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BIOFILTERS FOR URBAN RUNOFF POLLUTANTS

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Abstract

Biofilms were produced on three media with different physical and chemical characteristics to determine how media characteristics and the microbial community influence the properties of the biofilm and its effect on remediating runoff pollutants. Water, sediment and rocks from an urban stream were used as the inoculum to provide a source of microbes acclimated to runoff pollutants. The chemical make-up of the extracellular polymeric substances (EPS) produced by the microbial community affects electrostatic and hydrophobic interactions with the substrata as well as molecules in the surrounding environment. Thus the biofilm proteins and carbohydrates that make up the EPS and their hydrophobicities were assayed to determine their potential affects on remediating runoff pollutants. Based on these studies it appears that the medium affects the type of community that is attracted. The presence of degraders in this community seems to be the determining factor in contaminant degradation. The microbial community affects the EPS that is produced and it appears that the presence of EPS or the media itself may also affect degradation rate. The rate of degradation may also be affected by other factors such as diffusion of contaminant through the EPS, abiotic reactions or physiological state of the microbes. Further studies are needed to investigate these factors.

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Chapter 1. Introduction

PROBLEM

The Ala Wai watershed has an area of 42.4 km² (or 16.3 mi²) and is located on the south side of Oahu, Hawaii. Dense residential areas (about 55% of the land area) fill the backs of the valleys down to the ocean. Interspersed are many small businesses, schools and recreation areas. The heavily utilized H-1 freeway runs through the middle with a multitude of land arteries connecting to and from it. Daily vehicular traffic far exceeds the resident population of the watershed. From the many districts of Honolulu, runoff water is funneled via 66 major and minor outfalls directly into streams that empty into a two-mile water way called the Ala Wai canal (Lum 1992). The Ala Wai discharges into a harbor located between two popular beach destinations, Ala Moana and Waikiki. While impervious surfaces facilitate transportation and efficiently convey storm water to the canal, they have significantly altered natural ecosystems.

Large volumes of water carrying high concentrations of deposited contaminants rapidly flow toward receiving waters. Runoff from the Ala Wai watershed consists of petroleum hydrocarbons and metals from vehicular traffic and wear of asphalt. The runoff also contains high sediment loads from the forests, construction sites and homes. Nutrients from gardening and agricultural activities and animal waste are also pollutants that wash off from the roads during wet weather. Residents, county governments and golf course workers use herbicides to control weeds in yards, public parks and golf courses near roadways. Because of this unregulated use, pesticides from urban areas can occur in high concentration in water bodies especially after rain events due to impervious surfaces that lead water directly to streams or the ocean (Revitt et al. 2002, Blanchoud et

al. 2004).

There are many examples of water bodies polluted by runoff that have caused noticeable changes to the ecology and health of the wildlife (Ellis et al. 1997, Van Metre et al. 2000, Foster et al. 2000). The Ala Wai is considered an impaired water body under the Clean Water Act for several pollutants including nitrogen, phosphorous, sediment, turbidity, metals, pathogens, and pesticides (HIDOH, 2002). The sedimentation rate in the Ala Wai canal is approximately 7,000 to 8,000 m³/yr. Organic carbon enters the canal at a rate of about 14 g C/m²d and particulate nitrogen input is 0.8 g N/m²d. Seventy percent of the carbon is respired while 18% is sedimented; the remaining 12% exits the canal into the harbor (Laws et al. 1993). In an early nineties water quality study of the canal, inorganic ammonia, nitrate and phosphate concentrations increased after rain events to levels well above nutrient limitation (Laws et al 1994).

Heavy metals of concern to the environment and human health are also a problem. Roadside sediments were determined to have total concentrations of Pb, Sb, and Zn well above background levels (Sutherland and Tolosa, 2000), and increased levels of As, Cd, Pb, Zn, and Cu in streams after rain events were determined to be of anthropogenic origin (DeCarlo et al., 2002). Despite the canal's classification as a recreational waterway, signs posted by the Hawaii Department of Health advise against the consumption of fish from the canal due to lead, DDE, dieldrin and endosulfan.

The canal was built in 1927 as the major drainage basin in what used to be swamplands of Waikiki and Ala Moana. Today, along the two-mile man-made stretch of water are several canoe and kayaking clubs, a golf course, numerous apartment buildings and hotels, the convention center, two public parks, boat harbors and a popular surf break

at the mouth. The canal has provided immeasurable economic benefit over the decades and has been an important landmark for residents. Not only is it necessary to maintain its function as a drainage basin, but as a safe recreational body of water and aesthetic enhancement of the city.

Since the waters of the canal and the beaches serve many thousands of recreationers and is eventually released into the ocean, clean up of the contributing waters has been a concern for the city and state governments of Honolulu. The Hawaii Coastal Non-point Pollution Control Program recommends specific management measures for urban areas in Hawaii. One such measure involves “modifying existing surface water runoff management structures, where possible, to address water quality”. The Storm Water Quality Branch of the City and County of Honolulu is assessing the effectiveness of storm drain filters as a management measure. These filters, which contain an adsorbent material, are able to trap petroleum hydrocarbons in addition to debris that enters the storm drain system.

Traps that fit into storm drains are available on the market. Their purpose is to trap large debris and to collect sediment from road runoff and prevent them from entering water bodies. Several beachside communities have already had success with installing traps into their storm drains however filter bedding must be changed periodically in order to maintain optimal performance of the filters. Currently, the ability to biodegrade dissolved molecules or organic contaminants has not been incorporated into this technology. This capability for the traps would decrease the need to replace the filter material and reduce the amount of hazardous waste requiring disposal.

In this project, three different filter bedding material will be tested for their ability to support a biofilm for the capture of particles, uptake of nutrients and bioremediation of a polycyclic aromatic hydrocarbon (PAH), a common pollutant of oil combustion. Biofilms in streambeds have been shown to remove nutrient and organic pollutants from the water column (Gantzer et al. 1991; Carey et al. 1984). This process or ability has been attributed to the adhesiveness of EPS (Passow 2002), hydrophobic (Fang and Zheng 2004) and ion exchange (Freeman et al. 1995) properties of EPS, and heightened activity of a dense assemblage of organisms (Wolfaardt, 2000). Previous studies have revealed the presence of diverse microbial populations within the Ala Wai canal that have the potential to biodegrade anthropogenic organic compounds. Therefore biofilters produced from these indigenous microorganisms have enormous potential to remediate urban runoff which is multi-phasic and chemically varied.

BIOFILTERS

It has been shown that biofilms are able to absorb a variety of pollutants either as dissolved ions, organic molecules or solid particles from water. For example, heavy metals in storm water runoff were removed by a biofilter. Water from Hamilton Harbor, Ontario, Canada was flowed over a column of gravel to develop a biofilter. Highway runoff was collected and flowed through the biofilter at a rate of approximately 1 L/h. Runoff samples contained metals mainly in particulate form. To determine removal efficiency of dissolved metals, a solution of Cu, Pb and Zn was prepared and flowed through the filter. Influent and effluent concentrations of heavy metals were measured. Removal of the metals, either in dissolved or suspended solid form, was greater than

90%. On analysis of the biofilm, it was found that a significant amount of the metals were in the biomass. A filter without biofilm adsorbed greater than 90% of lead and zinc however the average copper removed by clean gravel was only 45%. It was noted that over the length of the experiment the effectiveness of the biofilter did not diminish (Lau et al. 2000).

Safferman et al. (2002) focused on the removal of ethylene glycol from flowing water. Reactors consisted of 6-inch diameter piping filled with volcanic rock amended with a water holding compound and slow-release fertilizers. Ninety percent of an average of 192 mg/L influent concentration of ethylene glycol was removed. Problems encountered were: 1) heavy biofilm growth which had to be broken up manually and 2) reduced removal rates for two days after flows were stopped for 4-8 days.

In the case of road runoff, hydrocarbons and other organics are often associated with particles thus if the solids were captured by the biofilter the problem of reduced uptake efficiencies after dry periods may be prevented.

Microbes growing on a nutrient poor organic substrate can uptake nutrients, common pollutants in surface waters. Columns packed with straw were inoculated and nutrient rich solutions were percolated through. Ammonia uptake by the biofilter and degradation of the substrate (straw) was detected. The gelatinous polymer developed by the microorganisms colonized on the straw also removed suspended clay particles. Clay concentrations of 300-400 mg/L were reduced by 66-75%. Straw was found to be a good biofilter media because of its high hydraulic conductivity and low susceptibility to clogging. Additionally, its large surface area was conducive to microbial attachment allowing it to efficiently remove nutrients and suspended particles from water (Diab et al.

1993).

CFT Treatment Systems, Inc. uses a bed of modified leaf compost in a storm water filter apparatus. Leaf compost was selected based on its permeability, ion exchange properties and ability to absorb organics. According to the company it produces results more consistent than vegetative swales or detention ponds. Based on measurements made on a prototype installed in an urban area of Oregon over the course of 3 years, 81% of oil and grease, 84% of petroleum hydrocarbons, 56% nutrients, 89% of TSS and 68-93% of metals were removed from stormwater runoff. The drawback of using compost is that it will be degraded by the organisms that colonize it resulting in a deterioration of the substratum and eventual release of the material increasing chemical oxygen demand (COD) in the effluent.

Biofilters have been studied for use in a variety of applications and its absorptive capacity depends greatly on the biofilms produced by microorganisms. Although these studies have demonstrated that biofilms can efficiently uptake inorganic and organic pollutants, the variables that affect the development of the biofilm for this purpose are not well understood.

BIOFILMS

Biofilms are a collection of organisms encased within a slime produced by the resident microbes. The slime is often referred to as extracellular polymeric substances (EPS) consisting of polysaccharides, proteins, nucleic acids, lipids and other biomolecules. Table 1 is a listing of concentration ranges of EPS components. Due to its adhesive nature, a biofilm may also contain organic debris and mineral particles. The

matrix is heterogeneous with many pores acting as a strainer (Wimpenny et al. 1993).

Table 1. Components of EPS and their concentration ranges (Flemming and Wingender 2001)

EPS Component	Content
Polysaccharide	40-95%
Protein	<1-60%
Lipids	<1-40%
Nucleic Acids	<1-10%

The majority of microorganisms on Earth's surface make their home in biofilms. The highly hydrated layer provides protection from desiccation, extreme pH, temperature, or nutrient concentrations, and toxic chemicals. Close proximity of cells also promotes genetic exchange within the community. Its complex chemical nature adsorbs ions and organics from fluid making it a nutritionally favorable situation as natural environments are often deficient in most necessary nutrients. Surface associated microbial populations have metabolic rates higher than planktonic populations (Wardell 1983, Yamaguchi et al. 1997).

Microbial extracellular polymers have been widely studied because they have been implicated in pathogenic infections. They also promote corrosion of metals and overgrowth can cause fouling of pipes. However, EPS has been used for our benefit in the food, cosmetic and pharmaceutical industries as a texturizer. Biofilms are key in nutrient recycling in aquatic environments and have therefore been adopted in the wastewater industry for remediation of pollutants.

The functional groups of the extracellular polymers give biofilms its reactive properties and determine its structural stability. The overall charge of EPS is negative resulting from various carboxyl, sulfate or phosphate groups of the polysaccharide.

Aspartic acid and glutamic acid in proteins and the phosphate of nucleic acids are also anionic. These moieties give EPS a large cation exchange capacity and durability as the incorporation of multivalent cations such as Ca^{2+} , Mg^{2+} and Fe^{3+} form cross-linkages between the polymers. Yet biofilms are highly complex allowing anionic and hydrophobic interactions. Amino sugars have been detected and there are the basic amino acids: histidine, lysine and arginine. Hydrophobic amino acids are alanine, leucine, valine and phenylalanine (Decho 1990; Flemming et al. 2000). Figure 1 depicts some of the interactions that can occur between EPS molecules.

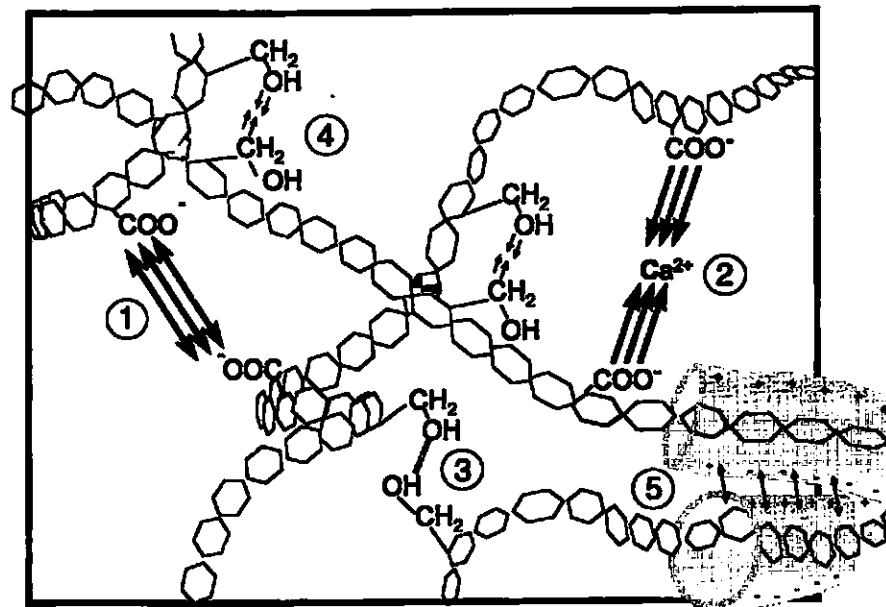


Figure 1. Interactions between EPS molecules. 1) Repulsion of two carboxylic groups; 2) attraction of two carboxylic groups by a divalent cation; 3) hydrogen bond; 4) electrostatic attraction; 5) dispersion forces (Flemming et al. 2000).

These types of interactions are also thought to play a role in biofilm formation.

To understand factors that may affect biofilm formation, the general processes of initial biofilm development onto an inert substratum should be reviewed. These steps have been

well studied and are as follows: a submerged surface will become coated by an assortment of organic colloids, inorganic particulates, molecules and ions either of environmental or microbial origin. This layer modifies interfacial interactions and is called a conditioning film. At the water-solid interface, hydrodynamic forces are important in influencing transport of the components of the conditioning film to the medium surface. Additionally, forces including Brownian motion, Lifshitz-van der Waals interactions, electrostatic forces and polar interactions affect the development at the molecular level. Transport of molecules to the surface happens rapidly in comparison to microbial cells (Busscher and van der Mei 2000).

Similar to the conditioning film, individual or aggregated cells adhere by a combination of physico-chemical forces often explained partially by the DLVO (Derjaguin, Landau, Verwey, Overbeek) theory of colloid stability. Lifshitz-van der Waals forces are generally attractive and weakly hold a cell close to the substratum, at smaller distances electrostatic interactions may be attractive or repulsive (Bos et al. 1999; Hermansson 1999). Van Oss et al. (1986) extended the DLVO explanation to include Lewis acid-base interactions that are influential at close approach of less than 5 nm. Another theory used to model microbial adhesion is based on thermodynamics where the free energy of adhesion is viewed to be in a process of equilibrium with the surface free energies of the microbial surface, substratum and surrounding liquid. Attachment is favorable when the free energy of adhesion is negative (Bos et al. 1999). However, the DLVO and thermodynamic approaches cannot always predict microbe-substrata interactions as cell surfaces are structurally and chemically extremely complex (Fletcher 1996). An organism's inherent mobility or, for certain bacteria, appendages such as

fimbriae, fibrils and holdfasts mediate adhesion. Infrequently co-adhesion arises when a sessile organism slows the approach of another suspended cell to speed its attachment. There are no covalent bonds but the additive effects of all of the interactions described above result in attraction energies exceeding those of covalent C-C bonds. Once microbes adhere, EPS is excreted and cells begin to multiply. Due to some environmental or physiological factor cells may detach or sections of the biofilm slough off (Busscher and van der Mei 2000; Rosenberg and Kjelleberg 1986). See figure 2 for a diagram of these processes.

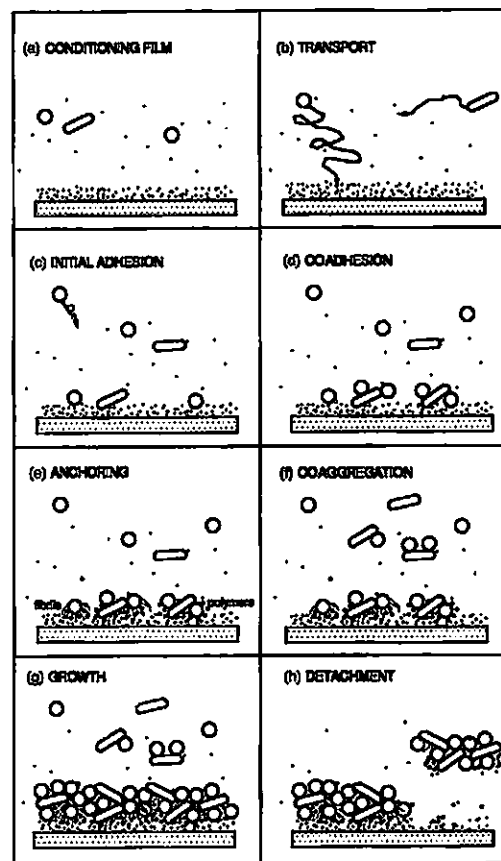


Figure 2. Steps in biofilm formation (Busscher and van der Mei 2000).

Substrata characteristics are thought to influence the numbers and types of

bacteria that attach and subsequent biofilm development. In a study by Picanco et al., the influence of material porosity on microbial attachment was tested using 4 different media, polyurethane foam, PVC, refractory brick and a high porosity ceramic in an up-flow fixed-bed anaerobic reactor. It was shown that polyurethane foam and the high porosity ceramic supported more biomass because of increased porosity. However, the different supports provided specialized conditions for the attachment of distinct types of microbes (2001). Atomic force microscopy was used to measure the adhesion forces between bacteria and glass or polystyrene. An attractive force was observed in 40% of the measurements for glass and 50% for polystyrene (Yongsunthon and Lower, 2006). Rijnaarts et al. also performed adhesion experiments on hydrophilic glass and hydrophobic Teflon. For seven moderately hydrophobic strains of pseudomonas and coryneform bacteria, interaction with Teflon was attractive even after applying shear force. In more hydrophilic bacterium/substratum combinations, steric interaction prevented permanent attachment (1995).

The extracellular matrix that is excreted has been observed to vary depending on species or strain, physiological state of the cell and environmental conditions. A bacterium may produce particular polymers during different stages of growth or as the result of a change in carbon source (Sutherland 2001, Rodrigues 2005, Veiga et al. 1997). Physical properties can also be altered with a different nutrient concentration. Nitrogen limitation resulted in enhanced polymer production but poor adhesion (Kjelleberg 1994). In experiments, *Vibrio proteolytica* produces an EPS containing different components depending on the surface upon which it adheres (Paul and Jeffrey 1985).

The production of EPS allows cells to absorb otherwise insoluble hydrophobic

molecules (Rodrigues et al. 2005). Removal of hydrophobic contaminants such as 2,4- and 3,4-dichlorophenol and phthalates has been attributed to biofilms in the aquatic environment (Carey et al. 1984; Fang and Zheng 2004).

OBJECTIVE

Material surfaces influences the amounts and types of microbes that they attract. Once cells adhere, the EPS produced will vary depending on the species or strain, their physiological state and environmental conditions, consequently affecting the characteristics of the biofilm. EPS have been shown to take up particles and molecules from aquatic systems. Based on these previously observed properties, it is apparent that producing biofilms on different types of media that are inoculated with a unique microbial community will result in unique biofilm characteristics and remedial properties. The objective of this project is to determine how the media characteristics and the microbial community affect the development and remedial properties of the biofilm.

The media chosen were a plastic aquarium particulate filter (AF), lava rock (LR) and a hydrocarbon absorbing material called, Smart Sponge® (SS). No previous studies of biofilms produced on the aquarium filter or the Smart Sponge® have been found in the literature despite having good potential as biofilter bedding material. Biofilters made of lava rock, on the other hand, have been studied and have demonstrated good remediation capabilities. Thus, biofilms were produced on three different media, two with relatively hydrophobic surfaces (AF and SS) and another with a highly charged surface (LR). They were compared in terms of biofilm characteristic and ability to remediate urban runoff

pollutants. This investigation will contribute to our understanding of the interactions between EPS components and environmental contaminants that mediate biofilter performance. A basic understanding of these processes will assist in finding and optimizing a lightweight and compact biofilter that can operate within stormdrain traps to mitigate the release of pollutants into natural waters.

Chapter 2. Biofilm Production and Characterization

As reviewed in Chapter 1, the structural and chemical properties of a substratum will have an effect on the types of organisms that adhere to it, and the source of microbes will influence the make up of the community, its physiological features and EPS properties. This chapter will introduce the three different media that were selected as a support for biofilm growth and the microbial inoculum source. Several methods were chosen to evaluate the characteristics of the biological matrix produced and they will also be described.

MEDIA

Because there are several mechanisms for attachment, medium characteristics will have an important effect on biofilm development. Selection of a porous material can provide spaces for microbial growth to be maintained even while water surrounding the material may be flowing rapidly. Good hydraulic conductivity is important to allow flow through the material without causing a backup. Also, since adhesion of organic molecules and microbial cells are affected by van der Waals, electrostatic and polar attractions, the surface properties of the medium will affect biofilm development. The three materials tested were: 1) aquarium particulate filter (AF), 2) lava rock (LR) and 3) hydrocarbon absorbing sponge (SS).

An aquarium particulate filter was selected because of its ability to trap small particles while maintaining good hydraulic conductivity. The network of plastic fibers has a wool-like texture. A similar filter found on the internet was said to made of

polyester (<http://www.drsfostersmith.com/product/Shop.cfm?N=2004>). Polyester has a relatively hydrophobic character that would attract hydrophobic organic compounds in the water as well as non-polar regions of microbial cells. To my knowledge, no studies have been published involving biofilms on this medium.

Lava cinder is relatively lightweight and very porous compared to other types of rock. It is composed of silica and metal oxides providing a charged surface for microbial attachment. Gravel has been used extensively in wastewater treatment facilities in trickling filters however lava cinder has an added benefit of being porous. In a comparison between lava rock, marble, granite, limestone and silica pebbles, lava rock far out performed the others with a water holding capacity of 40% and a drying time of 17.9 days (Safferman et al. 1999). Lava rocks provide a high surface area for microbes to colonize upon that could result in better biofiltering efficiency.

Smart Sponge® is a commercial hydrocarbon absorbent medium composed of a proprietary mixture of polymers that AbTech Industries uses in their storm drain filter. AbTech claims that its oleophilic and hydrophobic properties allow it to absorb more than 50% of total petroleum hydrocarbons from water when influent concentrations are 8-30 mg/L (Minton 2002). One problem with this product is that it needs to be replaced every few months as it reaches its capacity to hold hydrocarbons. If it is possible to establish a biodegrading community within the sponge, perhaps the biofilters' lifespan can be extended. No literature was found describing biofilms produced on this medium. Pictures of the three media are shown in figure 3.



Figure 3. Media used as biofilm support. Left: aquarium filter, middle: lava rock, right: Smart Sponge®.

INOCULUM

Microbial communities that have been repeatedly exposed to polluted runoff are theoretically acclimated to road deposited chemicals and able to metabolize them. In general, it is agreed that hydrocarbon-utilizing microorganisms are ubiquitous in the environment and that microbial communities near hydrocarbon sources have a high composition of degraders. Mulkins-Philips and Stewart reported that in some marine environments with a history of oil spills, 100% of the heterotrophic bacteria were hydrocarbon degraders (1974). Microbial biofilms were formed using water, rocks and sediment of a stream that runs through an urbanized area of Honolulu. The site selected for the collection of inoculum was the Manoa-Palolo Stream at the lowest part of the watershed where it runs under the busy H-1 freeway and 6-lane Kapiolani Boulevard just before the water becomes brackish. This location is exposed to the greatest variety of runoff pollutants without the influence of salt on the microbial community. Water and a small amount of sediment was scooped with a bucket at the stream bank, transferred to gallon-sized glass jars and taken to the lab to be used that same day, unless indicated

otherwise.

This water was plated on three different heterotrophic bacterial culture media (Nutrient Agar, Plate Count Agar and 1/2 Tryptic Soy Agar, all from Difco) to estimate the number of viable microorganisms. Serial dilutions of the collected water were made in phosphate buffer (3.7 mM K_2HPO_4 , 2.6 mM KH_2PO_4 and 0.4 mM $MgSO_4$) (RTDF 1999). Plates were incubated at 28°C for 60 hours. Colony counts were approximately 10^4 CFU in a milliliter of water.

By a most probable number (MPN) test, the stream water was found to have long chain and branched chain degraders. 400 ug of one hydrocarbon (pristine, tetracosane, phenanthrene, or pyrene) was added to sterilized glass test tubes. Four and a half milliliter of sterile Bushnell-Haas (BH) broth was prepared as described in the Difco Manual (Difco Laboratories 1998) and pipetted into each tube. Stream water was collected and sediment was allowed to settle in the refrigerator at 4°C overnight. Fifty milliliters from the top of settled stream water was centrifuged at 3000 rpm for 20 min. Supernatant was poured off and replaced with phosphate buffer (recipe described above). The tube was vigorously shaken for two minutes and centrifuged again. This was repeated twice more to remove any dissolved organic carbon. Cells were resuspended in 50 mL of buffer solution then serially diluted to obtain four dilutions of stream microbes: 10^0 , 10^{-1} , 10^{-2} , and 10^{-3} . One half milliliter of a dilution was transferred to each test tube. There were five replicates for each dilution and each hydrocarbon. The negative control received cells in BH broth without hydrocarbon and the positive control received cells in BH broth containing 540 ppm glucose. Only the undiluted suspension (10^0) was used in inoculating the controls. The tubes were incubated at 27° C and shaken once a day

(Monday through Friday) for 4 weeks. Turbid tubes indicated that cells were growing on the hydrocarbon substrate. All positive controls appeared turbid and all negative controls were clear. No growth was seen for either PAH (phenanthrene and pyrene). According to a table of most probable number values (Trolldenier 1995), there were 8 organisms/mL of stream water able to degrade pristane (branched chain) and 7,000 organisms/mL of stream water able to degrade tetracosane (straight chain).

