coated sand, the goethite coated sand, and the aluminum-coated sand, respectively (Figure 1A). In the uncoated glass wool the fraction eluted was 1.4×10^{-2} and decreased to 8.7×10^{-4} and 3.0×10^{-5} for the ferrihydrite coated glass wool and the aluminum-coated glass wool, respectively (Figure 1A). The uncoated glass wool retained more cells than the uncoated sand but a significant portion of cells were not captured. Importantly, the metal oxide coatings greatly improved retention on the filter material (reduced the amount of cells eluted from the column) over glass wool or sand alone (Figure 1A). The aluminum-coated glass wool had the lowest amount of cells eluted and the most retained among the examined filter materials. For the aluminum-coated glass wool filters, only one of the four effluent samples had a detectable level of *E. coli* in the effluent. This indicated almost complete retention relative to the resolution of the analysis. These results led to the aluminum-coated glass wool being chosen as the material for further testing.

3.2. Aluminum Glass Wool Laboratory Testing

3.2.1. Variable Groundwater Conditions—The aluminum-coated glass wool filters were further tested under oligotrophic *E. coli* cell concentrations to mimic a variety of field conditions. The filters were the regular AGW as a control, AGW at pH 6, AGW at pH 8, a short 3 cm filter, a long 10cm filter, a low flow rate filter (1.2 mL min^{-1}), and a high flow rate filter (9.8mL min^{-1}) (Figure 1B). Few cells were eluted from the aluminum-coated glass wool filters and 54% of the samples were below the detection limit of 0.01 cells mL^{-1}. The fraction of cells eluted from the aluminum-coated glass wool control filter was 5.2×10^{-8} and increased to 1.9×10^{-7} and 1.6×10^{-7} for the pH 6 and pH 8 filters, respectively. The fraction

of *E. coli* cells eluted for the short core increased to 2.3×10^{-5} and for the long core to 4.3×10^{-7}, the high flow rate core increased to 7.1×10^{-7} and the low flow rate to 4.5×10^{-7}. The short, 3 cm filter, eluted the most *E. coli* and indicated that a shorter filter is not sufficient for capturing enough biomass and saturation capacity of the aluminum-coated glass wool may have been reached (Figure 1B). The long 10cm filter did not improve retention, as it was more difficult to pack and pressure also increased within the column causing leaks and potentially preferential flow paths. Further testing could be conducted to optimize filter length. However, no matter the condition, the aluminum-coated glass wool filter removed a significant amount of the cells.

3.2.2. PLFA Extractions—Three filters (experiments from 3.2.1) were extracted and analyzed for PLFAs; the aluminum-coated glass wool control, the pH 6 and the pH 8 filter, along with aluminum-coated glass wool not used in experiments as a control. Aluminum-coated glass wool that was baked but not used in column experiments was extracted and contained no detectable carbon. The amount of *E. coli* cells retained on the filters, based on the influent and effluent concentrations were 2.3×10^{9} cells. The average sum of total PLFAs was 20±5 μg and the individual PLFAs ranged from 0.88±0.04 μg for 13:0 to 3.4±1.4 μg for 16:0 (Table 1). This data could be utilized to estimate the required volumes to filter to collect enough biomass for CSRA of total and individual PLFAs in the field. The volume estimates require utilizing the ratio of the amount each PLFA to the number of cells retained on the filter and the predicted aqueous concentration of cells. This assumes the same filtering and extraction efficiency and that the environmental samples have a distribution of PLFAs similar to that of *E. coli*. In an oligotrophic sample with a typical concentration of $\sim10^{5}$ cells mL^{-1} (ONSTOTT et al., 1999), a 20 μg sample for total PLFAs would require 23 liters and a 100 μg samples would require 120 liters. For individual PLFAs larger volumes would be required, the largest volumes were for 13:0, a 20 μg sample would require 520 liters and a 100 μg sample would require 2,600 liters. The smallest volumes were for 16:0, a 20 μg sample would require 130 liters and a 100 μg sample would require 670 liters.

3.2.3. Sorption Capacity Testing—Experiments with 0.1 and 1.0 μm microspheres and *E. coli* were performed to determine the filtering capacity of the aluminum-coated glass wool and if enough colloids could be sorbed onto the material for CSRA of cellular biomass. For the 0.1 μm microspheres, no microsphere breakthrough was observed for approximately the first 20 pore volumes, microsphere concentrations increased to injection levels by 50 pore volumes (Figure 2a, Table 2). Within the first 20 pore volumes, 2.3×10^{13} microspheres were retained in the filter material. Since the filter was run to capacity, the maximum amount of total PLFAs that could be captured on the filter can be estimated using the *E. coli* PLFA extraction data (Tables 1 and 2) . Scaling these results using the filter volume of a field-size filter would predict the capture of 1.5×10^{15} microspheres and converting to total lipid amounts from the extracted PLFAs would predict the retention of 1.3×10^{7}μg of total PLFAs (Table 2).

