AN ABSTRACT OF THE THESIS OF

Kristi M. Christensen for the degree of Master of Science in Chemical Engineering presented on June 22, 2007.

Title: Uptake and Metabolism of Polycyclic Aromatic Hydrocarbons by the Marine Alga *Acrosiphonia coalita*

Abstract approved:

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Gregory L. Rorrer

The purpose of this research was to examine the ability of the cold water, green, marine, macroalga *Acrosiphonia coalita* to take up and metabolize polycyclic aromatic hydrocarbons (PAH) from seawater. Axenic suspension tissue cultures of the alga were contacted with seawater containing PAH in sealed experimental vessels. Uptake time courses and equilibrium partitioning were examined. To determine if uptake was passive or active, equilibrium partitioning was also examined for uptake by heat-killed *A. coalita* tissue. Additionally, the seawater and biomass were monitored for the formation of PAH metabolites. The two model PAH compounds used in this study were naphthalene and phenanthrene.

The results of this study indicate that *A. coalita* did remove PAH from seawater, but that uptake was passive and the PAH was not metabolized. Both phenanthrene and naphthalene were taken up very quickly by the alga. Equilibrium partitioning between the seawater and biomass was achieved within 24 hours. Additionally, both compounds were found to partition linearly between *A. coalita* tissues and seawater. *A. coalita* removed significantly more phenanthrene than naphthalene from the seawater. The equilibrium partition coefficients for phenanthrene and naphthalene partitioning into living *A. coalita* tissue were 0.171 ± 0.0083 L/g FW and 0.0500 ± 0.0025 L/g FW, respectively. Naphthalene partitioning into heat-killed *A. coalita*

tissue was equivalent to naphthalene partitioning into living *A. coalita* tissue, indicating that uptake was likely passive. No compounds were detected in the seawater or biomass that could be identified as a products of PAH degradation. Additionally, all unidentified compounds that were present in the experiments with PAH and biomass were also present in the control experiments without PAH. The absence of detectable products of degradation indicates that the PAH was not metabolized.

Uptake and Metabolism of Polycyclic Aromatic Hydrocarbons by the Marine Alga *Acrosiphonia coalita*

by
Kristi M. Christensen

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APPROVED:

Major Professor, representing Chemical Engineering

Chair of the Department of Chemical Engineering

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Kristi M. Christensen, Author

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Uptake and Metabolism of Polycyclic Aromatic Hydrocarbons by the Marine Alga *Acrosiphonia coalita*

Introduction

Polycyclic aromatic hydrocarbons (PAH) are pervasive environmental pollutants that pose significant health risks to humans and other organisms. Many of these compounds are known to be carcinogenic, mutagenic, and toxic [1]. PAH are of particular concern when released into marine environments. They tend to adsorb to marine sediments creating areas with high PAH concentrations. PAH concentrations in sediments can be 1000 greater than surrounding aqueous concentrations [2]. They are also known to bioaccumulate in marine organisms. PAH compounds have been detected in a wide variety of finfish, marine mammals, and mollusks [2]. The primary sources of PAH in the marine environment are anthropogenic, such as petroleum spills, vehicular emissions, industrial processes, and combustion of fossil fuels [2].

Because of the abundance of these compounds and their potential health risks, there has been significant interest in PAH remediation methods. Bioremediation offers an attractive alternative to traditional remediation methods, because it is relatively inexpensive and does not require removal of sediment to on land treatment sites. Algal species have been shown to take up PAH compounds when in proximity to sediments containing PAH [3], and they are also believed to possess enzymes capable of detoxification of PAH compounds [4-6]. However, studies of their use for PAH bioremediation are limited.

The studies that have been conducted indicate that the ability of algae to take up and metabolize PAH depends on several factors. First, metabolism is algal species dependent. For example, it has been determined that the green algae species *Selenastrum capricornutum*, *Scenedesmus acutus*, and *Ankistrodesmus braunii* can almost complete metabolize benzo[a]pyrene, while other species such as *Chlamydomonas reinhardtii*, *Ochromonas malamensis*, *Anabaena flosaquae*, and *Euglena gracilis* could not metabolize benzo[a]pyrene at all [7]. Additionally, the algae species *Enteromorpha intestinalis*, *Cladophora glomerata*, and *Chara aspera* were shown to metabolize significantly more benzo[a]pyrene than the species *Fucus*

vesiculosus [8]. Second, removal is dependent on the PAH compound present. The algae species *Chlorella vulgaris, Scenedesmus platydiscus, Scenedesmus quadricauda, and Selenastrum capricornutum* were tested on their ability to remove and degrade flouranthrene and pyrene. It was found that flouranthrene was removed in higher amounts than pyrene by all species except *Scenedesmus platydiscus*. Additionally, the presence of both compounds resulted in removal that was equivalent to or greater than the removal of either single compound [9]. Additional factors that affect PAH metabolism by algae are type of illumination, the dose of PAH, phototoxicity of the PAH and metabolites, culture medium, and growth phase [7, 10].

This study examines the ability of the green, cold water, marine macroalga *Acrosiphonia coalita* to take up and degrade phenanthrene and naphthalene. To date, most studies have examined the ability of microalgae to remove PAH, but little work has been done with seaweeds. *A. coalita* was chosen because green algae have been shown to be effective at degrading PAH [7, 8, 10]. Additionally, an axenic tissue culture of this species was developed and available for experimental use [11]. The use of an axenic culture produces results that are not complicated by the presence of bacteria or other epiphytic organisms normally associated with algal seaweed cultures and field collected samples. The overall objectives of this study are to determine:

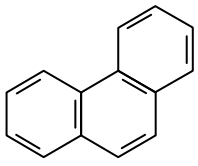
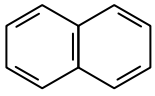
1. The time course for PAH uptake by *Acrosiphonia coalita* tissue culture of two model PAH compounds, naphthalene and phenanthrene;
2. Equilibrium partitioning of naphthalene and phenanthrene between *Acrosiphonia coalita* and seawater;
3. Metabolites of PAH metabolism by *Acrosiphonia coalita*;
4. Physical model for PAH uptake.

Materials and Methods

Materials

Two PAH compounds were used in this study. Pure reagent grade (98%) phenanthrene was purchased from Sigma-Aldrich (CAS #: 85-01-8), while certified reagent grade naphthalene was purchased from Fisher Scientific (CAS #: 91-20-3). Both PAH compounds are white crystalline solids. Physical properties for these compounds are presented in Table 1.

Table 1. Physical properties of phenanthrene and naphthalene

Property	Phenanthrene	Naphthalene	Reference
Molecular Formula	$C_{14}H_{10}$	$C_{10}H_8$	12
Molecular Weight (g/mol)	178.23	128.17	13
Boiling Point (ºC)	339.0	218.0	12
Melting Point (ºC)	101.0	80.2	12
Water Solubility at 20 ºC (mg/L)	6.2	33	13
Seawater Solubility at 25 ºC (mg/L)	0.71	22	14
Henry's Law Constant at 20 ºC (Dimensionless)	1.5×10^{-3}	4.9×10^{-2}	13
Henry's Law Constant at 20 ºC (atm-m^3/mol)	3.5×10^{-5}	1.15×10^{-3}	13
Density (g/cm^3)	0.98	1.16	12
Vapor Pressure at 20 ºC (atm)	8.9×10^{-7}	3×10^{-4}	13
Octanol-Water Partitioning Coefficient (Log K_{ow})	4.57	3.36	13
Structure			14

An ethanol bridge was used for PAH addition to liquid medium. PAH stock solutions were prepared by dissolving PAH compounds into 100% ethanol to achieve a

concentration of 10 mg PAH/mL ethanol. PAH stock solutions were added directly to the culture medium. The amount of stock solution added was determined based on the desired initial PAH concentration in the seawater medium.

The only exception to the ethanol bridge technique was the addition of naphthalene to experiments with high naphthalene concentrations. For these experiments, approximately 20 mg of naphthalene was added to 1.0 L of seawater medium. The solution was mixed for approximately 48 hours using a stir bar and stir plate. Then the solution was filtered under vacuum through a 0.22 μm polyethersulfone bottle top filter to remove any undissolved naphthalene. The resulting naphthalene concentration in the seawater was measured by HPLC analysis.

The initial PAH concentrations in the seawater medium used for experiments were determined based on the solubility of the PAH compounds in seawater. The solubility of phenanthrene in seawater is reported to be approximately 0.71 mg/L at 25 ºC [14]. Therefore, the initial phenanthrene concentrations for uptake experiments were set just below the solubility limit at 0.6 mg/L. For phenanthrene equilibrium partitioning, the initial phenanthrene concentrations in the seawater were varied from 0 to 0.7 mg/L. Because naphthalene is significantly more soluble in seawater than phenanthrene, initial concentrations were varied accordingly. Naphthalene has a reported solubility in seawater of approximately 22.0 mg/L at 25.0 ºC [14]. The initial concentration for naphthalene uptake experiments was determined to be approximately 14 mg/L in the seawater medium. Two concentration ranges were examined for naphthalene equilibrium partitioning. High concentration naphthalene partitioning was tested over the PAH concentration range of 0 to 16 mg/L, while low concentration naphthalene partitioning experiments examined initial PAH concentrations ranging from 0 to 1.0 mg/L.

Culture Maintenance

The *A. coalita* algal biomass used for this study was grown in semidifferentiated suspension tissue cultures. The development of this culture is described by Rorrer and Zhi [11]. Cultures were maintained in autoclaved natural seawater containing Provasoli's enriched seawater (PES) medium. Approximately 0.1 g fresh weight (FW) of biomass was suspended in 80 mL of medium in a sterile 250 mL flask. Flasks were capped with foam stoppers to maintain sterility while allowing free exchange of carbon dioxide and oxygen. The cultures were kept at a light intensity of approximately 50 $\mu E/m^2$-sec on a 16 hour light/8 hour dark photoperiod in an incubator at 12.0 ºC. To promote gas transfer, flasks were manually swirled for approximately 5 sec each day. To ensure sufficient nutrient availability, the medium was completely replaced approximately 17 days after subculture.

The alga was subcultured every 30 to 35 days. Subculture consisted of blending the culture in a 200 mL autoclaved glass blending jar. The lid of the blending jar was designed to be compatible with an Osterizer blender and was fitted with a sealed shaft assembly with two double-edged razor blades. For each subculture cycle, 200 mL of PES medium containing approximately 1.6 g FW biomass were sealed in the blending jar and blended with the Osterizer blender for 3-5 seconds on "liquefy" speed. Then the biomass suspension was dispensed to four 50 mL centrifuge tubes. The biomass was washed twice by centrifuging, decanting, and then re-suspending in fresh medium. Finally, the biomass was suspended in approximately 160 mL fresh medium. 10 mL of this suspension was added to each of 16 sterile 250 mL flasks. The total volume in the flask was brought to 80 mL with medium. Alga material used for experimental purposes was typically obtained 25-35 days after subculture.

Images of the culture are presented in Figures 1a-1c. Figure 1a shows the culture as it appears in suspension culture in culture flasks. Figure 1b is a microscopic image at 10x magnification. This image illustrates how the culture grows in small clumps with linear branches shooting out in all directions. The image shown in Figure 1c is a

microscopic image at 40x magnification. This image shows the filamentous chains of cells with some branching near the ends of the chains.

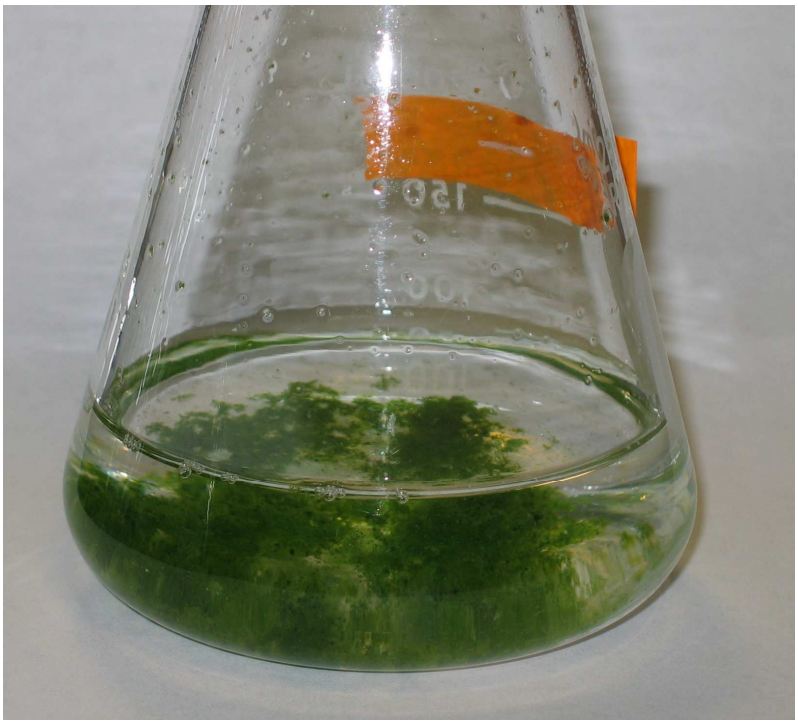


Figure 1a. *A. coalita* biomass suspension culture in culture flasks.

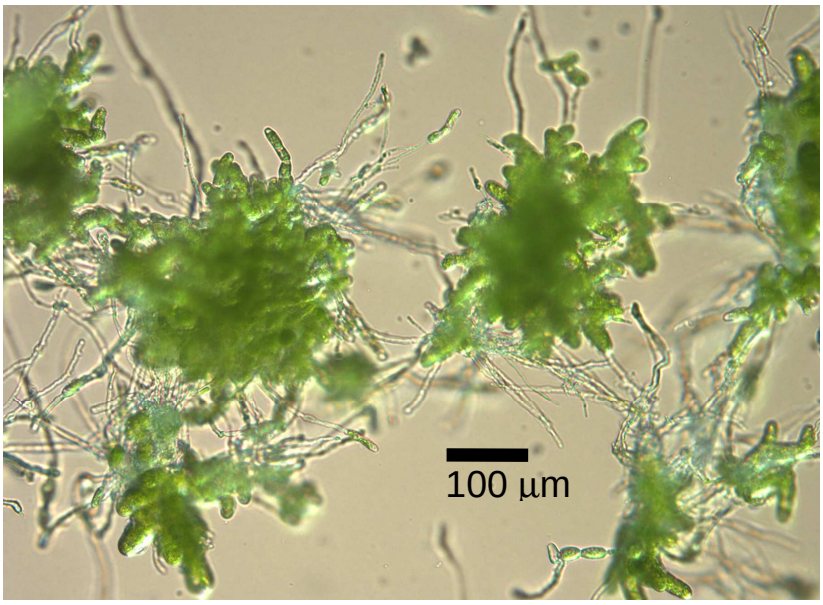


Figure 1b. *A. coalita* suspension culture magnification 10x

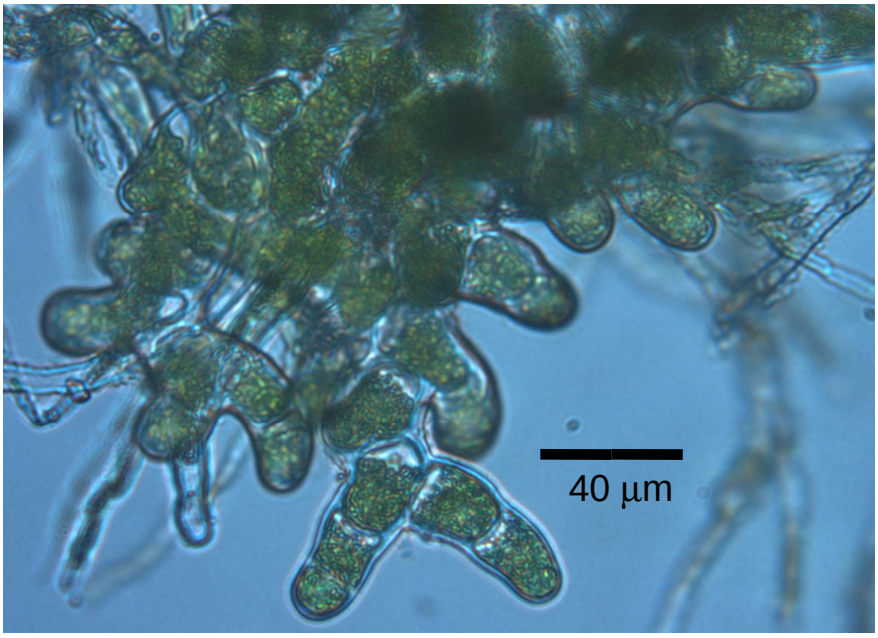


Figure 1c. *A. coalita* suspension culture magnification 40x

Uptake Experiments

The time course of PAH uptake by *A. coalita* was examined in glass bioreactors with sealed gas headspaces. The reactors were sealed to prevent the loss of PAH due to volatilization. Two bioreactor designs were used. Schematics of these bioreactors are presented in Figures 2a and 2b. The first reactor was a 2 L Belco reactor. This reactor was equipped with a stir shaft assembly with a 2 inch magnetic stir bar and Teflon fins for mixing. The second reactor design used medium bottles as the reactor vessel and held a volume of either 1 or 2 L. The bottle reactors were mixed with 2 inch stir bars not connected to stir shafts. Both reactor types were fit with sample ports that allowed sampling of the biomass and seawater as a function of time. The ports consisted of 3/8 inch glass tubing fit through the reactor lid using a stainless steel Swagelock bulk head union. The glass tubing was connected to a short length of silicon tubing that was clamped during experimentation to seal the sample port. The other end of the silicon tubing was connected to a shorter length of 3/8 inch glass tubing fit through a #3 rubber stopper. The rubber stopper was fit to a 50 mL filter flask that was connected to a syringe by a short length of silicon tubing.