Bacteria require nutrients and a carbon source for growth and since the selected materials are lacking in both, the stream water will serve not only as an inoculum, but also a source of the needed compounds for microbial establishment. Stream water was sent to the Agricultural Diagnostics Services Center at the University of Hawaii at Manoa for analysis of nutrients and carbon. Nitrate, nitrite and ammonia were measured using colorimetric methods with autoanalyzers, the total Kjeldahl nitrogen method was used for total nitrogen and EPA method 6010B was used to measure phosphorous. Average concentrations of three dry weather samples are listed in table 2.

Table 2. Nutrient concentrations of stream water.

Nutrient	Concentration (ug/mL)
Organic Carbon	823
Phosphorous	0.02
Ammonium nitrogen	0.012
Nitrite nitrogen	0.005
Nitrate nitrogen	0.29

The literature states that an optimal C: N: P molar ratio for microbial growth is around 100:10:1 (Maier et al. 2000, Chandy and Angles 2001). While the C: N: P ratio of the

stream water is far from optimal, 106,000: 35: 1, biofilms do grow on the streambed and it is not unusual as microbial attachment has been suggested to be a strategy for survival in nutrient limited conditions (Davies 2000). It should be noted that the samples submitted contained a small amount of sediment that was scooped up with the water.

METHODS

Biofilm Production

Media units were made to be approximately the same volume. An aquarium filter unit had four stacked pieces held together by monofilament fishing line with dimensions of $5 \times 5 \times 8.9 = 222.5 \text{ cm}^3$ (or $2 \times 2 \times 3.5 = 14 \text{ in}^3$). About 75 g lava rocks in a mesh bag had dimensions of $8.9 \times 5 \times 5 = 222.5 \text{ cm}^3$ (or $3.5 \times 2 \times 2 = 14 \text{ in}^3$). One whole Smart Sponge® propellet weighed about 37.5 g and had a cylindrical shape with a volume of $\pi 2.2^2 \times 7.6 = 118 \text{ cm}^3$ (or $\pi 0.875^2 \times 3 = 8.2 \text{ in}^3$) but to increase surface area it was broken up into chunks and placed into a mesh bag, dimensions were $8.9 \times 5 \times 6.3 = 286.1 \text{ cm}^3$ (or $3.5 \times 2 \times 2.5 = 17.5 \text{ in}^3$). Units were suspended in a glass aquarium tank where stream water trickled over them from a container full of stream rocks. Water flowed down the media, into the tank then pumped up back into the container with rocks. Flow to each unit was about 10 mL/s. A diagram of the biofilm developing tank can be seen in figure 4. Circulation of the water was continuous and each week a portion (~40%) of the water was removed from the tank and replaced with an amount of freshly collected stream water. In a separate tank, biofilms were produced in the same way as described above except that phenanthrene was spiked onto the media. On the day following water replacement, units were removed and water was allowed to drain off. One whole unit

was emptied into a glass dish and 16 drops of phenanthrene dissolved in acetone (1 drop = 0.02 mL) or toluene (1 drop = 0.022 mL) was pipetted. On the first through fifth weeks, the amount of spiked phenanthrene gradually increased between 0.625 to 3.125 ug in each drop of acetone. This was done in effort to prevent toxicity to the microbes colonizing the media. After that, toluene was the solvent and phenanthrene ranged from 2.86 to 3.15 ug/drop. The pieces of media were tossed to distribute the PAH and evaporate some of the solvent (although media were not allowed to dry completely) then transferred to its mesh bag and returned to the tank. For the aquarium filter, each of the four pieces received four drops of spiking solution. The addition of the PAH served two purposes, to acclimate the organisms to the hydrocarbon and to provide a source of carbon.

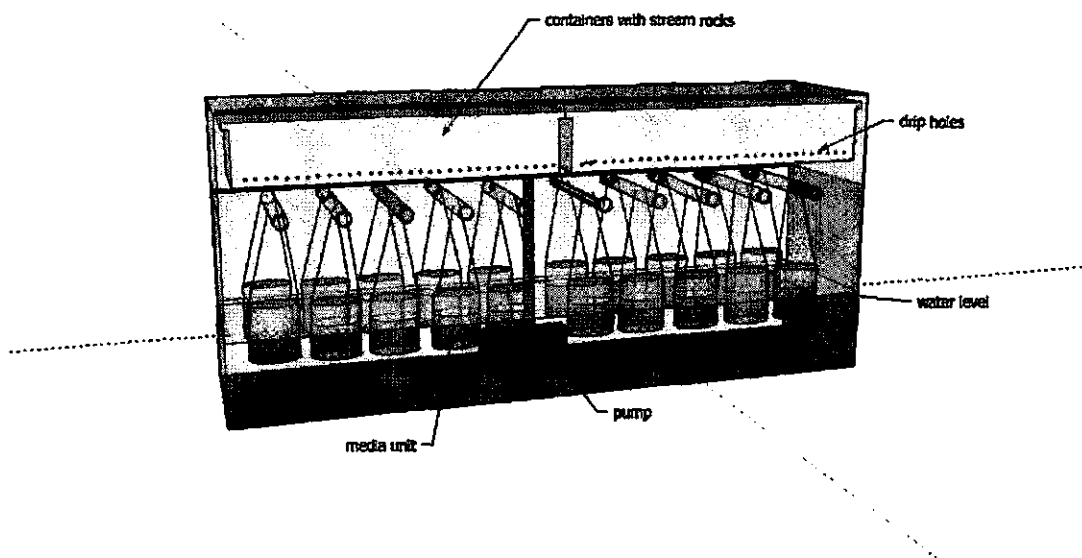


Figure 4. Schematic diagram of biofilm developing tank.

The source of carbon can affect the properties of the excreted EPS. It has been shown for instance, sorption of toluene caused an increase in uronic acids, the carboxylated form of sugars, causing an increased capacity for cations (Flemming 1995). Tanks were indoors, water temperature ranged from 24 to 30°C and pH was about 8.1.

Scanning Electron Microscopy of Biofilms

Scanning electron microscopy (SEM) allows the 3-dimensional visualization of micrometer sized surface structures. This technique was used to characterize the media surface at the scale of a microorganism as well as determine the extent of the biological coverage of the material.

Pieces of media that had been developing biofilms for 98 days were collected from the tank. Lava rock pieces about 15 mm wide and 12 mm tall were selected and Smart Sponge® and aquarium filter pieces were cut to those dimensions. Stream rocks were collected from the Manoa-Palolo stream a day before the fixation process was started and stored in the refrigerator. The samples were placed in a fixative solution of 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at 4°C for four days. Glutaraldehyde slowly penetrates tissues and reacts with protein and also to some degree with nucleic acids, carbohydrates and lipids to form permanent cross-links. Glutaraldehyde reagent was removed in three successive washes in 0.1 M sodium cacodylate for 20 min with agitation. Wash buffer was removed and a postfixation solution of 0.1% osmium tetroxide was added to the media. Osmium tetroxide reacts primarily with unsaturated bonds of fatty acids and becomes black as it is reduced to provide contrast. Containers were placed on a rotator for one hour. Following

postfixation, samples were washed with cacodylate buffer for 10 min then dehydrated in a series of ethanol solutions of increasing concentration: 30%, 50%, 70%, 85%, 95%, 100%. There were two changes in each dilution with 10 min of soaking on a rotator. Samples were placed into an envelope of optical lens tissue then soaked in 100% ethanol with three changes of fresh ethanol every 15 min. Envelopes were placed into the chamber of a Tousimis critical point dryer with a purge time of 20 min. Pieces were mounted on stubs using silver paste and allowed to set overnight in a sealed container with dessicant. Samples were sputter coated by a Hummer 6.2 Sputter System from Anatech Ltd. then viewed by a Hitachi S-4800 SEM. The Biological Electron Microscope Facility at the University of Hawaii at Manoa provided all methods and instrumentation.

Bacterial Community Characterization

After biofilms had been forming for 108 days, media samples (not amended with phenanthrene) were collected from the tank. Stream rocks (SR) were collected from the Manoa-Palolo stream one day prior to use and stored at 4°C. DNA was extracted based on a protocol described by Stewart and Via (1993). Approximately 5 mL of substrate was placed in 10 mL of Tris-EDTA (TE) or CTAB buffer and incubated at 65°C for 10 min. Ten milliliters of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the samples, mixed by inversion for 3 min, and centrifuged at 4000 g for 2 min. The upper water phase was collected and precipitated with an equal volume of 2-propanol for 5 min. This solution was centrifuged at 12,000 g for 15 min at 4°C. The supernatant was discarded and the pellet was allowed to air dry for 10 min before being re-suspended in

500 uL of sterile water. Non-soluble debris was pelleted by brief centrifugation and the supernatant was transferred to a new tube. Fifty microliters (or 0.1 volumes) of 3 M sodium acetate (pH 5.2) and 1 mL (or 2 volumes) of 95% ethanol were added to the extract and incubated at -20°C overnight. Samples were then centrifuged at 12,000 g for 10 min and the supernatant removed. Pellets were washed with 1 mL of cold 70% ethanol and centrifuged at 12,000 g for 2 min. Following removal of the supernatant, the pellet was air-dried as above and re-suspended in 50 uL of sterile water. Agarose (0.8% w/v) gel electrophoresis was used to quantify DNA recovery and indicated CTAB buffer was more effective than the TE buffer for DNA isolation (Figure 5).

DNA of the 16S rRNA gene was amplified from DNA isolated with the CTAB buffer by polymerase chain reaction (PCR) using the 27F and 1,492R primers (Lane 1991). The 50 uL PCR reactions were composed of 25 uL of 2X ImmoMix buffer (BioLine), 10 pmol of each primer, and approximately 30 ng of DNA. The PCR reactions were preheated at 95 °C for 7 min followed by 30 cycles of 95°C for 1 min, 53 °C for 1 min, 72 °C for 1.5 min, and a final incubation at 72°C for 7 min. An agarose gel of the PCR products in figure 6 that shows that the DNA was in good condition and an ample amount was obtained for each biofilm. The amplicons were ligated into the pGEM-T Easy vector (Promega) following the manufacturers instructions. The ligation reactions were used to transform *E. coli* DH5alpha cells, which underwent selection on MacConkey medium amended with 50 ppm ampicillin. Bacterial colonies containing recombinant plasmids were individually cultured overnight in LB medium amended with 50 ppm ampicillin. Plasmids were extracted using the Qiaprep Spin Miniprep kit (Qiagen, Valencia, CA) following the manufacturers instructions. Plasmid DNA was sequenced

with the T7 primer at the Center for Genomic, Proteomic and Bioinformatic Research at the University of Hawaii at Manoa. Sequences were compared with GenBank accessions using the blastn algorithm at the NCBI website (www.ncbi.nlm.nih.gov).

Lanes: 1 2 3 4 5 6 7 8 9

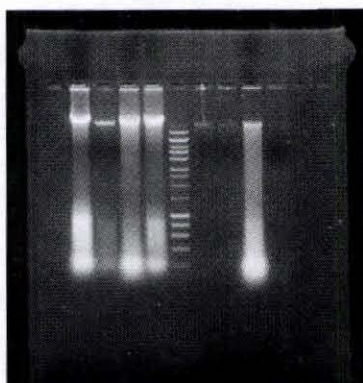


Figure 5. Agarose gel electrophoresis of CTAB and TE buffer-based extracts. Lanes in order from left to right: AF-CTAB (1), LR-CTAB (2), SR-CTAB (3), SS-CTAB (4), DNA ladder (5), AF-TE (6), LR-TE (7), SR-TE (8) and SS-TE (9).

Lanes: 1 2 3 4 5 6

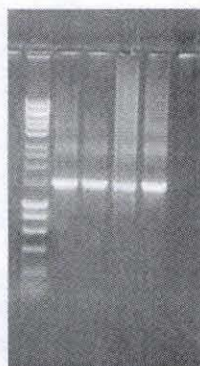


Figure 6. Agarose gel electrophoresis of PCR products from CTAB buffer-based extract. Lanes in order from left to right: DNA ladder (1), AF (2), LR (3), SR (4), SS (5) and water used to dilute samples (6).

EPS Protein and Carbohydrate Quantification

The two most common components of the extracellular polymeric matrix are

carbohydrates and proteins (Flemming and Wingender 2001, Tsuneda 2003). EPS was extracted from the media and these components were quantified to give an indication of the properties of the biofilms produced on different media.

EPS Extraction from Media: Extraction of the biofilm attached to the media was assisted by cation exchange resin (CER) modified from a procedure described by Frolund et al. (1996). This method removes cations in the biofilm such as Ca^{2+} and Mg^{2+} that form bridges between polymers to stabilize the matrix. Once these supportive bonds are broken, agitation causes the EPS to slough off. This method was chosen because it does not chemically change the polymeric substances nor does it add interfering compounds to the extract that would affect analyses of the biomolecules.

One half of a medium unit was portioned out. Loose particles were removed by gently swirling the material in 200 mL distilled water twice, then rinsed off with another 200 mL distilled water. Cation exchange resin, Dowex Marathon C, Na^+ form, 20-50 mesh (Sigma, Saint Louis, MO), was washed prior to use by placing 4 g into a 50-mL Erlenmyer flask with 25 mL phosphate buffer and swirled for one hour. The buffer was replaced with a fresh amount, and then drained off. Rinsed material was transferred to a 500-mL Erlenmyer flask along with the CER and 50 mL of 8.3 mM phosphate buffer at pH 7.2 (Sigma, Saint Louis, MO). The flasks were covered then placed on a platform rotator at 200 rpm for 14 hours at 5°C. The slurry was poured into a Teflon centrifuge tube and centrifuged for 1 min at 12,000 g at 5°C to remove the resin. Supernatant was collected and centrifuged for 15 min at 12,000 g at 5°C, this was done twice to remove particulates and bacterial cells (Frolund et al. 1996). Supernatants were collected in a

glass test tube and stored at 4°C until the EPS was analyzed, usually within 2 days.

Anthrone Carbohydrate Assay: Anthrone reagent based on methods described by Dreywood (1946) and Raunkjaer et al. (1994) was used to determine total carbohydrates in the EPS extracts. Concentrations are expressed as glucose equivalents. Anthrone reagent was prepared no more than a day before the analysis was performed; anthrone powder was dissolved in concentrated H₂SO₄ at a concentration of 0.2% then placed into the refrigerator. Standards were prepared by diluting a 100 ug/mL glucose solution in phosphate buffer to concentrations between 1 and 30 ug/mL. For the analysis, one milliliter of sample or standard was pipetted into a glass test tube followed by 1.75 mL of anthrone reagent. The mixture was mixed by vortexing followed by incubation in a 100°C water bath for 14 min. The tubes were then transferred to a cold water bath for 5 min. Absorbance was measured at 625 nm. Concentrations were calculated using a five point standard curve made using standards prepared on the day of analysis.

Modified Lowry Protein Assay: The importance of quantifying biofilm proteins is that they are exoenzymes involved in metabolizing sorbed compounds. Bacteria have also been found to excrete transporter proteins and DNA-binding protein regulators (Tremoulet et al. 2002). Additionally, proteins that maintain the structure of the biofilm may be present (Dignac 1998).

Total proteins were measured using the Modified Lowry Protein Assay Kit from Pierce. The manufacturer's procedures were followed. Bovine serum albumin standards were prepared by diluting a 2 mg/mL solution (provided) in phosphate buffer to make concentrations between 1 and 30 ug/mL. Concentrations were calculated using a five

point standard curve made using standards prepared on the day of analysis.

EPS Hydrophobicity Characterization

Proteins are the main contributor to hydrophobic properties of a biofilm. Some of the hydrophobic amino acids are alanine, leucine, glycine, valine and phenylalanine.

Hydrophobicity can assist in adhesion of the cell to a surface as well as attracting organic molecules to the biofilm. At distances of about 10 nm attractive forces including hydrophobic interactions between the cell and inert surface predominate and form a reversible attractive interaction (Jones et al. 1996)

Phthalates removal by activated sludge from a municipal wastewater treatment plant was investigated by Fang and Zheng. Each gram of activated sludge maximally adsorbed 0.73 mg of diethyl phthalate and 17.6 mg dibutyl phthalate. The hydrophobic property of the EPS was attributed to a relatively high content of protein and humic substances (2004).

Hydrophobic interaction chromatography (HIC) was developed originally for the separation of proteins (Rosenberg and Kjelleberg 1986) and has since been used for bacterial and yeast cells and lipopolysaccharides (Stenstrom 1989; Kozel 1983; Muck 1999). In this project, hydrophobic and hydrophilic exopolymers extracted from the three different media were separated using agarose beads with covalently bonded octyl groups. The hydrophobic fraction of the carbohydrates and proteins of extracted biofilm was determined to speculate about the kind of interactions the biofilm may have with contaminants.

The EPS extraction method was slightly different for HIC than is described

above: 3/8 of a media unit was extracted in a 1000-mL Erlenmyer flask. A larger volume increased mixing.

Columns were prepared by plugging 5-mL glass serological pipets with glass wool. The pipets were washed with 5 mL of phosphate buffer three times then packed with 1 mL of octyl-Sepharose CL-4B (Sigma). The packed column was washed with 3x5 mL of phosphate buffer. Columns were prepared in triplicate. Five milliliters of extract was run through the column and followed by 1 mL of phosphate buffer. Approximately 6 mL of eluant was collected. Eluants contained the hydrophilic fraction of the EPS and were analyzed for carbohydrates and proteins using assays described above.

RESULTS AND DISCUSSION

SEM of Biofilms

The strands of the aquarium filter were smooth and intertwined or fused to other strands. Biological coverage on this material was by far the most extensive with areas almost completely covered with a 2-5 μ m-thick film that had entrapped mineral debris (vermiculite or metal sheets) from the water. The matrix was spongy and microbial cells can be seen enveloped within it along with tightly attached filaments (Figures 7 and 8). Hydrophobic polymer surfaces are known to rapidly adsorb proteins from aqueous solutions (Brash 1969) allowing a speedy formation of a conditioning film followed by adhesion of microbial surfaces. After a layer of cells had attached, the production of sticky EPS likely enhanced the accumulation of more cells and debris.

The bare lava rock surface varied from smooth to cratered with rigid edges to lumpy. Lava rock surfaces seen were the least colonized of the media. Biological

material, either organisms or debris was sparse (Figures 9 and 10). Hydroxyl groups of the silica and metal oxides likely produce a highly negative charge at the surface repelling negatively charged microbial cells. According to the general steps in biofilm formation, a mass of cells must first accumulate before polysaccharides are excreted and the community is anchored onto the substratum surface. A denser assemblage of microbes may need to accumulate before a fully formed biofilm can be observed on this material.

The Smart Sponge® was the most difficult to visualize by SEM due to an incompatibility of the material with the microscopic method. From the images that were clear, some observation could be made. The uncolonized Smart Sponge® surfaces were varied from porous and smooth to irregular and rough. Filamentous organisms, cells and debris were seen mostly in pores probably for protection from the forces of the flowing water. In some areas, biological coverage is estimated to be about 3%, in others it was less (Figures 11 and 12). This hydrophobic surface seems to attract cells but sheer forces of the flowing water may be slowing the interconnection of the biomass to completely cover the medium surface.

During the fixation and drying processes, the samples were in vials submerged in reagents and rotating. This agitation may have caused damage to or slaughtering off of the biofilms. Therefore, the SEM pictures taken may underestimate the biofilm coverage. However, clearly the aquarium filter had the most extensive and stable biofilms. The aquarium filters have a wool-like structure that is capable of entrapping particles from the water giving it an inherent advantage over the other media. It also appears to have the most surface area upon which to colonize.

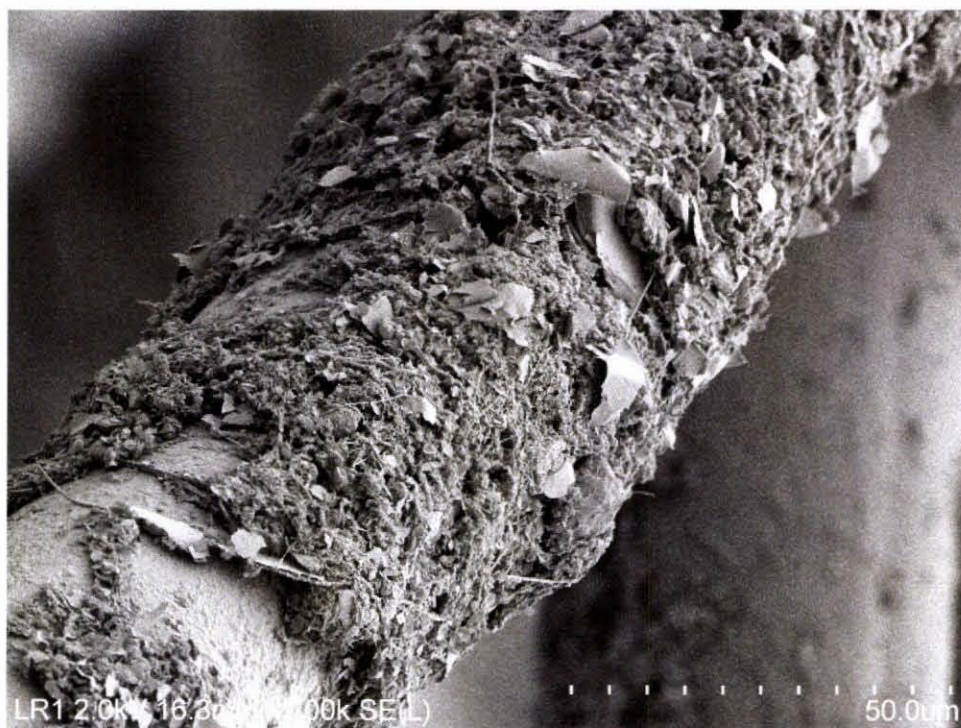


Figure 7. SEM of aquarium filter biofilms



Figure 8. SEM of aquarium filter biofilms

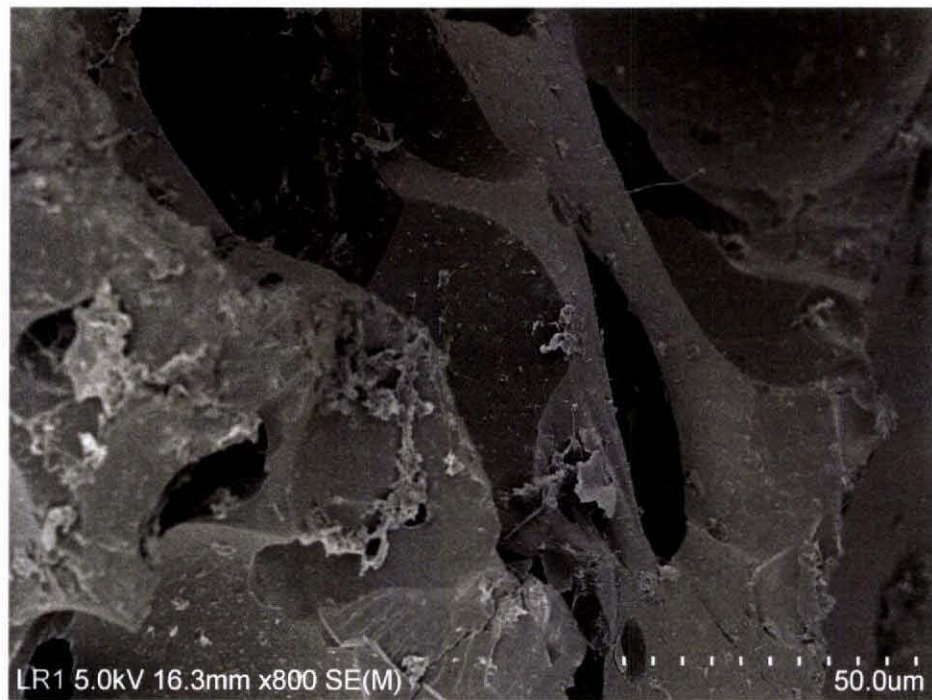


Figure 9. SEM of lava rock biofilms



Figure 10. SEM of lava rock biofilms

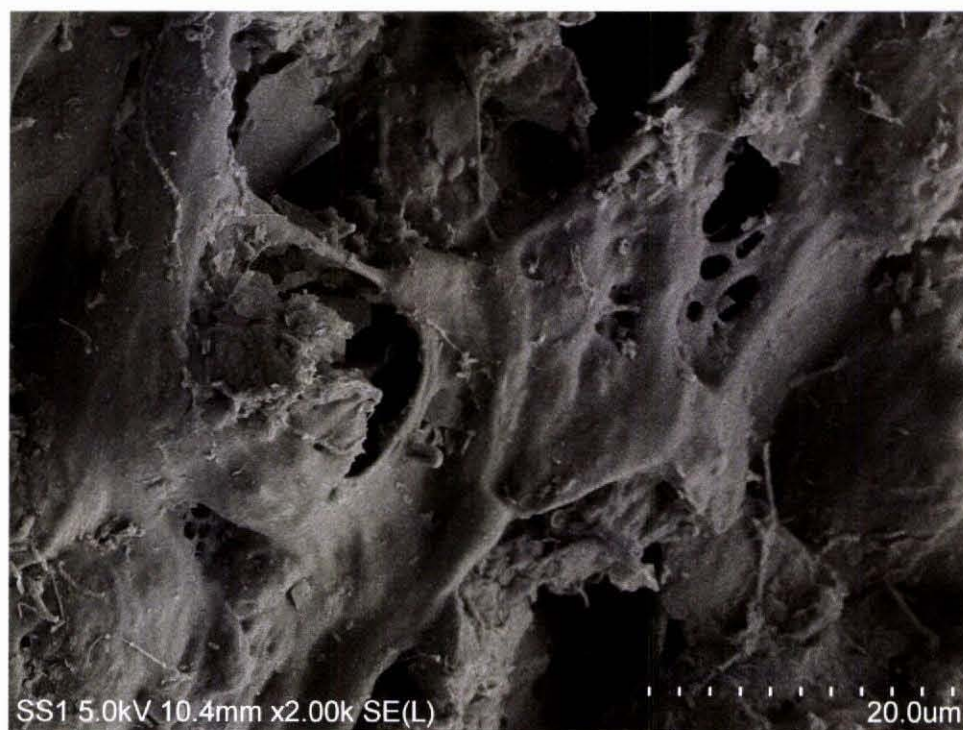


Figure 11. SEM of Smart Sponge biofilms.