For the 1.0 μm microspheres, no microsphere breakthrough was observed and 1.6×10^{11} microspheres were retained in the filter material (Figure 2b, Table 2). It was observed by color change in the column that the microspheres initially spread through 50% of the column

but then stopped progressing even as more microspheres were injected. Given the stop in migration through the column and no microspheres observed in the effluent, it was hypothesized that filter ripening was occurring within the column and the experiment was stopped. Scaling these results to a field-size filter would predict the retention of at least 6.5×10^{12} microspheres and converting to total lipid amounts would predict the retention of 5.5×10^{4}μg of total PLFAs (Table 2).

The *E. coli* experiments were repeated 4 times. Results are shown for experiment 3. At 45 pore volumes concentrations started to increase to near injection values (Figure 2C, Table 2). The filter retained 4.1×10^{11} cells. The flow rate decreased over time and the effluent cells never reached injected concentrations (C/C_o=0.96) but if filter ripening was occurring it was not as obvious as with the 1.0 μm microspheres. Three of the four columns were similar with the second column retaining fewer cells; on average $2.5\pm1.5\times10^{11}$ cells were captured. Scaling the four cores to a field-size filter would predict the capture of $1.0\pm0.6\times10^{13}$ *E. coli* and converting to total PLFAs would predict the retention of $8.5\pm5\times10^{4}$ μg of total PLFAs (Table 2). The first *E. coli* filter was utilized for SEM analysis.

3.2.4. SEM and XRD Analysis—SEM analysis and XRD was performed on the first *E. coli* filter that was run to failure. Images of the filter material indicated that the *E. coli* attached to the aluminum coatings which were attached to the glass wool (Figure 3). Significantly more *E. coli* were always observed where aluminum coatings were present (Figure 3-1). For example, near the influent where only clean glass wool was placed, fewer *E. coli* attached. EDS spectra showed that the glass wool surfaces were dominated by Si when no coatings were present and Al when coatings were present. These results further indicated that the coatings were critical for retaining biomass within the filter. It also indicated that the coatings were a source of charged surface area that acted as a filter to retain bacteria. XRD analysis of the coatings indicated that they are mainly amorphous (Figure 3-3).

3.3. Aluminum-Coated Glass Wool Filter Field Testing

The aluminum-coated glass wool filter was scaled up to enable collection of samples in the field (see 2.2). The radius of the filter was increased to enable filtering of larger volumes at a higher flow rate but with the same flux (flow rate normalized by area). In addition, the filter housing was only constructed from stainless steel parts and the aluminum-coated glass wool was baked after coating and then baked again after being placed in the filter housing. No PLFAs were detected in the process control filter after it was extracted indicating that during the process of attaching and detaching the filter assembly from the manifold in the mine and processing the filter in the lab, no detectable microbial contamination occurred. The planktonic total cell concentrations were $2.6\pm0.4\times10^{4}$ and $7.9\pm0.3\times10^{3}$ cells mL^{-1}, respectively in BH1 and BH2, determined by direct total cell counts. In the flowing filters, the filtered volumes were 1,100 L and 5,956 L for BH1 and BH2, respectively. The flow rate in BH1 had decreased significantly during the 5-day period, whereas that of BH2 had not. The total PLFAs extracted were 250 μg and 450 μg for BH1 and BH2, respectively. Utilizing *E. coli* extraction data (Table 1) and assuming a similar amount of total PLFAs in the environmental samples, the amount of total PLFAs correspond to 2.7×10^{4} and 8.9×10^{3} cells

mL^{-1} for BH1 and BH2, respectively, showing close agreement between direct counts and PLFA estimates.

4. DISCUSSION and CONCLUSIONS

Multiple filter materials were tested to select the optimum filter material for collecting microbes for compound specific radiocarbon analysis (CSRA) of cellular biomass. The goal was to develop a filter that could be baked to remove organic contaminants, that would run autonomously under diverse field settings, and from which lipids could be easily extracted in the lab. Aluminum-coated glass wool was the most efficient material, retaining the most biomass in laboratory testing and met all these criteria. SEM images indicated that the aluminum coatings were critical for bacterial attachment. In experiments focused on bacterial transport, aluminum oxides have been shown to be positively charged, favorable sites for bacterial retention along with Fe(III) (hydr)oxides (Hall et al., 2005). A benefit of aluminum is that it does not have a redox couple indicating it would not preferentially support the growth of subsets of microbial populations. For example, in many subsurface environments Fe(III) reduction is common (Nealson and Saffarini, 1994). The Fe(III) (hydr)oxide based filters could potentially become an energy source and alter the community structure during sampling. In summary, after laboratory testing it appeared that the aluminum-coated glass wool filters met all the criteria and could potentially work in the field.