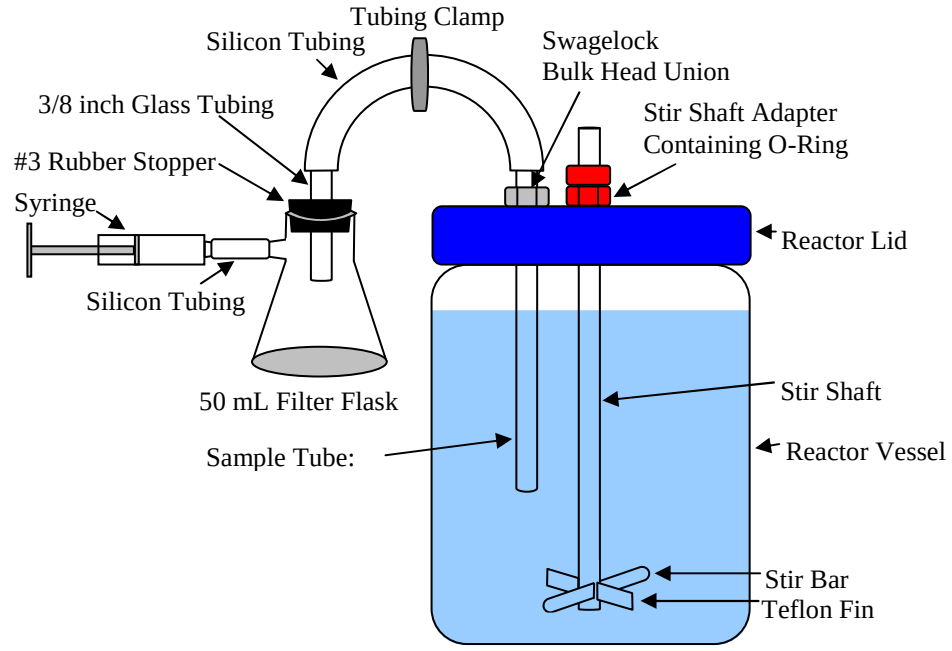


Figure 2a. Bioreactor Design: Belco Reactor

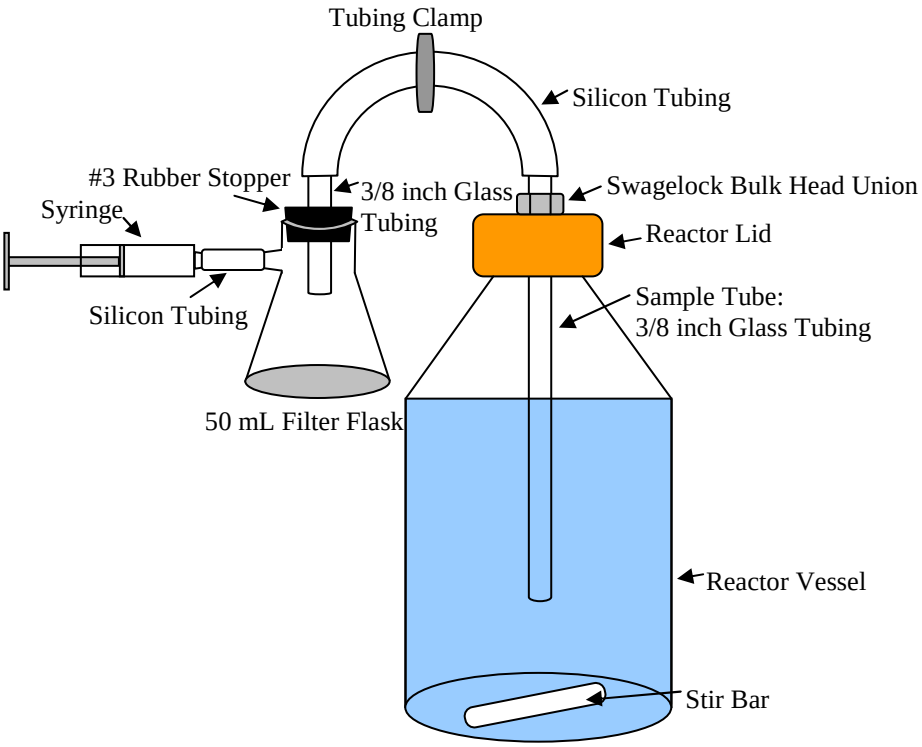


Figure 2b. Bioreactor Design: Bottle Reactor

Prior to experimentation, the glass reactor vessel of the bioreactor was passivated by soaking in an aqueous solution of 20% nitric acid for a minimum of 24 hours. After passivation, the reactor vessel was rinsed several times with dionized water and then rinsed with acetone. Finally, the sample port was assembled, the reactor was sealed, and the entire reactor was sterilized by autoclaving.

One naphthalene uptake experiment and three phenanthrene uptake experiments were conducted. Conditions for these experiments and relevant control experiments are listed in Table 2. Biomass inoculation varied between the experiments. For phenanthrene uptake experiments 1 and 2, biomass was transferred from culture flasks to the bioreactors containing fresh culture medium and allowed to adapt for 24 hours before phenanthrene addition. In the third phenanthrene uptake experiment, biomass was added directly from culture flasks to medium containing phenanthrene. Biomass was also added directly from culture flasks to medium containing PAH for the naphthalene uptake experiment. All experiments were conducted at 12.0 ºC, with light intensities ranging from 80-150 $\mu E/m^2$-s on a 16 hour light/8 hour dark photoperiod. Reactors were well mixed using a stir plate at approximately 150 rpm.

PAH uptake was monitored for a period of 120 hours. Samples were drawn from the reactors into the 50 mL filter flask by removing the clamp from the silicon tubing and creating a vacuum using the syringe. After samples were collected in the filter flask, the biomass was immediately separated from the seawater by filtering through a nylon mesh filter. The time of sampling, seawater volume, biomass fresh weight, temperature, and seawater pH were all measured and recorded. An extraction was performed on the biomass, and PAH concentrations in the seawater and biomass were measured by HPLC analysis.

Table 2. Uptake experimental conditions

Experiment	Experiment ID	Reactor Type	Initial Biomass Density (g FW/L)	Initial PAH Conc. In Seawater (mg/L)
Phenanthrene Control	AC205	2 L Belco	NA	0.45
Phenanthrene Uptake Trial 1	AC206	2 L Belco	1.0	0.62
Phenanthrene Uptake Trial 2	AC207	2 L Belco	1.5	0.61
Phenanthrene Control	AC208	2 L Bottle	NA	0.55
Phenanthrene Uptake Trial 3	AC222	1 L Bottle	1.0	0.60
Naphthalene Control	AC212	1 L Bottle	NA	13.9
Naphthalene Uptake	AC212	1 LBottle	1.3	14.8

Equilibrium Partitioning Experiments

Equilibrium partitioning experiments were conducted using 20 mL glass vials sealed with Teflon-lined caps. Prior to experimentation, glass vials were passivated by soaking in an aqueous solution of 20% nitric acid for a minimum of 24 hours. Then the vials were rinsed several times with deionized water, rinsed with acetone, and finally sterilized by autoclaving.

Experiments were conducted by adding biomass and seawater containing PAH to the glass vials, sealing the vials, and allowing equilibrium to be achieved. During experimentation, the vials were secured in place on their sides on an orbital shaker and mixed at 162 rpm. Lights were placed directly above the vials. Light intensities ranged from 50 to 90 $\mu E/m^2$-s with a 16 hour light/8 hour dark photoperiod. Temperature was maintained at 12.0 ºC. Vials were sampled after 120 hour approximately midway into the light cycle. When sampled, the biomass was separated from the seawater by filtering through a nylon mesh filter. A biomass extraction was performed, and the concentration in each phase was measured by HPLC analysis.

A summary of the equilibrium partitioning experiments is presented in Table 3. For all experiments, a range of PAH concentrations was achieved by varying the initial PAH concentration in the seawater. Two trials were conducted to determine the partitioning of phenanthrene between living *A. coalita* tissue and seawater. Each trial tested 7 initial phenanthrene concentrations, with a minimum of two vials at each concentration. The initial biomass density for phenanthrene partitioning experiments was 20 mg FW in 20 mL seawater medium. One high concentration naphthalene partitioning experiment and two low concentration naphthalene partitioning experiments were conducted with living *A. coalita* tissue. All naphthalene partitioning experiments tested 5 initial naphthalene concentrations with vials prepared in duplicate. The initial biomass density for high concentration naphthalene partitioning was 60 mg FW in 20 mL medium, while the initial biomass density for low concentration naphthalene partitioning was 20 mg FW in 20 mL medium.

Equilibrium partitioning for PAH partitioning between seawater and heat-killed *A. coalita* tissue was measured in the same manner as PAH partitioning between seawater and living *A. coalita* tissue, except that the alga was heat-killed prior to the addition of medium containing PAH. To heat-kill the alga, the biomass was collected from culture flasks. The biomass and spent medium were separated by filtration under vacuum using a filter funnel and filter paper. Excess water was removed from the biomass by gently pressing the biomass between sterile paper towels. Then, 20 mg FW biomass was weighed into each 20 mL vial. The vials were capped with Teflon lined lids and autoclaved for 20 minutes at 121 ºC and 20 psi. Once out of the autoclave, the vials were allowed to cool, and finally the medium with PAH was added. Partitioning of PAH between seawater and heat-killed *A. coalita* tissue was tested for phenanthrene and low concentration naphthalene. Two trials were conducted for each PAH.

Table 3. Equilibrium experimental conditions

Experiment	Alga Condition	Experiment ID	Initial Biomass Density (g FW/L)	Initial PAH Conc. Range (mg/L)	# of Initial Conc. Tested
Phenanthrene Partitioning Trial 1	Living	AC215	1.0	0-0.51	7
Phenanthrene Partitioning Trial 2	Living	AC220	1.0	0.11-0.90	7
Phenanthrene Partitioning Trial 1	Heat-Killed	AC220	1.0	0.11-0.90	7
Phenanthrene Partitioning Trial 2	Heat-Killed	AC221	1.0	0.08-0.70	7
High Conc. Naphthalene Partitioning	Living	AC214	3.0	0-16	5
Low Conc. Naphthalene Partitioning Trial 1	Living	AC218	1.0	0.14-0.78	5
Low Conc. Naphthalene Partitioning Trial 2	Living	AC223	1.0	0.17-0.94	5
Low Conc. Naphthalene Partitioning Trial 1	Heat-Killed	AC221	1.0	0.22-0.93	5
Low Conc. Naphthalene Partitioning Trial 2	Heat-Killed	AC223	1.0	0.17-0.94	5

Desorption Experiments

To examine desorption, phenanthrene was first adsorbed to algal biomass using the same reactor design and experimental conditions as described for uptake experiments. However, for this experiment samples were not taken as a function of time, so the reactor lid was not fitted with a sample port. Phenanthrene uptake occurred for a period of 48 hours. After 48 hours, the biomass was separated from the seawater by filtration under vacuum using a filter funnel and filter paper. Excess water was removed from the biomass by pressing dry with sterile paper towels. Then the biomass was transferred to fresh medium.

Desorption was examined in 20 mL glass vials with conditions similar to those described for equilibrium partitioning experiments. However, to achieve a range of PAH concentrations, the initial biomass density in the vials was varied. The range of biomass densities examined was 0 to 200 mg FW per 20 mL of seawater medium. Biomass containing PAH and fresh medium were added to the vials, then the vials were sealed and allowed to equilibrate for a period of 120 hours. At the end of the experiment, the liquid and biomass were separated by filtering through a nylon mesh filter. The relative concentrations in each phase were measured by HPLC analysis.

Equilibrium Isotherms

The partitioning of PAH between biomass and seawater was described by the equation:

$$q_{Af} = k C_{Af}$$

where q_{Af} is the final concentration of PAH in the biomass, C_{Af} is the final concentration of PAH in the seawater, and k is a partition coefficient. Units for C_{Af} are mg PAH/L seawater, while q_{Af} is given in either mg PAH/g FW or mg PAH/g dry weight (DW). The units for the partition coefficient are dependent on the units of q_{Af} and are either L seawater/g FW or L seawater/g DW.

HPLC Sample Preparation

Seawater samples and biomass samples were prepared for HPLC analysis by separate procedures. Preparation of seawater samples consisted of diluting 0.5 mL seawater sample with 2 mL of acetonitrile and then filtering through a 0.45 μm nylon syringe filter. Extractions were performed to quantify the amount of PAH concentrated in the biomass. PAH compounds were extracted from biomass samples by adding approximately 1 mL of acetonitrile per 20 mg of biomass. The extraction was allowed to proceed for no less than 36 hours in a sealed vial. During the extraction period the vials were sonicated for a minimum of 90 minutes in a Branson 1200 sonicator. The samples were then filtered through 0.45 μm nylon syringe filters to separate the biomass from the solvent. The solvent was analyzed by HPLC.

HPLC Analysis

PAH concentrations in both the seawater and the algal biomass were quantified by HPLC analysis. Concentration was measured using a Dionex DX-300 series HPLC system with a Dionex variable UV wavelength detector. A Waters Spherisorb ODS2 column was used for separation using isocratic elution at 1.0 mL/min with 80:20 acetonitrile:water. Samples from phenanthrene experiments were monitored at a wavelength of 249 nm, while the samples from naphthalene experiments were monitored at 214 and 254 nm. The detector output was converted to concentration values by external calibration curves.

Metabolite Detection

Aqueous samples for metabolite detection were generated in 1 L glass bottle reactors under the same conditions as described for uptake experiments. Two reactors were run simultaneously. The first reactor contained both phenanthrene and biomass, while the second reactor contained only phenanthrene. In addition to the reactor generated samples, an additional control sample was produced from spend medium from culture flasks.

To ensure that metabolites were present in quantities detectible by GC-MS, aqueous samples were concentrated using 6 mL Supelco supelclean LC-18 solid phase extraction (SPE) columns. First, the samples were filtered through 0.2 μm polyethersulfone bottle top filters. Then approximately 200 mL of the filtrate was loaded onto the SPE column. The column was rinsed with deionized water to remove salts, and finally the sample was eluted with 3.5 mL of acetonitrile. The sample was then derivatized and analyzed by GC-MS.

Biomass extracts were also analyzed for the presence of metabolites. The biomass containing PAH that was generated in the first reactor was collected from the bottle top filter during the aqueous concentration process. Additionally, a biomass sample that was not exposed to phenanthrene was collected from culture flasks. A biomass extraction was performed. The extracts were derivatized and analyzed by GC-MS.

All samples for GC-MS analysis were derivatized with N-methyl-N-trimethylsilyltriflouroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS). 0.5 mL of sample was first dried over anhydrous sodium sulfate. Next, the majority of the acetonitrile was removed by blowing down the samples to approximately 0.1 mL under nitrogen. Then 0.125 mL of the derivatization reagent was added and allowed to react for approximately 15 minutes. Finally, the sample was brought back up to a volume of approximately 0.5 mL with ethyl acetate. The sample was filtered through a 0.45 μm nylon syringe filter and analyzed by GC-MS.

GC-MS Analysis

Metabolite detection was performed using GC-MS analysis. A Hewlett Packard HP 6890 series GC-MS was used with a DB5-MS 0.25 mm x 30 m capillary column with a film thickness of 0.25 μm. The carrier gas was helium at a flowrate of 0.9 mL/min. The temperature program was set to range from 60 ºC to 300 ºC, increasing by 10 ºC per minute. A 1 μL sample was injected using splitless injection with an injector at 300 ºC. Chromatograms were created from the total ion count measured by an HP

5972 quadrupole MS detector using Chemstation version 1701 BA. Compound identification was performed using the NIST98 mass spectral library.