Figure 12. SEM of Smart Sponge biofilms.

Bacterial Community Characterization

Conventional laboratory culture methods to characterize bacteria are extremely limited as organisms' physiology may be altered by the growth medium or its specific needs may not be able to be replicated in the lab. Molecular biology techniques give a more complete representation to complicated environmental samples. This has been shown in the biofilm extracts. Of the 36 sequences obtained from the AF biofilm, four phyla are represented (Figure 13). From the LR biofilms, 40 sequences were obtained representing six different phyla (Figures 14). Of the 40 sequences obtained from the SS biofilm, seven different phyla are represented (Figure 15). Finally, there were 41 sequences from the stream rock biofilm and six phyla are represented (Figure 16). There were very few sequences that were the same, within a biofilm and between them, indicating high bacterial diversity. Wolfaardt et al. points out two main properties of a biofilm involved in remediation: "1) spatial organization of the cells and 2) the establishment of a stable microenvironment through the production of EPS." These components will lead to a diverse community able to degrade a wide range of substrates (2000). It appears that all of these media are capable of supporting communities that provide these properties.

The photosynthetic *Cyanobacteria* were present in stream rock biofilms but not in any other because the rocks were collected from an outdoor environment where the light intensity is much greater than indoor lighting. Most sequences from all biofilms were of the *Proteobacteria* phylum, which is the largest and most phylogenetically diverse. However, bacterial communities in all of the biofilms were distinct suggesting that the media being tested influences the microbial attachment.

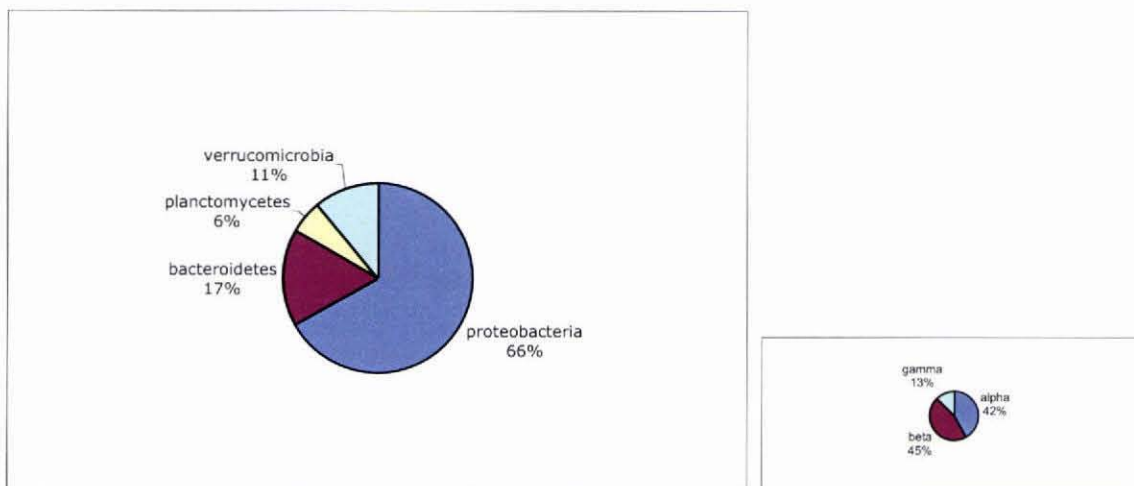


Figure 13. Phyla and proteobacteria subclass representations of sequences from aquarium filter.

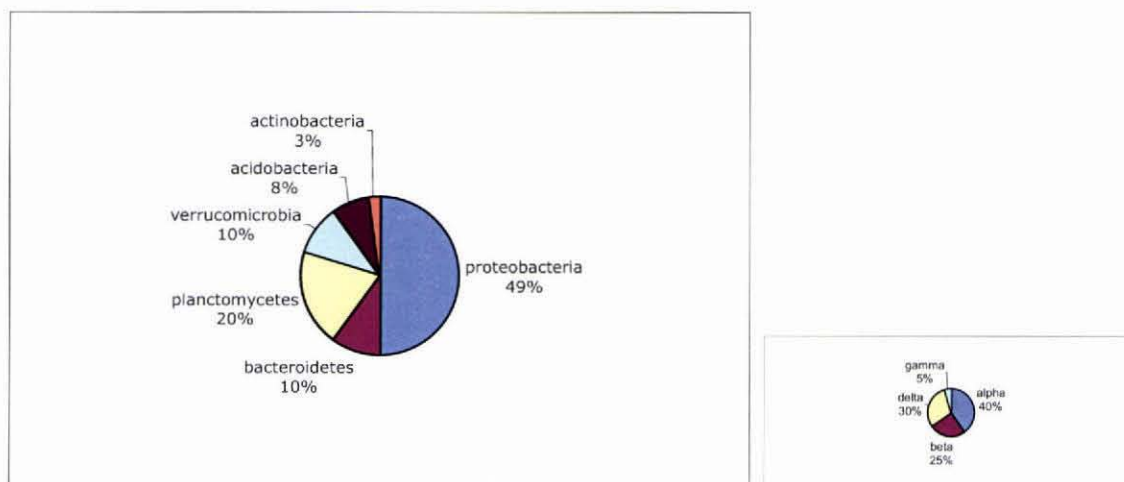


Figure 14. Phyla and proteobacteria subclass representations of sequences from lava rock.

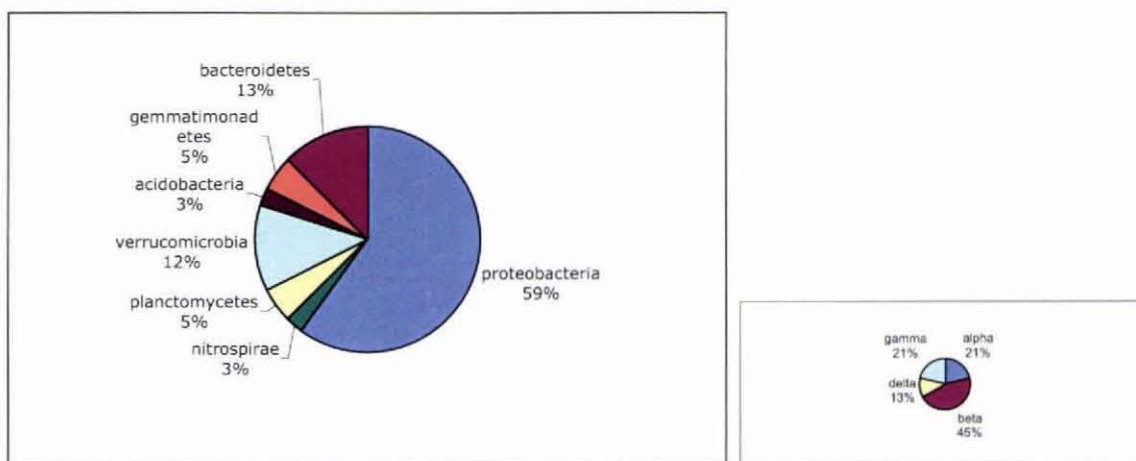


Figure 15. Phyla and proteobacteria subclass representations of sequences from Smart Sponge®.

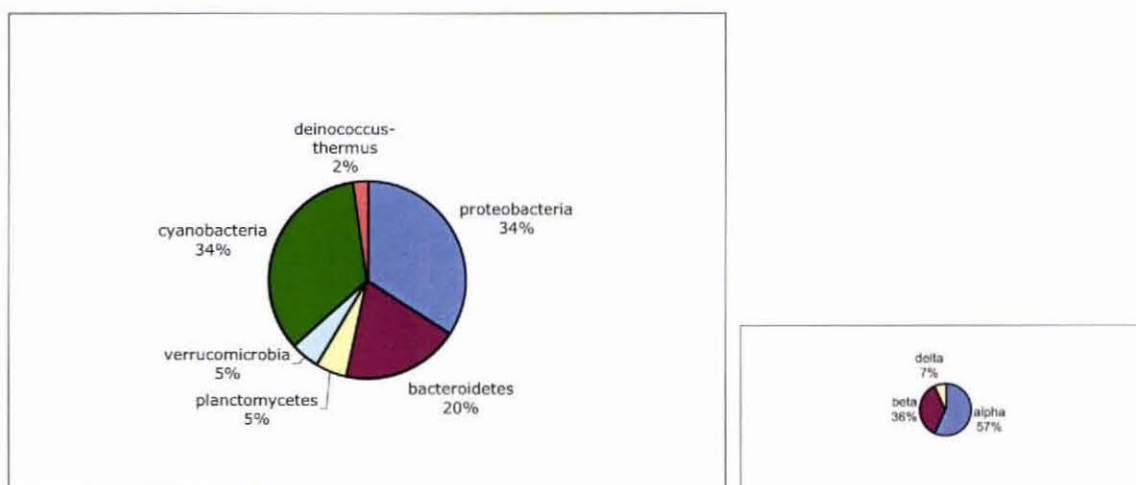


Figure 16. Phyla and proteobacteria subclass representations of sequences from stream rocks.

Sphingomonads of the *alpha*-proteobacteria sub-phyla are often isolated from contaminated environments. They have been shown to have a wide range of catabolic capabilities and are prominent in bioremediation (Leys et al. 2004). There are several clones from the biofilms with sequence similarity to strains of sphingomonad species reported to degrade environmental pollutants. Three sequences from AF were similar to

those of sphingomonad degraders. There were sequence similarities to one sphingomonad degrader and also a *Dechlormonas* species in LR and from stream rock, which served as the inoculum, there were two sequences similar to sphingomonad degraders and one to a *beta-proteobacterium* capable of metabolizing a dioxin. Table 3 lists the clones that had sequence similarity to bacteria with abilities to degrade pollutants and their purported abilities.

Table 3. Listing of biofilm clones with sequences similarity to those of reported pollutant degraders.

Clone	Sub-phyla	Activity	Accession
AF2(9/16)	<i>alpha-proteobacteria</i>	toluene, xylene, CH ₃ Br degrader	AY858797
AF2t(9/22)	<i>alpha-proteobacteria</i>	carbofuran degrader	U52146
AF7(10/21)	<i>alpha-proteobacteria</i>	chlorophenol degrader	AF367204
LR5(10/21)	<i>alpha-proteobacteria</i>	monochloroacetic acid degrader	AF532188
LR25(11/4)	<i>beta-proteobacteria</i>	(per)chlorate-reducing	AF170356
SR2t(9/22)	<i>beta-proteobacteria</i>	cis-dichloroethylene degrader	AF408397
SR8t(9/22)	<i>alpha-proteobacteria</i>	dibenzo-p-dioxin metabolizer	AB021492
SR8(10/21)	<i>alpha-proteobacteria</i>	high molecular weight PAH	AF502400

According to Liu et al., adhesion is highly facilitated if cells and substratum are hydrophobic. Adhesion onto a hydrophilic material increases with increasing cell hydrophobicity while adhesion is unfavorable when cells and substratum are hydrophilic (2004). It should therefore follow that a hydrophobic substratum would assist adhesion of hydrophilic microorganisms. The hydrophobic surface of AF assisted adhesion of many cells including a high concentration of sphingomonads, a group that has been found to be hydrophilic (Cunliffe and Kertesz 2006).

EPS Protein and Carbohydrate Quantification

Concentrations of protein and carbohydrates within the EPS were measured as these are thought to affect the attractive forces, stage of development and strength of the

biofilm.

Concentrations of the EPS from the biofilms not amended with phenanthrene are shown in figure 17 and those of the biofilms amended with phenanthrene are shown in figure 18. The most noticeable observation is the absence of carbohydrates from EPS extracts of lava rock. According to the generally accepted sequence of steps involved in initial biofilm formation, non-capsular extracellular polysaccharides are produced by microbes after attachment and to a substratum and aggregation of cells. The lava rock surface is highly charged due to its composition of silica and metal oxides thus repulsion by acidic hydroxyl groups may be the dominant interaction between cells and the surface. Proteins are known to collect on surfaces to form a conditioning film prior to bacterial adhesion and are the likely source of proteins detected in extracts of LR. Therefore, it is probable that biofilms had not yet been established on the LR surface.

Total protein and carbohydrate concentrations varied highly between replicates, which seems to be a common observation from many investigators. Multi-species biofilms are tremendously complex, therefore, “within any biofilm or floc, there will almost certainly be wide variations and any samples analyzed will inevitably reveal wide variations in composition.” Microenvironments within a biofilm may alter the physiological state of subpopulations of the same species causing differences in the composition or physical properties of excreted EPS (Sutherland, 2001).

Despite high degrees of variability, a few generalizations can be made. It is clear the proteins are a significant part of the matrix. Studies have shown that calcium and magnesium ion bridges between EPS molecules have a higher association with proteins versus polysaccharides. This contributes to the strength of the biofilm (Dignac et al.

1998).

Proteins can also contribute to stability in other indirect ways. During active polysaccharide synthesis, bacteria are also producing polysaccharide lyases but compartmentalization within the cell prevents lysis activity. Cell lysis or some other trigger can release the enzymes causing disintegration of the polysaccharide and sloughing (Laspidou and Rittman 2002). It has been hypothesized that in times of starvation, populations within the biofilm community will utilize the exopolysaccharides as a carbon source (Freeman and Lock 1995). Proteins are less likely than exopolysaccharides to be metabolized thus contributing to a more stable biofilm.

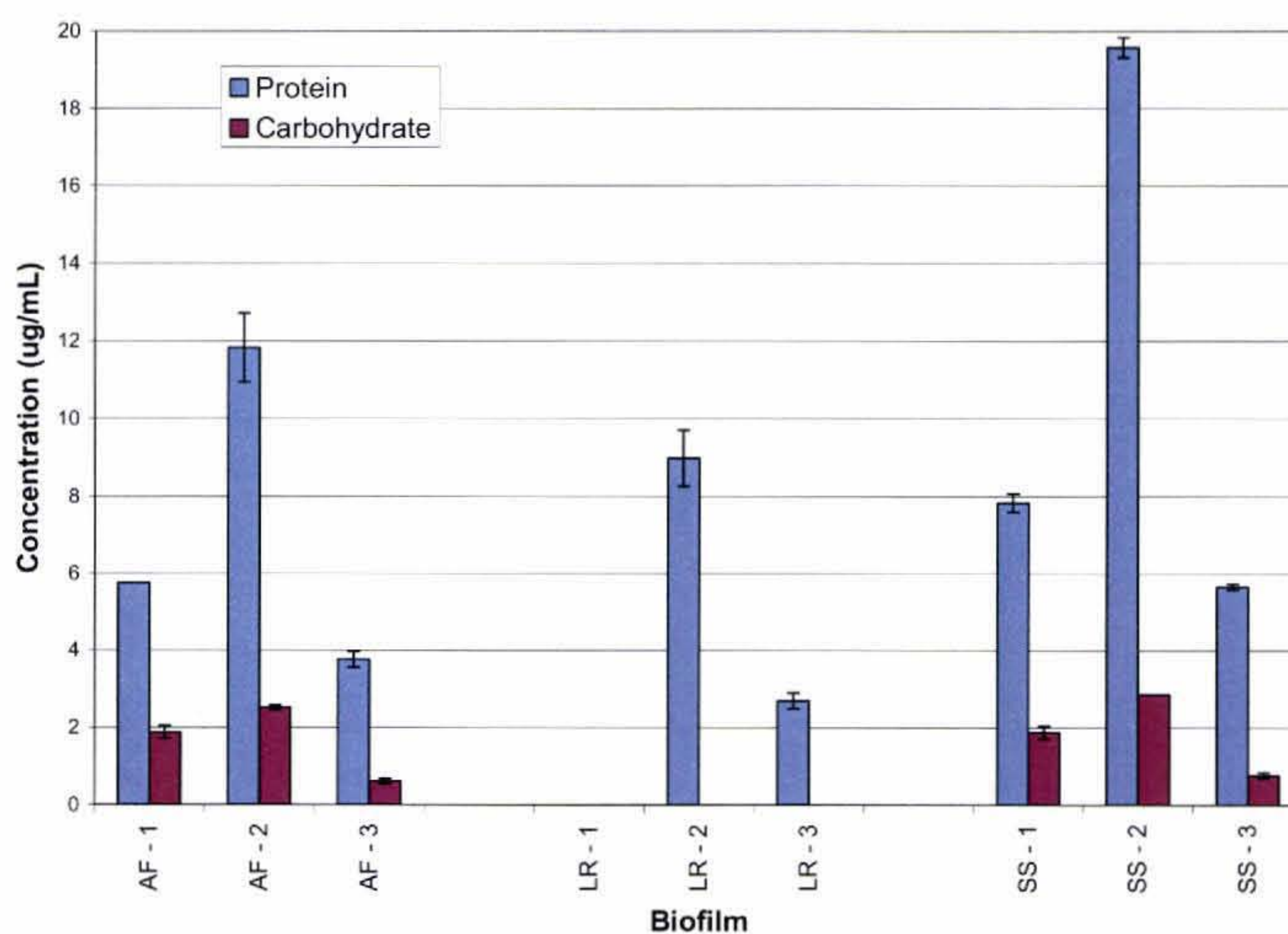


Figure 17. Protein and carbohydrate concentrations of EPS from biofilms not amended with phenanthrene. Three replicate extracts from the three media are shown, AF: aquarium filter, LR: lava rock, SS: Smart Sponge®.

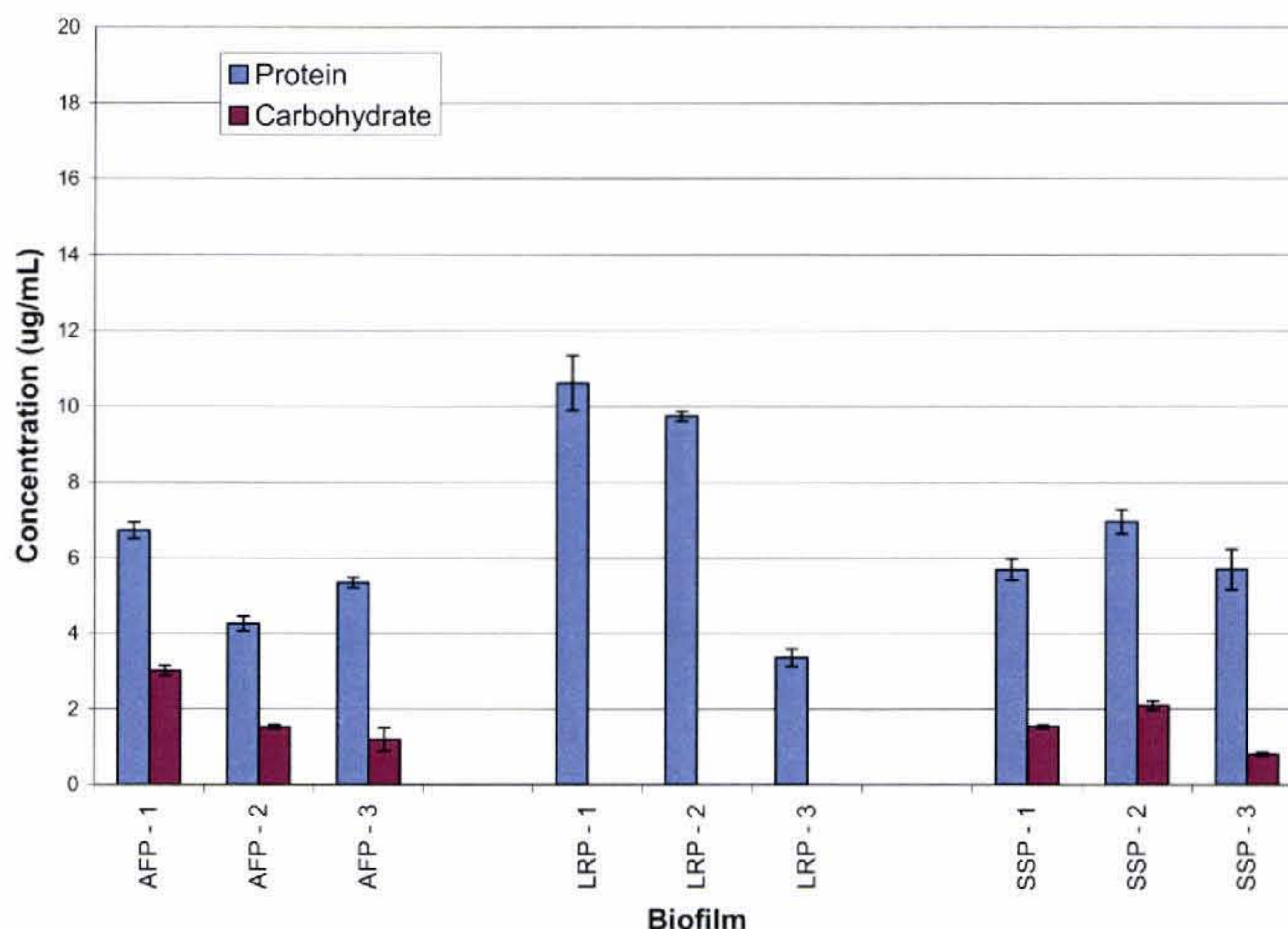


Figure 18. Protein and carbohydrate concentrations of EPS from biofilms amended with phenanthrene. Three replicate extracts from the three media are shown, AF: aquarium filter, LR: lava rock, SS: Smart Sponge®.

EPS Hydrophobicity Characterization

The ratio of hydrophilic carbohydrates to the total amount of carbohydrates was always above 0.83 (see figure 19). Hydrophobic interaction chromatography confirmed the literature that describes the hydrophilicity of carbohydrates in EPS. Hydroxyl, carboxyl, sulfate, phosphate and amino groups of the sugar molecules make polysaccharides hydrophilic, an important characteristic for maintaining hydration within the biofilm and ion exchange for dissolved nutrient uptake.

A larger proportion of hydrophobic proteins were expected. In a wastewater floc, EPS extracts contained almost an equal percentage of hydrophobic and hydrophilic amino acids (Dignac 1998). In the AF, LR and SS biofilms 75-100% of the proteins were

hydrophilic (see figure 20). In proteins, hydrophobic amino acids are often found on the interior of a protein while the exposed ones that interact with the aqueous environment are usually hydrophilic. Because whole or at least fragments of proteins are measured in the Lowry assay, interactions with the chromatographic packing are likely with the exterior portions of the proteins. Interactions of whole proteins are a more accurate measure of the character of EPS rather than the amino acid content as interactions with surfaces and molecules will, at least initially, be at the exterior of a biofilm. It is interesting to note that AF biofilms grown with or without phenanthrene produced the greatest amount of hydrophobic proteins. Hydrophobicity of a biofilm has been attributed to the protein fraction within the EPS. Although the majority of the biochemicals in the biofilm are hydrophilic, Van der waals attractive forces of the hydrophobic groups on bacterial surfaces may overcome the resultant electrical repulsion leading to adhesion (Bunt et al. 1995). The slightly more hydrophobic biofilm of AF would also be expected to be able to absorb more hydrophobic contaminants as was shown in studies by Fang and Zheng (2004).

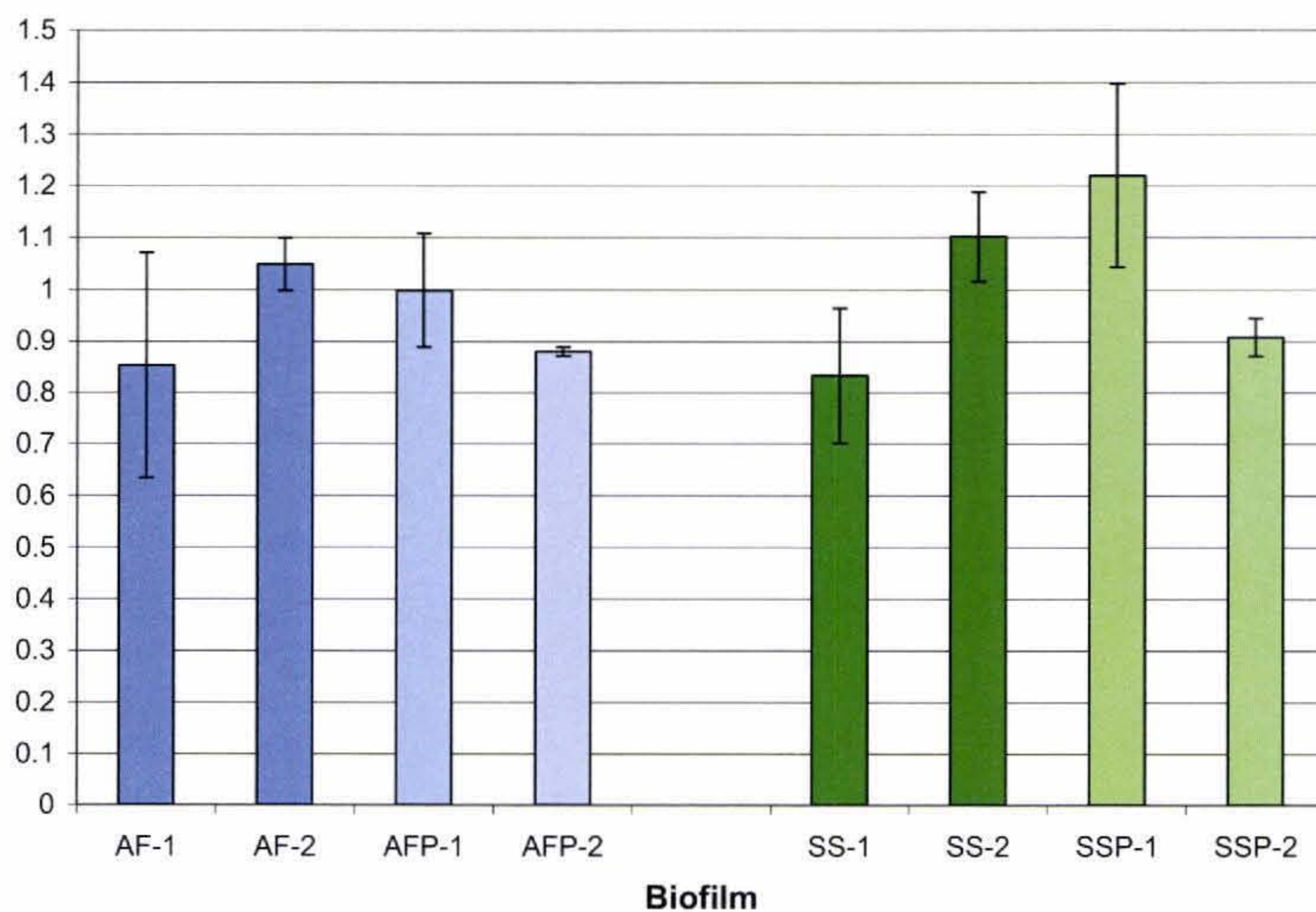


Figure 19. Ratios of hydrophilic to total carbohydrates from biofilms grown on AF: aquarium filter without phenanthrene, AF-P: aquarium filter with phenanthrene, SS: Smart Sponge® without phenanthrene, SS-P: Smart Sponge® with phenanthrene. 1 and 2 represent replicate extracts.