The aluminum-coated glass wool filter utilizes electrostatic interactions to capture biomass and not size exclusion utilized in normal flow filtration. With normal flow filtration, a size cutoff of a filter is provided and it captures all particles greater than that size. As the filter captures material, the filter clogs, pressure increases, and flow rate decreases. It is this clogging process that makes collecting sufficient biomass for CSRA on filter paper difficult. With the aluminum-coated glass wool filter, capture occurs through electrostatic interactions and is probabilistic. The filter can capture colloids until all sites are filled. If filter ripening occurs as with the 1.0 μm microspheres then all particles over time would get captured. However, this appears to only happen with the larger microspheres. Therefore, the results could become biased by only capturing certain size microbes, differentially capturing different species of bacteria, and at some point during filtration, ceasing to capture particles. The aluminum-coated glass wool captured 0.1 μm microspheres and thus could potentially also capture colloids less than 0.1μm, thus capturing colloids smaller than a traditional 0.2 μm filter (Somerville et al., 1989). Future tests will focus on more diverse types of bacteria but considering the agreement between the amount of total PLFAs extracted from field samples and total counts, this indicates that the majority of the microbial population was captured. During field sampling the aluminum-coated glass wool filter could become saturated with cells, however this is unlikely, considering the predicted sorption capacity for the field scale filter is 1.0×10^{13} *E. coli* cells, which in oligotrophic water sampled in this study with 1×10^{4} cells mL^{-1} would require 1×10^{6} liters to be filtered. Therefore, the aluminum-coated glass wool filters have ample filter capacity and should not erroneously sample subpopulations.

CSRA is continually improving with minimum sample sizes decreasing. However, larger sample sizes will decrease errors and are always sought. The goal of this work was not to address the issues with analysis of small sample sizes (Santos et al., 2010), but to develop a filter that can collect sufficient biomass for CSRA. Each individual project will need to address the issue of sample size. Two sample sizes were chosen for illustrative purposes, 20 μg which represented a small sample and 100 μg which represented a larger sample with smaller errors (Table 2). For both the microspheres and *E. coli*, the aluminum-coated glass wool filter had ample filter capacity to capture significantly more biomass then required for CSRA (Table 2). In addition, the calculations already incorporated any inefficiencies in the method since the calculations were based on the extraction of *E. coli* captured on the filter. Even with the least abundant PLFA, 12:0, the filter capacity of the glass wool was large enough that sample sizes greater than 1,000 μg should be achievable (Table 2). The limiting factor with the glass wool filters becomes collection of enough cellular biomass from oligotrophic waters and not the filter capacity. However, this limitation can be overcome by longer durations of pumping and higher flow rates with larger diameter filters. The diameter of the filter becomes limiting given the amount of filter material and extraction requirements. The 4.1cm ID filter was chosen as a compromise between flow rates and amount of filter material.

The filter was field tested in the Beatrix Gold Mine of South Africa. The filter was plumbed into a manifold on a flowing borehole and then left unattended for 5 days during filtering. The process control filter that was brought to the mine and through which no flow occurred had no detectable carbon. The flowing filters were extracted and enough biomass was extracted for CSRA of total PLFAs. This indicates that even under difficult test conditions, the filter met all of the requirements. In summary, we have developed an aluminum-coated glass wool filter that is easy to make and can be baked to remove organic carbon contaminants. The filter can be easily plumbed into most sampling equipment and can be left to run autonomously. The filter material is easily extracted and was designed for CSRA but is also applicable for analysis of PLFA distributions, stable isotopes of cellular biomass, and potentially collection for nucleic acids. This filter should greatly expand the number of samples and locations available for CSRA of cellular biomass.

Acknowledgments

We are grateful to Karl Rose and Khathide Sokhela of Beatrix Gold Mine for arranging the logistical support for the two mine trips and to Prof. Esta vanHeerden for the use of her laboratory at the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, J. Kirby, J. Cheung, R. Silvern, and the Environmental Measurements Class at Barnard College for laboratory support, A. van Geen and B. Bostick for technical support, and M. Borchardt for technical advice. The travel costs to and from Beatrix Gold Mine in South Africa were supported by a grant from the Sloan Foundation to TCO administered by the Deep Carbon Observatory at the Carnegie Institute of Washington. Other field trip costs and analyses of the filter material were supported by an NSF grant EAR-0948659 to TCO from the Geobiology and Low Temperature Geochemistry Program. Laboratory work was supported by the Superfund Research Program (NIEHS Grant ES010349), NIH/Forgarty International Center Grant 5R01TW8066-2, and NSF CMMI-0928249.