Results

Uptake

Figures 3 and 4 present the time course data for phenanthrene uptake by *A. coalita* obtained from two repeat uptake experiments. The phenanthrene concentration in the seawater decreased very quickly, and phenanthrene concentration in the biomass increased proportionately. After the initial increase in the PAH concentration in the biomass, the PAH concentration decreased slightly and then remained approximately constant for the remainder of the experiment. This trend was mirrored by the PAH concentration in the seawater. Figures 5 and 6 present seawater pH vs. time profiles for phenanthrene uptake experiments 1 and 2, respectively. The variations in PAH concentration coincided with fluctuations in pH.

To further investigate the relationship of pH and uptake, a third phenanthrene uptake experiment was conducted. The time course PAH concentration data for this experiment is presented in Figure 7a, the material balance as a function of time is presented in Figure 7b, and the pH vs. time profile is presented in Figure 8. The frequency of sampling was increased for this experiment. Samples were taken every four hours for a period of 72 hours. The seawater pH varied with photoperiod. The PAH concentration in the biomass initially varied with pH, but stabilized after approximately 24 hours. The material balance revealed that the measured amount of PAH in both the liquid phase and in the biomass remained approximately constant throughout the experiment, even though PAH was removed from the reactor during sampling. Also, the amount of PAH that was unaccounted for decreased with time. The rate of decrease in the amount unaccounted for was approximately inversely proportional to the rate of PAH removed from the reactor through sampling. The reasons for this observation are unknown.

Figure 9 presents the time course PAH concentration data for naphthalene uptake by *A. coalita*, and Figure 10 presents the seawater pH vs. time profile for naphthalene uptake. The uptake profile of naphthalene was very similar to the uptake profile of

phenanthrene. The uptake occurred primarily in the first 24 hours. The PAH concentration in the biomass increased rapidly before decreasing and then remained constant for the rest of the experiment. The primary difference observed between the uptake experiments for naphthalene and phenanthrene was the seawater pH vs. time profile. The seawater pH in phenanthrene uptake experiments initially increased then remained relatively constant, varying with photoperiod. The seawater pH in the naphthalene uptake experiment also initially increased, but then steadily decreased for the remainder of the experiment. While the initial fluctuations in the naphthalene concentrations profile coincided with variations in pH, the naphthalene concentrations in the seawater and in the biomass remained relatively constant after the first 24 hours as the pH continued to decrease.

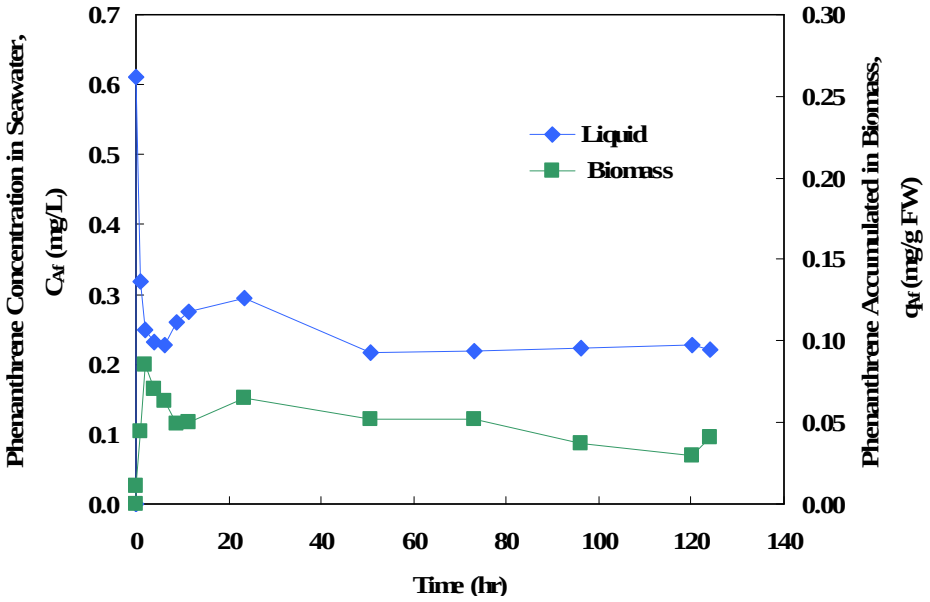


Figure 3. Uptake of phenanthrene by *A. coalita* tissue culture, Trial 1.

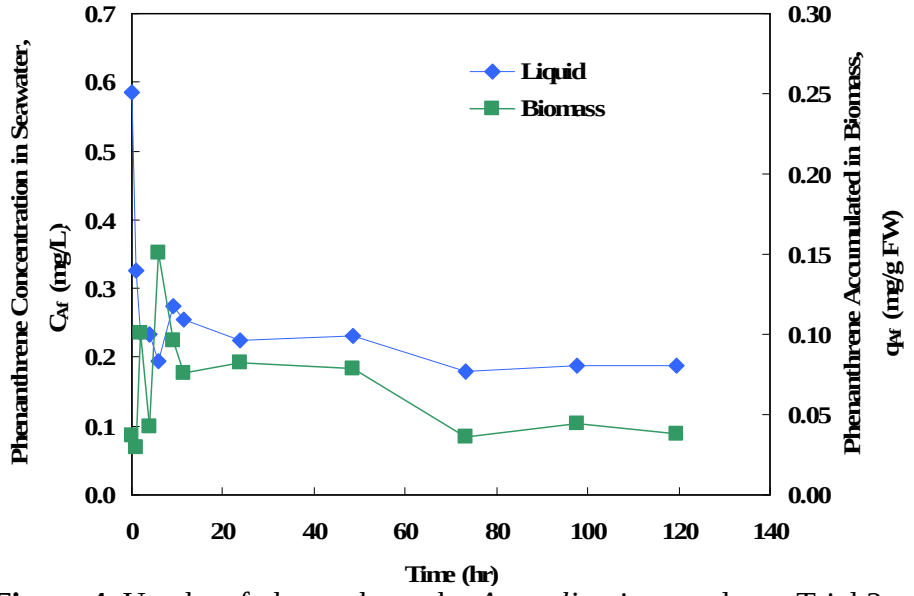


Figure 4. Uptake of phenanthrene by *A. coalita* tissue culture, Trial 2.

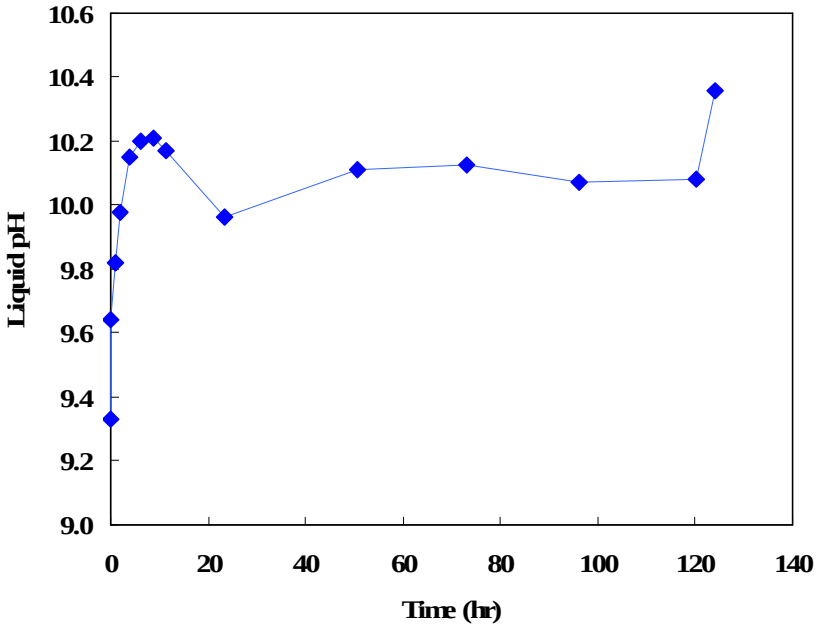


Figure 5. Seawater pH as a function of time for phenanthrene uptake, Trial 1.

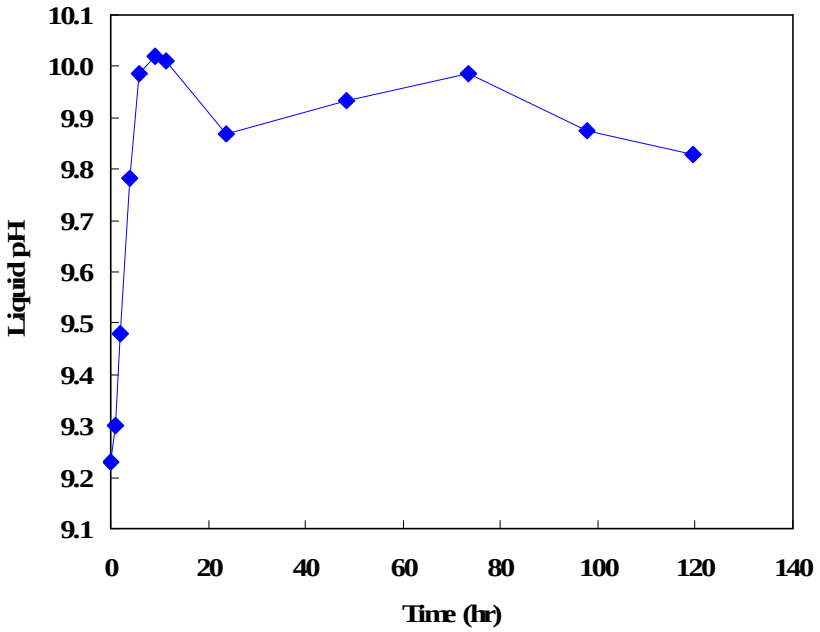


Figure 6. Seawater pH as a function of time for phenanthrene uptake, Trial 2.

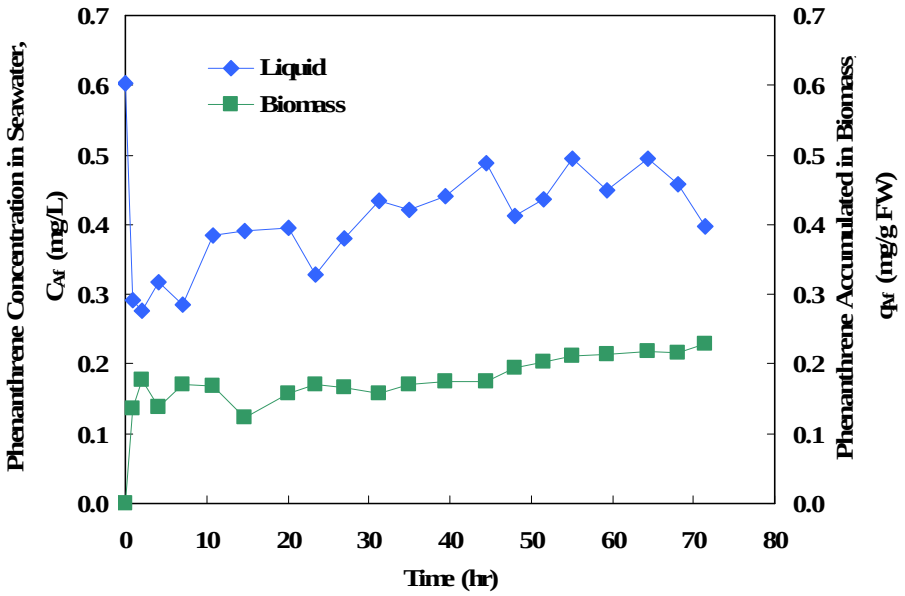


Figure 7a. Uptake of phenanthrene by *A. coalita* tissue culture, Trial 3.

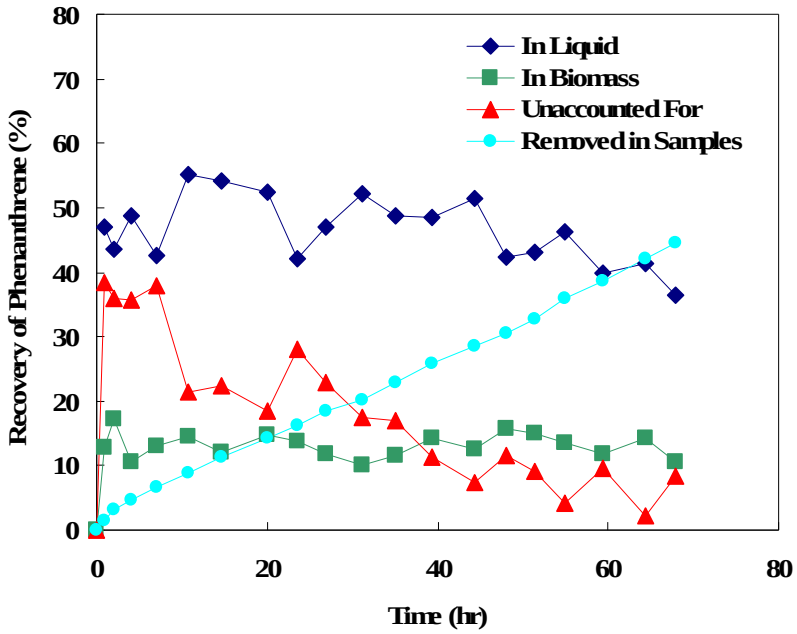


Figure 7b. PAH material balance: uptake of phenanthrene by *A. coalita* tissue culture, Trial 3.

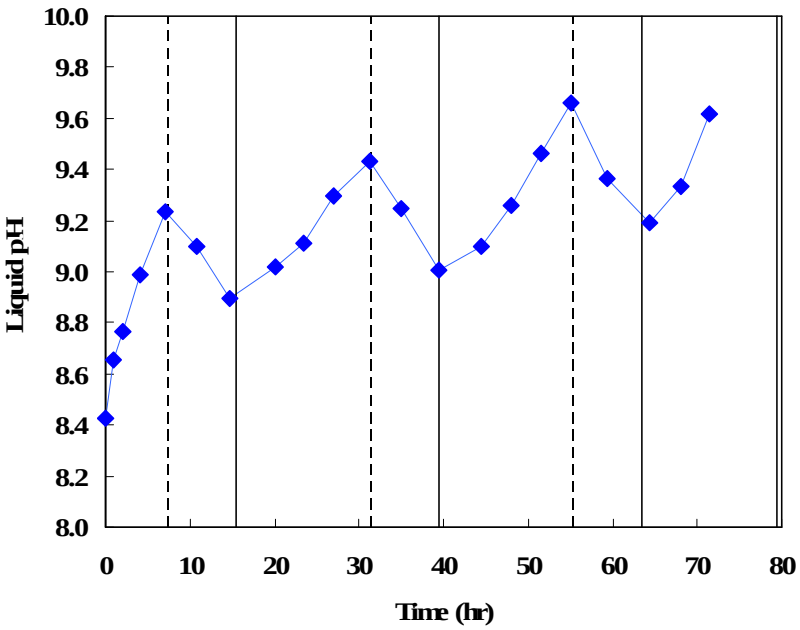


Figure 8. Seawater pH as a function of time for phenanthrene uptake, Trial 3. Solid lines indicate start of light period. Dashed lines indicate start of dark period.

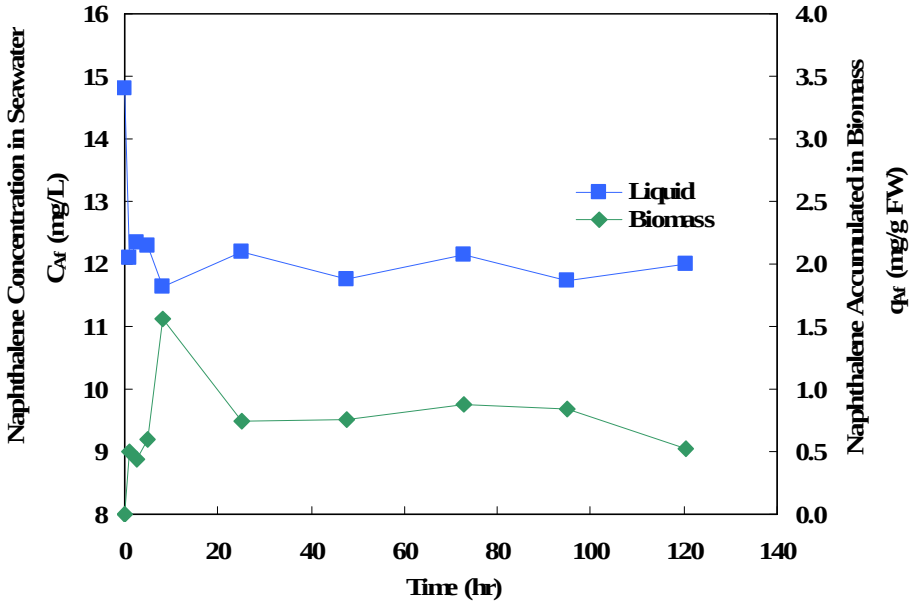


Figure 9. Uptake of naphthalene by *A. coalita* tissue culture.