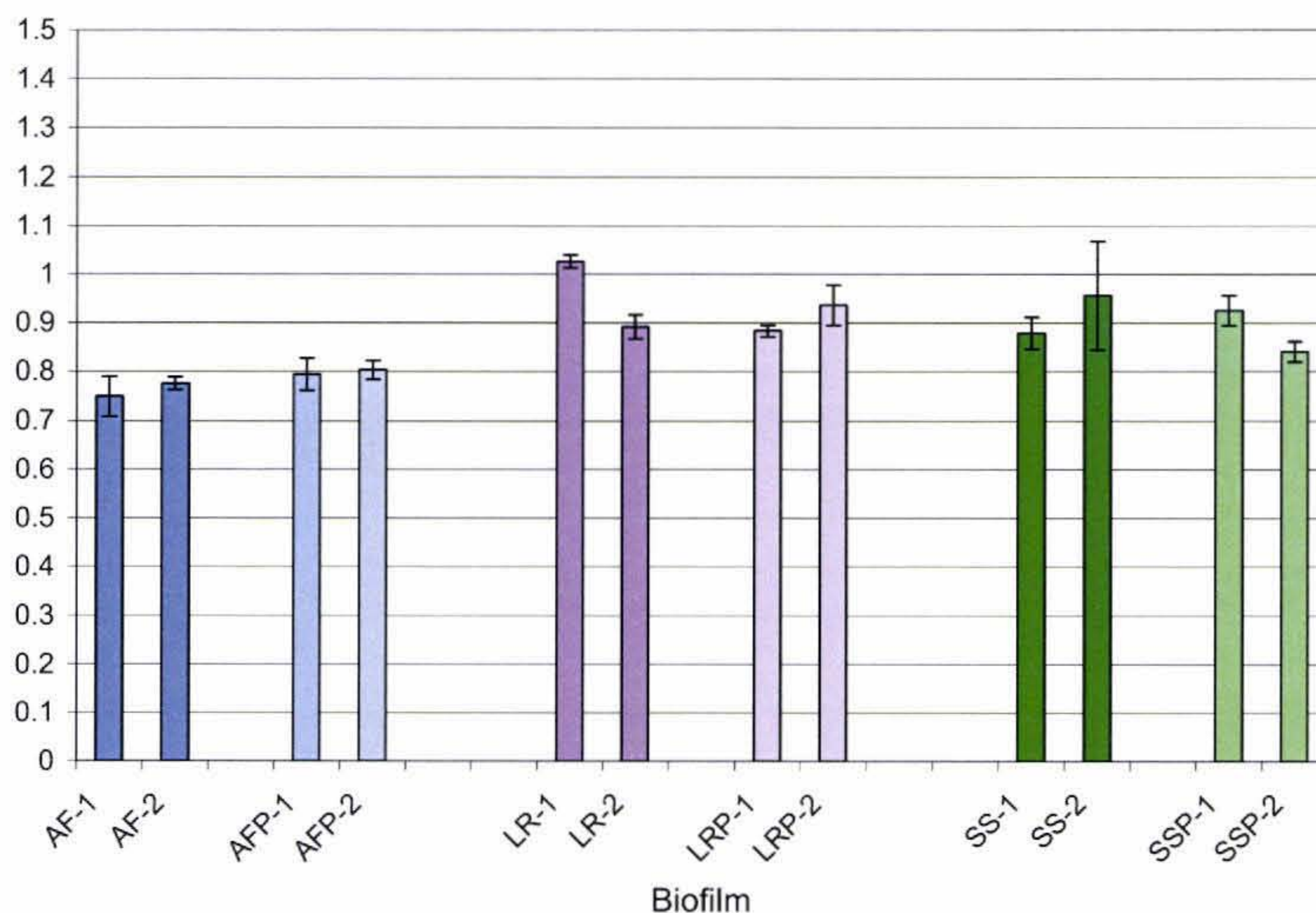


Figure 20. Ratios of hydrophilic to total proteins from biofilms grown on AF: aquarium filter without phenanthrene, AF-P: aquarium filter with phenanthrene, LR: lava rock without phenanthrene, LR-P lava rock with phenanthrene, SS: Smart Sponge® without phenanthren, SS-P: Smart Sponge® without phenanthrene. 1 and 2 represent replicate extracts.

Chapter 3. Biofilter Performance

Several experiments were conducted to determine how well the biofilm on each medium performed in biofiltering urban stormwater runoff. The objectives of the methods described in this section were to: 1) evaluate the extent of nutrient removal from water by the biologically active media, 2) test the ability of each biofilter to capture suspended solids, 3) assess the availability of hydrocarbons associated with sediment and 4) characterize the ability of biofilms on different substrata to degrade a polycyclic aromatic hydrocarbon (PAH). In addition, the affect of acclimating the biofilm to the contaminant prior to degradation tests was examined.

METHODS

Nutrient Removal

Nutrient concentrations of the waters contributing to the Ala Wai canal must be reduced to be in compliance with state regulations. According to the Hawaii Department of Health, wet season concentrations of nitrate- plus nitrite-nitrogen are not to exceed 0.07 mg/L in natural freshwater bodies (DOH 2004), however, storm samples of the Manoa and Palolo streams were higher by a at least a factor of 10 (Ikeno 1996). Dissolved phosphorous from storm samples of the streams were at least twice as high compared to non-storm samples reaching levels of around 0.1 mg/L (Ikeno 1996) while Hawaii Administrative Rules state that total phosphorous is not to exceed 0.05mg/L (DOH 2004).

For the nutrient removal test, a solution containing 0.15 mg/L nitrite-nitrogen, 1

mg/L ammonia-nitrogen and 0.5 mg/L phosphorous was prepared. Fresh non-inoculated media and media with biofilm were rinsed with tap water then with deionized water. Length of biofilm formation was 26 days. At approximately 3 mL/s, 200 mL of nutrient solution was flowed over the biofilters and the second 100 mL of the filtrate was collected. Filtrates as well as the initial nutrient solution were analyzed by Hach water analysis reagents (Loveland, CO). Quantification of ammonia-nitrogen was based on the Nessler method, nitrite-nitrogen used a diazotization method and ortho phosphate was measured by an ascorbic acid method. Standard solutions of each nutrient were also purchased from Hach Company.

Particle Capture

While nutrients do occur as dissolved ions in runoff water, according to the literature, the majority of the pollutants are in the solid fraction. Inorganic phosphorous in natural soils is strongly adsorbed onto mineral surfaces and most nitrogen is in the form of organic compounds such as proteins and humic acids (Brady and Weil 1999). In the Ala Wai canal, deposited particulate nitrogen from terrestrial origin was calculated to be 0.7 g N/m² d (Laws et al. 1994). In an urban residential area of Wisconsin the measured dissolved phosphorous in storm runoff accounted for only 15 – 40% of the total phosphorous (Waschbsch et al. 1999). Dry road surface sediment was sieved and analyzed for total nitrogen and total phosphorous. Less than 25% of the phosphorous and less than 10% of the nitrogen could be dissolved by water. The authors recommend that to effectively remove most of the N and P from runoff, a treatment system must be able to capture particles (Vaze and Chiew 2002).

Due to the hydrophobicity of hydrocarbons, it is expected that most of the HCs in runoff will be adsorbed to particles. K_{OC} is the measure of the affinity of a compound to organic carbon in soil and sediment. Low molecular weight PAHs such as phenanthrene (3-ring) have K_{OC} values in the area of 10^3 and medium molecular weight PAHs such as pyrene (4-ring) have K_{OC} values around 10^4 (ATSDR 1995). Field samples of hydrocarbons confirm this physical property. In the literature, runoff from several different storm events 75-95% of aromatic hydrocarbons were associated with particulates (MacKenzie and Hunter 1979; Eganhouse and Kaplan 1981).

Considering this information, it is important that the media selected for the biofilter are able to retain particles. This will be accomplished with the help of a microbial biofilm.

Biofilms were developed on the media for 19 days by circulating stream water over stream rocks then onto the units. Roadside sediment was collected, dried and sieved. Preparation of the suspension was done to increase the low-density/organic proportion. PAHs have a greater affinity to this fraction of sediment (Murakami et al. 2005 and Rockne et al. 2002) therefore I wanted to focus on the interception of lighter particles. To make the suspension of particles, about 8 g of the fine sediment was combined with about a liter of water and vigorously shaken. The dense particles were allowed to settle to the bottom of the flask for a minute, the top 600 mL was poured into another flask then divided into three 200-mL portions. One aliquot was used to measure the initial amount of sediment in the solution; the other two aliquots of the solution were poured over media units at approximately 20 mL/s. The filtrate was collected for mass and optical density (transmittance at 420nm) measurements. Solids from each filtrate

were collected on a filter paper by vacuuming the water away in a Bushner funnel. The filter paper and eluted particles were placed in an oven at 110° C overnight, cooled in a humidifier, then weighed. Initial weight for each of the filter papers was subtracted to obtain the weight of the solids. There were 5 replicates for each media type.

PAH Degradation

Phenanthrene (3-ring) and pyrene (4-ring) are polycyclic aromatic hydrocarbons (PAHs), industrial and automotive products of incomplete combustion commonly found in urban runoff. PAHs are potentially mutagenic and carcinogenic. Studies have shown that prolonged inhalation exposure to PAHs causes respiratory diseases, reduced levels of serum immunoglobulins, and cancer in animal studies (ATSDR 1995). There may also be an enhanced toxicity of certain PAHs due to free radical generation in sunlight (Irwin et al. 1997). Certain PAH congeners are of particular concern to aquatic wildlife.

Phototoxicity can immediately affect respiration in fish and aquatic invertebrates as reactive oxygen species damage gill or skin membranes (Crosby 1998). Motelay-Massei et al. report that the most important source of PAHs for surface waters are those that are deposited onto streets (2006). Mitigating the release of PAHs to the aquatic environment is important and biofilters are a promising technology as toxins may be captured as well as mineralized.

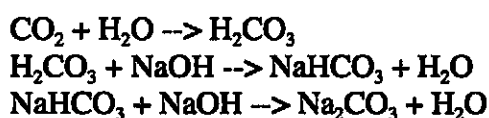
Bioavailability of PAH on sediment: Even though solids may be taken up by the biofilm, it is still not certain that the bacteria will be able to degrade the hydrocarbons. Sorption of organic compounds to particles is often very strong rendering it unavailable to

microbial attack, this is known as chemisorption (Smith et al. 1999). To test whether PAHs associated with roadside sediment can be accessed by microbes, a bioreactor study was conducted.

Roadside sediment was collected, oven dried at a low temperature and sieved. Ten grams of the sediment was weighed out into glass jars. The jars were covered with aluminum foil and autoclaved three times with at least 24 hours between each autoclaving. Spiking solutions were prepared with phenanthrene or pyrene plus the respective radiolabeled PAH in toluene. For jars receiving only one PAH, 20 μ L of spiking solution was delivered using a syringe; for jars receiving both, 10 μ L from each spiking solution was delivered so that the final concentration in each jar was 20 ppm PAH and about 10,000 DPM or 0.0047 μ Ci/g of carbon-14 activity. The spiking solvent was allowed to evaporate and the hydrocarbons equilibrated within the soil for 7 days. To prepare the inoculum, stream water and rocks were collected from the Manoa-Palolo stream. Slime was loosened from the rocks with a bristle brush and combined with stream water along with mineral medium. Live cell counts were made of the inoculum with a hemacytometer and a light microscope. Six milliliters of the inoculum solution was pipetted over the spiked sediment. About 5.7×10^7 live cells were added to each jar receiving inoculum. Sterile controls received autoclaved water and mineral medium.

The respired carbon dioxide was captured in 1.5 mL of a CO_2 -free solution of sodium hydroxide (0.5 N) in a small glass centrifuge tube placed in each jar (see chemical reaction below). Every few days the NaOH solution was removed and added to a 20-mL glass scintillation vial containing 15 mL of EcoLume Liquid Scintillation Cocktail (MP Biomedicals, Irvine, CA). 1.5 mL of water was pipetted into the centrifuge

tube then into the same scintillation vial to wash of residual solution. A fresh amount of NaOH solution was pipetted into the centrifuge tube. Scintillation counts were made of the mineralized radiolabeled carbon for 1 min using a Beckman LS 6500 Multi-Purpose Scintillation Counter. This method was extremely useful because sediment from the road, which is itself 6% organic carbon, may be used as an environment for growth while still getting direct information on biodegradation of the pollutant of interest.



To calculate the percent carbon respired, counts per minute (CPM) of a scintillation vial containing only 15mL of scintillation cocktail was subtracted from the CPM of each sample reading. CPM values were converted to disintegrations per minute (DPM) by dividing CPM by counting efficiency of the liquid scintillation counter. DPM numbers were multiplied by the number of carbon atoms in the jar per DPM, divided by the carbon atoms per jar then multiplied by 100 to get a percentage. This calculation overestimates the carbon respired because it assumes that every carbon on the PAH molecule is equally degraded, however, for phenanthrene the labeled carbon is in the #9 position which is exposed and sterically more accessible to attack by bacteria than other positions. The same is true for pyrene as the labeled 4,5, 9 and 10 positions are also on the outsides of the molecule (Figure 21).

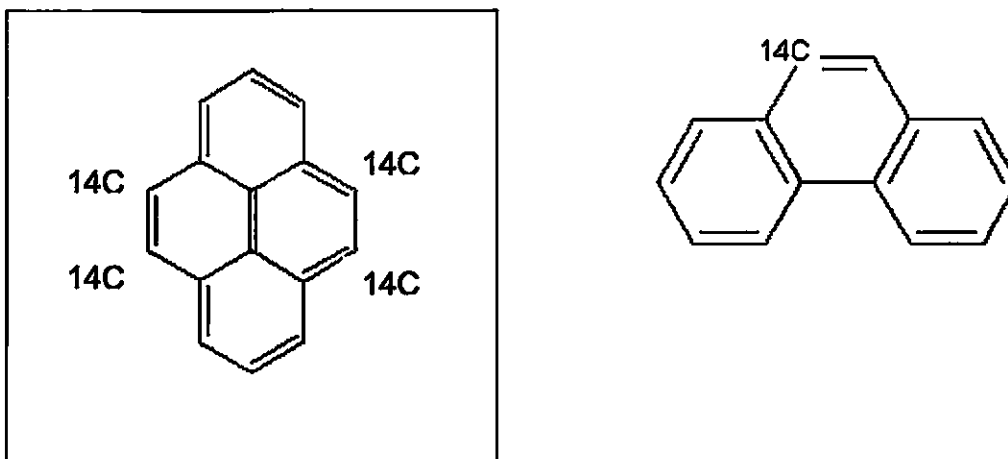


Figure 21. Carbon-14 labeled pyrene (left) and phenanthrene (right) showing location of labeling.

Biodegradation by biofilm on media: Biodegradation of a PAH by the biofilms produced on each media was determined by adding C-14 labeled phenanthrene to media with biofilm and sterile media. Additionally, media with biofilm that had been previously exposed to doses of phenanthrene were also spiked to determine whether a biofilm acclimated to the PAH could enhance degradation.

Approximately one fourth of the media from three replicate units of each media was weighed out. The material was placed in a clean dish with 200 mL of sterile deionized water then swirled to remove any non-attached particles. Washing was repeated twice then transferred to a sterile centrifuge tube for transport to the fume hood designated for radioactive work. For the sterile control, media of approximately the same mass as the corresponding biofilter samples were wrapped in aluminum foil and autoclaved for 30 min twice with 24 h between autoclavings. The sterilized media was removed from the foil and emptied into to a clean glass dish to be washed with sterile deionized water before being placed in a sterile centrifuge tube.

In a fume hood designated for radioactive work, autoclaved glass jars received 1 mL of toluene containing 50 ug of phenanthrene (Sigma, Saint Louis, MO) and approximately 108,000 DPM of phenanthrene-9-C14 with an activity of 8.2 mCi/mmol (Sigma, Saint Louis, MO). The solution was swirled around as the solvent evaporated to coat the bottom of the jar with the PAH. Once the toluene had evaporated off, 3 mL of mineral medium was pipetted into each jar and the media were placed into the jars. Jars were capped and shaken vigorously. A sterilized glass centrifuge tube containing 1.5 mL a 0.5N CO₂-free NaOH solution was placed in each jar. As the bacteria metabolized the phenanthrene, respired carbon dioxide was captured in the solution of sodium hydroxide.

On days 5, 9, 14, 20, 26, 30 and 35, the respired carbon-14, was measured by liquid scintillation counting. The NaOH solution was pipetted out of the centrifuge tube then into 12 mL of liquid scintillation cocktail in a scintillation vial. One and a half milliliters of deionized water was added to the centrifuge tube to suspend any residual alkaline solution then was pipetted into the same scintillation vial. A fresh amount of sodium hydroxide solution was replaced in the tube. Each sample was counted for 1 min in a Beckman LS6500 Multi-Purpose Scintillation Counter.

In this experiment, we eliminated the need for transport of the contaminant to the biofilms and placed the PAH in direct contact with the biofilm, thus minimizing the effect of this dynamic process on the degradation by biofilm communities on different media.

RESULTS AND DISCUSSION

Nutrient Removal

Dissolved nutrient removal by new media without biofilms and media with biofilms was determined. In each case, more nutrients were taken up by inoculated media than the non-inoculated therefore removals can be attributed to the biofilms and not the media themselves (see figures 22-24). Small amounts of nitrite and ammonia actually leached out of new media causing an increase in concentration in the effluent. Only a small amount of the negatively charged nitrite was removed probably due to repulsion from the anionic biofilm. For the cationic ammonia, removal was between 13 and 25%. The lava rock itself was able to absorb some ammonia probably by cation exchange interactions with hydroxyl groups at its surface. Uptake of the polyanion, phosphate, was 5 to 50%. Biofilm uptakes of the dissolved ions from most effective to least were the aquarium filter, lava rock then Smart Sponge®.

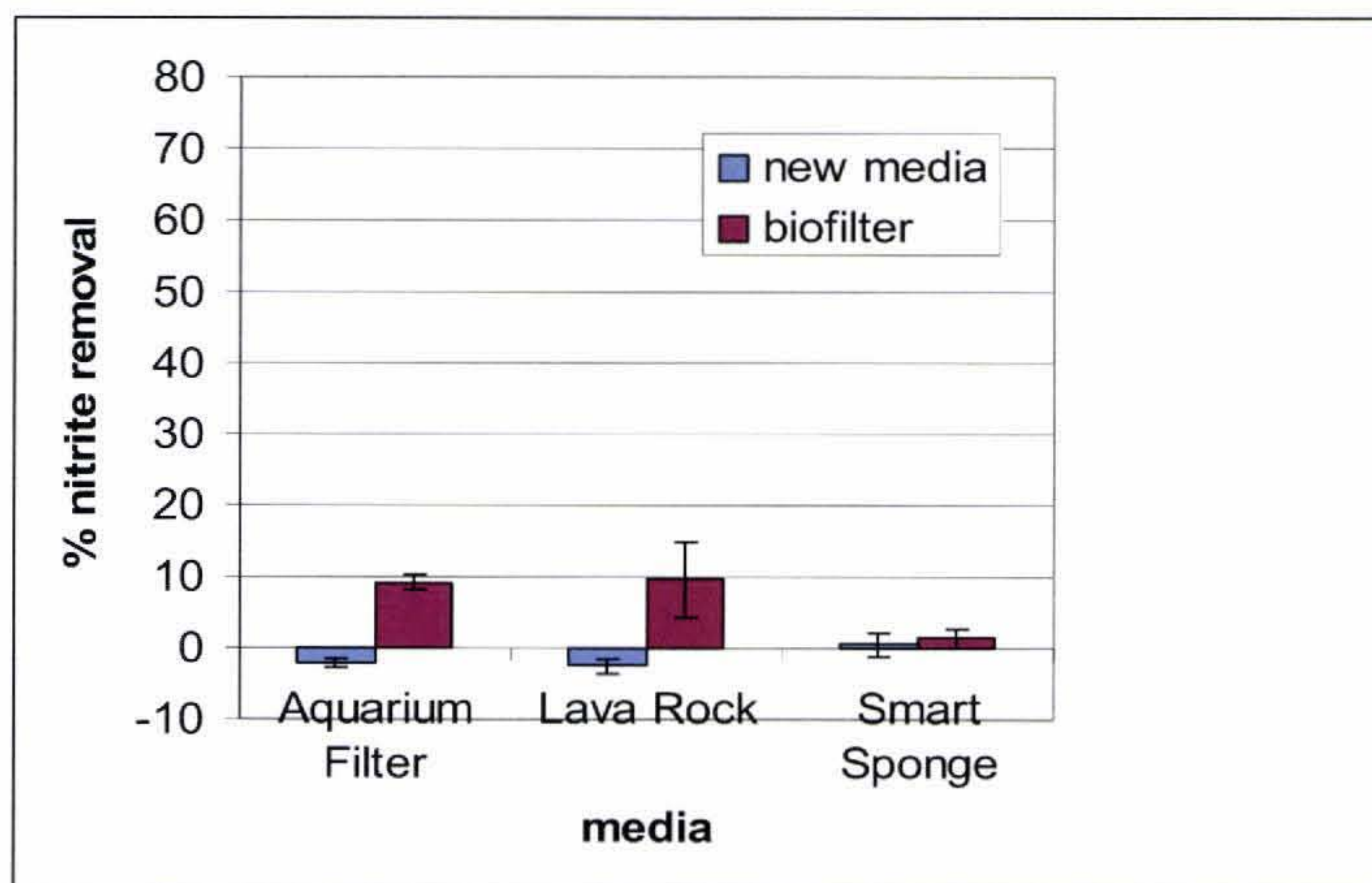


Figure 22. Nitrite removal by fresh media and media with biofilm.

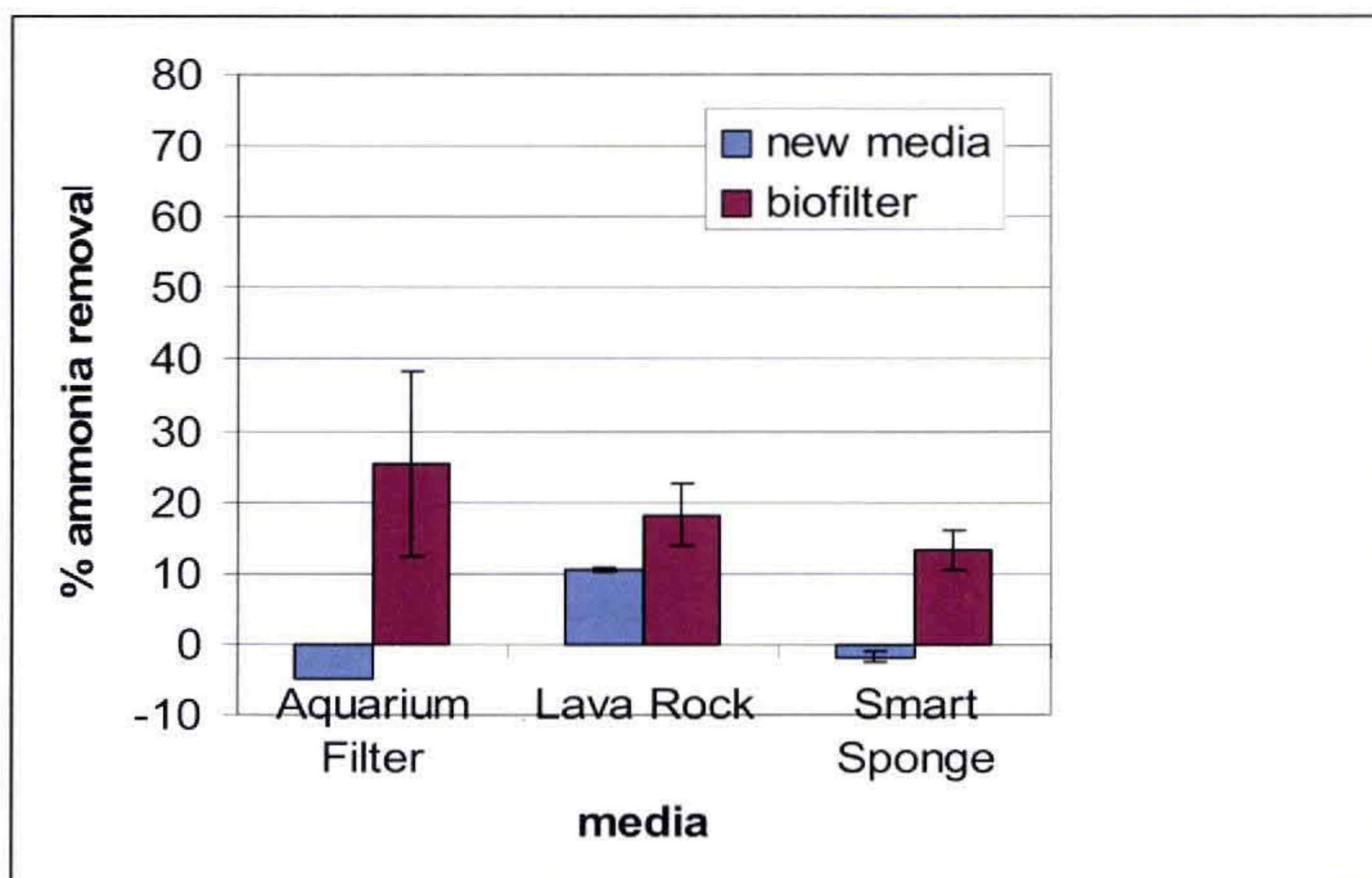


Figure 23. Ammonia removal by fresh media and media with biofilm.