References

Borgonie G, Garcia-Moyano A, Litthauer D, Bert W, Bester A, van Heerden E, Moller C, Erasmus M, Onstott TC. Nematoda from the terrestrial deep subsurface of South Africa. Nature. 2011; 474:79–82. [PubMed: 21637257]

Brady AL, Slater G, Laval B, Lim DS. Constraining carbon sources and growth rates of freshwater microbialites in Pavilion Lake using 14C analysis. Geobiology. 2009; 7:544–555. [PubMed: 19702837]

Brooks SC, Taylor DL, Jardine PM. Reactive transport of EDTA-complexed cobalt in the presence of ferrihydrite. Geochimica et Cosmochimica Acta. 1996; 60:1899–1908.

Cherrier J, Bauer JE, Druffel ERM, Coffin RB, Chanton JP. Radiocarbon in marine bacteria: Evidence for the ages of assimilated carbon. Limnology and Oceanography. 1999; 44:730–736.

Dong H, Kostka JE, Kim J. Microscopic Evidence for Microbial Dissolution of Smectite. Clays and Clay Minerals. 2003; 51:502–512.

Green CT, Scow KM. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeol. J. 2000; 8:126–141.

Guckert JB, Antworth CP, Nichols PD, White DC. Phospholipid, Ester-Linked Fatty-Acid Profiles as Reproducible Assays for Changes in Prokaryotic Community Structure of Estuarine Sediments. FEMS Microbiol. Ecol. 1985; 31:147–158.

Hall JA, Mailloux BJ, Onstott TC, Scheibe TD, Fuller ME, Dong H, DeFlaun MF. Physical versus chemical effects on bacterial and bromide transport as determined from on site sediment column pulse experiments. J. Contam. Hydrol. 2005; 76:295–314. [PubMed: 15683885]

Hill VR, Kahler AM, Jothikumar N, Johnson TB, Hahn D, Cromeans TL. Multistate Evaluation of an Ultrafiltration-Based Procedure for Simultaneous Recovery of Enteric Microbes in 100-Liter Tap Water Samples. Appl Environ Microbiol. 2007; 73:4218–4225. [PubMed: 17483281]

Knappett PSK, Layton A, McKay LD, Williams D, Mailloux BJ, Huq MR, Alam MJ, Ahmed KM, Akita Y, Serre ML, Sayler GS, van Geen A. Efficacy of Hollow-Fiber Ultrafiltration for Microbial Sampling in Groundwater. Ground Water. 2011; 49:53–65. DOI: 10.1111/j.1745-6584.2010.00712.x [PubMed: 20497484]

Lambertini E, Spencer SK, Bertz PD, Loge FJ, Kieke BA, Borchardt MA. Concentration of Enteroviruses, Adenoviruses, and Noroviruses from Drinking Water by Use of Glass Wool Filters. Appl. Environ. Microbiol. 2008; 74:2990–2996. [PubMed: 18359827]

Mills CT, Amano Y, Slater GF, Dias RF, Iwatsuki T, Mandernack KW. Microbial carbon cycling in oligotrophic regional aquifers near the Tono Uranium Mine, Japan as inferred from delta C-13 and Delta C-14 values of in situ phospholipid fatty acids and carbon sources. Geochimica et Cosmochimica Acta. 2010; 74:3785–3805.

Murphy EM, Ginn TR, Chilakapati A, Resch CT, Philips JL, Wietsma TW, Spadoni CM. The influence of physical heterogeneity on microbial degradation and distribution in porous media. Water Resour. Res. 1997; 33:1087–1103.

Nealson KH, Saffarini D. Iron and manganese in anaerobic respiration: Environmental significance, physiology, and regulation. Annual Reviews of Microbiology. 1994; 48:311–343.

Onstott, T., Phelps, TJ., Kieft, TL., Colwell, FS., Balkwill, D., Fredrickson, J., Brockman, FJ. A global perspective on the microbial abundance and activity in the deep subsurface. In: Seckbach, J., editor. Enigmatic microorganisms and life in extreme environments. Kluwer Academic Publishers; 1999.

Pearson A, McNichol AP, Benitez-Nelson BC, Hayes JM, Eglinton TI. Origins of lipid biomarkers in Santa Monica Basin surface sediment: a case study using compound-specific [Delta]14C analysis. Geochimica et Cosmochimica Acta. 2001; 65:3123–3137.

Petsch ST, Eglinton TI, Edwards KJ. 14C–Dead Living Biomass: Evidence for Microbial Assimilation of Ancient Organic Carbon During Shale Weathering. Science. 2001; 292:1127–1131. [PubMed: 11283356]

Santos GM, Southon JR, Drenzek NJ, Ziolkowski LA, Druffel E, Xu XM, Zhang DC, Trumbore S, Eglinton TI, Hughen KA. Blank Assessment for Ultra-Small Radiocarbon Samples: Chemical Extractions and Separation Versus AMS. Radiocarbon. 2010; 52:1322–1335.