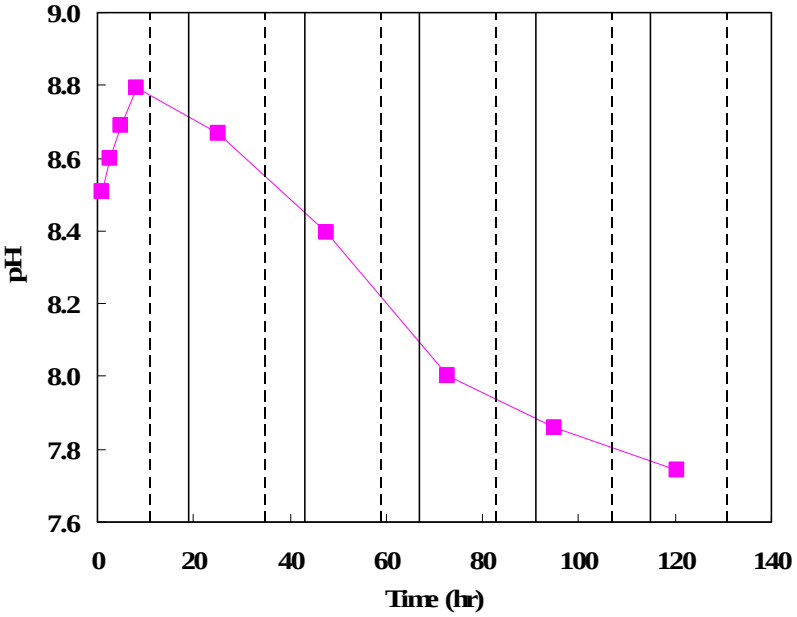


Figure 10. Seawater pH as a function of time for naphthalene uptake. Solid lines indicate start of light period. Dashed lines indicate start of dark period

Equilibrium Partitioning of PAH between Living A. coalita Tissue and Seawater

Figures 11, 12, 13, and 14 present the equilibrium partitioning of two PAH compounds between seawater and living *A. coalita* tissue culture. Specifically, Figure 11 shows the partitioning of phenanthrene, Figure 12 presents naphthalene partitioning data at high naphthalene concentrations, Figure 13 shows partitioning of naphthalene at low naphthalene concentrations, and Figure 14 compares partitioning of naphthalene and phenanthrene. The results of these experiments are summarized in Table 4. While all isotherms were linear, comparison of the partition coefficients showed that the living algal biomass removed significantly more phenanthrene than naphthalene from seawater. The partition coefficient for phenanthrene partitioning between living *A. coalita* tissue and seawater was 0.171 ± 0.0083 L/g FW. In comparison, the coefficients for naphthalene partitioning were 0.0470 ± 0.0017 L/g FW for the naphthalene concentration range of 0 to 16 mg/L, and 0.0500 ± 0.0025 L/g FW for naphthalene concentrations ranging from 0 to 1.0 mg/L.

The biomass continued to grow during PAH uptake experiments. The ratio of final biomass density at the end of the uptake experiment to initial biomass density (X_f/X_0) is presented in Table 4. Growth was not dependent on the initial concentration of PAH in the seawater. The growth was also approximately constant regardless of whether phenanthrene or naphthalene was present. An average growth value is reported for each experiment that represents the average growth from all the sample vials for that experiment.

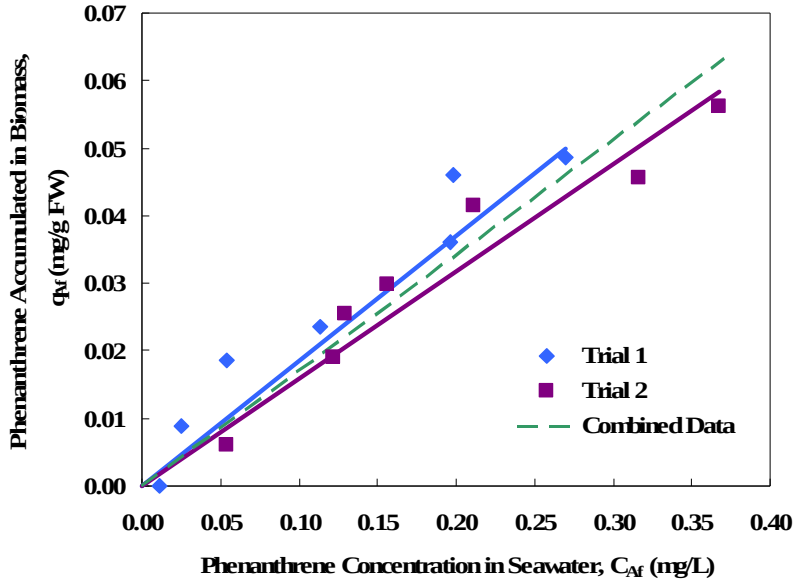


Figure 11. Equilibrium partitioning of phenanthrene between living *A. coalita* and seawater. Each point in Trial 1 represents the average concentrations measured in three replicated vials, while each point in Trial 2 represents the average of two vials. Trial 1: k = 0.186 ± 0.013 L/g FW, X_f/X_0 = 1.46 ± 0.11. Trial 2: k = 0.159 ± 0.0089 L/g FW, X_f/X_0 = 1.24 ± 0.11. Combined Data: k = 0.171 ± 0.0083 L/g FW, X_f/X_0 = 1.37 ± 0.15.

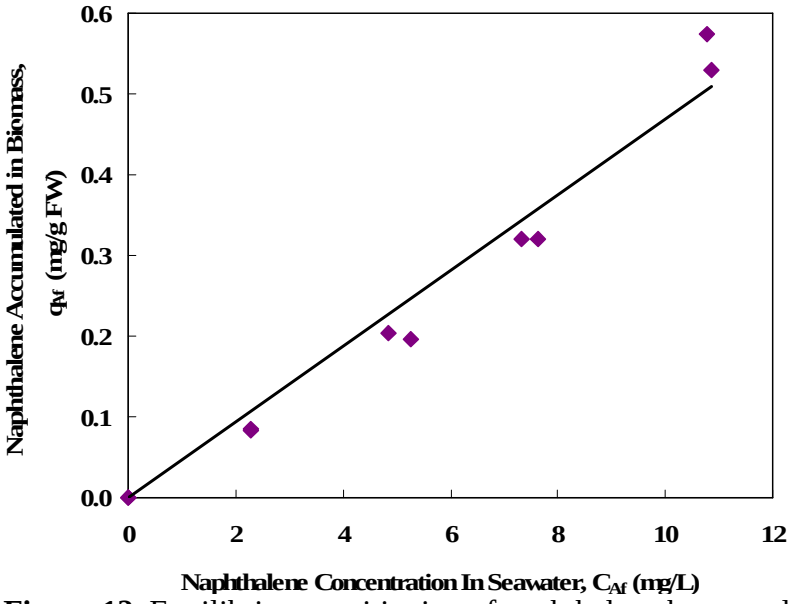


Figure 12. Equilibrium partitioning of naphthalene between living *A. coalita* and seawater at high naphthalene concentrations. k = 0.0470 ± 0.0017 L/g FW, X_f/X_0 = 1.32 ± 0.16.

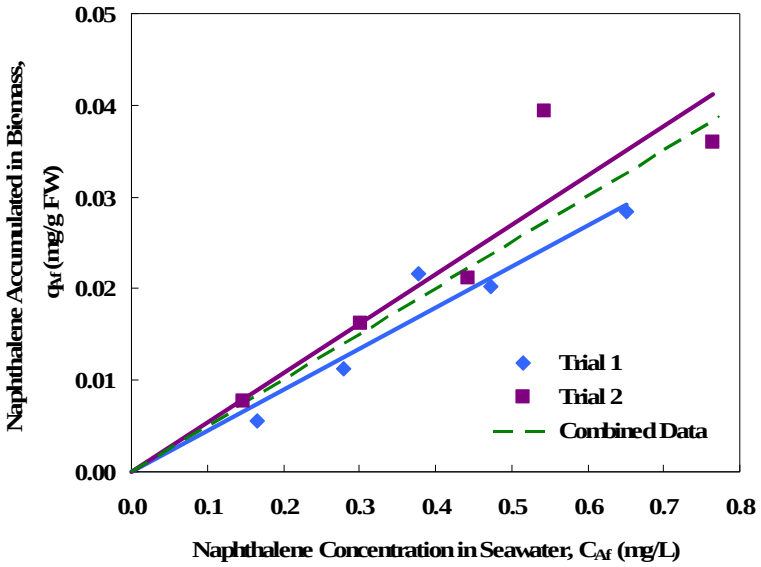


Figure 13. Equilibrium partitioning of naphthalene between living *A. coalita* and seawater at low naphthalene concentrations. Each point represents the average concentrations measured in duplicate sample vials. Trial 1: k = 0.0449 ± 0.0020 L/g FW, X_f/X_0 = 1.18 ± 0.11. Trial 2: k = 0.0539 ± 0.0041 L/g FW, X_f/X_0 = 1.33 ± 0.11. Combined Data: k = 0.0500 ± 0.0025 L/g FW, X_f/X_0 = 1.25 ± 0.13.

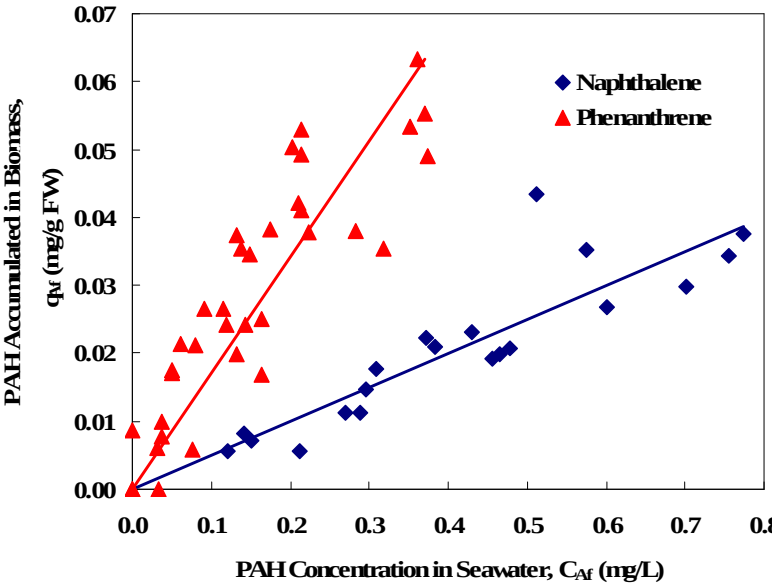


Figure 14. Comparison of equilibrium partitioning of phenanthrene and naphthalene between living *A. coalita* tissue and seawater. Naphthalene: k = 0.0500 ± 0.0025 L/g FW, X_f/X_0 = 1.25 ± 0.13. Phenanthrene: k = 0.171 ± 0.0083 L/g FW, X_f/X_0 = 1.37 ± 0.15.

Table 4. Comparison of PAH partitioning experimental results.

Experiment	Partition Coefficient [a] (L/g FW)	Partition Coefficient [a] (L/g DW)	(X_f/X_0) [b]
Phenanthrene Partitioning Living *A. coalita* Tissue, Trial 1	0.186 ± 0.013 $R^2 = 0.699$	0.518 ± 0.037 $R^2 = 0.698$	1.46 ± 0.11 n = 21
Phenanthrene Partitioning Living *A. coalita* Tissue, Trial 2	0.159 ± 0.0089 $R^2 = 0.812$	0.444 ± 0.025 $R^2 = 0.812$	1.24 ± 0.11 n = 14
Phenanthrene Partitioning Heat-Killed *A. coalita* Tissue, Trial 1	NA	1.82 ± 0.15 $R^2 = 0.614$	NA
Phenanthrene Partitioning Heat-Killed *A. coalita* Tissue, Trial 2	NA	1.98 ± 0.11 $R^2 = 0.841$	NA
High Conc. Naphthalene Partitioning Living *A. coalita* Tissue	0.0470 ± 0.0017 $R^2 = 0.970$	0.131 ± 0.0049 $R^2 = 0.970$	1.32 ± 0.16 n = 10
Low Conc. Naphthalene Partitioning Living *A. coalita* Tissue, Trial 1	0.0449 ± 0.0020 $R^2 = 0.896$	0.125 ± 0.0057 $R^2 = 0.896$	1.18 ± 0.11 n = 10
Low Conc. Naphthalene Partitioning Living *A. coalita* Tissue, Trial 2	0.0539 ± 0.0041 $R^2 = 0.757$	0.151 ± 0.011 $R^2 = 0.757$	1.33 ± 0.11 n = 10
Low Conc. Naphthalene Partitioning Heat-Killed *A. coalita* Tissue, Trial 1	NA	0.137 ± 0.0073 $R^2 = 0.875$	NA
Low Conc. Naphthalene Partitioning Heat-Killed *A. coalita* Tissue, Trial 2	NA	0.191 ± 0.0071 $R^2 = 0.937$	NA

Table 4. Continued

Experiment	Partition Coefficient [a] (L/g FW)	Partition Coefficient [a] (L/g DW)	(X_f/X_0) [b]
Phenanthrene Partitioning Living *A. coalita* Tissue, Trial 1 and 2 Combined	0.171 ± 0.0083 $R^2 = 0.728$	0.477 ± 0.023 $R^2 = 0.728$	1.37 ± 0.15 n = 35
Phenanthrene Partitioning Heat-Killed *A. coalita* Tissue, Trial 1 and 2 Combined	NA	1.92 ± 0.091 $R^2 = 0.788$	NA
Low Conc. Naphthalene Partitioning Living *A. coalita* Tissue, Trial 1 and 2 Combined	0.0500 ± 0.0025 $R^2 = 0.783$	0.140 ± 0.0071 $R^2 = 0.783$	1.25 ± 0.13 n = 20
Low Conc. Naphthalene Partitioning Heat-Killed *A. coalita* Tissue, Trial 1 and 2 Combined	NA	0.163 ± 0.0080 $R^2 = 0.794$	NA
Phenanthrene Desorption Living *A. coalita* Tissue	0.564 ± 0.014 $R^2 = 0.9203$	1.58 ± 0.038 $R^2 = 0.9203$	0.985 ± 0.066 n = 14

[a] Partition coefficients were determined by linear regression analysis. Error for partition coefficients is 1.0 standard error with 95% confidence.
[b] X_f is final biomass density, and X_0 is initial biomass density. Biomass growth is determined by averaging the values of (X_f/X_0) for all vials in an experiment. The error for this measurement is 1.0 standard deviation.

Equilibrium Partitioning of PAH between Heat-Killed A. coalita Tissue and Seawater

The equilibrium data for phenanthrene partitioning between seawater and heat-killed *A. coalita* tissue is presented in Figure 15, while Figure 16 compares phenanthrene partitioning into heat-killed *A. coalita* tissue to phenanthrene partitioning into living *A. coalita* tissue. The experiments are summarized in Table 4. The partition coefficients for phenanthrene partitioning into heat-killed *A. coalita* tissue were 1.82 ± 0.15 L/g DW and 1.98 ± 0.11 L/g DW for trials 1 and 2, respectively. The partition coefficient value for the combined data from both trials was 1.92 ± 0.091 L/g DW. This value is

significantly higher that the partition coefficient found for phenanthrene partitioning into living *A. coalita* tissue, which was 0.477 ± 0.023 L/g DW.