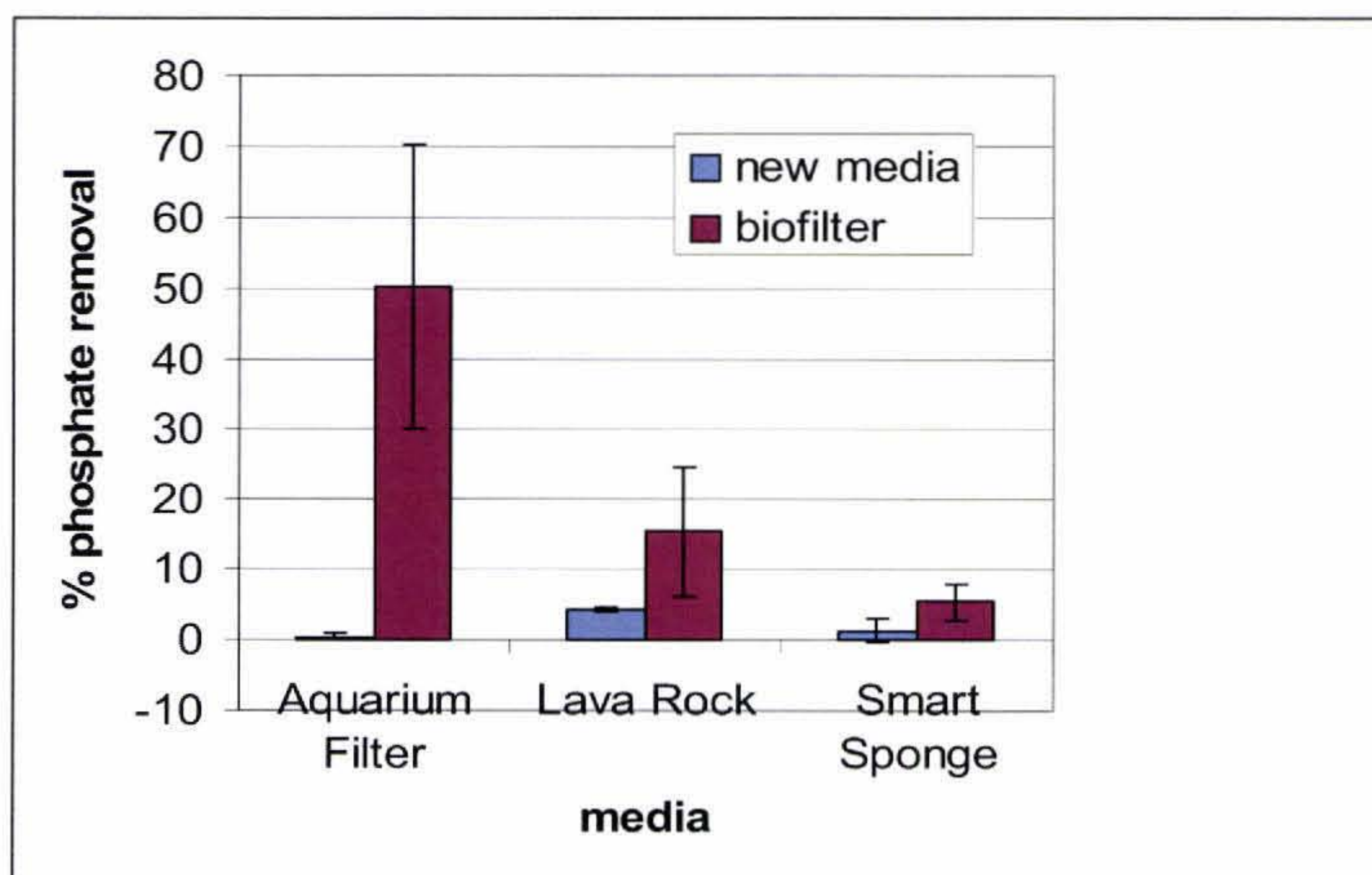


Figure 24. Phosphate removal by fresh media and media with biofilm.

As the nutrient solution comes into contact with the media surfaces, the dissolved

ions will react with charged groups that they encounter. From the lack of activity seen by the new AF and SS trials it can be assumed that these two materials have inert surfaces. For cation or multivalent anion uptake, AF and SS must be covered by a reactive biofilm. AF absorbed more ammonia and phosphate than SS because its biofilm was more complete. Although new LR removed some ammonia and phosphate, a biofilm does improve its reactivity.

Particle Capture

Table 4 shows the particle removal rates by each biofilter in terms of mass and optical density. The aquarium filter had the most extensive biofilm as seen by SEM and the greatest amount of particles captured, 42% by weight and improvement in optical density was 3.6%. A brown film had developed on the smart sponge especially toward the center of the unit; it removed about 27.8% of the particles by mass and increased transmittance by 1.8%. Lava rock filters removed 21.7% of solids and optical density was improved by 2.3% although an interconnected layer of slime could not be seen.

A well developed biofilm is important for capturing particles from water because not only does the EPS matrix itself hold solids but there are internal channels and protuberances that extend into the water column intercepting particles and slowing flow around the biofilm (Battin et al. 2003).

Table 4. Particle removal by biofilms grown on different media expressed as change in mass and reduced optical density.

	solids removed by mass (%)	standard deviation	solids removed by transmittance (%)	standard deviation
Aquarium filter	42.4	13.0	3.6	0.98
Lava rock	21.7	2.7	2.3	0.38
Smart Sponge	27.8	7.3	1.8	0.81

PAH Degradation

Bioavailability of PAH on Sediment: Seventy-seven percent of the phenanthrene, 58% of the pyrene and 66% of phenanthrene+pyrene had been captured as carbon dioxide by day 145 (Figure 25). The lag periods for the phenanthrene and phenanthrene+pyrene treatments were very short whereas a lag period of nearly 22 days was seen for the jars with pyrene only. The stream rock biofilm inoculum was initially the same in all jars and a population shift may have occurred as the bacterial community adjusted to the presence of pyrene. A large increase in respired PAH-carbon was seen in the two treatments with pyrene around day 32. The increased hydrophobicity and K_{OC} of pyrene along with an initial low density of pyrene degraders probably contributed to the observed lag period and reduced degradation rate. The degradation curve for the phenanthrene+pyrene treatment was in between those of the phenanthrene only and pyrene only treatments indicating that there were no synergistic or antagonistic interactions of the two compounds in this biofilm community. Degradation of the two PAHs in this system was additive. While degradation had leveled off, at each measurement, a small amount of respired C-14 was detected therefore continued contact of the bacteria with the contaminant would bring residual levels down even further.

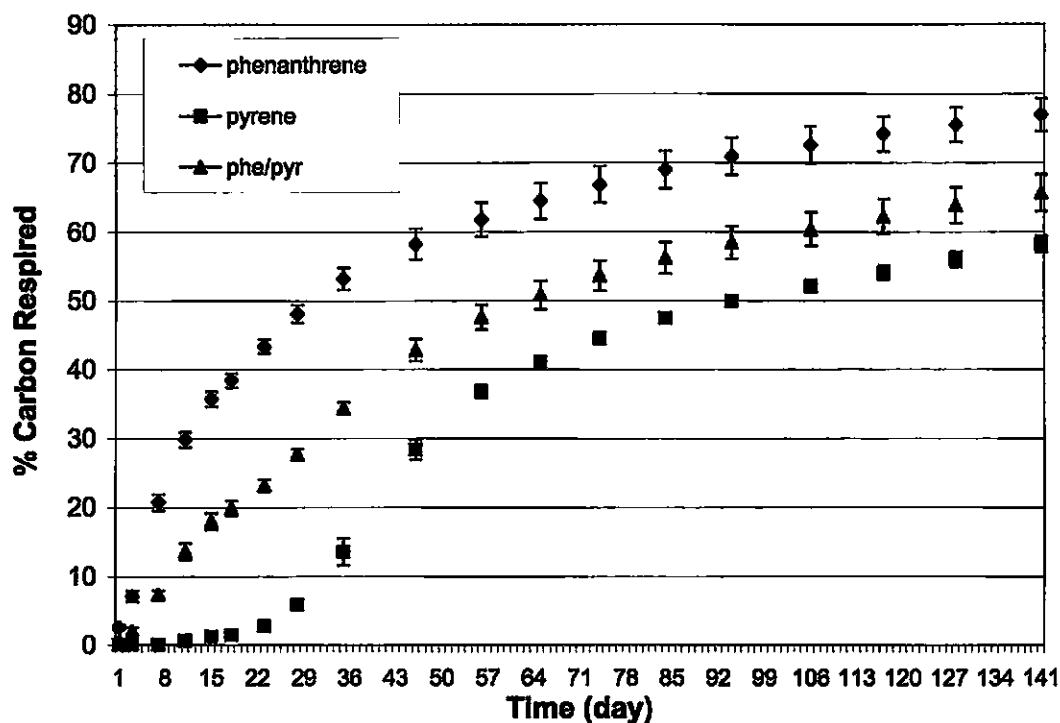


Figure 25. Biodegradation of PAHs by stream biofilm as percent carbon respired as carbon dioxide. Plots are an average of 3 measurements with bars representing standard deviations.

Sediments of aquatic systems are an important sink for toxic hydrophobic molecules, which may prevent stream biota from being exposed to potentially harmful pollutants, however, these results show that PAHs are available to bacteria. Capturing particles in a biofilter will allow the biofilm microbes to be in contact with sorbed contaminants to encourage decontamination rather than accumulation in the streambed.

Highly hydrophobic molecules are often inaccessible to charged bacterial cells as a nutrient source; however, Rodrigues et al. found that the production of EPS allowed *Pseudomonas putida* ATCC 17514 cells to aggregate onto a phenanthrene crystal and degrade it at a rate of 17.5 mg/g VSS/day. It was suggested that hydrogen bonding of the EPS to the aromatic ring may have made the PAH more available to the microbes within

the matrix (2005).

C-14 measurements may be reduced because they do not include carbon used for cell growth and maintenance. It is estimated that for every gram of glucose utilized, 0.4 g of it goes toward cell growth (Maier et al. 2000). Also, jars were opened at each sampling to access the NaOH solution and some CO₂ may have escaped.

Biodegradation by Biofilm on Media: Figures 26 and 27 shows the average percent phenanthrene-carbon mineralized by the different biofilters. “N” indicates biofilm developed without exposure to phenanthrene and “P” indicates biofilms that had been exposed to phenanthrene. Points represent an average of three replicates except for SS-N, which is an average of two replicates because the third showed degradation similar to the sterile control which may be due to incorrect labeling or biofilm detachment during rinsing. By day 35, SS-N, AF-N and LR-N had mineralized about 24, 42 and 52% of the phenanthrene carbon, respectively. AF-P, SS-P and LR-P had respired about 40, 42 and 46% of the phenanthrene carbon, respectively. Exposing the biofilms to phenanthrene eliminated or greatly reduced the lag period for all biofilms however it did not improve utilization of the compound in the long run for aquarium filter or lava rock biofilms. The Smart Sponge® film, on the other hand, nearly doubled the amount of degradation. The high degree of diversity seen in the biofilms appears to be very important for a biofilter that may encounter different kinds of environmental pollutants however a period of acclimation to a new carbon source is necessary.

Sterile controls of AF and SS are not shown because the amount of labeled CO₂ produced was very small. Abiotic degradation by the sterile lava rocks, as measured in

produced was very small. Abiotic degradation by the sterile lava rocks, as measured in the controls, was subtracted from the mineralization measured in the jars with biofilm.

It was expected that the more hydrophobic biofilm of AF would have the highest degradation rate, however the initial rate was fastest in LR. There are several potential explanations for this result. First, diffusion of the PAH molecule through the biofilm matrix to the cells may be a limiting factor. The undeveloped biofilm community of LR would not encounter this barrier. Second, at each measurement, small amounts of carbon-14 were detected in the CO₂-absorbing solution from the sterile LR jars. This

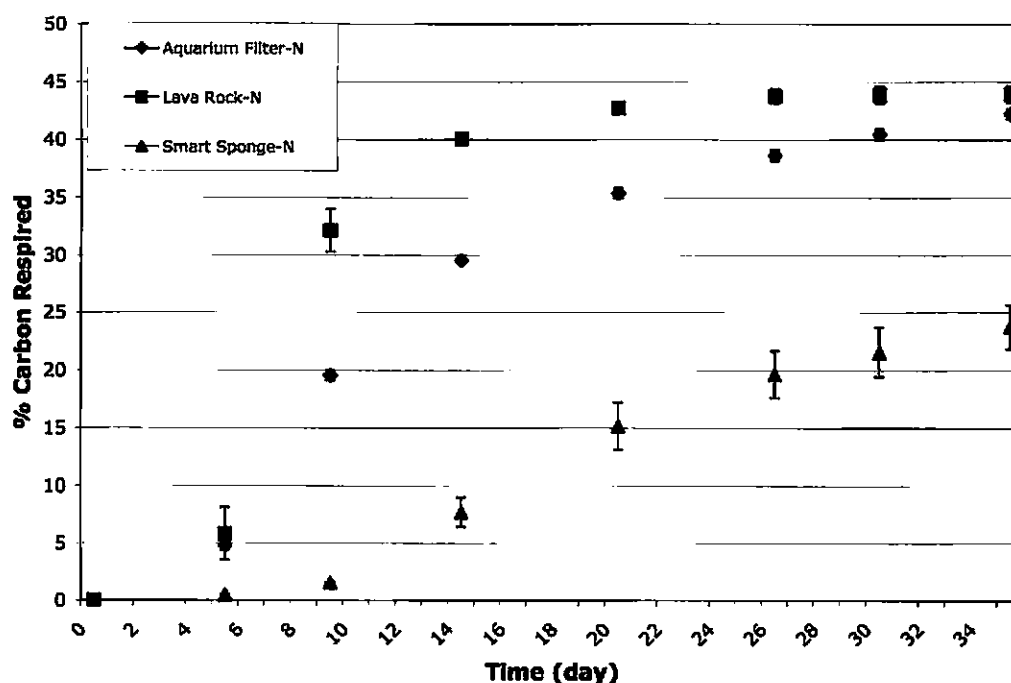


Figure 26. Biodegradation of phenanthrene by biofilms growing on three different media measured by percent carbon respired as carbon dioxide. Plots are an average of three measurements with bars representing standard deviations.

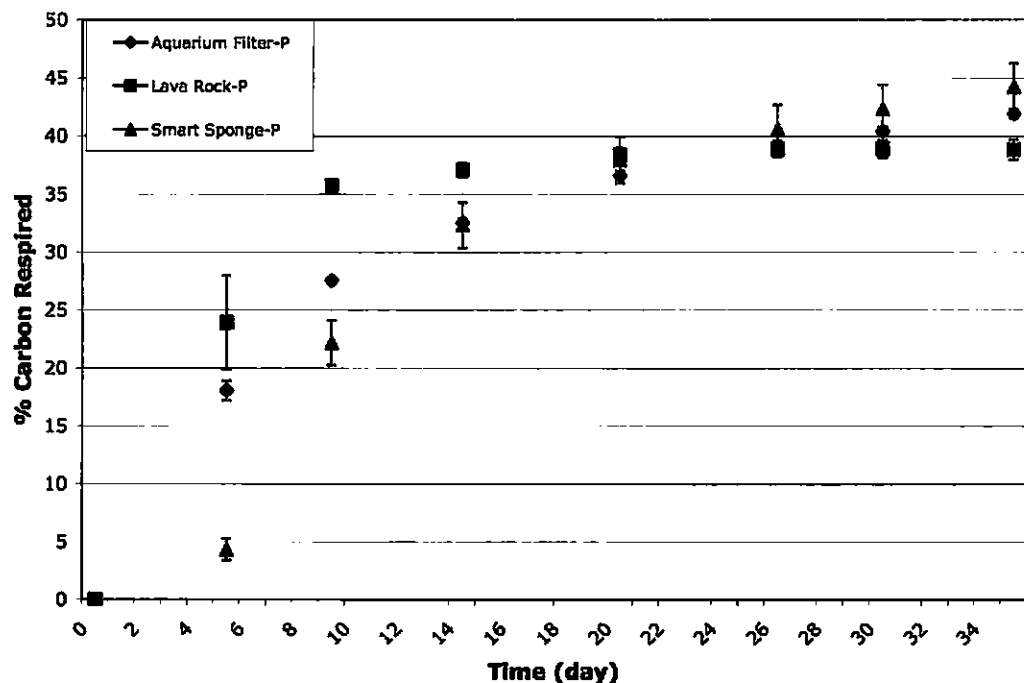


Figure 27. Biodegradation of phenanthrene by biofilms acclimated to phenanthrene growing on three different media as percent carbon respired as carbon dioxide. Plots are an average of three measurements with bars representing standard deviations.

abiotic reaction of the LR surface with phenanthrene may be converting the PAH into more accessible or metabolizable residues for biodegradation. A final possibility is that the biofilms on each media may be at different stages of development, and the cells in different physiological states (attachment, EPS production, multiplication, etc.), which possibly affects metabolism. Still, in a dynamic environment, biofilm hydrophobicity will play an important role in attracting molecules from the surrounding fluid.

Chapter 4. Summary and Conclusions

SUMMARY

Urban runoff pollution has greatly compromised aquatic ecosystems around the world including those picturesque, tropical waters upon which Hawaii bases its economy. During rain events the Ala Wai canal drains the central Honolulu watershed discharging road deposited nutrients and organic chemicals that can be toxic to aquatic life and even humans into the ocean. One method of mitigating the release of toxic compounds into open waters is to have a detention pond where contaminants can settle out or be utilized by bioremediating organisms. However, in cities where land is limited and expensive, this is not an option so stormdrain traps that capture debris are a possible solution. If a biological component capable of degrading organic contaminants is incorporated into these traps, the need to change the filter bedding and dispose of hazardous wastes would be reduced.

Biofilters use biofilms to remove and degrade compounds of interest from flowing systems. Biofilms are the focus of this project and can be thought of as an aggregation of a variety of microbes enveloped in a self-produced matrix of extracellular polymers attached to a surface. The extracellular polymeric substances (EPS) are composed mostly of proteins and carbohydrates but can include lipids, nucleic acids and other compounds and debris that have been adsorbed. The adsorption of solids and molecules as well as cellular attachment to a surface has been attributed to the chemical and physical properties of the EPS. It is thought that the substrata influence the amounts and types of bacteria that attach and that hydrophobicity of the media plays an important role in the initial adhesion process. The EPS produced depends on the species or strains

that colonize, their physiological states and the environmental conditions. Reactive sites of the biopolymers and the resident organisms engage in electrostatic and hydrophobic interactions with dissolved nutrients, organic molecules and solid particles in the surrounding fluid to incorporate them into the matrix.

The objective of this project was to determine how media characteristics and the microbial community influence the properties of the biofilm and its affect on remediating urban runoff pollutants. To do this, biofilms were produced on three different media with varying physical and chemical characteristics using stream water, sediment and rocks as the inoculum. The Manoa-Palolo stream was chosen because it runs through a highly urbanized part of Honolulu where the community had been repeatedly exposed to runoff contaminants and should be acclimated and potentially able to biodegrade them. The relatively hydrophobic media made of synthetic polymers were aquarium filter (AF) and a hydrocarbon absorbing material called Smart Sponge® (SS). And the highly charged medium was lava rock (LR). LR has been described in biofilter studies and has been shown to remediate environmental pollutants, however, no literature describing biofilms on AF or SS was found despite being lightweight and having potential as a biofilter support media. The results of this project will give insight into the effect of substratum on biofilm characteristics given the same inoculum and growth conditions. These characteristics have important implications when applied to biofiltration of runoff pollutants.

Visual assessment of the extent of biofilm coverage was accomplished by scanning electron microscopy. Biofilms several micrometers thick were seen on most AF surfaces while cells and biological material were scattered on the SS surfaces and

colonization on LR was even sparser.

DNA of bacteria obtained from each biofilter was sequenced. Like stream rocks, which served as a control, AF had three sequences with similarity to degrader species. Two sequences from LR also were related to degrader organisms while in SS there were none.

Hydrophobicity of the films was also assayed but most carbohydrates and proteins were hydrophilic. Most hydrophobic was the proteins extracted from AF and it could therefore be expected that AF would be able to adsorb more hydrophobic contaminants.

Several tests were done to assess the performance of the biofilters, mainly, nutrient removal, particle capture and hydrocarbon degradation. In terms of dissolved nutrient uptake, greatest levels of phosphate and ammonia were removed due to the overall negative charge that biofilms have. SS took up only small amounts of nutrients. AF absorbed the greatest amount of phosphate but otherwise AF and LR performed similarly. Only a small portion of particles were captured by any biofilter but AF, which had the most extensive biofilm, performed the best.

Hydrophobic molecules like polycyclic aromatic hydrocarbons (PAHs) in runoff will be sorbed onto solids. The results of this project demonstrated that phenanthrene and pyrene on roadside sediment could be degraded by stream biofilms. It was also found that when both of the PAHs were spiked onto the sediment together, degradation was additive.

Biodegradation of a PAH by biofilms on LR was the fastest, but by day 35 AF had caught up. SS had mineralized about half of the amount of PAH mineralized by the other two treatments; however, if the biofilms were previously acclimated to

phenanthrene the level of degradation was the same in all samples.

CONCLUSIONS

Biofilms were produced on three different media: aquarium filter (AF), Smart Sponge® (SS) and lava rock (LR) using stream rocks and water from an urban stream as the inoculum. LR was chosen because it had been used in biofilter studies and was found to produce good results. AF and SS are made of proprietary mixtures of synthetic polymers and are hydrophobic. No literature was found describing biofilms on these materials despite being lightweight and having good potential as biofilter support media.

The hydrophobic media (AF and SS) accelerated the process of biofilm development. Previous studies have shown that hydrophobic polymer surfaces rapidly adsorb proteins from aqueous solutions (Brash 1969), which allowed a speedy formation of a conditioning film followed by adhesion of microorganisms and the production of a sticky EPS that likely increased the accumulation of cells and particles. The wool-like structure of AF was conducive to microbial colonization as it provided protection from the forces caused by the flowing water; however, the coarse structure of SS may have inhibited complete biofilm coverage. The complex architecture of AF biofilms extended storage space and surface area for particle capture. As hydrophobic organic molecules would likely be sorbed onto sediment in urban runoff, the captured particles would provide an important supply of carbon. Uptake of cationic and polyanionic dissolved nutrients were also enhanced by a mature biofilm due to its overall negative charge.

Biological material was sparse on lava rock surfaces as seen by SEM, which agrees with the absence of carbohydrates in the EPS extracts, although attached

microorganisms did exist on the lava rocks. This was evident by the high levels of PAH biodegradation and the variety of bacterial sequences obtained from this medium. Lava rock is composed of silica and metal oxides that may repel negatively charged microbial cells. While it is assumed that biofilms can develop on any material, the sequence of biofilm formation is perhaps on different time scales for each medium. Greater repulsion forces slow the initial adhesion of a conditioning film, which in turn delays the attraction of microbes and the eventual excretion of EPS. Conditioning films are mainly composed of proteins whereas polysaccharides are produced by attached organisms, this would explain the detection of proteins in the EPS extracts but no carbohydrates in the LR biofilm.

The urban stream proved to be an appropriate inoculum source. A high number of degrader species in the biofilms turned out to be a key component for PAH degradation as well as a high degree of diversity that allowed the community to adapt to a carbon substrate following an acclimation period. The most favorable condition for microbial adhesion is for both cell and substratum to be hydrophobic and the least favorable is for both cell and substratum to be hydrophilic (Liu 2004). It should follow that a hydrophobic medium will promote the adhesion of hydrophobic as well as hydrophilic cells. Therefore, AF surfaces enhanced adhesion of many species including several of the hydrophilic sphingomonad group, a group important in degrading organic pollutants.

Hydrophobic PAH molecules sorbed onto solid particles were shown to be available for degradation to microbes of stream rock biofilms and when phenanthrene and pyrene were together in the same system, biodegradation was additive.

We presumed that in a dynamic environment the hydrophobic biofilm would increase the adsorption of hydrophobic contaminants and thus the overall degradation. However, in these degradation experiments, we set up a static situation where we minimized the need for transport of contaminant from the environment to the biofilm. Due to the proximity in these systems, the attraction between the biofilm and the contaminant would not be an important factor in the degradation process. In this case the more hydrophobic AF biofilm displayed a slower rate of degradation than the LR biofilm. The rate of degradation is possibly affected by one or more factors such as: diffusion of contaminant through the EPS to the organism; abiotic reactions from the lava rock; or physiological state of the microbes. Further studies are needed to investigate these factors.

Summing all of its traits, AF produced the most promising biofilters for remediation of urban runoff. These attributes included the hydrophobic surface for speedy formation of a complex biofilm, a relatively hydrophobic EPS matrix for attracting hydrophobic contaminants and presence of several degrader species.

Appendix A. Results of GenBank sequence alignments for aquarium filter biofilm bacteria.