Schwertmann, U., Cornell, RM. Iron oxides in the laboratory: preparation and characterization. Wiley-VCH; New York: 2000.

Slater GF, White HK, Eglinton TI, Reddy CM. Determination of Microbial Carbon Sources in Petroleum Contaminated Sediments Using Molecular 14C Analysis. Environmental Science & Technology. 2005; 39:2552–2558. [PubMed: 15884348]

Sobsey MD, Stauber CE, Casanova LM, Brown JM, Elliott MA. Point of Use Household Drinking Water Filtration: A Practical, Effective Solution for Providing Sustained Access to Safe Drinking Water in the Developing World. Environmental Science & Technology. 2008; 42:4261–4267. [PubMed: 18605542]

Somerville CC, Knight IT, Straube WL, Colwell RR. Simple, rapid method for direct isolation of nucleic acids from aquatic environments. Appl. Environ. Microbiol. 1989; 55:548–554. [PubMed: 2467621]

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, Wu D, Paulsen I, Nelson KE, Nelson W, Fouts DE, Levy S, Knap AH, Lomas MW, Nealson K, White O, Peterson J, Hoffman J, Parsons R, Baden-Tillson H, Pfannkoch C, Rogers Y-H, Smith HO. Environmental Genome Shotgun Sequencing of the Sargasso Sea. Science. 2004; 304:66–74. [PubMed: 15001713]

Vilaginès P, Sarrette B, Husson G, Vilaginès R. Glass wool for virus concentration at ambient water pH level. Water Sci. Technol. 1993; 27:299–306.

Wakeham SG, McNichol AP, Kostka JE, Pease TK. Natural-abundance radiocarbon as a tracer of assimilation of petroleum carbon by bacteria in salt marsh sediments. Geochimica et Cosmochimica Acta. 2006; 70:1761–1771.

White DC, Davis WM, Nickels JS, King JD, Bobbie RJ. Determination of the Sedimentary Microbial Biomass by Extractible Lipid Phosphate. Oecologia. 1979; 40:51–62. [PubMed: 28309603]

Highlights

- We developed a carbon free filter for radiocarbon analysis of cellular biomass.
- Made from Aluminum coated glass wool
- The filter can run autonomously to collect large samples from oligotrophic waters
- Utilized to collect samples from 1.3 km depth in a S. African goldmine
- This filter should greatly expand the collection of PLFAs for radiocarbon analysis.