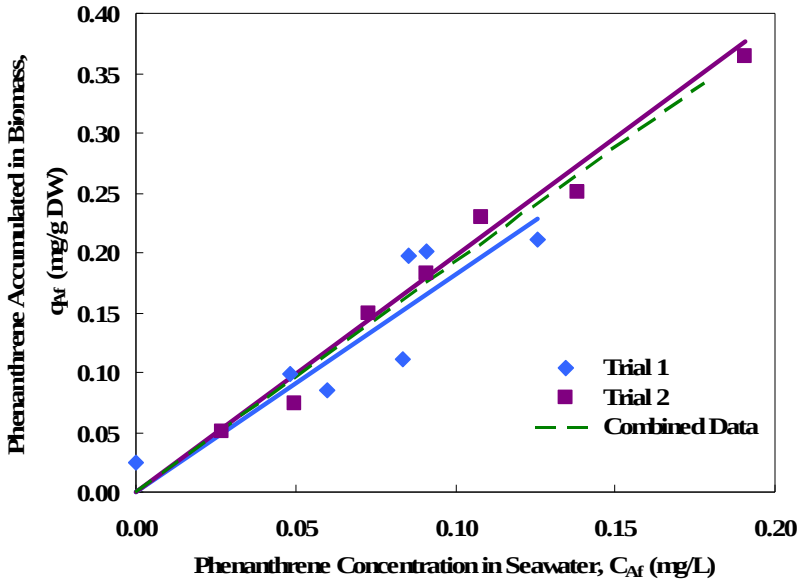


Figure 15. Equilibrium partitioning of phenanthrene between heat-killed *A. coalita* and seawater Each point represents the average concentrations measured in duplicate sample vials. Trial 1: k = 1.82 ± 0.15 L/g DW, Trial 2: k = 1.98 ± 0.11 L/g DW, Combined Data: k = 1.92 ± 0.091 L/g DW.

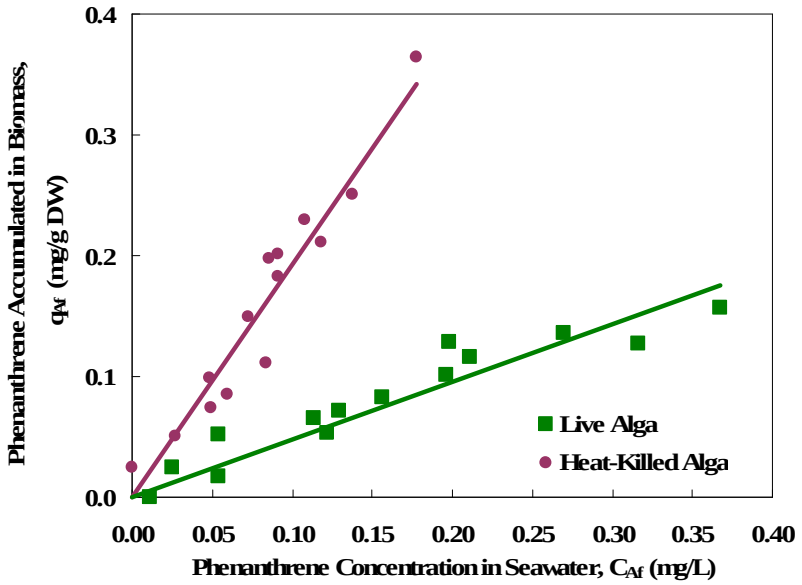


Figure 16. Comparison of phenanthrene equilibrium isotherms for partitioning into living and heat-killed *A. coalita* tissue. Heat-Killed *A. coalita* Tissue: k = 1.92 ± 0.091 L/g DW, Living *A. coalita* Tissue: k = 0.477 ± 0.023 L/g DW.

On the other hand, heat-killed *A. coalita* tissue removed approximately the same amount of naphthalene as living *A. coalita* tissue. The equilibrium isotherms for naphthalene partitioning between seawater and heat-killed *A. coalita* tissue are presented in Figure 17, while Figure 18 compares naphthalene partitioning into heat-killed *A. coalita* tissue to naphthalene partitioning into living *A. coalita* tissue. The coefficients for naphthalene partitioning into heat-killed *A. coalita* tissue were 0.137 ± 0.0073 L/g DW and 0.191 ± 0.0071 L/g DW in two separate trials. The partition coefficient for the combined data from both trials was 0.162 ± 0.0080 L/g DW. This was only slightly higher than the value of 0.140 ± 0.0071 L/g DW found for partitioning of naphthalene into living *A. coalita* tissue. A statistical analysis revealed that there was not a statistically significant difference between naphthalene partitioning into living *A. coalita* tissue compared to naphthalene partitioning into heat-killed *A. coalita* tissue.

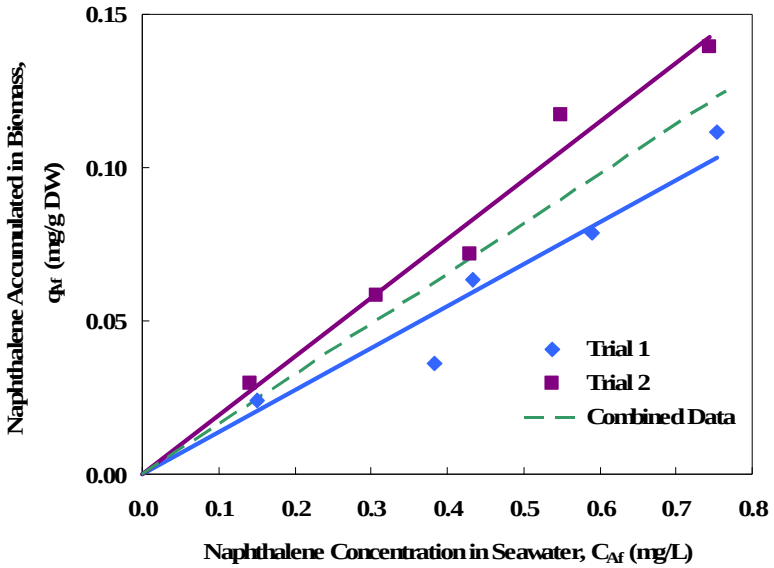


Figure 17. Equilibrium partitioning of naphthalene between heat-killed *A. coalita* and seawater Trial 1: k = 0.137 ± 0.0073 L/g DW, Trial 2: 0.191 ± 0.0071 L/g DW. Combined Data: k = 0.162 ± 0.0080 L/g DW.

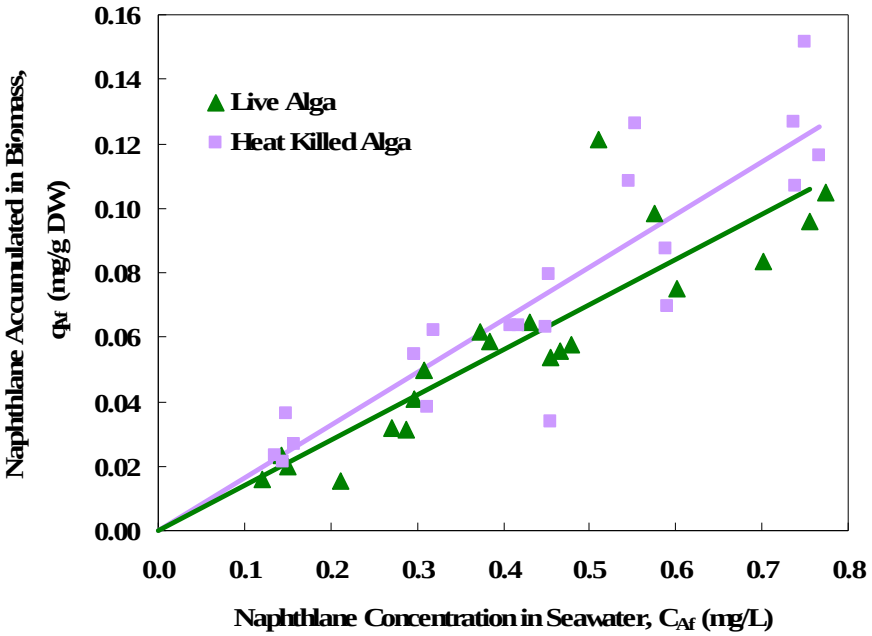


Figure 18. Comparison of naphthalene equilibrium isotherms for partitioning into living and heat-killed *A. coalita* tissue. Heat-Killed *A. coalita* Tissue: k = 0.163 ± 0.0080 L/g DW, Living *A. coalita* Tissue: k = 0.140 ± 0.0071 L/g DW.

Desorption

Figure 19 presents the phenanthrene desorption isotherm for phenanthrene desorbed from living *A. coalita* tissue into fresh medium initially containing no phenanthrene. The experiment is summarized in Table 4. Figure 20 compares phenanthrene uptake by living *A. coalita* tissue to phenanthrene desorption. The phenanthrene desorption isotherm did not result in the same PAH distribution as the uptake isotherm. The ratio of PAH recovered in the biomass to the PAH found in the seawater was higher in phenanthrene desorption partitioning experiments relative to phenanthrene uptake partitioning experiments. This resulted in a larger partition coefficient for desorption compared to uptake. The partition coefficient for desorption was 0.564 ± 0.014 L/g FW. This value was significantly higher than the coefficient of 0.171 ± 0.0083 L/g FW found for phenathrene uptake into living *A. coalita* tissue. An additional difference in this experiment was the biomass growth rate. The X_f/X_0 value was 0.985 ± 0.066. The biomass fresh weight actually slightly decreased. In all other partitioning experiments the biomass grew significantly. As with uptake experiments

the pH in the seawater increased over the course of the experiment. The initial pH was 8.62. The average final pH for all the vials in the experiment was 9.93 ± 0.074.

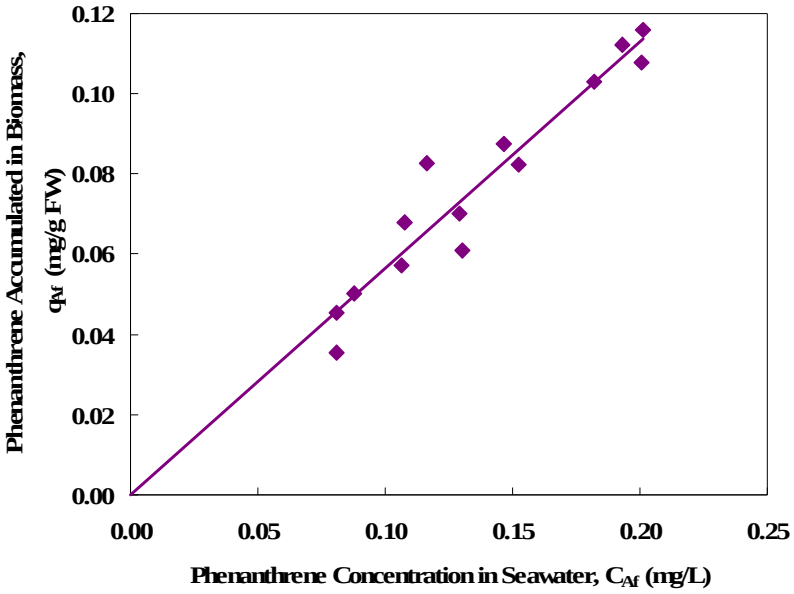


Figure 19. Phenanthrene desorption isotherm. k = 0.564 ± 0.014 L/g FW, X_f/X_0 = 0.985 ± 0.066.

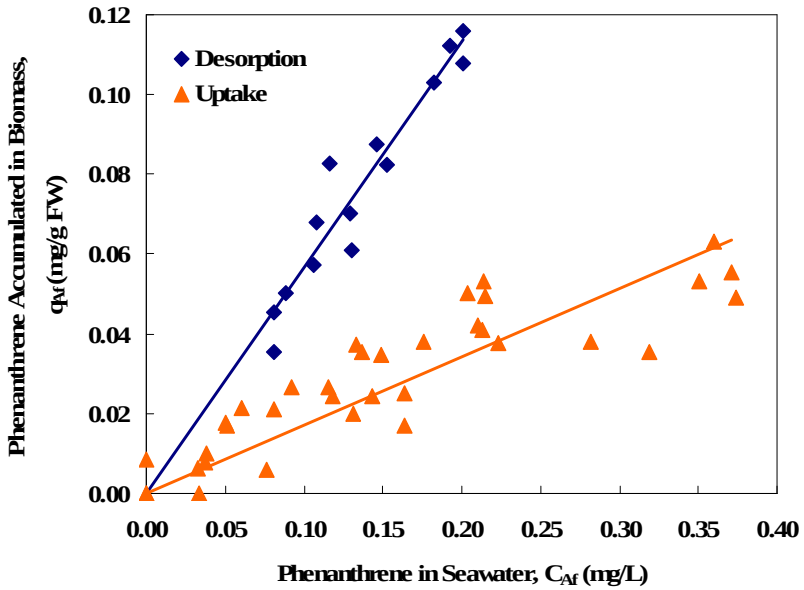


Figure 20. Comparison of phenanthrene desorption to phenanthrene uptake by living *A. coalita* Desorption: k = 0.564 ± 0.014 L/g FW, X_f/X_0 = 0.985 ± 0.066. Uptake: k = 0.171 ± 0.0083 L/g FW, X_f/X_0 = 1.37 ± 0.15.

Material Balance on PAH Compounds in Uptake Experiments

Table 5 presents the results of a material balance on the PAH compound for each uptake experiment. This table indicates the amount of PAH that was removed during sampling, the amounts that were recovered in both the seawater and the biomass at the end of the 120 hour experiment, as well as the amount that was unaccounted for. The amounts are given as a percentage of the initial measured amount of PAH added to the reactor vessel. To determine the amount lost to abiotic processes, control experiments were conducted in each reactor. The control experiments had PAH compounds in the seawater, but no biomass was present. The Belco reactor was used for phenanthrene uptake experiments 1 and 2. A phenanthrene control experiment in this reactor resulted in a loss of 46.0 % of the phenanthrene. Bottle reactors were used for phenanthrene uptake experiment 3 and the naphthalene uptake experiment. Control experiments in the bottle reactors had 7.9 % of phenanthrene unaccounted for and a negligible loss of naphthalene. The amount of PAH unaccounted for is significantly reduced if it is assumed that the same amount of PAH is lost to abiotic processes in uptake experiments as in control experiments.

Table 5. PAH mass balance: distribution of PAH in uptake experiments

Experiment	PAH in Samples (%)	PAH in Seawater (%)	PAH in Biomass (%)	PAH Unaccounted For (%)	PAH Unaccounted For Relative to Control (%)
Phenanthrene Uptake Experiment 1	9.3	30.2	8.2	52.3	6.3
Phenanthrene Uptake Experiment 2	11.8	25.7	10.6	51.8	5.8
Phenanthrene Uptake Experiment 3	45.4	30.2	17.5	6.9	-1.0
Naphthalene Uptake Experiment 1	21.6	61.6	4.6	12.2	12.2

Material Balance on PAH Compounds in Equilibrium Partitioning Experiments

Material balances on PAH compounds were performed for all PAH equilibrium partitioning experiments. Figure 21 compares the material balances for partitioning of phenanthrene, while Figure 22 compares the material balances for naphthalene partitioning. The percentage of the initial amount of PAH recovered in each phase was constant for any given experiment, regardless of initial PAH concentration. Therefore, the data was presented as an average percentage of the total amount of PAH found in each phase at the end of the experiment. Additionally, the material balance for a no biomass control experiment is presented in each figure.

There were some differences in the material balances for phenanthrene and naphthalene. The amount of naphthalene missing in all experiments with biomass was within error of the amount unaccounted for in the naphthalene control. Conversely, all phenanthrene trials had larger amounts of PAH unaccounted for compared to the control. Also, the distribution of PAH was approximately the same in all four

partitioning experiments with naphthalene. This result was expected because there was not a significant difference in the partition coefficients for naphthalene partitioning into living and heat-killed *A. coalita* tissue. On the other hand, because the partition coefficient for phenanthrene is significantly higher for partitioning into heat-killed *A. coalita* tissue than living *A. coalita* tissue, the amount of phenanthrene recovered from heat-killed biomass should have been higher than the amount recovered from living *A. coalita* biomass, but this result was not observed. The amount of phenanthrene recovered in the biomass was approximately the same for all phenanthrene experiments. Instead less of the phenanthrene was recovered in the liquid and more remained unaccounted for in phenanthrene experiments with heat-killed *A. coalita* tissue. An additional difference between experiments with phenanthrene and naphthalene was the distribution of PAH. The majority of the PAH was recovered in the liquid phase for naphthalene experiments, while phenanthrene experiments had higher amounts recovered in the biomass. All of the experiments represented in Figures 21 and 22 had the same initial biomass densities and initial PAH concentration range. The difference in PAH distribution was due to the difference in partitioning coefficients and amounts unaccounted for. The amount recovered in the biomass was less for naphthalene experiments than phenanthrene experiments, because naphthalene partitioned into *A. coalita* to a lesser extent than phenanthrene. It was not known why phenanthrene experiments had higher amounts of PAH unaccounted for relative to naphthalene experiments.