Sequence	Closest GenBank Taxon	Phylum	Accession	GenBank Score, Expect value, Identities
AF8t(9/22)	alpha proteobacterium SBR6alpha7	alpha proteobacteria	AY254888	Score = 860 bits (434), Expect = 0.0, Identities = 463/470 (98%)
AF17(10/21)	Azospirillum amazonense	alpha proteobacteria	Z29816	Score = 710 bits (358), Expect = 0.0, Identities = 515/566 (90%)
AF1(10/21)	Rhizobium indigoferae	alpha proteobacteria	AY034027	Score = 898 bits (453), Expect = 0.0, Identities = 720/803 (89%)
AF16(10/21)	Rhodobacter capsulatus	alpha proteobacteria	D16427	Score = 1255 bits (633), Expect = 0.0, Identities = 673/687 (97%)
AF31(11/4)	Rhodoplanes elegans	alpha proteobacteria	D25311	Score = 815 bits (411), Expect = 0.0, Identities = 727/831 (87%)
AF1(9/16)	Rhodovulum sp. CP-10	alpha proteobacteria	AB079682	Score = 432 bits (218), Expect = 4e-118, Identities = 341/382 (89%)
AF2(9/16)	Sphingomonadaceae bacterium Oxy6	alpha proteobacteria	AY858797	Score = 2284 bits (1152), Expect = 0.0, Identities = 1303/1352 (96%)
AF2t(9/22)	Sphingomonas CFO6	alpha proteobacteria	U52146	Score = 961 bits (485), Expect = 0.0, Identities = 554/577 (96%)
AF36(11/4)	Sphingomonas subterranea	alpha proteobacteria	AB025014	Score = 1421 bits (717), Expect = 0.0, Identities = 781/801 (97%)
AF7(10/21)	Sphingopyxis sp., C1	alpha proteobacteria	AF387204	Score = 1481 bits (747), Expect = 0.0, Identities = 788/799 (98%)
AF4t(9/22)	Flavobacterium ferrugineum	bacteroidetes	M62798	Score = 1059 bits (534), Expect = 0.0, Identities = 647/686 (94%)
AF5t(9/22)	Flexibacter sancti strain IFO 16034	bacteroidetes	AB078068	Score = 1033 bits (521), Expect = 0.0, Identities = 653/697 (93%)
AF5(10/21)	Uncultured Flexibacter sp. clone TM19_36	bacteroidetes	DQ279370	Score = 829 bits (418), Expect = 0.0, Identities = 544/586 (92%)
AF18(10/21)	Uncultured Flexibacter sp. clone TM19_36	bacteroidetes	DQ279370	Score = 757 bits (382), Expect = 0.0, Identities = 535/586 (91%)
AF10(10/21)	Uncultured Saprospiraceae clone MS-Tang1-L	bacteroidetes	AJ786331	Score = 476 bits (240), Expect = 8e-131, Identities = 339/372 (91%)
AF29(11/4)	uncultured Sphingobacteriales bacterium	bacteroidetes	AJ897701	Score = 700 bits (353), Expect = 0.0, Identities = 632/721 (87%)
AF1t(9/22)	Caldimonas sp. On1	beta proteobacteria	AY845052	Score = 1164 bits (587), Expect = 0.0, Identities = 635/651 (97%)
AF22(10/21)	Ideonella sp. 0-0013	beta proteobacteria	AB211233	Score = 1332 bits (672), Expect = 0.0, Identities = 747/772 (96%)
AF3(10/21)	Ideonella sp. 0-0013	beta proteobacteria	AB211233	Score = 1298 bits (655), Expect = 0.0, Identities = 743/771 (96%)
AF27(11/4)	Ideonella sp. B513	beta proteobacteria	AB049107	Score = 1447 bits (730), Expect = 0.0, Identities = 799/822 (97%)
AF8(10/21)	Methylophilus sp. ECd5	beta proteobacteria	AY436794	Score = 1283 bits (647), Expect = 0.0, Identities = 745/774 (96%)
AF32(11/4)	Neisseria sp. R-24680	beta proteobacteria	DQ006842	Score = 771, E value = 0.0
AF35(11/4)	Neisseria sp. R-24680	beta proteobacteria	DQ006842	Score = 902, E value = 0.0
AF13(10/21)	Ralstonia campinensis strain WS2	beta proteobacteria	AF312020	Score = 1078 bits (544), Expect = 0.0, Identities = 712/768 (92%)
AF9t(9/22)	Rubrivivax gelatinosus strain OK303	beta proteobacteria	AF487435	Score = 1669 bits (842), Expect = 0.0, Identities = 885/898 (98%)
AF20(10/21)	Uncultured Aquabacterium sp. clone DS130	beta proteobacteria	DQ234213	Score = 1340 bits (676), Expect = 0.0, Identities = 769/800 (96%)
AF5(9/16)	Uncultured Lautropia sp. clone 2.15	beta proteobacteria	DQ016725	Score = 1574 bits (794), Expect = 0.0, Identities = 1140/1258 (90%)
AF3t(9/22)	Aquamonas fontana strain: AQ11	gamma proteobacteria	AB120965	Score = 1495 bits (754), Expect = 0.0, Identities = 867/902 (96%)
AF33(11/4)	Thiobalkalivibrio halophilus	gamma proteobacteria	AY348464	Score = 930 bits (469), Expect = 0.0, Identities = 667/733 (90%)
AF24(11/4)	Uncultured Xanthomonas sp. clone TM17_46	gamma proteobacteria	DQ279336	Score = 1148 bits (579), Expect = 0.0, Identities = 793/855 (92%)
AF15(10/21)	Gemmata obscuriglobus	planctomycetes	X81957	Score = 1070 bits (540), Expect = 0.0, Identities = 638/668 (95%)
AF3(9/16)	planctomycete clone DEL26	planctomycetes	AJ816270	Score = 739 bits (373), Expect = 0.0, Identities = 460/487 (94%)
AF28(11/4)	Opitutus sp. VeSm13	verrucomicrobia	X99392	Score = 1213 bits (612), Expect = 0.0, Identities = 708/740 (95%)
AF23(10/21)	Opitutus sp. VeSm13	verrucomicrobia	X99392	Score = 1227 bits (619), Expect = 0.0, Identities = 689/711 (96%)
AF30(11/4)	Uncultured Verrucomicrobia clone 12-30	verrucomicrobia	AF351215	Score = 771 bits (389), Expect = 0.0, Identities = 649/734 (88%)
AF19(10/21)	Uncultured Verrucomicrobia clone JAB SMS 108	verrucomicrobia	AY694682	Score = 1292 bits (652), Expect = 0.0, Identities = 729/752 (96%)
AF4(9/16)		eukaryote		
AF12(10/21)	no match			

Appendix B. Results of GenBank sequence alignments for lava rock biofilm bacteria.

Sequence	Closest GenBank Taxon	Phylum	Accession	GenBank Score, Expect value, Identities
LR7(9/22)	Acidobacterium	acidobacterium	AY124343	Score = 1096 bits (553), Expect = 0.0, Identities = 757/824 (91%)
LR4(10/21)	Uncultured Acidobacteriaceae clone P27	acidobacterium	DQ263491	Score = 783 bits (395), Expect = 0.0, Identities = 458/475 (96%)
LR6(9/22)	Uncultured Acidobacterium sp	acidobacterium	AJ538052	Score = 823 bits (415), Expect = 0.0, Identities = 523/551 (94%)
LR8(9/22)	uncultured actinomycete clone AOF9	actinobacteria	AJ555218	Score = 971 bits (490), Expect = 0.0, Identities = 629/674 (93%)
LR3(10/21)	Agrobacterium sanguineum	alpha proteobacterium	AB082105	Score = 1328 bits (670), Expect = 0.0, Identities = 748/774 (96%)
LR37(11/4)	Azospirillum sp. LOD4	alpha proteobacterium	AY283791	Score = 888 bits (448), Expect = 0.0, Identities = 690/768 (89%)
LR34(11/4)	Azospirillum sp. WAI-19	alpha proteobacterium	AY563022	Score = 860, E value = 0.0
LR6(10/21)	Candidatus Alysiosphaera europaea	alpha proteobacterium	AY428766	Score = 882 bits (445), Expect = 0.0, Identities = 637/700 (91%)
LR5(9/16)	Methylocella tundratae	alpha proteobacterium	AJ563928	Score = 1933 bits (975), Expect = 0.0, Identities = 1309/1415 (92%)
LR17(10/21)	Porphyrobacter dokdonensis strain DSW-74	alpha proteobacterium	DQ011529	Score = 1388 bits (700), Expect = 0.0, Identities = 757/776 (97%)
LR5(10/21)	Sphingomonas sp. DB-1	alpha proteobacterium	AY947654	Score = 1275 bits (643), Expect = 0.0, Identities = 757/794 (95%)
LR35(11/4)	Uncultured Skermanella sp. clone BL004B35	alpha proteobacterium	AY806019	Score = 860 bits (434), Expect = 0.0, Identities = 631/697 (90%)
LR3(9/22)	Cytophagales ESR 5	bacteroidetes	AF268289	Score = 662 bits (334), Expect = 0.0, Identities = 521/578 (90%)
LR4(9/22)	Flavobacterium sp. GPTSA100-9	bacteroidetes	AY904351	Score = 1447 bits (730), Expect = 0.0, Identities = 829/862 (96%)
LR14(10/21)	Uncultured Flexibacter sp. clone TM19_36	bacteroidetes	DQ279370	Score = 755 bits (381), Expect = 0.0, Identities = 515/557 (92%)
LR26(11/4)	Uncultured Flexibacteraceae clone LIUU-3-160	bacteroidetes	AY509276	Score = 737 bits (372), Expect = 0.0, Identities = 534/585 (91%)
LR8(10/21)	Acidovorax sp. 'smarlab 133815'	beta proteobacterium	AY093698	Score = 1503 bits (758), Expect = 0.0, Identities = 788/798 (98%)
LR25(11/4)	Dechloromonas sp. SIUL	beta proteobacterium	AF170356	Score = 1447 bits (730), Expect = 0.0, Identities = 820/850 (96%)
LR1(10/21)	Ideonella sp. 0-0013	beta proteobacterium	AB211233	Score = 1308 bits (660), Expect = 0.0, Identities = 744/772 (96%)
LR16(10/21)	Uncultured Comamonadaceae bacterium	beta proteobacterium	AB114618	Score = 1372 bits (692), Expect = 0.0, Identities = 761/777 (97%)
LR9(9/22)	Uncultured Sterolibacterium sp. clone TM1_20	beta proteobacterium	DQ279355	Score = 1152 bits (581), Expect = 0.0, Identities = 651/673 (96%)
LR3(9/16)	Uncultured delta proteobacterium clone Syt 5	delta proteobacteria	AM040101	Score = 1023 bits (516), Expect = 0.0, Identities = 702/764 (91%)
LR4(9/16)	Bdellovibrio sp. Gunpowder	delta proteobacterium	AF084853	Score = 1562 bits (788), Expect = 0.0, Identities = 1149/1267 (90%)
LR27(11/4)	Bdellovibrio sp. W, strain ATCC 27047	delta proteobacterium	AJ292518	Score = 829 bits (418), Expect = 0.0, Identities = 725/820 (88%)
LR2(9/22)	Uncultured delta proteobacterium clone VHS-B3-63	delta proteobacterium	DQ394954	Score = 642 bits (324), Expect = 0.0, Identities = 516/577 (89%)
LR18(10/21)	Uncultured Desulfobacteraceae clone cLaKi-JM22	delta proteobacterium	AJ582686	Score = 908 bits (457), Expect = 0.0, Identities = 692/765 (90%)
LR19(10/21)	Uncultured Pelobacter sp. clone U3P26	delta proteobacterium	DQ173799	Score = 684 bits (345), Expect = 0.0, Identities = 478/521 (91%)
LR5(9/22)	Lysobacter brunescens	gamma proteobacterium	AB161360	Score = 1558 bits (788), Expect = 0.0, Identities = 846/866 (97%)
LR38(11/4)	Nostocoida limicola III strain Ben222	planctomycetes	AF244749	Score = 1055 bits (532), Expect = 0.0, Identities = 680/727 (93%)
LR1(9/22)	Planctomycetes sp.	planctomycetes	X81954	Score = 1548 bits (780), Expect = 0.0, Identities = 871/900 (96%)
LR24(11/4)	planctomycete clone AKYH674	planctomycetes	AY921762	Score = 898 bits (453), Expect = 0.0, Identities = 625/681 (91%)
LR23(10/21)	planctomycete clone CY0ARA030H03	planctomycetes	BX294821	Score = 888 bits (448), Expect = 0.0, Identities = 659/720 (91%)
LR10(9/22)	Planctomycetes GMD16E07	planctomycetes	AY162118	Score = 692 bits (349), Expect = 0.0, Identities = 588/665 (88%)
LR2(9/16)	Uncultured planctomycete clone B4	planctomycetes	AY266450	Score = 607 bits (306), Expect = 5e-170, Identities = 480/538 (89%)
LR2(10/21)	uncultured planctomycete clone GR61	planctomycetes	AY150894	Score = 862 bits (435), Expect = 0.0, Identities = 682/763 (89%)
LR10(10/21)	uncultured planctomycete clone: Y164	planctomycetes	AB116499	Score = 912 bits (460), Expect = 0.0, Identities = 665/728 (91%)
LR13(10/21)	Opitutur sp. VeSm13	verrucomicrobia	X99392	Score = 1146 bits (578), Expect = 0.0, Identities = 729/778 (93%)
LR21(10/21)	Opitutur sp. VeSm13	verrucomicrobia	X99392	Score = 1255 bits (633), Expect = 0.0, Identities = 741/777 (95%)
LR9(10/21)	Uncultured Verrucomicrobium sp. JAB SASS clone 103	verrucomicrobia	DQ084307	Score = 900 bits (454), Expect = 0.0, Identities = 646/703 (91%)

LR11(10/21)	Verrucomicrobia bacterium YNPRH34A	verrucomicrobia	AF465851	Score = 1047 bits (528), Expect = 0.0, Identities = 682/732 (93%)
LR28(11/4)	no match			
LR1(9/16)	no match			
LR31(11/4)	no match			

Appendix C. Results of GenBank sequence alignments for Smart Sponge® biofilm bacteria.

Sequence	Closest GenBank Taxon	Phylum	Accession	GenBank Score, Expect value, Identities
SS5(9/18)	Acidobacteria bacterium clone AKYG636	acidobacteria	AY922046	Score = 2109 bits (1064), Expect = 0.0, Identities = 1232/1280 (96%)
SS3(9/18)	Acidospaera rubrifaciens	alpha proteobacterium	D86512	Score = 2212 bits (1116), Expect = 0.0, Identities = 1375/1451 (94%)
SS5(10/21)	Erythrobacter sp. MBIC4117	alpha proteobacterium	AB035544	Score = 1423 bits (718), Expect = 0.0, Identities = 779/798 (97%)
SS21(9/22)	Ochrobactrum sp. R-25055	alpha proteobacterium	AM084018	Score = 595 bits (300), Expect = 9e-167, Identities = 428/470 (91%)
SS16(10/21)	Rhodovibrio sodomensis	alpha proteobacterium	AJ318524	Score = 767 bits (387), Expect = 0.0, Identities = 523/567 (92%)
SS26(11/4)	Sphingomonas adhaesiva	alpha proteobacterium	D16146	Score = 1031 bits (520), Expect = 0.0, Identities = 571/588 (97%)
SS18(10/21)	Flavobacterium ferrugineum	bacteroidetes	M62798	Score = 1009 bits (509), Expect = 0.0, Identities = 708/773 (91%)
SS8(10/21)	Flavobacterium ferrugineum	bacteroidetes	M62798	Score = 959 bits (484), Expect = 0.0, Identities = 701/772 (90%)
SS20(10/21)	Flexibacter sp. clone TM19_36	bacteroidetes	DQ279370	Score = 797 bits (402), Expect = 0.0, Identities = 540/586 (92%)
SS25(11/4)	Sphingobacteriales clone JAB SHC 57	bacteroidetes	AY694526	Score = 1007 bits (508), Expect = 0.0, Identities = 629/668 (94%)
SS15(10/21)	Uncultured Bacteroidetes clone AKYG501	bacteroidetes	AY921927	Score = 753 bits (380), Expect = 0.0, Identities = 623/704 (88%)
SS38(11/4)	Aquabacterium sp. clone DS130	beta proteobacterium	DQ234213	Score = 1471 bits (742), Expect = 0.0, Identities = 823/850 (96%)
SS6(10/21)	Aquabacterium sp. MG67	beta proteobacterium	AJ746124	Score = 1316 bits (664), Expect = 0.0, Identities = 750/776 (96%)
SS21(10/21)	beta proteobacterium clone LIUU-9-233	beta proteobacterium	AY509483	Score = 1106 bits (558), Expect = 0.0, Identities = 723/778 (92%)
SS27(11/4)	Hydrogenophaga pseudoflava	beta proteobacterium	AF078770	Score = 1598 bits (806), Expect = 0.0, Identities = 839/850 (98%)
SS30(11/4)	Ideonella sp. 0-0013	beta proteobacterium	AB211233	Score = 1473 bits (743), Expect = 0.0, Identities = 788/803 (98%)
SS4(9/22)	Ideonella sp. 0-0013	beta proteobacterium	AB211233	Score = 1368 bits (700), Expect = 0.0, Identities = 775/800 (96%)
SS12(10/21)	Leptothrix sp. MBIC3364	beta proteobacterium	AB015048	Score = 1322 bits (667), Expect = 0.0, Identities = 722/739 (97%)
SS9(10/21)	Methylophilus sp. ECd5	beta proteobacterium	AY436794	Score = 1304 bits (658), Expect = 0.0, Identities = 748/776 (96%)
SS33(11/4)	Rubrivivax gelatinosus strain OK303	beta proteobacterium	AF487435	Score = 1544 bits (779), Expect = 0.0, Identities = 830/847 (97%)
SS4(10/21)	Rubrivivax gelatinosus strain OK303	beta proteobacterium	AF487435	Score = 1146 bits (578), Expect = 0.0, Identities = 678/706 (95%)
SS35(11/4)	Uncultured Comamonadaceae clone M10Ba46	beta proteobacterium	AY360635	Score = 1516 bits (765), Expect = 0.0, Identities = 816/833 (97%)
SS3t(9/22)	Nannocystis exedens	delta proteobacterium	AB084253	Score = 1447 bits (730), Expect = 0.0, Identities = 781/798 (97%)
SS32(11/4)	uncultured delta proteobacterium clone F1	delta proteobacterium	DQ003171	Score = 654 bits (330), Expect = 0.0, Identities = 536/602 (89%)
SS1(9/18)	Uncultured delta proteobacterium clone KY221	delta proteobacterium	AB116509	Score = 1241 bits (626), Expect = 0.0, Identities = 897/985 (91%)
SS31(11/4)	Marinomonas sanya	gamma proteobacterium	AB242868	Score = 642 bits (324), Expect = 0.0, Identities = 516/580 (88%)
SS1t(9/22)	Nevskia ramosa	gamma proteobacterium	AJ001343	Score = 954 bits (481), Expect = 0.0, Identities = 655/709 (92%)
SS36(11/4)	Nevskia ramosa	gamma proteobacterium	AJ001010	Score = 1005 bits (507), Expect = 0.0, Identities = 691/751 (92%)
SS2(9/18)	Nevskia ramosa	gamma proteobacterium	AJ001343	Score = 1243 bits (627), Expect = 0.0, Identities = 934/1031 (90%)
SS11(10/21)	Uncultured Methylobacter sp. clone C22	gamma proteobacterium	AY090650	Score = 823 bits (415), Expect = 0.0, Identities = 454/467 (97%)
SS10(10/21)	Gemmatimonadetes bacterium clone AKYG1555	gemmatimonadetes	AY921894	Score = 955 bits (482), Expect = 0.0, Identities = 648/698 (92%)
SS10t(9/22)	Uncultured Gemmatimonadetes clone W2a-1F	gemmatimonadetes	AY192277	Score = 1124 bits (567), Expect = 0.0, Identities = 816/867 (91%)
SS2(10/21)	Nitrospira sp. strain GC86	nitrospirae	Y14844	Score = 1493 bits (753), Expect = 0.0, Identities = 791/803 (98%)
SS28(11/4)	Gemmata obscuriglobus	planctomycetes	X81957	Score = 783 bits (395), Expect = 0.0, Identities = 662/748 (88%)
SS4(9/18)	Pirellula sp.	planctomycetes	X81947	Score = 1695 bits (855), Expect = 0.0, Identities = 1332/1474 (90%)
SS5t(9/22)	Opitutus sp. VeSm13	verrucomicrobia	X89392	Score = 1562 bits (788), Expect = 0.0, Identities = 873/900 (97%)
SS19(10/21)	Uncultured Verrucomicrobia clone EB1106	verrucomicrobia	AY395425	Score = 712 bits (359), Expect = 0.0, Identities = 621/707 (87%)
SS6t(9/22)	Verrucomicrobia bacterium clone JAB SMS 44	verrucomicrobia	AY694594	Score = 839 bits (423), Expect = 0.0, Identities = 704/797 (88%)
SS7t(9/22)	Verrucomicrobia bacterium clone VC12	verrucomicrobia	AY211073	Score = 1356 bits (684), Expect = 0.0, Identities = 846/900 (94%)

SS9(9/22)	Verrucomicrobium sp.JAB SASS clone 103	verrucomicrobia	DQ084307	Score = 819 bits (413), Expect = 0.0, Identities = 547/589 (92%)
SS37(11/4)	no match			
SS24(11/4)	no match			
SS13(10/21)	no match			

Appendix D. Results of GenBank sequence alignments for stream rock biofilm bacteria.

Sequence	Closest GenBank Taxon	Phylum	Accession	GenBank Score, Expect value, Identities
SR4(9/22)	alpha proteobacterium clone AKYG694	alpha proteobacteria	AY921823	Score = 910 bits (459), Expect = 0.0, Identities = 588/631 (93%)
SR7(10/21)	Defluviobacter sp. s82	alpha proteobacteria	AY972458	Score = 1326 bits (669), Expect = 0.0, Identities = 769/801 (96%)
SR8(10/21)	Novosphingobium pentaromativorans strain US6-1	alpha proteobacteria	AF502400	Score = 1332 bits (672), Expect = 0.0, Identities = 768/800 (96%)
SR18(10/21)	Rhodobacter gluconicum	alpha proteobacteria	AB077986	Score = 1310 bits (661), Expect = 0.0, Identities = 772/801 (96%)
SR9(9/22)	Rhodobacter sp. TCR1 3	alpha proteobacteria	AB017796	Score = 1532 bits (773), Expect = 0.0, Identities = 870/901 (96%)
SR10(10/21)	Rhodovulum inexpectatum strain FRR-10T	alpha proteobacteria	AF465833	Score = 1215 bits (613), Expect = 0.0, Identities = 704/733 (96%)
SR8(9/22)	Sphingomonas sp. IC145	alpha proteobacteria	AB196253	Score = 1562 bits (788), Expect = 0.0, Identities = 873/900 (97%)
SR5(10/21)	Uncultured Sphingomonas sp. clone KL-2-4-7	alpha proteobacteria	AF408323	Score = 944 bits (476), Expect = 0.0, Identities = 683/751 (90%)
SR1(9/22)	Arcoella aquatica strain NO-502T	bacteroidetes	AJ535729	Score = 856 bits (331), Expect = 0.0, Identities = 466/511 (91%)
SR17(10/21)	Bacteroidetes bacterium clone LIUU-9-137	bacteroidetes	AY509313	Score = 460 bits (232), Expect = 3e-126, Identities = 346/384 (90%)
SR33(11/4)	Flavobacterium sp. CC-JY-6	bacteroidetes	DQ239767	Score = 1166 bits (631), Expect = 0.0, Identities = 748/802 (93%)
SR5(9/16)	Flexibacteraceae bacterium clone LIUU-3-217	bacteroidetes	AY509280	Score = 589 bits (297), Expect = 5e-165, Identities = 462/517 (89%)
SR34(11/4)	Uncultured Bacteroidetes clone Bih116	bacteroidetes	AJ318142	Score = 454 bits (229), Expect = 2e-124, Identities = 408/464 (87%)
SR2(9/16)	Uncultured Cytophaga sp. clone JTB251	bacteroidetes	AB015265	Score = 1441 bits (727), Expect = 0.0, Identities = 1133/1263 (89%)
SR5(9/22)	Uncultured Flexibacter sp. clone	bacteroidetes	DQ279370	Score = 1009 bits (509), Expect = 0.0, Identities = 740/817 (90%)
SR16(10/21)	Uncultured Flexibacter sp. clone TM19_36	bacteroidetes	DQ279370	Score = 1053 bits (531), Expect = 0.0, Identities = 708/767 (92%)
SR4(9/16)	Aquaspirillum delicatum	beta proteobacteria	AF078756	Score = 2409 bits (1215), Expect = 0.0, Identities = 1420/1484 (95%)
SR23(10/21)	Leptothrix discophora (strain SP-6)	beta proteobacteria	L33974	Score = 1493 bits (753), Expect = 0.0, Identities = 788/797 (98%)
SR7(9/22)	Rubrivivax gelatinosus	beta proteobacteria	D16213	Score = 1580 bits (797), Expect = 0.0, Identities = 869/893 (97%)
SR2(9/22)	Variovorax sp. HI-14	beta proteobacteria	DQ205307	Score = 1112 bits (561), Expect = 0.0, Identities = 648/677 (95%)
SR28(11/4)	Variovorax sp. KS2D-23	beta proteobacteria	AB198432	Score = 1419 bits (716), Expect = 0.0, Identities = 779/800 (97%)
SR37(11/4)	Chamaesiphon subglobosus PCC 7430	cyanobacteria	AY170472	Score = 1130 bits (570), Expect = 0.0, Identities = 770/836 (92%)
SR25(11/4)	cyanobacterium clone SIMO-845	cyanobacteria	AY712382	Score = 815 bits (411), Expect = 0.0, Identities = 471/491 (95%)
SR22(10/21)	Cyanothece sp. WH 8902	cyanobacteria	AY620238	Score = 1039 bits (524), Expect = 0.0, Identities = 732/796 (91%)
SR10(9/22)	Gloeotheca sp. clone TRK02	cyanobacteria	AY874086	Score = 1164 bits (587), Expect = 0.0, Identities = 828/903 (91%)
SR24(11/4)	Gloeotheca sp. clone TRK02	cyanobacteria	AY874086	Score = 1164 bits (587), Expect = 0.0, Identities = 828/903 (91%)
SR38(11/4)	Gloeotheca sp. clone TRK02	cyanobacteria	AY874086	Score = 1164 bits (587), Expect = 0.0, Identities = 828/903 (91%)
SR1(9/16)	Gloeotheca sp. SK40	cyanobacteria	AB067576	Score = 1499 bits (756), Expect = 0.0, Identities = 1203/1351 (89%)
SR30(11/4)	Leptolyngbya foveolarum	cyanobacteria	X84808	Score = 1090 bits (550), Expect = 0.0, Identities = 768/837 (91%)
SR31(11/4)	Leptolyngbya foveolarum	cyanobacteria	X84809	Score = 1090 bits (550), Expect = 0.0, Identities = 768/837 (91%)
SR4(10/21)	Leptolyngbya foveolarum	cyanobacteria	X84808	Score = 1041 bits (525), Expect = 0.0, Identities = 733/800 (91%)
SR21(10/21)	Oscillatoria spongeliae 39P1	cyanobacteria	AY615503	Score = 922 bits (465), Expect = 0.0, Identities = 677/745 (90%)
SR32(11/4)	Spirulina major OBB36S18	cyanobacteria	AJ839890	Score = 908 bits (458), Expect = 0.0, Identities = 698/774 (89%)
SR15(10/21)	Stanieria cyanosphaera	cyanobacteria	AF132931	Score = 1126 bits (568), Expect = 0.0, Identities = 731/780 (93%)
SR2(10/21)	Uncultured Stanieria sp. clone Sc02	cyanobacteria	DQ058832	Score = 920 bits (464), Expect = 0.0, Identities = 683/756 (90%)
SR20(10/21)	Melothermus cerbereus, strain GY-5	delta proteobacteria	Y13595	Score = 858 bits (432), Expect = 0.0, Identities = 540/576 (93%)
SR14(10/21)	Polyangium celulosum strain Sc02007-3	delta proteobacteria	AY252114	Score = 920 bits (464), Expect = 0.0, Identities = 718/800 (89%)
SR3(9/22)	Planctomyces sp. (strain 599)	planctomycetes	AJ231189	Score = 1209 bits (610), Expect = 0.0, Identities = 821/890 (92%)
SR9(10/21)	Uncultured Pietermaritzburg bacterium Y14-6	planctomycetes	AF312220	Score = 1348 bits (679), Expect = 0.0, Identities = 785/804 (97%)

SR6(10/21)	Opitutatus sp. VeGlc2	verrucomicrobia	X99390	Score = 920 bits (484), Expect = 0.0, Identities = 570/604 (94%)
SR3(9/16)	Uncultured Verrucomicrobia clone PIS48	verrucomicrobia	AY770729	Score = 1894 bits (1006), Expect = 0.0, Identities = 1225/1297 (94%)
SR36(11/4)		eukaryote		

Appendix E. Raw data for carbohydrate quantification.