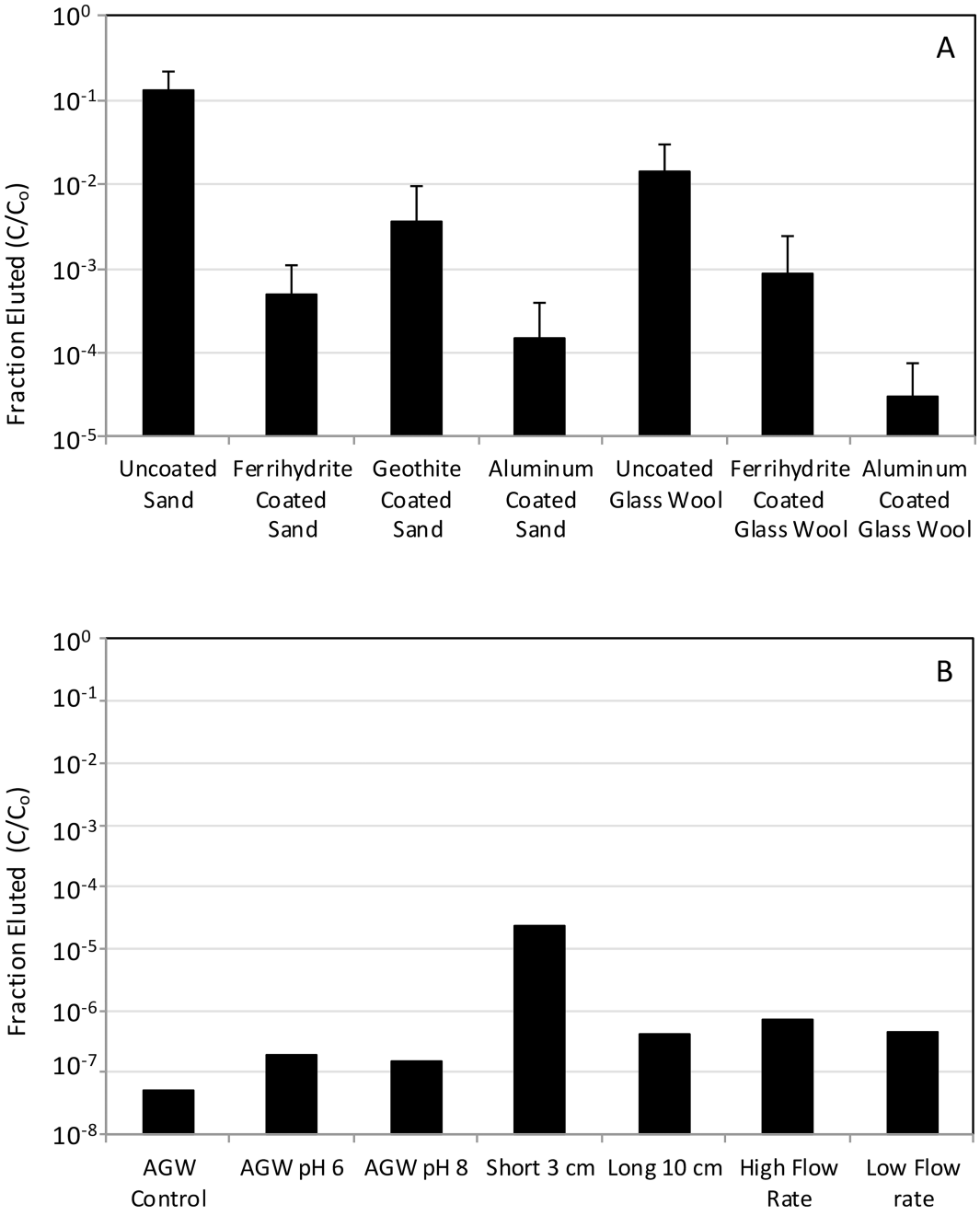

Figure 1.

A) The fraction of cells eluted (C/C_o) from laboratory low concentration *E. coli* columns. The column pore volumes were approximately 2.4 mL. The influent *E. coli* concentration was of 7.2×10^5 cells mL^{-1} and the columns were run for 29.25 hours with an average flow rate of 6 mL min^{-1}. The values represent the average of four samples and the error bars are the standard deviations. Non-detectable concentrations were taken as half the lowest detectable concentration for statistical purposes. B) The fraction of cells eluted from laboratory low concentration *E. coli* columns that mimic a range of field conditions. The average cell influent was 2.9×10^5 cells mL^{-1} and the average flow rate was 5.4 mL min^{-1}

with the high flow rate at 9.8 mL min^{-1} and the slow flow rate at 1.2 mL min^{-1}. The pH 6 and pH 8 columns were AGW with the pH adjusted with HCl and NaOH, respectively. Larger sample sizes were used in the experiments conducted in B as compared to A and thus lowered the detection limit.