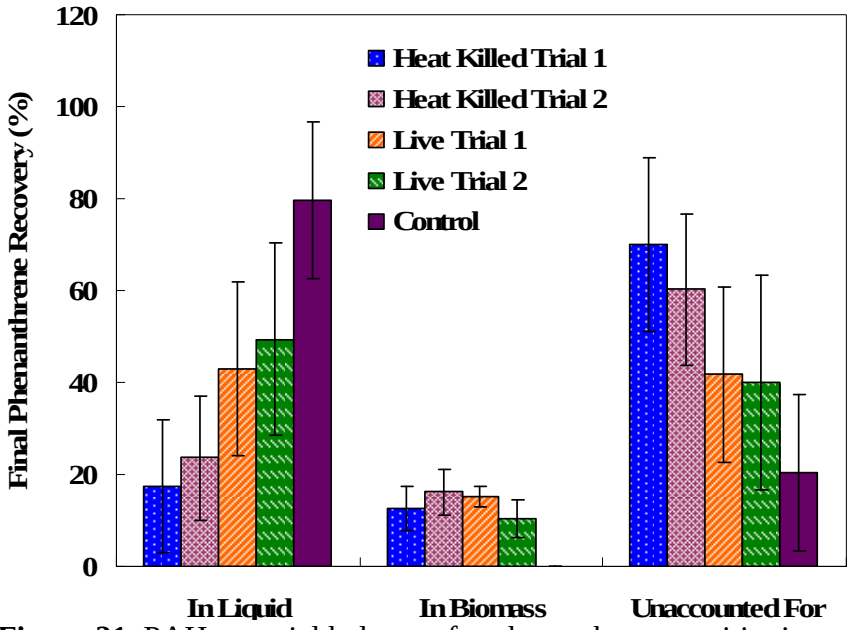


Figure 21. PAH material balances for phenanthrene partitioning experiments. Given as the percentage of the initial amount of PAH added that was recovered in each phase. Error bars represent 1.0 standard deviation.

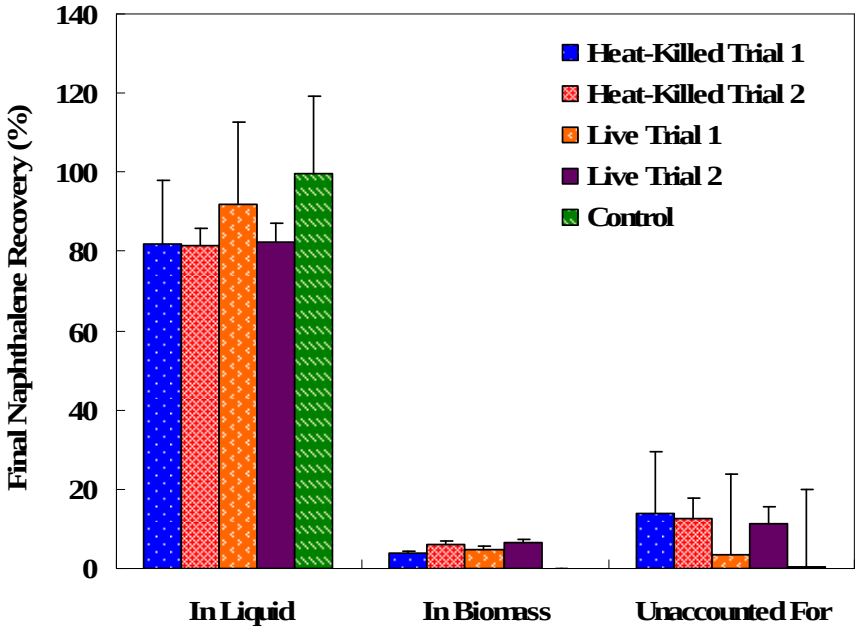


Figure 22. PAH material balances for naphthalene partitioning experiments. Given as the percentage of the initial amount of PAH added that was recovered in each phase. Error bars represent 1.0 standard deviation.

Recovery of PAH using Multiple Biomass Extractions

To determine if multiple extractions of the biomass would result in a higher recovery of PAH, a biomass extraction study was performed. The material balance for this experiment is presented in Figure 23. The experiment was conducted by adding approximately 12 mg FW of biomass to each of two 20 mL vials. 10 mL of medium containing phenanthrene was also added to each vial. The vials were sealed and mixed on an orbital shaker overnight. After 24 hours, the seawater and biomass were separated by filtration through a nylon mesh filter. The liquid phase was sampled, and the total amount of phenanthrene in the liquid phase was determined by HPLC analysis. The biomass was collected in a 4 mL vial. The biomass was extracted 3 times with 0.5 mL of acetonitrile. The acetonitrile was separated from the biomass and analyzed by HPLC after each extraction.

The results of the biomass extraction study indicate that the majority of the extractable PAH in the biomass is recovered in the first biomass extraction. The amount of PAH recovered in the liquid phase was approximately 33 % of the initial amount of PAH added to the vials. The first biomass extraction recovered 38.6 % and 56.0 % of the total PAH in vials 1 and 2, respectively. The second extraction recovered an additional 2.80 % of the PAH in vial 1 and 4.59 % in vial 2. The PAH recovery in the third biomass extraction was less than 0.35 % of the total mass of PAH for both vials. The amount of PAH that was not recovered was 25.3 % in the first vial and 5.75 % in the second vial. Because there was very little PAH in the third biomass extraction, the missing PAH was assumed to be bound in a non-extractable form or to be lost due to abiotic processes. The large difference in the amount of PAH unaccounted for between the two vials illustrates the large error associated with PAH recovery.

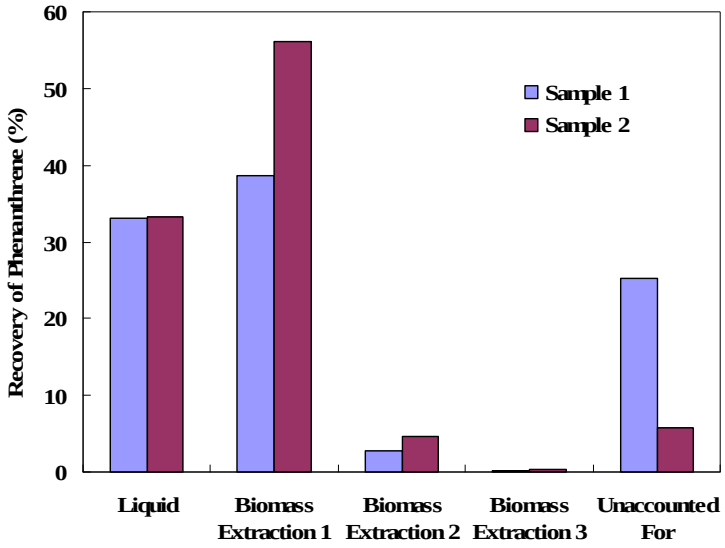


Figure 23. PAH material balance with multiple biomass extractions

Metabolite Detection

GC-MS analysis was performed to detect and identify any metabolites present in the aqueous phase or in biomass extracts. Figure 24 compares chromatograms for aqueous samples, while the chromatograms for biomass extraction samples are presented in Figure 25. Major peaks and peaks of interest are identified in the chromatograms and summarized in Table 6. Peaks 1, 2, 7-9, 12, and 13 were found in all aqueous and biomass extraction samples including the control samples without phenanthrene. Peak 1 was identified as a component of the derivatization reagent used for this experiment, while peak 2 was identified as an ingredient found in PES nutrient stock. Peaks 7-9, 12, and 13 were not identifiable. However, since they were present in control samples without phenanthrene, they are not products of phenanthrene degradation. Peak 11 was found in all aqueous phase samples, but not detected in biomass extracts. This peak was identified as 1, 8-octanediylbis trimethyl silane, and is not a possible product of phenanthrene degradation.

Peak number 4, with a retention time of 15.320 minutes, was identified as phenanthrene. A detailed view of this peak is presented in Figure 26. As expected, this peak only occurred in samples when phenanthrene was added. The total ion count

for this peak was less in the aqueous sample from the reactor with biomass compared to the control, indicating that the presence of the biomass reduced the amount of phenanthrene in the liquid.

Peaks 10 and 14 were investigated because they appeared only in the aqueous sample from the reactor with biomass and did not appear in the control. However, upon closer examination, peak 14 was identified as phthalate ether, a chemical commonly added to plastics, and peak 10 was identified as 1, 1'-sulfonylbis 4-chlorobenzene, a pesticide. The source of these compounds in the sample was not known. However, to verify if these compounds were contaminants, a second sample was prepared from the aqueous phase of an independent phenanthrene uptake trial. The sample generation, preparation, and analysis for the second sample was identical to the procedures used in the first analysis. Peaks 10 and 14 were not detected in the second sample.

The remaining peaks were primarily found in the biomass extracts. Peaks 5 and 6 were found exclusively in the biomass, while peak 3 was found in both aqueous and biomass samples but was detected in much larger amounts in the biomass. Peak 5 could not be identified. However, peak 3 was identified as glycerol modified by the derivitazation agent. Glycerol is a primary component found in lipids. Peak 6 was identified as 9, 12, 15-octadecatrienoic acid methyl ester, a fatty acid. The identification of these peaks was important, because lipids and fatty acids are likely binding sites for PAH compounds.

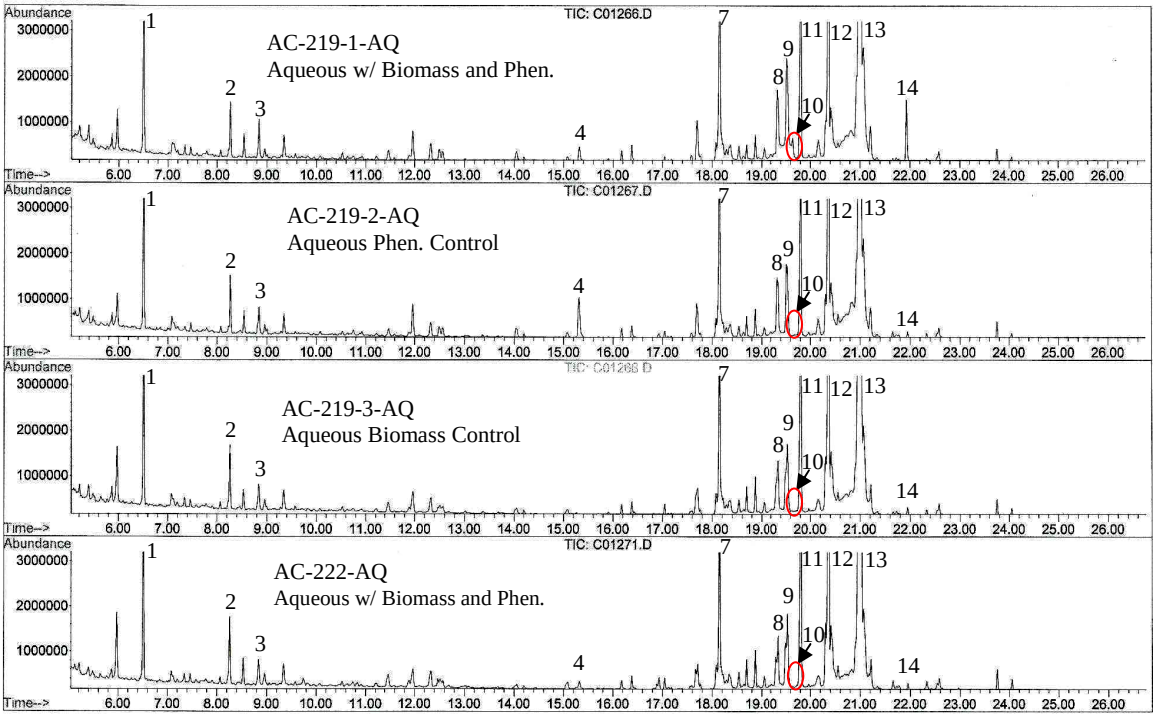


Figure 24. Comparison of GC-MS chromatograms for metabolite detection in aqueous samples.

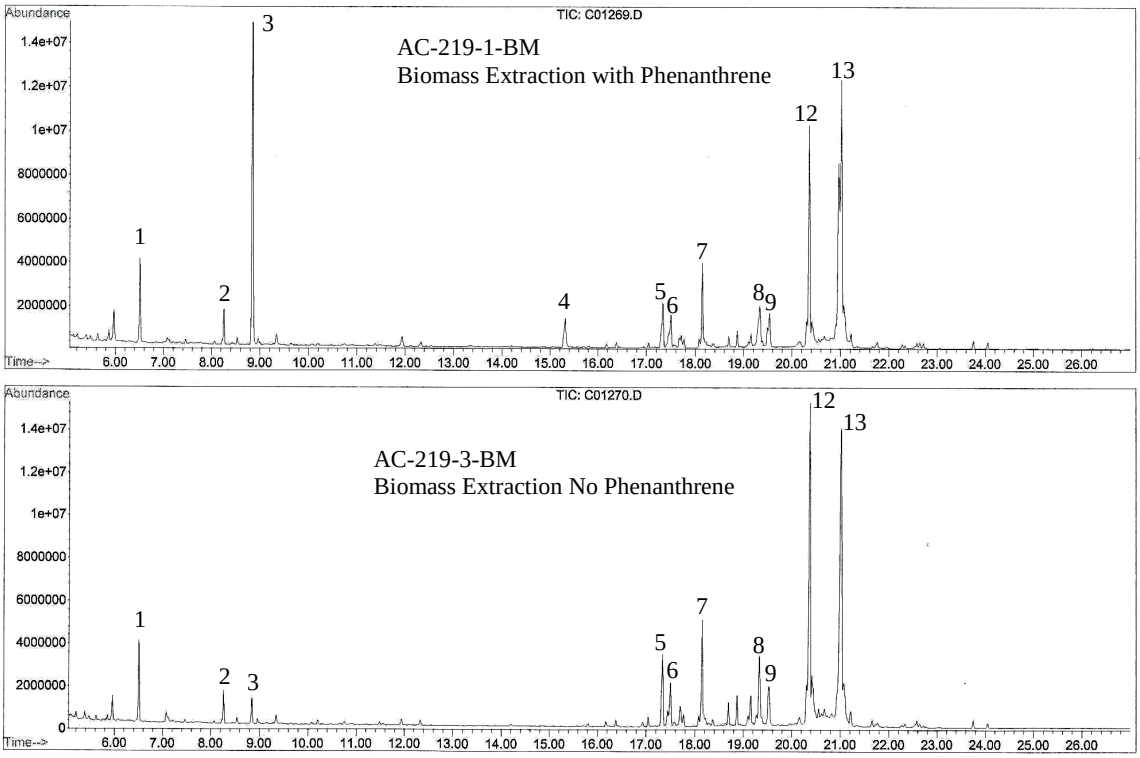


Figure 25. Comparison of GC-MS chromatograms for metabolite detection in biomass extraction samples.

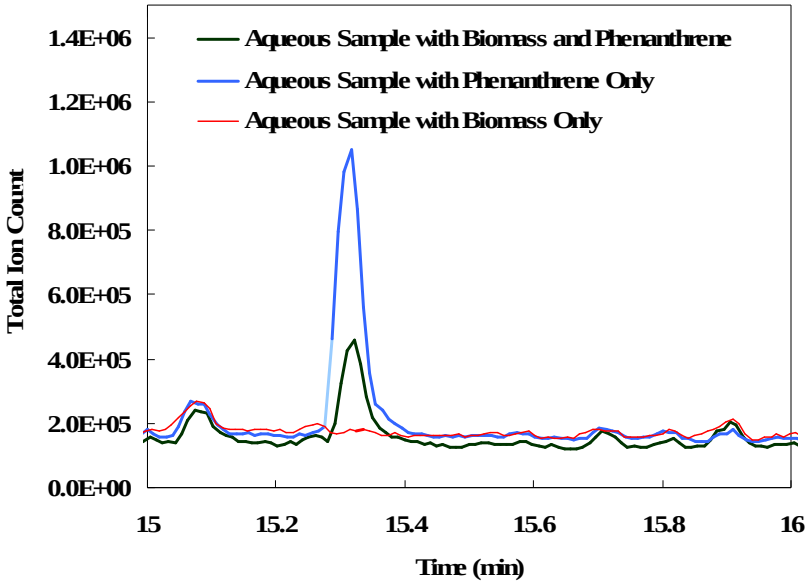


Figure 26. Phenanthrene peaks detected by GC-MS in aqueous samples.

Table 6. Summary of major peaks and peaks of interest found by GC-MS analysis. Relative abundance of each ion is given in parentheses.