Carbohydrate Quantification: EPS Extract 1						
standard	abs	abs-blk	ave			
Blank	0.004	0.0050	0.0000			
Blank	0.005					
Blank	0.006					
0.5ug/mL	0.013	0.0080	0.0080			
0.5	0.012	0.0070				
0.5	0.014	0.0090				
1	0.020	0.0150	0.0150			
1	0.020	0.0150				
1	0.020	0.0150				
6	0.081	0.0760	0.0760			
6	0.079	0.0740				
6	0.083	0.0780				
10	0.135	0.1300	0.1295			
10	0.134	0.1290				
sample	abs	abs-C	conc (ug/mL)	media correction	ave conc	stdev
C - A	0.005	0.006				
C - B	0.004					
C - C	0.010					
AF - A	0.030	0.024	1.77		1.88	0.33
AF - B	0.028	0.022	1.62			
AF - C	0.036	0.030	2.24			
LR - A	0.038	0.032	2.40	-5.57	-5.36	0.30
LR - B	0.039	0.033	2.48	-5.49		
LR - C	0.045	0.039	2.95	-5.02		
SR - A	0.073	0.067	5.14		5.30	0.34
SR - B	0.072	0.066	5.06			
SR - C	0.080	0.074	5.69			
SS - A	0.029	0.023	1.70		1.88	0.32
SS - B	0.029	0.023	1.70			
SS - C	0.036	0.030	2.24			

Appendix E. Raw data for carbohydrate quantification. (Continued)

Carbohydrate Quantification: EPS Extract #2						
standard	abs	ave abs	abs - blink			
Blank	0.006		0.0040	0.0000		
Blank	0.002					
Blank	0.004					
0.5 ug/mL	0.009		0.0103	0.0063		
0.5 ug/mL	0.011					
0.5 ug/mL	0.011					
1 ug/mL	0.020		0.0190	0.0150		
1 ug/mL	0.019					
1 ug/mL	0.018					
6 ug/mL	0.077		0.0843	0.0803		
6 ug/mL	0.085					
6 ug/mL	0.091					
10 ug/mL	0.128		0.1347	0.1307		
10 ug/mL	0.137					
10 ug/mL	0.139					
sample	abs	abs-C	conc (ug/mL)	media correction	ave	stdev
AF	0.036	0.0320	2.39		2.52	0.117
AF	0.038	0.0340	2.55			
AF	0.039	0.0350	2.62			
LR	0.070	0.0660	4.99	-2.98	-2.95	0.117
LR	0.072	0.0680	5.15	-2.82		
LR	0.069	0.0650	4.92	-3.05		
SR	0.260	0.2640	20.13		20.54	0.385
SR	0.274	0.2700	20.59			
SR	0.278	0.2740	20.90			
SS	0.042	0.0380	2.85		2.85	0.000
SS	0.042	0.0380	2.85			
SS	0.042	0.0380	2.85			
C	0.005	average C =				
C	0.002	0.0040				
C	0.005					

Appendix E. Raw data for carbohydrate quantification. (Continued)

Carbohydrate Quantification: EPS Extract #3						
standard	abs(625nm)	ave abs	abs - b1nk			
blank	0.000	0.002	0.0000			
blank	0.000					
blank	0.004					
blank	0.003					
0.5ug/mL	0.012	0.012	0.0106			
0.5ug/mL	0.013					
0.5ug/mL	0.012					
1ug/mL	0.017	0.017	0.0156			
1ug/mL	0.017					
1ug/mL	0.018					
6ug/mL	0.085	0.083	0.0809			
6ug/mL	0.081					
6ug/mL	0.082					
10ug/mL	0.134	0.131	0.1293			
10ug/mL	0.128					
10ug/mL	0.131					
sample	abs	abs-C	conc (ug/mL)	media correction	ave	stdev
AF	0.017	0.0123	0.76		0.61	0.143
AF	0.016	0.0113	0.69			
AF	0.013	0.0083	0.45			
AF	0.014	0.0093	0.53			
LR	0.042	0.0373	2.72	-5.25	-4.98	0.226
LR	0.049	0.0443	3.26	-4.71		
LR	0.046	0.0413	3.03	-4.94		
LR	0.045	0.0403	2.95	-5.02		
SS	0.016	0.0113	0.69		0.76	0.156
SS	0.016	0.0113	0.69			
SS	0.016	0.0113	0.69			
SS	0.020	0.0153	1.00			
C	0.003	average C = 0.0048				
C	0.004					
C	0.004					
C	0.008					

Appendix E. Raw data for carbohydrate quantification. (Continued)

Carbohydrate Quantification: Extract #1-P						
standard	abs(625nm)	abs-blk	ave			
blank	0.002	0.002	0			
blank	0.002					
blank	0.002					
0.5 ug/mL	0.011	0.009	0.0083			
0.5 ug/mL	0.010	0.008				
0.5 ug/mL	0.010	0.008				
1 ug/mL	0.016	0.014	0.0157			
1 ug/mL	0.020	0.018				
1 ug/mL	0.017	0.015				
6 ug/mL	0.083	0.081	0.0767			
6 ug/mL	0.074	0.072				
6 ug/mL	0.079	0.077				
10 ug/mL	0.135	0.133	0.1307			
10 ug/mL	0.125	0.123				
10 ug/mL	0.138	0.136				
sample	abs	abs-C	conc(ug/g)	media correction	ave	stdev
control	0.005	0.004				
control	0.003					
control	0.004					
AF	0.042	0.0380	2.86		3.01	0.27
AF	0.048	0.0440	3.32			
AF	0.042	0.0380	2.86			
LR	0.101	0.0970	7.44	-0.53	-0.40	0.16
LR	0.105	0.1010	7.76	-0.21		
LR	0.102	0.0980	7.52	-0.45		
SS	0.026	0.0220	1.61		1.54	0.08
SS	0.024	0.0200	1.46			
SS	0.025	0.0210	1.54			

Appendix E. Raw data for carbohydrate quantification. (Continued)

Carbohydrate Quantification: EPS Extract #2-P						
standard	abs	abs-blk	ave			
blank	0.007	0.0077	0.0000			
blank	0.006					
blank	0.010					
0.5 ug/mL	0.011	0.0033	0.0057			
0.5 ug/mL	0.013	0.0053				
0.5 ug/mL	0.016	0.0083				
1 ug/mL	0.017	0.0093	0.0100			
1 ug/mL	0.015	0.0073				
1 ug/mL	0.021	0.0133				
6 ug/mL	0.068	0.0603	0.0600			
6 ug/mL	0.062	0.0543				
6 ug/mL	0.073	0.0653				
10 ug/mL	0.110	0.1023	0.1060			
10 ug/mL	0.109	0.1013				
10 ug/mL	0.122	0.1143				
sample	abs	abs-C	conc(ug/mL)	media correction	ave	stdev
control	0.007	0.0067				
control	0.006					
control	0.007					
AF	0.021	0.0143	1.40		1.53	0.11
AF	0.023	0.0163	1.60			
AF	0.023	0.0163	1.60			
LR	0.086	0.0793	7.60	-0.37	-0.12	0.24
LR	0.089	0.0823	7.88	-0.09		
LR	0.091	0.0843	8.07	0.10		
SS	0.026	0.0193	1.88		2.10	0.24
SS	0.031	0.0243	2.36			
SS	0.028	0.0213	2.07			

Appendix E. Raw data for carbohydrate quantification. (Continued)

Carbohydrate Quantification: EPS Extract #3-P						
standard	abs	abs-blk	ave			
blank	0.007	0.0067	0.0000			
blank	0.007					
blank	0.006					
0.5 ug/mL	0.014	0.0073	0.0067			
0.5 ug/mL	0.013	0.0063				
0.5 ug/mL	0.013	0.0063				
1 ug/mL	0.019	0.0123	0.0143			
1 ug/mL	0.026	0.0193				
1 ug/mL	0.018	0.0113				
6 ug/mL	0.080	0.0733	0.0730			
6 ug/mL	0.079	0.0723				
6 ug/mL	0.080	0.0733				
10 ug/mL	0.131	0.1243	0.1230			
10 ug/mL	0.130	0.1233				
10 ug/mL	0.128	0.1213				
sample	abs	abs-C	conc(ug/mL)	media correction	ave	stdev
control	0.012	0.011				
control	0.01					
control	0.011					
AF	0.022	0.011	0.84		1.20	0.62
AF	0.035	0.024	1.91			
AF	0.022	0.011	0.84			
LR	0.049	0.038	3.06	-4.91	-4.89	0.13
LR	0.051	0.04	3.22	-4.75		
LR	0.048	0.037	2.97	-5.00		
SS	0.021	0.01	0.76		0.81	0.09
SS	0.023	0.012	0.92			
SS	0.021	0.01	0.76			

Appendix F. Raw data for protein quantification.

Protein Quantification: EPS Extract 1

blank - a	0.023	0.0227	0.0000
blank - b	0.023		
blank - c	0.022		
0.98 - a	0.025	0.0023	0.0027
0.98 - b	0.026	0.0033	
0.98 - c	0.025	0.0023	
1.9 - a	0.029	0.0063	0.0060
1.9 - b	0.029	0.0063	
1.9 - c	0.028	0.0053	
7.8 - a	0.048	0.0253	0.0250
7.8 - b	0.048	0.0253	
7.8 - c	0.047	0.0243	
31.25 - a	0.145	0.1223	0.1200
31.25 - b	0.143	0.1203	
31.25 - c	0.14	0.1173	
125 - a	0.419	0.3963	0.3983
125 - b	0.423	0.4003	

sample	abs	abs-C	conc	media correction	ave	stdev
AF - a	0.044	0.021	5.76		5.76	0.00
AF - b	0.044	0.021	5.76			
AF - c	0.044	0.021	5.76			
LR - a	0.038	0.015	3.88	-2.69	-2.58	0.18
LR - b	0.038	0.015	3.88	-2.69		
LR - c	0.039	0.016	4.19	-2.38		
SR - a	0.072	0.049	14.50		15.02	0.65
SR - b	0.073	0.050	14.81			
SR - c	0.076	0.053	15.75			
SS - a	0.049	0.026	7.32		7.84	0.48
SS - b	0.051	0.028	7.94			
SS - c	0.052	0.029	8.25			
C - a	0.022	0.023				
C - b	0.023					
C - c	0.023					

Appendix F. Raw data for protein quantification. (Continued)

Protein Quantification: EPS Extract #2						
Standard	abs	ave abs	ave - blk	stdev		
blank	0.025	0.0207	0.0000	0.005		
blank	0.021					
blank	0.016					
0.98 ug/mL	0.026	0.0227	0.0020	0.004		
0.98 ug/mL	0.024					
0.98 ug/mL	0.018					
1.9 ug/mL	0.027	0.0247	0.0040	0.004		
1.9 ug/mL	0.027					
1.9 ug/mL	0.020					
7.8 ug/mL	0.037	0.0337	0.0130	0.004		
7.8 ug/mL	0.035					
7.8 ug/mL	0.029					
31.25 ug/mL	0.089	0.0900	0.0693	0.001		
31.25 ug/mL	0.091					
31.25 ug/mL	0.090					
Sample	abs	abs - C	conc ug/mL	media correction	ave	stdev
AF	0.049	0.0293	13.61		11.83	1.79
AF	0.045	0.0253	11.83			
AF	0.041	0.0213	10.04			
LR	0.052	0.0323	14.96	8.39	8.98	1.44
LR	0.057	0.0373	17.19	10.62		
LR	0.051	0.0313	14.51	7.94		
SR	0.142	0.1223	55.20		53.86	2.72
SR	0.143	0.1233	55.64			
SR	0.132	0.1123	50.73			
SS	0.063	0.0433	19.87		19.58	0.52
SS	0.061	0.0413	18.98			
SS	0.063	0.0433	19.87			
C	0.024	average C =				
C	0.022	0.0197				
C	0.013					

Appendix F. Raw data for protein quantification. (Continued)

Protein quantification: EPS extract #3			
standard	abs	ave abs	abs - blink
blank	0.023	0.0237	0.0000
	0.024		
	0.024		
0.98	0.027	0.0263	0.0027
	0.026		
	0.026		
1.95	0.030	0.0293	0.0057
	0.029		
	0.029		
7.81	0.042	0.0467	0.0230
	0.049		
	0.049		
31.25	0.135	0.1380	0.1143
	0.138		
	0.141		

sample	abs	abs - C	conc (ug/mL)	media correction	ave	stdev
AF	0.036	0.0117	3.67		3.76	0.413
	0.038	0.0137	4.21			
	0.035	0.0107	3.40			
LR	0.057	0.0327	9.35	2.78	2.69	0.413
	0.055	0.0307	8.81	2.24		
	0.058	0.0337	9.62	3.05		
SS	0.043	0.0187	5.57		5.66	0.156
	0.044	0.0197	5.84			
	0.043	0.0187	5.57			
control	0.025	average C = 0.0243				
	0.024					
	0.024					

Appendix F. Raw data for protein quantification. (Continued)

Protein Quantification: EPS Extract #1-P						
sample	abs	abs-blank	ave			
blank	0.023	0.0232	0.0000			
blank	0.021					
blank	0.023					
blank	0.025					
blank	0.024					
0.98 ug/mL	0.027	0.0038	0.0041			
0.98 ug/mL	0.027	0.0038				
0.98 ug/mL	0.028	0.0048				
1.95 ug/mL	0.033	0.0098	0.0105			
1.95 ug/mL	0.033	0.0098				
1.95 ug/mL	0.035	0.0118				
7.81 ug/mL	0.050	0.0268	0.0281			
7.81 ug/mL	0.051	0.0278				
7.81 ug/mL	0.053	0.0298				
31.25 ug/mL	0.137	0.1138	0.1153			
31.25 ug/mL	0.140	0.1168				
sample	abs	abs - C	conc (ug/mL)	media correction	ave	stdev
control	0.022	0.0237				
control	0.022					
control	0.027					
AF	0.049	0.0253	6.83		6.73	0.432
AF	0.047	0.0233	6.26			
AF	0.050	0.0263	7.11			
LR	0.080	0.0563	15.58	9.01	10.61	1.450
LR	0.090	0.0663	18.41	11.84		
LR	0.087	0.0633	17.56	10.99		
SS	0.045	0.0213	5.70		5.70	0.565
SS	0.043	0.0193	5.13			
SS	0.047	0.0233	6.26			

Appendix F. Raw data for protein quantification. (Continued)

Protein Quantification: EPS Extract #2-P						
standard	abs	abs-blank	ave			
blank	0.023	0.0237	0.0000			
blank	0.024					
blank	0.024					
0.98 ug/mL	0.027	0.0033	0.0037			
0.98 ug/mL	0.027	0.0033				
0.98 ug/mL	0.028	0.0043				
1.95 ug/mL	0.030	0.0063	0.0070			
1.95 ug/mL	0.031	0.0073				
1.95 ug/mL	0.031	0.0073				
7.81 ug/mL	0.052	0.0283	0.0300			
7.81 ug/mL	0.053	0.0293				
7.81 ug/mL	0.056	0.0323				
31.25 ug/mL	0.143	0.1193	0.1223			
31.25 ug/mL	0.148	0.1243				
31.25 ug/mL	0.147	0.1233				
sample	abs	abs-C	conc (ug/mL)	media correction	ave	stdev
control	0.026	0.0253				
control	0.025					
control	0.025					
AF	0.040	0.0147	3.83		4.25	0.39
AF	0.042	0.0167	4.34			
AF	0.043	0.0177	4.59			
LR	0.090	0.0647	16.57	10.00	9.75	0.25
LR	0.088	0.0627	16.06	9.49		
LR	0.089	0.0637	16.32	9.75		
SS	0.050	0.0247	6.38		6.97	0.64
SS	0.052	0.0267	6.89			
SS	0.055	0.0297	7.65			

Appendix F. Raw data for protein quantification. (Continued)

Protein Quantification: EPS Extract #3-P						
standard	abs	abs-blk	ave			
blank	0.026	0.0267	0.0000			
blank	0.026					
blank	0.028					
0.98 ug/mL	0.030	0.0033	0.0033			
0.98 ug/mL	0.029	0.0023				
0.98 ug/mL	0.031	0.0043				
1.95 ug/mL	0.031	0.0043	0.0053			
1.95 ug/mL	0.032	0.0053				
1.95 ug/mL	0.033	0.0063				
7.81 ug/mL	0.053	0.0263	0.0293			
7.81 ug/mL	0.055	0.0283				
7.81 ug/mL	0.060	0.0333				
31.25 ug/mL	0.140	0.1133	0.1153			
31.25 ug/mL	0.140	0.1133				
31.25 ug/mL	0.146	0.1193				
sample	abs	abs-C	conc (ug/mL)	media correction	ave	stdev
control	0.028	0.0277				
control	0.028					
control	0.027					
AF	0.046	0.0183	5.08		5.34	0.269
AF	0.047	0.0193	5.34			
AF	0.048	0.0203	5.61			
LR	0.062	0.0343	9.39	2.82	3.35	0.467
LR	0.065	0.0373	10.19	3.62		
LR	0.065	0.0373	10.19	3.62		
SS	0.049	0.0213	5.88		5.70	1.089
SS	0.052	0.0243	6.69			
SS	0.044	0.0163	4.54			

Appendix G. Data for hydrophobic interaction chromatography of carbohydrates in EPS.

Carbohydrate - extract 1

sample	Concentration (ug/mL)	ratio	average ratio	1/2 stdev
AF	0.94			
AF A	1.00	1.07	0.814	0.116
AF B	0.58	0.62		
AF C	0.71	0.75		
LR	0.49			
LR A	1.14	2.31	2.163	0.067
LR B	1.01	2.07		
LR C	2.14	2.11		
SS	1.73			
SS A	1.43	0.83	0.833	0.050
SS B	1.61	0.93		
SS C	1.27	0.73		
AF-P	1.75			
AF-P A	1.56	0.89	0.986	0.093
AF-P B	1.52	0.87		
AF-P C	2.11	1.20		
LR-P	-0.94			
LR-P A	-0.40			
LR-P B	-0.08			
LR-P C	-0.06			
SS-P	2.22			
SS-P A	2.28	1.03	1.220	0.136
SS-P C	3.14	1.41		

Carbohydrate - extract 2

sample	Concentration (ug/mL)	ratio	average ratio	1/2 stdev
AF	1.67			
AF-A	1.94	1.16	1.049	0.051
AF-B	1.61	0.96		
AF-C	1.72	1.03		
LR	0.45			
LR-A	1.16	2.58	1.674	0.439
LR-B	0.37	0.82		
LR-C	0.73	1.62		
SS	1.88			
SS-A	1.87	1.00	1.102	0.086
SS-B	2.45	1.30		
SS-C	1.90	1.01		
AFP	3.35			
AFP-A	2.91	0.87	0.880	0.008
AFP-B	2.91	0.87		
AFP-C	3.01	0.90		
LRP	0.27			
LRP-A	-0.67			
LRP-B	-0.54			
LRP-C	-0.24			
SSP	1.55			
SSP-A	1.38	0.89	0.908	0.037
SSP-B	1.53	0.99		
SSP-C	1.30	0.84		

Appendix H. Data for hydrophobic interaction chromatography of proteins in EPS.

Protein - extract 2

sample	Concentration (ug/mL)	ratio	ave	1/2 stdev
AF	6.42			
AF A	4.85	0.76	0.776	0.013
AF B	5.17	0.81		
AF C	4.91	0.77		
LR	4.65			
LR A	4.07	0.88	0.892	0.010
LR B	4.12	0.89		
LR C	4.25	0.91		
SS	7.05			
SS A	5.38	0.76	0.956	0.112
SS B	8.46	1.20		
SS C	6.37	0.90		
AF-P	9.70			
AF-P A	7.72	0.80	0.803	0.019
AF-P B	7.47	0.77		
AF-P C	8.19	0.84		
LR-P	7.05			
LR-P A	6.30	0.89	0.937	0.022
LR-P B	6.61	0.94		
LR-P C	6.92	0.98		
SS-P	5.91			
SS-P A	4.79	0.81	0.841	0.021
SS-P B	5.26	0.89		
SS-P C	4.87	0.82		

Protein - extract 1

sample	Concentration (ug/mL)	ratio	average	1/2 stdev
AF	4.40			
AF A	2.90	0.66	0.749	0.041
AF B	3.58	0.81		
AF C	3.42	0.78		
LR	1.45			
LR A	1.45	1.00	1.027	0.013
LR B	1.48	1.02		
LR C	1.53	1.06		
SS	6.62			
SS A	5.48	0.83	0.879	0.032
SS B	5.67	0.86		
SS C	6.30	0.95		
AF-P	6.15			
AF-P A	4.43	0.72	0.794	0.033
AF-P B	5.06	0.82		
AF-P C	5.18	0.84		
LR-P	4.60			
LR-P A	4.04	0.88	0.883	0.011
LR-P B	4.18	0.91		
LR-P C	3.97	0.86		
SS-P	8.14			
SS-P A	7.88	0.97	0.925	0.030
SS-P C	7.18	0.88		

Appendix I. Raw data and calculations for nutrient removal test.

NITRITE media with biofilm					
sample	abs	conc(ug/mL)	removal	ave	stdev
Initial	0.154	0.15			
AF1	0.136	0.13	11.2%	9.2%	0.022
AF2	0.144	0.14	6.2%		
AF4	0.137	0.14	10.6%		
AF5	0.140	0.14	8.7%		
LR1	0.150	0.15	2.5%	9.7%	0.107
LR3	0.144	0.14	6.2%		
LR4	0.147	0.14	4.4%		
LR5	0.113	0.11	25.5%		
SS1	0.150	0.15	2.5%	1.4%	0.027
SS2	0.147	0.14	4.4%		
SS3	0.157	0.15	-1.9%		
SS4	0.153	0.15	0.6%		

NITRITE new media					
sample	abs	conc(ug/mL)	removal	ave	stdev
Initial	0.340	0.15			
AF-new 1	0.351	0.15	-2.9%	-2.1%	0.012
AF-new 2	0.345	0.15	-1.2%		
LR-new 1	0.344	0.15	-0.9%	-2.5%	0.022
LR-new 2	0.355	0.15	-4.1%		
SS-new 1	0.331	0.14	2.7%	0.5%	0.032
SS-new 2	0.347	0.15	-1.8%		

Explanation of abbreviations:

AF: aquarium filter, LR: lava rock, SS: Smart Sponge®, #: biofilter label, abs: spectrophotometric absorbance, conc: concentration, ave: average, stdev: standard deviation.

AMMONIA media with biofilm					
sample	abs	conc(ug/mL)	removal	ave	stdev
Initial	0.172	0.64			
AF1	0.081	0.30	53.3%	25.3%	0.260
AF2	0.167	0.62	2.9%		
AF4	0.166	0.62	3.5%		
AF5	0.101	0.37	41.6%		
LR1	0.146	0.54	15.2%	18.2%	0.088
LR3	0.121	0.45	29.9%		
LR4	0.140	0.52	18.8%		
LR5	0.157	0.58	8.8%		
SS1	0.145	0.54	15.8%	13.3%	0.055
SS2	0.138	0.51	19.9%		
SS3	0.158	0.59	8.2%		
SS4	0.156	0.58	9.4%		

AMMONIA new media					
sample	abs	conc(ug/mL)	removal	average	stdev
Initial	0.324	1.23			
AF-new 1	0.339	1.29	-4.7%	-4.9%	0.002
AF-new 2	0.340	1.29	-5.0%		
LR-new 1	0.292	1.11	10.1%	10.6%	0.007
LR-new 2	0.289	1.10	11.0%		
SS-new 1	0.333	1.27	-2.8%	-1.7%	0.016
SS-new 2	0.326	1.24	-0.6%		

Appendix I. Raw data and calculations for nutrient removal test. (Continued)

PHOSPHATE media with biofilm

sample	abs	conc(ug/mL)	removal	ave	stdev
Initial	0.074	0.27			
AF1	0.020	0.06	77.5%	50.2%	0.403
AF2	0.079	0.29	-7.2%		
AF4	0.038	0.13	51.7%		
AF5	0.019	0.06	79.0%		
LR1	0.062	0.22	17.2%	15.4%	0.184
LR3	0.047	0.16	38.8%		
LR4	0.078	0.28	-5.7%		
LR5	0.066	0.24	11.5%		
SS1	0.068	0.25	8.6%	5.4%	0.052
SS2	0.075	0.27	-1.4%		
SS3	0.067	0.24	10.0%		
SS4	0.071	0.26	4.3%		

PHOSPHATE

new media

sample	abs	conc(ug/mL)	init ave	removal	ave	stdev
Initial 1	0.115	0.44	0.43			
Initial 2	0.111	0.43				
AF-new 1	0.112	0.43		0.9%	0.5%	0.007
AF-new 2	0.113	0.43		0.0%		
LR-new 1	0.109	0.42		3.7%	4.2%	0.007
LR-new 2	0.108	0.41		4.7%		
SS-new 1	0.109	0.42		3.7%	1.4%	0.033
SS-new 2	0.114	0.44		-0.9%		

Appendix J. Raw data and calculations for particle capture test.