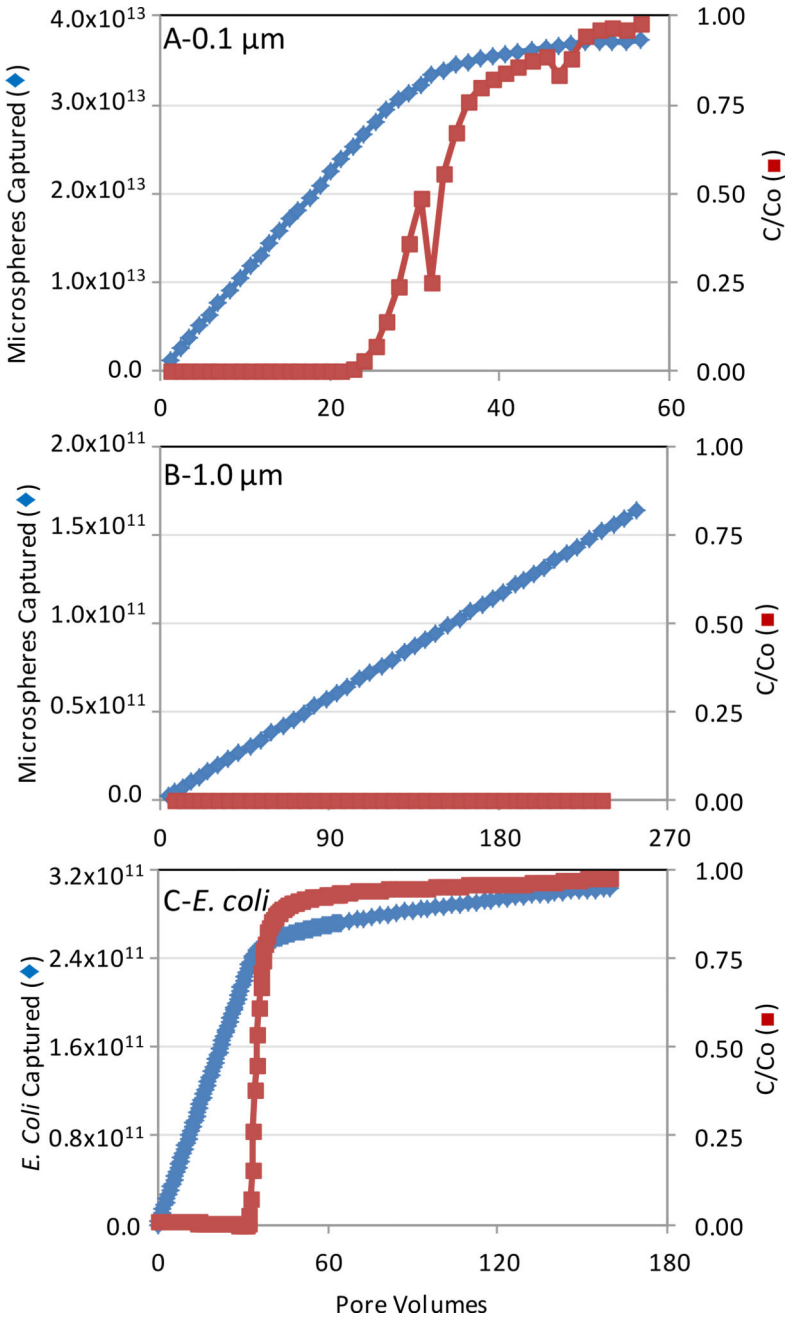

Figure 2.

Dimensionless effluent concentrations, C/C_o, (red squares ■) and the amount of microspheres or *E. coli* captured (blue triangles ◆) versus pore volumes on aluminum-coated glass wool filters during laboratory testing. A) Injection of 0.1 µm microsphere at 3.6×10^{11} microspheres mL^{-1}, B) Injection of 1.0µm microsphere at 2.7×10^{8} microspheres mL^{-1}, and C) Injection of *E. coli* at 3.04×10^{9} cells mL^{-1}(Experiment 3). These filter experiments were utilized to predict the amount of lipids retained in field filters (Table 2)