Peak #	Retention Time (min)	Mass Signal (m/z) and Relative Abundance	Peak ID	Quality of Match	Comments
1	6.506	174 (100), 73 (73), 128 (33), 59 (31), 86 (27)	1,1,1-trimethyl-N-propyl-N-(trimethylsilyl)-silanamine	81	Component of derivatization agent
2	8.269	174 (100), 73(83), 147 (31), 58 (26)	N, N-bis(trimethylsilyl)-beta Alanine	72	A component of PES nutrient stock
3	8.843	73 (100), 147 (51), 58 (33), 205 (30), 117 (28)	Trimethylsilyl ether of glycerol	64	Glycerol modified by the derivatization
4	15.320	178 (100), 58 (27), 77 (16), 152(11)	Phenanthrene	96	PAH added to seawater medium
5	17.332	73 (100), 79 (80), 77 (44), 75 (72), 91 (47), 93 (39), 67 (39), 117 (32)	Unidentified	NA	Only found in biomass extraction samples
6	17.500	75 (100), 73 (89), 79 (76), 67 (48), 77 (45), 55 (37), 93 (35), 95 (30), 129 (27)	9, 12, 15-octadecatrienoic acid, methyl ester	55	Only found in biomass extraction samples
7	18.152	55 (100), 69 (59), 122 (58), 136 (47), 83 (34), 56 (32)	Unidentified	NA	Found in all samples

Table 6. Continued

Peak #	Retention Time (min)	Mass Signal (m/z) and Relative Abundance	Peak Identification	Quality of Match	Comments
8	19.331	75 (100), 73 (84), 131 (76), 144 (56), 128 (46), 116 (46), 55 (42)	Unidentified	NA	Found in all samples
9	19.519	131 (100), 75 (48), 73 (45), 116 (36)	Unidentified	NA	Found in all samples
10	19.638	159 (100), 75 (62), 111 (41), 50 (26), 58 (26)	1, 1'-sulfonylbis 4-chlorobenzene	94	Found only in the aqueous sample with both biomass and phenanthrene
11	19.806	147(100), 133 (33)	1, 8-octanediylbis trimethyl silane	83	In liquid samples only
12	20.361	73 (100), 147 (70), 203 (55), 216 (27), 75 (25), 55 (24)	Unidentified	NA	Found in all samples
13	21.024	75 (100), 73 (90), 131 (93), 128 (55), 144 (74), 116 (53), 55 (41), 338 (25)	Unidentified	NA	Found in all samples
14	21.950	149 (100), 57 (33), 167 (27)	Bis (2-ethylhexyl) phthalate	78	Found only in the aqueous sample with both biomass and phenanthrene

Lipid Analysis of Acrosiphonia coalita

To determine if PAH partitioning was due to uptake into the lipid layer of the algal cell, measured partition coefficients were compared to theoretical partition coefficients for PAH compounds partitioning between lipid and water. The theoretical coefficient for PAH partitioning between lipid and water can be estimated from the octanol-water partitioning coefficient of the PAH compound. This relationship is described by the equation:

$$K_{lipw} = 0.0032 K_{ow}^{0.9} \quad [12]$$

where K_{lipw} is the partition coefficient for the partitioning of PAH between water and lipid in units of L/g lipid, and K_{ow} is the octanol-water partition coefficient. Using this relationship, the theoretical partition coefficients for phenanthrene and naphthalene partitioning between water and lipid are 41.5 L/g lipid and 3.38 L/g lipid, respectively.

To compare the theoretical partition coefficient values to measured values, the lipid content of *A. coalita* was measured. The procedure used for determination of the lipid fraction is described by Hellebust and Craigie [15]. The lipid analysis indicated that 0.507 % of the fresh weight of *A. coalita* tissue was lipid. Based on the lipid fraction in the alga, the measured partition coefficients were 33.7 L/g lipid and 9.85 L/g lipid for phenanthrene and naphthalene partitioning into living *A. coalita* tissue, respectively.

Discussion

The uptake of PAH by tissue cultures of *A. coalita* occurred very quickly and was affected by changes in seawater pH. The fast rate of uptake was in agreement with other studies from the literature. An examination of the uptake kinetics of phenanthrene accumulation by marine diatoms revealed that short term steady state was achieved within 30 minutes [15], and the uptake of phenanthrene by dead tissues of the brown seaweed *Sargassum hemiphyllum* reached equilibrium within 24 hours [16]. It was determined that the uptake rate of phenanthrene by *S. hemiphyllum* was proportional to agitating speeds, but that the maximum sorption capacity remained constant once equilibrium was achieved [16].

The variation in pH with photoperiod was due to a shift in the carbon equilibrium. The carbon equilibrium can be described by [17]:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+ \leftrightarrow HCO_3^- \leftrightarrow CO_2 + OH^-$$

During the light phase CO_2 was consumed by photosynthesis. Consumption of CO_2 caused a shift in the equilibrium resulting in the dissociation of carbonate and the release of hydroxyl groups. During the dark cycle, CO_2 was released due to respiration processes such as the oxidation of sugars in the energy pathways of glycolysis and the Krebs cycle [17]. The released CO_2 reacted with free hydroxyl group to form carbonate, reducing the pH.

The reasons why pH affected uptake are unclear. It is possible that the change in pH affected binding of PAH to the algal cell constituents. However, PAH compounds are nonionic, so their physical properties should not be greatly affected by pH change. On the other hand, the nature of the organic material present in the algal cell is complex. For example, polysaccharides and proteins are found in algal cell walls. These components can have sulfate, carboxyl, and phosphate groups which can be charged [17]. A change in pH could affect the affinity of PAH to these compounds. Other experiments have shown a relationship between the uptake of PAH by algae and pH. However, an increase in pH usually caused a decrease in the adsorption capacity. For

example, the removal of phenanthrene by dead tissue of the *S. hemiphyllum* was decrease under constant alkalinity compared to removal at neutral pH levels [16].

The seawater pH vs. time profile was the primary difference observed between the time course experiments for uptake of naphthalene and uptake of phenanthrene. The seawater pH in phenanthrene uptake trials initially increased, and then remained relatively high throughout the experiment. The seawater pH in the naphthalene uptake trial also increased initially, but then decreased for the rest of the experiment. The drop in pH in the naphthalene experiment was possibly due to an increased toxic effect on the alga caused by the increased PAH concentration in the seawater for this experiment. The initial PAH concentration for naphthalene uptake was 14.8 mg/L compared to only 0.6 mg/L for phenanthrene uptake. Naphthalene has been shown to inhibit photosynthesis in the Acrosiphonia species *Acrosiphonia sonderi*, *Acrosiphonia arcta*, and *Acrosiphonia centralis*, and photosynthesis was stopped completely in *Acrosiphonia sonderi* in supersaturated concentrations of naphthalene [18]. It is possible that the decrease in pH was due to a decrease in photosynthetic activity caused by the toxic effects of elevated naphthalene concentration.

All equilibrium isotherms for uptake of PAH by *A. coalita* were linear. The partition coefficients for phenanthrene and naphthalene uptake by living and heat-killed *A. coalita* tissues are listed in Table 4. Compared to values found in the literature, the amount of PAH accumulated in *A. coalita* tissue was much lower that what was observed for other algal species. Bioaccumulation of phenanthrene for various microalgal species has been found to range from 8.3 to 38.2 L/g DW [15]. The partitioning coefficient found in this study for uptake of phenanthrene by living *A. coalita* tissue was 0.477 L/g DW. However, microalgae have much higher surface area to volume ratios compared to macroalgae. This might suggest adsorption to the cell surface. If this were the mechanism of uptake, then it would be expected that microalgae would take up more PAH per gram than macroalgae.

The partition coefficient for phenanthrene uptake was 3.4 times higher than the partition coefficient for naphthalene uptake by living *A. coalita* tissue. The difference in the partitioning coefficients of the two compounds is due to differences in their physical properties (Table 1). PAH uptake is likely caused by hydrophobic interactions with components of the algal cell. The octanol/water partition coefficient (K_{ow}) of a compound is a good indicator of hydrophobicity, and PAH accumulation into organic matter increases with higher values of K_{ow} [12, 13].

The differences between PAH partitioning into living *A. coalita* compared to heat-killed *A. coalita* are more subtle. Naphthalene partitioning into the live alga tissue was approximately the same as partitioning into the heat-killed *A. coalita* tissue. Meanwhile, the partition coefficients for phenanthrene uptake into living and heat-killed *A. coalita* tissues were not equivalent. The coefficient was significantly higher for phenanthrene partitioning into heat-killed *A. coalita* tissue. However, the phenanthrene recovery in the biomass was approximately the same from both living and heat-killed tissues for all phenanthrene partitioning experiments (Figure 21). The increase in the partition coefficient for uptake by heat-killed *A. coalita* tissue was caused by a decrease in the amount recovered in the liquid phase and an increase in the amount of phenanthrene unaccounted for. Because of the large amount of phenanthrene missing in partitioning experiments with heat-killed tissue, comparisons between uptake of phenanthrene by heat-killed and living tissues cannot be made.

The most likely cause for the loss of phenanthrene in experiments with heat-killed tissue is that some of the phenanthrene was bound to dissolved organic matter (DOM) in the liquid phase. Figure 27 is a microscopic image of *A. coalita* after the heat treatment process. The process of heat-killing the alga modified the algal structure. Some of the cell walls were ruptured. The organic material from inside the cell was likely released into the liquid medium, producing a large amount of DOM.
To determine if the PAH was bound to DOM in the liquid, the HPLC chromatograms for aqueous samples from phenanthrene partitioning experiments were closely

examined. If PAH was adsorbed to DOM that passed through the HPLC column, but did not interact with the stationary phase of the column, there would be an increase in the area of the void peak. The average area of the void peak from the HPLC analysis of aqueous samples for each phenanthrene partitioning experiment is presented in Table 7. The void peak area was significantly lower in the two phenanthrene partitioning trials with living *A. coalita* tissue compared to the second phenanthrene partitioning trial with heat-killed *A. coalita* tissue. However, the void peak was much smaller in the first phenanthrene partitioning trial with heat-killed tissue compared to all other trials. Figure 28a presents an HPLC chromatogram from an aqueous sample from the first trial of phenanthrene partitioning into heat-killed alga. For comparison, Figure 28b presents an HPLC chromatogram of an aqueous sample from Trial 1 of phenanthrene partitioning into living *A. coalita* tissue. There is a second peak that occurs just after the void peak in all HPLC chromatograms from Trial 1 of phenanthrene partitioning into heat-killed tissue. This peak does not appear in any of the other phenanthrene partitioning experiments. The appearance of a second peak just after the void peak in Trial 1 of phenanthrene uptake by heat-killed tissue and the increase in the void peak area in Trial 2 of phenanthrene uptake by heat-killed tissue might suggest that the PAH is passing through the HPLC column adsorbed to DOM.

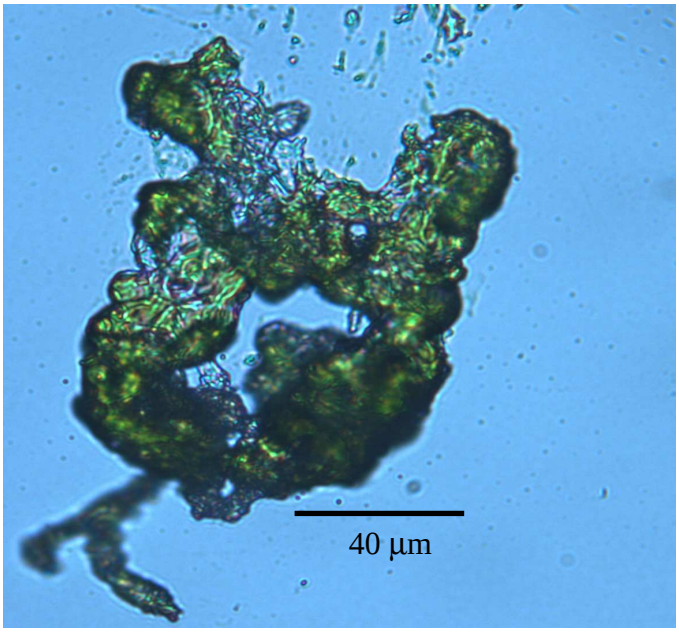


Figure 27. Degradation of algal structure after heat treatment, 40x magnification

Table 7. Comparison of void peak areas from the HPLC analysis of liquid samples from phenanthrene partitioning experiments with living and heat-killed *A. coalita* tissues.

Experiment	Void Peak Area
Phenanthrene Partitioning Living *A. coalita* Tissue, Trial 1	38.4 ± 7.1
Phenanthrene Partitioning Living *A. coalita* Tissue, Trial 2	21.9 ± 8.4
Phenanthrene Partitioning Heat-Killed *A. coalita* Tissue, Trial 1	11.5 ± 1.5
Phenanthrene Partitioning Heat-Killed *A. coalita* Tissue, Trial 2	50.7 ± 4.5

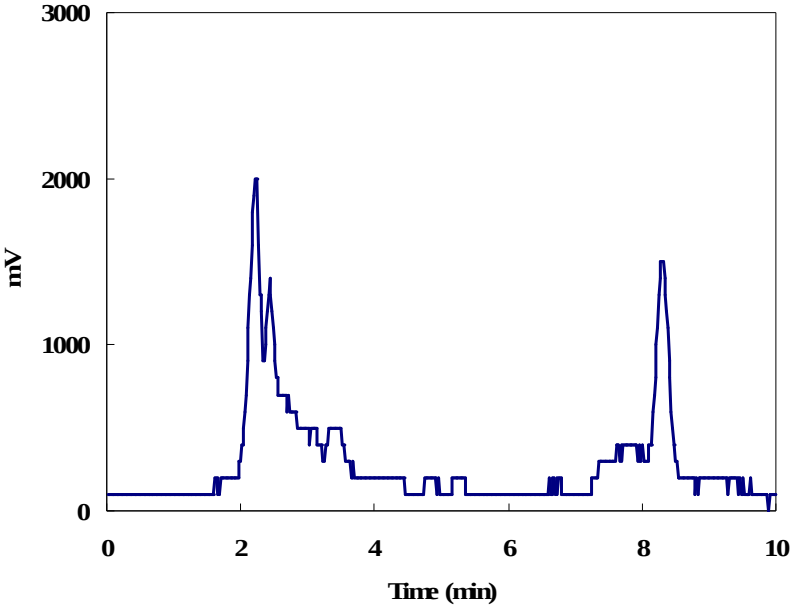


Figure 28a. HPLC Sample Chromatogram: aqueous sample from Trial 1 of phenanthrene partitioning into heat-killed *A. coalita* tissue. HPLC File: 220p41.ASC.

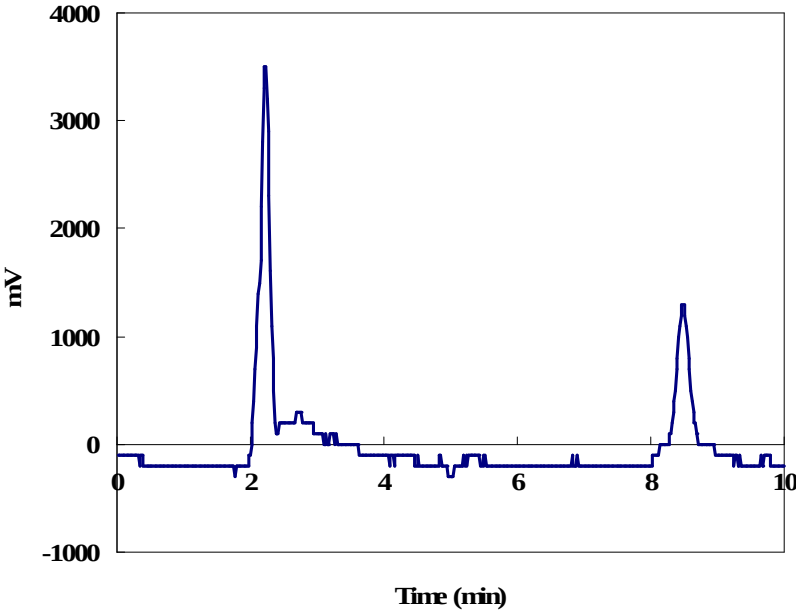


Figure 28b. HPLC Sample Chromatogram: aqueous sample from Trial 1 of phenanthrene partitioning into living *A. coalita* tissue. HPLC File: 215p56.ASC.