Particle capture - optical density

Sample	Initial %T	final %T	change	% change	ave	stdev
AF 1	84.7	89.2	4.5	0.053	0.044	0.024
AF 2	79.4	81.1	1.7	0.021		
AF 3	80.3	84.0	3.7	0.046		
AF 4	81.5	83.2	1.7	0.021		
AF 5	79.7	86.0	6.3	0.079		
LR 1	85.5	87.0	1.5	0.018	0.029	0.010
LR 2	76.8	79.3	2.5	0.033		
LR 3	79.0	81.5	2.5	0.032		
LR 4	80.5	83.9	3.4	0.042		
LR 5	80.0	81.7	1.7	0.021		
SS 1	86.0	89.0	3.0	0.035	0.022	0.020
SS 2	76.4	79.7	3.3	0.043		
SS 3	81.0	82.2	1.2	0.015		
SS 4	80.4	79.7	-0.7	-0.009		
SS 5	80.1	82.2	2.1	0.026		

Particle capture - weight of solids

Sample	Initial solids	final solids	change	%capture	ave	stdev
AF 1	0.0371	0.0266	0.0105	28.3	42.4	13.0
AF 2	0.0636	0.0419	0.0217	34.1		
AF 3	0.0497	0.0241	0.0256	51.5		
AF 4	0.0491	0.0304	0.0187	38.1		
AF 5	0.0529	0.0212	0.0317	59.9		
LR 1	0.0389	0.0310	0.0079	20.3	21.7	2.1
LR 2	0.0713	0.0541	0.0172	24.1		
LR 3	0.0636	0.0484	0.0152	23.9		
LR 4	0.0467	MD				
LR 5	0.0445	0.0362	0.0083	18.7		
SS 1	0.0289	0.0226	0.0063	21.8	27.8	7.3
SS 2	0.1164	0.0770	0.0394	33.8		
SS 3	0.0497	0.0311	0.0186	37.4		
SS 4	0.0491	0.0386	0.0105	21.4		
SS 5	0.0445	0.0335	0.0110	24.7		

Appendix K. Raw data and calculations for bioavailability of PAHs on sediment.

	day 5				day 7				day 11			
	CPM	DPM	DPM-blnc	%C	count	DPM	DPM-blnc	%C	count	DPM	DPM-blnc	%C
Phen - 1	73	75.3	0.2		71	73.2	-1.9		81	83.5	-13.4	
Phen - 2	71	73.2	-1.9		81	83.5	8.5		71	73.2	-23.7	
Phen - 3	84	86.6	11.5		60	61.9	-13.2		78	80.4	-16.5	
Phen + 1	2551	2629.9	2554.8	2.8	4168	4296.9	4221.9	4.6	11517	11873.2	11776.3	12.7
Phen + 2	2858	2946.4	2871.3	3.1	5093	5250.5	5175.5	5.6	13338	13750.5	13653.6	14.7
Phen + 3	1745	1799.0	1723.9	1.9	3396	3501.0	3426.0	3.7	12022	12393.8	12296.9	13.3
Pyr - 1	81	83.5	8.5		80	82.5	7.4		76	78.4	-18.6	
Pyr - 2	57	58.8	-16.3		61	62.9	-12.2		55	56.7	-40.2	
Pyr - 3	67	69.1	-6.0		64	66.0	-9.1		66	68.0	-28.9	
Pyr + 1	42	43.3	-31.8	0.0	58	59.8	-15.3	0.0	143	147.4	50.5	0.1
Pyr + 2	47	48.5	-26.6	0.0	72	74.2	-0.8	0.0	124	127.8	30.9	0.0
Pyr + 3	50	51.5	-23.5	0.0	73	75.3	0.2	0.0	134	138.1	41.2	0.0
Ph/Py - 1	62	63.9	-11.1		64	66.0	-9.1		57	58.8	-38.1	
Ph/Py - 2	67	69.1	-6.0		69	71.1	-3.9		75	77.3	-19.6	
Ph/Py - 3	84	86.6	11.5		78	80.4	5.4		76	78.4	-18.6	
Ph/Py + 1	434	447.4	372.4	0.4	924	952.6	877.5	1.0	4200	4329.9	4233.0	4.7
Ph/Py + 2	805	829.9	754.8	0.8	1525	1572.2	1497.1	1.7	4589	4730.9	4634.0	5.2
Ph/Py + 3	630	649.5	574.4	0.6	1295	1335.1	1260.0	1.4	5622	5795.9	5699.0	6.4
Blnk	72.8	75.1			72.8	75.1			94	96.9		
Bkg	18	18.6			25	25.8			27	27.8		
C-14 UNQ	95995	98963.9			95655	98613.4			94684	97612.4		

Explanations of abbreviations:

Phen: phenanthrene, Pyr: pyrene, Ph/Py: phenanthrene and pyrene, +: with biofilm, -: sterile, 1,2,3: replicates, CPM: counts per minute, DPM: disintegrations per minute, %C: percent PAH carbon respired, blnc: scintillation cocktail only, bkg: background, C-14 UNQ: unquenched carbon-14 standard, MD: missing data.

Appendix K. Raw data and calculations for bioavailability of PAHs on sediment. (Continued)

	day 15				day 19				day 22			
	CPM	DPM	DPM-blk	%C	CPM	DPM	DPM-blk	%C	CPM	DPM	DPM-blk	%C
Phen - 1	64	66.0	-9.3		94	96.9	21.6		65	67.0	2.1	
Phen - 2	73	75.3	0.0		70	72.2	-3.1		76	78.4	13.4	
Phen - 3	73	75.3	0.0		MD	MD	MD		39	40.2	-24.7	
Phen + 1	7868	8111.3	8036.1	8.7	4695	4840.2	4764.9	5.1	2295	2366.0	2301.0	2.5
Phen + 2	8179	8432.0	8356.7	9.0	5070	5226.8	5151.5	5.6	2218	2286.6	2221.6	2.4
Phen + 3	8805	9077.3	9002.1	9.7	6328	6523.7	6448.5	7.0	2995	3087.6	3022.7	3.3
Pyr - 1	50	51.5	-23.7		79	81.4	6.2		72	74.2	9.3	
Pyr - 2	81	83.5	8.2		75	77.3	2.1		69	71.1	6.2	
Pyr - 3	67	69.1	-6.2		61	62.9	-12.4		75	77.3	12.4	
Pyr + 1	765	788.7	713.4	0.8	537	553.6	478.4	0.6	372	383.5	318.6	0.4
Pyr + 2	354	364.9	289.7	0.3	584	602.1	526.8	0.6	368	379.4	314.4	0.4
Pyr + 3	516	532.0	456.7	0.5	538	554.6	479.4	0.6	230	237.1	172.2	0.2
Ph/Py - 1	61	62.9	-12.4		78	80.4	5.2		84	86.6	21.6	
Ph/Py - 2	50	51.5	-23.7		65	67.0	-8.2		68	70.1	5.2	
Ph/Py - 3	62	63.9	-11.3		72	74.2	-1.0		MD	MD	MD	
Ph/Py + 1	4567	4708.2	4633.0	5.2	3997	4120.6	4045.4	4.5	1930	1989.7	1924.7	2.1
Ph/Py + 2	5120	5278.4	5203.1	5.8	3744	3859.8	3784.5	4.2	1738	1791.8	1726.8	1.9
Ph/Py + 3	6830	7041.2	6966.0	7.8	3890	4010.3	3935.1	4.4	1493	1539.2	1474.2	1.6
Blnk	73	75.3			73	75.3			63	64.9		
Bkg	26	26.8			44	45.4			14	14.4		
C-14 UNQ	95159	98102.1			95402	98352.6			94812	97744.3		

Appendix K. Raw data and calculations for bioavailability of PAHs on sediment. (Continued)

	day 27				day 32				day 39			
	CPM	DPM	DPM-blk	%C	CPM	DPM	DPM-blk	%C	CPM	DPM	DPM-blk	%C
Phen - 1	77	79.4	12.4		78	80.4	9.3		86	88.7	22.7	
Phen - 2	66	68.0	1.0		65	67.0	-4.1		79	81.4	15.5	
Phen - 3	73	75.3	8.2		98	101.0	29.9		71	73.2	7.2	
Phen + 1	4753	4900.0	4833.0	5.2	4048	4173.2	4102.1	4.4	3828	3946.4	3880.4	4.2
Phen + 2	4814	4962.9	4895.9	5.3	4971	5124.7	5053.6	5.4	5008	5162.9	5096.9	5.5
Phen + 3	3947	4069.1	4002.1	4.3	3919	4040.2	3969.1	4.3	5105	5262.9	5196.9	5.6
Pyr - 1	82	84.5	17.5		78	80.4	9.3		74	76.3	10.3	
Pyr - 2	58	59.8	-7.2		81	83.5	12.4		75	77.3	11.3	
Pyr - 3	53	54.6	-12.4		76	78.4	7.2		56	57.7	-8.2	
Pyr + 1	1542	1589.7	1522.7	1.8	2934	3024.7	2953.6	3.4	5346	5511.3	5445.4	6.3
Pyr + 2	753	776.3	709.3	0.8	1651	1702.1	1630.9	1.9	4961	5114.4	5048.5	5.8
Pyr + 3	997	1027.8	960.8	1.1	3737	3852.6	3781.4	4.4	9228	9513.4	9447.4	10.9
Ph/Py - 1	61	62.9	-4.1		83	85.6	14.4		61	62.9	-3.1	
Ph/Py - 2	60	61.9	-5.2		77	79.4	8.2		63	64.9	-1.0	
Ph/Py - 3	62	63.9	-3.1		48	49.5	-21.6		73	75.3	9.3	
Ph/Py + 1	3283	3384.5	3317.5	3.7	4063	4188.7	4117.5	4.6	5457	5625.8	5559.8	6.2
Ph/Py + 2	2773	2858.8	2791.8	3.1	4139	4267.0	4195.9	4.7	6878	7090.7	7024.7	7.8
Ph/Py + 3	2704	2787.6	2720.6	3.0	3830	3948.5	3877.3	4.3	5382	5548.5	5482.5	6.1
Blnk	65	67.0			69	71.1			64	66.0		
Bkg	20	20.6			16	16.5			14	14.4		
C-14 UNQ	95790	98752.6			95215	98159.8			94841	97774.2		

Appendix K. Raw data and calculations for bioavailability of PAHs on sediment. (Continued)

	day 50					day 60					day 69				
	CPM	DPM	DPM-blink	%C		CPM	DPM	DPM-blink	%C		CPM	DPM	DPM-blink	%C	
Phen - 1	51	52.6	2.1			63	64.9	16.5			71	73.2	7.2		
Phen - 2	48	49.5	-1.0			56	57.7	9.3			66	68.0	2.1		
Phen - 3	43	44.3	-6.2			48	49.5	1.0			80	82.5	16.5		
Phen + 1	3265	3366.0	3315.5	3.6		2858	2946.4	2897.9	3.1		2194	2261.9	2195.9	2.4	
Phen + 2	5497	5667.0	5616.5	6.1		3712	3826.8	3778.4	4.1		2617	2697.9	2632.0	2.8	
Phen + 3	5007	5161.9	5111.3	5.5		3210	3309.3	3260.8	3.5		2669	2751.5	2685.6	2.9	
Pyr - 1	45	46.4	-4.1			60	61.9	13.4			68	70.1	4.1		
Pyr - 2	40	41.2	-9.3			42	43.3	-5.2			63	64.9	-1.0		
Pyr - 3	39	40.2	-10.3			52	53.6	5.2			61	62.9	-3.1		
Pyr + 1	12248	12626.8	12576.3	14.5		8067	8316.5	8268.0	9.5		3986	4109.3	4043.3	4.7	
Pyr + 2	13479	13895.9	13845.4	16.0		7630	7866.0	7817.5	9.0		3829	3947.4	3881.4	4.5	
Pyr + 3	11805	12170.1	12119.6	14.0		5730	5907.2	5858.8	6.8		3141	3238.1	3172.2	3.7	
Ph/Py - 1	41	42.3	-8.2			45	46.4	-2.1			74	76.3	10.3		
Ph/Py - 2	55	56.7	6.2			33	34.0	-14.4			73	75.3	9.3		
Ph/Py - 3	33	34.0	-16.5			42	43.3	-5.2			57	58.8	-7.2		
Ph/Py + 1	6123	6312.4	6261.9	7.0		3692	3806.2	3757.7	4.2		2471	2547.4	2481.4	2.8	
Ph/Py + 2	8887	9161.9	9111.3	10.2		4706	4851.5	4803.1	5.4		3225	3324.7	3258.8	3.6	
Ph/Py + 3	7078	7296.9	7246.4	8.1		4138	4266.0	4217.5	4.7		2913	3003.1	2937.1	3.3	
Blink	49	50.5				47	48.5				64	66.0			
Bkg	15	15.5				24	24.7				24	24.7			
C-14 UNQ	95313	98260.8				95430	98381.4				95219	98163.9			

Appendix K. Raw data and calculations for bioavailability of PAHs on sediment. (Continued)

	day 78					day 88					day 98				
	CPM	DPM	DPM-blink	%C		CPM	DPM	DPM-blink	%C		CPM	DPM	DPM-blink	%C	
Phen - 1	41	42.3	16.5			57	58.8	-5.2			82	84.5	4.1		
Phen - 2	37	38.1	12.4			42	43.3	-20.6			62	63.9	-16.5		
Phen - 3	48	49.5	23.7			49	50.5	-13.4			72	74.2	-6.2		
Phen + 1	2124	2189.7	2163.9	2.3		2064	2127.8	2063.9	2.2		1839	1895.9	1815.5	2.0	
Phen + 2	2332	2404.1	2378.4	2.6		2263	2333.0	2269.1	2.4		1854	1911.3	1830.9	2.0	
Phen + 3	1993	2054.6	2028.9	2.2		1692	1744.3	1680.4	1.8		1617	1667.0	1586.6	1.7	
Pyr - 1	30	30.9	5.2			50	51.5	-12.4			62	63.9	-16.5		
Pyr - 2	35	36.1	10.3			53	54.6	-9.3			58	59.8	-20.6		
Pyr - 3	28	28.9	3.1			46	47.4	-16.5			68	70.1	-10.3		
Pyr + 1	3050	3144.3	3118.6	3.6		2780	2866.0	2802.1	3.2		2476	2552.6	2472.2	2.9	
Pyr + 2	2947	3038.1	3012.4	3.5		2678	2760.8	2696.9	3.1		2155	2221.6	2141.2	2.5	
Pyr + 3	2603	2683.5	2657.7	3.1		2245	2314.4	2250.5	2.6		1782	1837.1	1756.7	2.0	
Ph/Py - 1	50	51.5	25.8			47	48.5	-15.5			59	60.8	-19.6		
Ph/Py - 2	42	43.3	17.5			51	52.6	-11.3			63	64.9	-15.5		
Ph/Py - 3	35	36.1	10.3			48	49.5	-14.4			55	56.7	-23.7		
Ph/Py + 1	2227	2295.9	2270.1	2.5		2133	2199.0	2135.1	2.4		1806	1861.9	1781.4	2.0	
Ph/Py + 2	2667	2749.5	2723.7	3.0		2468	2544.3	2480.4	2.8		2113	2178.4	2097.9	2.3	
Ph/Py + 3	2481	2557.7	2532.0	2.8		2431	2506.2	2442.3	2.7		2108	2173.2	2092.8	2.3	
Blink	25	25.8				62	63.9				78	80.4			
Bkg	16	16.5				18	18.6				18	18.6			
C-14 UNQ	95580	98536.1				95457	98409.3				94628	97554.6			

Appendix K. Raw data and calculations for bioavailability of PAHs on sediment. (Continued)

	day 110				day 121				day 132			
	CPM	DPM	DPM-blink	%C	CPM	DPM	DPM-blink	%C	CPM	DPM	DPM-blink	%C
Phen - 1	89	91.8	11.3		67	69.1	-5.2		67	69.1	0.0	
Phen - 2	69	71.1	-9.3		69	71.1	-3.1		75	77.3	8.2	
Phen - 3	84	86.6	6.2		71	73.2	-1.0		71	73.2	4.1	
Phen + 1	1758	1812.4	1732.0	1.9	1768	1822.7	1748.5	1.9	1413	1456.7	1387.6	1.5
Phen + 2	1537	1584.5	1504.1	1.6	1320	1360.8	1286.6	1.4	1228	1266.0	1196.9	1.3
Phen + 3	1365	1407.2	1326.8	1.4	1407	1450.5	1376.3	1.5	1229	1267.0	1197.9	1.3
Pyr - 1	63	64.9	-15.5		81	83.5	9.3		58	59.8	-9.3	
Pyr - 2	68	70.1	-10.3		68	70.1	-4.1		80	82.5	13.4	
Pyr - 3	66	68.0	-12.4		64	66.0	-8.2		66	68.0	-1.0	
Pyr + 1	2198	2266.0	2185.6	2.5	1911	1970.1	1895.9	2.2	1912	1971.1	1902.1	2.2
Pyr + 2	1912	1971.1	1890.7	2.2	1620	1670.1	1595.9	1.8	1506	1552.6	1483.5	1.7
Pyr + 3	1627	1677.3	1596.9	1.8	1660	1711.3	1637.1	1.9	1688	1740.2	1671.1	1.9
Ph/Py - 1	69	71.1	-9.3		78	80.4	6.2		53	54.6	-14.4	
Ph/Py - 2	66	68.0	-12.4		64	66.0	-8.2		58	59.8	-9.3	
Ph/Py - 3	71	73.2	-7.2		68	70.1	-4.1		58	59.8	-9.3	
Ph/Py + 1	1626	1676.3	1595.9	1.8	1542	1589.7	1515.5	1.7	1384	1426.8	1357.7	1.5
Ph/Py + 2	1915	1974.2	1893.8	2.1	1762	1816.5	1742.3	1.9	1534	1581.4	1512.4	1.7
Ph/Py + 3	1829	1885.6	1805.2	2.0	1666	1717.5	1643.3	1.8	1591	1640.2	1571.1	1.8
Blink	78	80.4			72	74.2			67	69.1		
Bkg	19	19.6			20	20.6			22	22.7		
C-14 UNQ	95321	98269.1			95057	97996.9			95415	98366.0		

Appendix K. Raw data and calculations for bioavailability of PAHs on sediment. (Continued)

	day 145			
	CPM	DPM	DPM-blk	%C
Phen - 1	51	52.6	18.6	
Phen - 2	40	41.2	7.2	
Phen - 3	56	57.7	23.7	
Phen + 1	1515	1561.9	1527.8	1.6
Phen + 2	1230	1268.0	1234.0	1.3
Phen + 3	1301	1341.2	1307.2	1.4
Pyr - 1	60	61.9	27.8	
Pyr - 2	45	46.4	12.4	
Pyr - 3	57	58.8	24.7	
Pyr + 1	1970	2030.9	1996.9	2.3
Pyr + 2	1801	1856.7	1822.7	2.1
Pyr + 3	1911	1970.1	1936.1	2.2
Ph/Py - 1	55	56.7	22.7	
Ph/Py - 2	48	49.5	15.5	
Ph/Py - 3	43	44.3	10.3	
Ph/Py + 1	1518	1564.9	1530.9	1.7
Ph/Py + 2	1633	1683.5	1649.5	1.8
Ph/Py + 3	1624	1674.2	1640.2	1.8
Bink	33	34.0		
Bkg	21	21.6		
C-14 UNQ	94888	97822.7		

Appendix L. Raw data and calculations for degradation of PAH by biofilms.

sample	day 5			day 9			day 14			day 20		
	CPM	DPM-blk	%C	CPM	DPM-blk	%C	CPM	DPM-blk	%C	CPM	DPM-blk	%C
blank	44			37			38			30		
AF-1-S	48	2.6	0.0	94	58.8	0.1	70	33.0	0.0	89	40.2	0.0
AF-2-S	48	2.6	0.0	121	88.8	0.1	53	15.5	0.0	60	30.9	0.0
AF-3-S	48	0.6	0.0	97	61.9	0.1	67	29.9	0.0	49	19.6	0.0
LR-4-S	1214	1216.5	1.3	1295	1307.2	1.4	1208	1205.2	1.3		1167.2	1.2
LR-5-S	1022	1018.6	1.1	1128	1135.1	1.2	1126	1120.6	1.2		1167.2	1.2
SS-1-S	49	3.6	0.0	108	73.2	0.1	48	10.3	0.0	53	23.7	0.0
SS-2-S	50	4.6	0.0	94	58.8	0.1	47	9.3	0.0	55	25.8	0.0
SS-3-S	48	0.6	0.0	92	56.7	0.1	51	13.4	0.0	44	14.4	0.0
AF-10-N	5038	4992.6	4.6	14700	15116.5	14.0	11025	11326.8	10.5	5766	5913.4	5.5
AF-12-N	4592	4546.6	4.2	16964	17450.5	16.2	9812	10076.3	9.3	5718	5863.9	5.4
AF-14-N	5994	5948.6	5.5	14898	15320.6	14.2	MD	MD	MD	7013	7199.0	6.7
LR-7-N	4065	4019.6	3.7	31565	32503.1	30.2	10129	10403.1	9.7	3344	3416.5	3.2
LR-9-N	13305	13259.6	12.3	27646	28462.9	26.4	5381	5508.2	5.1	3517	3594.8	3.3
LR-11-N	5472	5426.6	5.0	27524	28337.1	26.3	13372	13746.4	12.8	4801	4918.6	4.6
SS-8-N	611	565.6	0.5	1085	1080.4	1.0	4615	4718.6	4.4	6731	6808.2	6.4
SS-9-N	514	468.6	0.4	1243	1243.3	1.2	8302	8519.6	7.9	8979	9225.8	8.6
SS-11-N	58	12.6	0.0	71	35.1	0.0	61	23.7	0.0	55	25.8	0.0
AF-3-P	20952	20906.6	19.4	9069	9311.3	8.6	5578	5711.3	5.3	4441	4547.4	4.2
AF-6-P	20122	20076.6	18.6	9389	9641.2	8.9	4926	5039.2	4.7	4406	4511.3	4.2
AF-7-P	17432	17386.6	16.1	11329	11641.2	10.8	5331	5456.7	5.1	4112	4208.2	3.9
LR-1-P	31721	31675.6	29.4	8817	9051.5	8.4	2840	2888.7	2.7	2426	2470.1	2.3
LR-2-P	32444	32398.6	30.1	7691	7890.7	7.3	2611	2652.6	2.5	2330	2371.1	2.2
LR-6-P	17090	17044.6	15.8	24807	25536.1	23.7	2665	2914.4	2.7	2381	2423.7	2.2
SS-5-P	7022	6976.6	6.5	19102	19854.6	18.2	11113	11417.5	10.6	5842	5991.8	5.6
SS-6-P	3375	3329.6	3.1	21940	22580.4	21.0	10219	10495.9	9.7	6155	6314.4	5.9
SS-14-P	3711	3685.6	3.4	15066	15493.8	14.4	10819	10908.2	10.1	5757	5904.1	5.5

Explanation of abbreviations:

AF: aquarium filter, LR: lava rock, SS: Smart Sponge®, S: sterile, N: no PAH, P: spiked with phenanthrene, #: biofilter label, CPM: counts per minute, DPM: disintegrations per minute, blk = blank scintillation cocktail only, %C: percent phenanthrene carbon respired.

Appendix L. Raw data and calculations for degradation of PAH by biofilms. (Continued)

sample	day 26			day 30			day 35		
	CPM	DPM-blank	%C	CPM	DPM-blank	%C	CPM	DPM-blank	%C
blank	34			27			39		
AF-1-S	40	8.2	0.0	71	45.4	0.0	66	27.8	0.0
AF-2-S	62	28.9	0.0	85	59.8	0.1		MD	MD
AF-3-S	55	21.6	0.0	91	66.0	0.1	69	30.9	0.0
LR-4-S		1167.2	1.2		1167.2	1.2		1167.2	1.2
LR-5-S		1167.2	1.2		1167.2	1.2		1167.2	1.2
SS-1-S	33	-1.0	0.0	75	49.5	0.0	75	37.1	0.0
SS-2-S	34	0.0	0.0	80	54.8	0.1	77	39.2	0.0
SS-3-S	49	15.5	0.0	70	44.3	0.0	65	26.8	0.0
AF-10-N	3391	3460.8	3.2	1944	1976.3	1.8	1922	1941.2	1.8
AF-12-N	3342	3410.3	3.2	2018	2052.6	1.9	1947	1967.0	1.8
AF-14-N	3557	3632.0	3.4	1826	1854.8	1.7	1865	1882.5	1.7
LR-7-N	1919	1943.3	1.8	1059	1063.9	1.0	1217	1214.4	1.1
LR-9-N	2117	2147.4	2.0	1116	1122.7	1.0	1115	1109.3	1.0
LR-11-N	2403	2442.3	2.3	1389	1404.1	1.3	1143	1138.1	1.1
SS-8-N	4697	4807.2	4.5	1907	1938.1	1.8	2707	2750.5	2.6
SS-9-N	4744	4855.7	4.5	2145	2183.5	2.0	1992	2013.4	1.9
SS-11-N	47	13.4	0.0	84	58.8	0.1	61	22.7	0.0
AF-3-P	2370	2408.2	2.2	1592	1613.4	1.5	1645	1655.7	1.5
AF-6-P	2347	2384.5	2.2	1575	1595.9	1.5	1449	1453.6	1.3
AF-7-P	2721	2770.1	2.6	1546	1568.0	1.5	1711	1723.7	1.6
LR-1-P	1597	1611.3	1.5	987	989.7	0.9	1027	1018.6	0.9
LR-2-P	1535	1547.4	1.4	1066	1071.1	1.0	732	714.4	0.7
LR-6-P	1814	1835.1	1.7	1246	1256.7	1.2	1269	1268.0	1.2
SS-5-P	2976	3033.0	2.8	1819	1850.5	1.8	1928	1947.4	1.8
SS-6-P	2769	2819.6	2.6	1829	1857.7	1.7	1957	1977.3	1.8
SS-14-P	2729	2778.4	2.6	1823	1851.5	1.7	2088	2112.4	2.0

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