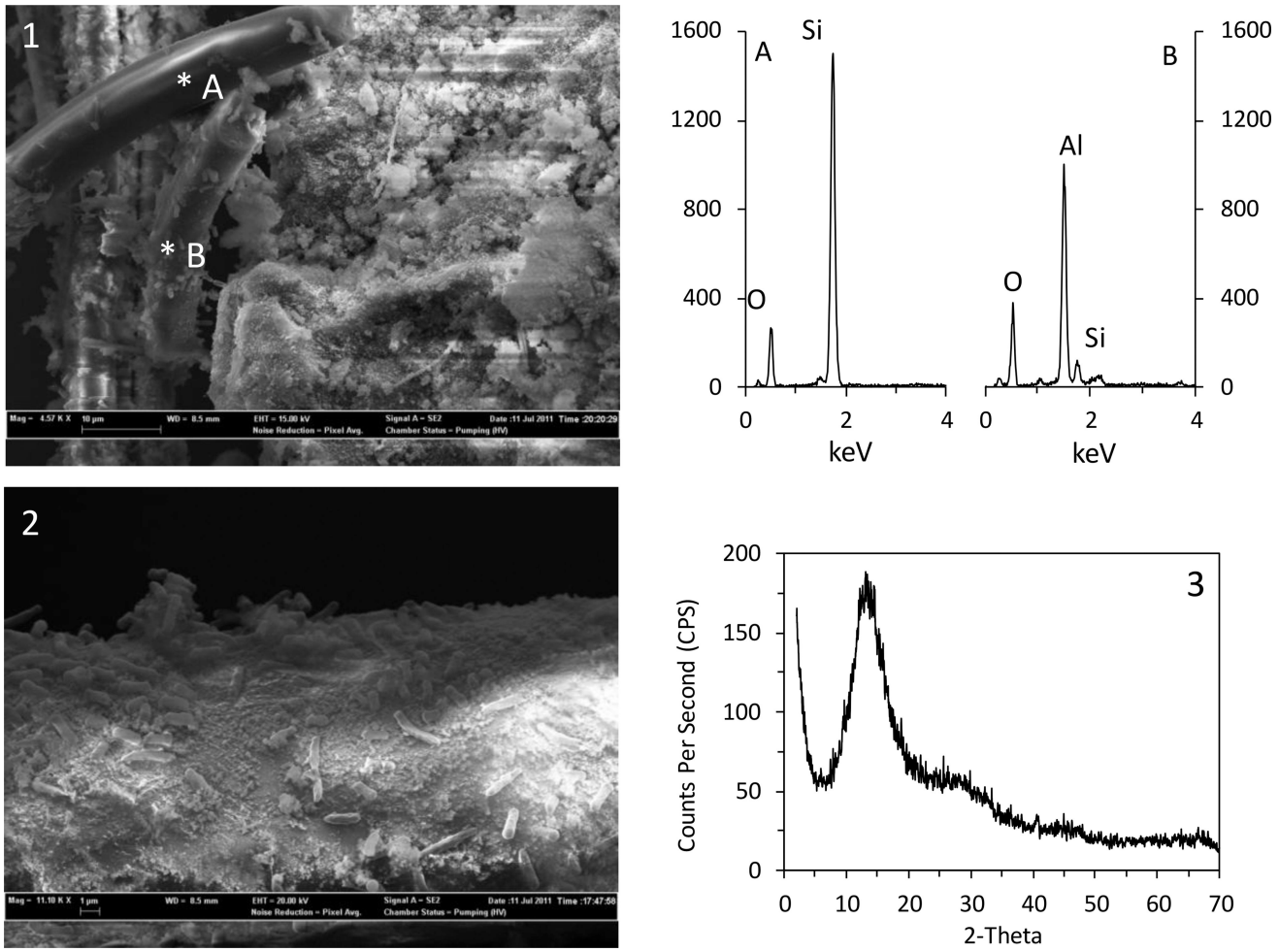

Figure 3.

SEM images of aluminum-coated glass wool from the high concentration *E. coli* experiment. 1) Image of an uncoated (A) and coated (B) glass wool surfaces and the matching EDS spectra. Few cells were observed to attach to uncoated surfaces. The uncoated glass wool (A) is dominated by Si whereas the coated glass wool (B) was dominated Al. 2) The image shows *E. coli* in direct contact with aluminum that was coating the glass wool surface. This was observed to occur throughout the filter. 3) XRD of aluminum-coated glass wool from the high concentration *E. coli* experiment. The XRD pattern shows that there was amorphous material in the sample. The XRD pattern showed a broad halo peak indicating that the structure of the Al-coated glass wool was amorphous. The glass wool was transparent in the XRD due to glass having an unordered structure.

Table 1

Average amount of individual and total PLFAs extracted from the three laboratory filters (Control, pH 6, and pH 8). The filters retained 2.3×10^9 cells. The quantities of each PLFA were then used to estimate filtering requirements from an Oligotrophic water source with 1×10^5 cells mL^{-1}.

PLFA	Average Amount of PLFA extracted from Filter (μg)	Ratio of PLFA to cells (μg $cell^{-1}$)	Volume for 20μg in Oligotrophic water (Liters)	Volume for 100 μg in Oligotrophic water (Liters)
12:0	0.90±0.08	3.9×10^{-10}	510	2600
13:0	0.88±0.04	3.8×10^{-10}	520	2600
14:0	0.92±0.07	4.0×10^{-10}	500	2500
16:0	3.4±1.4	1.5×10^{-10}	130	670
16:1	2.9±1.1	1.3×10^{-10}	160	800
cyc 17:0	2.2±0.6	9.7×10^{-10}	210	1000
18:0	1.6±0.1	7.0×10^{-10}	280	1400
trans 18:1	1.6±0.1	6.9×10^{-10}	290	1400
cis 18:1	3.1±1.7	1.4×10^{-10}	150	740
cycl 19	1.6±0.2	7.1×10^{-10}	280	1400
TOTAL	20±5	8.5×10^{-10}	23	120

Table 2

Colloids captured during laboratory experiments, predicted amount of colloids captured on a field filter, predicted amount of 12:0 PLFA extracted from a field filter, and predicted amount of total PLFAs extracted from a field filter. The 1.0 μm microspheres concentrations are underestimates as complete breakthrough was never observed in the laboratory column. The predicted amounts were scaled by comparing the radius of the laboratory and field based columns and assuming identical fluxes and capture efficiencies. The *E. coli* high concentration experiment is the average of four columns. The amount of 12:0 PLFAs and total PLFAs are based on the computed μg cell^{-1} from the filter extracts and assuming the same amounts in a field sample (Table 1). The 12:0 PLFA was chosen as an example as this was the least abundant PLFA from the laboratory testing. These were the maximum amounts, assuming that a filter was pumped until clogging, which is probably unrealistic in the field but indicated that the filter has enough filtering capacity for isotopic analyses.

Column Experiment	Colloids retained in Laboratory Filter	Predicted Colloids Retained in Field Filter	Predicted 12:0 PLFA amount (μg)	Predicted Total PLFA Amount (μg)
0.1 μm microspheres	3.7×10^{13}	1.5×10^{15}	5.8×10^{5}	1.3×10^{7}
1.0 μm microspheres	1.6×10^{11}	6.5×10^{12}	2.5×10^{3}	5.5×10^{4}
E. coli high concentration	$2.5\pm1.5\times10^{11}$	$1.0\pm0.6\times10^{13}$	$3.9\pm2.3\times10^{3}$	$8.5\pm5.0\times10^{4}$