Physical models for the uptake of PAH compounds by *A. coalita* tissue are presented in Figures 29a and 29b. Figure 29a illustrates all possible fates of PAH compounds, while Figure 29b shows the likely fates based on the results of this study. The proposed mechanism of PAH accumulation is passive uptake into the hydrophobic components in the algal cell. The uptake is believed to be passive because partitioning of naphthalene into heat-killed alga tissues was the same as partitioning into living tissues. Additionally, the theoretical values for phenanthrene and naphthalene partitioning between water and lipid were relatively close to the measured partitioning values for partitioning into the lipid fraction of living *A. coalita* tissue. Based on the lipid fraction, the partition coefficients for phenanthrene and naphthalene partitioning into living *A. coalita* tissue were estimated to be 33.7 L/g lipid and 9.85 L/g lipid, respectively. The theoretical water-lipid partition coefficients were 41.5 L/g lipid for phenanthrene partitioning and 3.38 L/g lipid for naphthalene partitioning. Aromatic hydrocarbons are known to enter the lipophilic layer of the algal cell membrane [17]. Also, fatty acids are commonly found in algal lipid membranes [19]. The fatty acid 12, 15-octadecatrienoic acid methyl ester was detected in the biomass extracts by GC-MS analysis. Fatty acids are composed of organic carbon and are likely sources for PAH adsorption.

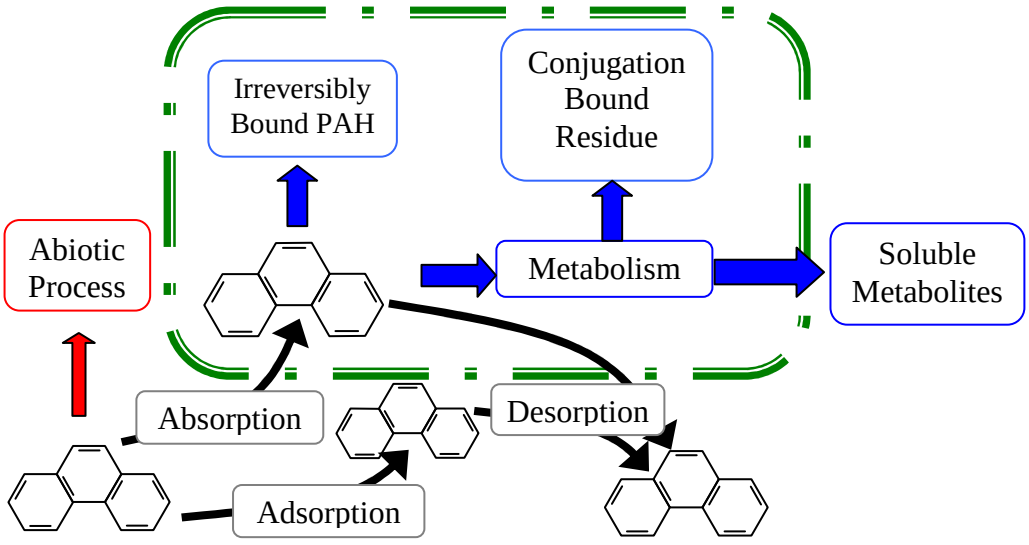


Figure 29a. Physical model demonstrating all possible fates of PAH compounds in contact with algal cells. Dashed line indicates the algal cell wall.

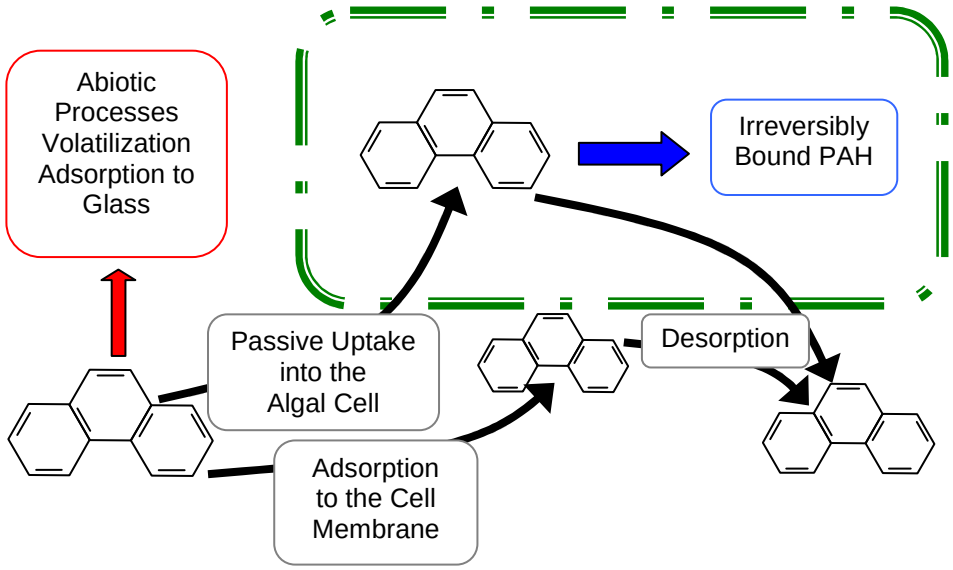


Figure 29b. Physical model demonstrating likely fates of PAH compounds in contact with algal cells. Dashed line indicates the algal cell wall.

PAH was unaccounted for in control experiments (Figures 21 and 22). This indicates that some of the PAH was lost to abiotic processes. The most likely causes for abiotic loss are volatilization or adsorption to the surface of glass. Previous studies have also shown abiotic losses of PAH. In a study of the removal of fluoranthene and pyrene by algae, the abiotic losses over a period of seven days ranged from 9.7 to 16.3 percent of the total PAH added [9]. Additionally, studies have observed abiotic losses of phenanthrene of approximately 19% after 29 days of experimentation [10], and abiotic losses of benzo[a]pyrene of 0.5 and 1.0 percent in just 30 and 60 minutes, respectively [8].

There were higher losses of PAH in all experiments with biomass relative to control experiments. A possible explanation for this difference is that the extraction procedures were not sufficient to extract all bound PAH. The biomass extraction study indicated that some additional PAH was recovered with multiple biomass extractions, but that there was still a portion of PAH unaccounted for (Figure 23). Acetonitrile was used as the extraction solvent for all biomass extractions because

phenanthrene solubility in this solvent is relatively high [20]. Also, acetonitrile was used because this solvent was most compatible with the HPLC system used for sample analysis. Use of a different solvent would have required a solvent replacement prior to HPLC analysis. It was thought that the amount of phenanthrene that would be lost in the solvent replacement process would outweigh any benefits of using a different extraction solvent.

Because no metabolites were detected in the aqueous samples or in the biomass extracts by GC-MS analysis, it is unlikely that the PAH were metabolized. Several studies have identified metabolites due to degradation of PAH compounds by algae. The identified metabolites of benzo[a]pyrene degradation are dihydrodiols and quinones [7]. Compounds identified as metabolites of phenanthrene include 1-naphthalene-carboxylic acid, 2-naphthalene-propanioc acid, 3H-benzo[f]chromene-2-carboxylic acid, dihydroxy-dihydro-phenanthrene, 4-(1-hydroxynaphthalen-2-yl)-2-oxobutanoic acid, 4-(1-hydroxynaphthalen-2-yl)-4-oxobutanoic acid, (Z) 2-hydroxy-4-(1-hydroxynaphthalen-2-yl)-but-3-enoic acid, and aromatic acid [10]. No compounds similar to the reported metabolites were detected in this study. It has been shown that only certain algal species posses the ability to degrade PAH, while others species do not [7, 8]. This study indicates that the species *Acrosiphonia coalita* does not posses the ability to metabolize PAH compounds.

In conclusion, the uptake of PAH by the alga *A. coalita* was likely the result of passive adsorption to the hydrophobic components of the algal cell membrane. The uptake occurred at a very fast rate and varied with pH. The equilibrium partitioning coefficients for phenanthrene and naphthalene partitioning into living *A. coalita* tissue were 0.171 ± 0.0083 L/g FW and 0.0500 ± 0.0025 L/g FW, respectively. The difference in uptake for the two compounds was likely due to their relative hydrophobicities. The adsorption of naphthalene by heat-killed *A. coalita* tissue was equivalent to the adsorption by living *A. coalita* tissue, supporting the theory of passive adsorption. The recovery of phenanthrene was the same for living and heat-

killed *A. coalita* tissue in phenanthrene partitioning experiments, but 60-70% of the PAH was unaccounted for in phenanthrene partitioning experiments with heat-killed *A. coalita* tissue. Due to the large amount of PAH not recovered in the experiments with heat-killed tissue, the results of this experiment could not be compared to partitioning into living *A. coalita*. Finally, no PAH metabolites were detected in this study. The absence of detectable metabolites indicates that the PAH was not likely metabolized.

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Appendices

Appendix A: Procedures

Acrosiphonia coalita Subculture Procedure

Materials:

1. 25 mL Graduated Cylinder
2. 1000 mL Graduated Cylinder
3. 50 X PES Nutrient Solution
4. 3 L Seawater
5. 16, 250 mL Erlenmeyer Flasks Capped with Foam Stoppers
6. Paper towels
7. Filter Flask with Filter, Filter Paper and Filter Adapter
8. 200 mL Glass Blending Jar Equipped with Razor Blade Cutting Adapter
9. Osterizer Blender
10. Spatulas
11. 500 mL Beaker
12. 2, 1 L Glass Bottles
13. 100 mL Graduated Cylinder
14. 4-50 mL Centrifuge Tubes
15. 5 mL Pipette with Pipette Tips

Procedure:

Note: This procedure should be done using proper plant tissue culturing techniques. If possible, steps should be performed in the laminar flow hood, and all reasonable precautions should be taken to maintain sterile conditions.

1. Prepare Medium
    a. Sterilize approximately 2 L of seawater. Allow 24 hours for the seawater to cool and equilibrate prior to use.
    b. If not in stock, prepare and sterilize 50 X PES nutrient solution.

c. Prepare culture medium by adding 20 mL ± 0.5 mL of 50 X PES nutrient solution per 1 L of seawater. Transfer culture medium to sterile 1 L medium bottles. Prepare 2 L culture medium.

2. Using a 100 mL graduated cylinder, add 70 mL ± 5 mL culture medium to each of the 16, 250 mL flasks. Place the flasks in an incubator at a temperature of 12.0 °C to allow the medium to cool.
3. Transfer the flasks containing the *A. coalita* to be subcultured to the laminar flow hood. Decant the majority of the spent medium from each flask. Then combine the remaining contents into a single flask. Use a spatula to transfer any biomass that remains in the flasks or stuck to the flask walls.
4. Filter the suspension to separate the biomass from the liquid medium using filter paper and a filter funnel under vacuum.
5. Remove excess liquid by gently pressing the biomass between sterile paper towels.
6. Weigh approximately 1.6 g FW of biomass and transfer to a 500 mL beaker.
7. Bring the total volume in the beaker to 200 mL using fresh culture medium.
8. Transfer the suspension to the blender cup. Use a spatula to transfer any biomass remaining in the beaker.
9. Cap the blender cup with the lid fitted with the razor blade cutting assembly.
10. Blend for 3 to 5 seconds using the Osterizer blender on “liquefy” speed.
11. Carefully remove the lid and razor blade assembly.
12. Dispense the suspension evenly to 4, 50 mL centrifuge tubes.
13. Centrifuge the mixture for 5 minute at 1000 rpm.
14. Decant the supernatant.
15. Refill the centrifuge tube with fresh medium, and re-suspend the biomass by capping the tube and shaking.
16. Repeat steps 13 through 15 for a total of two washes.
17. After the final wash, re-suspend the biomass in approximately 20 mL of medium per centrifuge tube.

18. Transfer the suspension from the centrifuge tubes to the 500 mL beaker and bring the total volume in the beaker to 160 mL with fresh medium.
19. Transfer the 16 prepared flasks from the 12.0 ºC incubator back to the laminar flow hood.
20. Using a 5 mL pipette transfer approximately 10 mL of the biomass suspension from the beaker to each of the flasks. Note: To avoid clogging pipette tips with biomass, the ends of the tips must be cut off to create a wider opening.
21. Gently swirl the flasks, and place back in the incubator.
22. Clean-up all glassware, and dispose of excess biomass.
23. Log subculture information in the subculture data log.

Culture Maintenance between Subcultures:

1. Maintained the cultures in an incubator at 12.0 ºC.
2. Maintain a light intensity of approximately 50 $\mu E/m^2$-s on a 16 hour light/8 hour dark photoperiod.
3. Gently swirl each flask every day for approximately 5 sec.
4. Replace medium approximately half way through the subculture cycle. The alga should be subcultured every 30 to 35 days, and the medium should be replaced between day 15 and 18. To replace medium:
    a. Prepare 2 L of culture medium as described above.
    b. Decant spent medium from each of the culture flasks.
    c. Measure and transfer 80 mL of fresh medium to each flask.
    d. Gently swirl the flasks, and then return them to the incubator.

Uptake of PAH by Acrosiphonia coalita

Experiment Procedure:

1. Sterilize seawater by autoclaving. Allow 24 hours for the seawater to cool and equilibrate prior to use.
2. Prepare the bioreactor vessel.
    a. Passivate the inner glass surface of the reactor vessel by filling the reactor with an aqueous solution of 20% nitric acid. Allow the acid to remain in the reactor for a minimum of 24 hour.
    b. Drain the acid from the reactor and rinse several times with deionized water to remove any residual acid.
    c. Rinse the inner surface of the reactor vessel with acetone to remove any organic soluble compounds. Dispose of the acetone wash in the organic waste container, and allow sufficient time for the reactor to dry completely. Note: Acetone remaining in the reactor will volatilize. The reactor should be left in the chemical fume hood until completely dry.
3. Assemble the sample port.
    a. Fit a 3/8” stainless steel Swagelock bulk head union through the reactor lid. The lid should be pre-drilled with an appropriate sized opening.
    b. Secure a 10” length of 3/8” diameter glass tubing through the lid using the bulk head union fitting.
    c. Connect the outer end of the glass tubing to a 10” length of silicon tubing.
    d. Fit a 3” long piece of glass tubing through a #3 rubber stopper drilled with an appropriately sized hole.
    e. Connect the free end of the silicon tubing to the outer end of the 3” length of glass tubing.
4. Sterilize the reactor.
    a. Place a stir bar into the reactor.

   b. Screw the reactor lid with sample port onto the reactor.
   c. Seal the reactor by clamping the silicon tubing with a metal clamp.
   d. Autoclave for approximately 30 minutes.
5. Prepare culture medium.
   a. If not in stock, prepare and sterilize 50 X PES nutrient solution.
   b. Add 20 mL ± 0.5 mL of 50 X PES nutrient solution per 1 L of seawater.
   c. Transfer culture medium directly to sterilize reactor.
6. Add biomass to the reactor.
   a. Biomass used for experimental purposes should be obtained 25 to 35 days after subculture.
   b. Transfer the culture flasks from the incubator to the laminar flow hood, and separate the biomass from the culture medium by filtration using a filter funnel and filter paper under vacuum.
   c. Remove excess liquid from the biomass by gently pressing between sterile paper towels.
   d. Weigh approximately 1.0 to 1.5 g FW biomass. Record the exact mass.
   e. Transfer the biomass to the reactor.
7. Allow 24 hours for the biomass to adapt to the reactor vessel.
8. Add PAH
   a. Prepare PAH stock solution by dissolving PAH compound in 100 % pure ethanol to create a solution with a concentration of approximately 10 mg PAH/mL.
   b. Calculate the amount of stock solution required to achieve the desired initial PAH concentration in the seawater.
   c. Add the calculated amount of stock solution using a mechanical pipette.

Experiment Conditions:

1. Maintain experiments in incubators at 12.0 ºC.
2. Light intensity is 80-150 μE/m^2-s on a 16 hour light/8 hour dark photoperiod.

3. The reactors are well mixed using a 1.5 inch stir bar and stir plate set to 150 rpm.

Sampling:

1. Place the rubber stopper connected to the sample port into a 50 mL filter flask.
2. Attach the filter flask to a 50 mL syringe with a small piece of tubing.
3. Remove the clamp from the silicon tubing.
4. Slightly loosen the lid of the reactor to break the reactor seal.
5. While the reactor is mixing, using the syringe to create a vacuum and draw a sample from the reactor into the 50 mL filter flask.
6. After sampling, tighten to reactor lid and replace the clamp on the silicon tubing to re-seal the reactor.
7. Record the time and date of sampling as well as the temperature of the incubator.
8. Measure and record the total volume removed from the reactor.
9. Transfer a 0.5 mL sample of the seawater to a 4 mL vial. Dilute the sample with 2 mL acetonitrile.
10. Separate the biomass from the seawater by filtering through a nylon mesh filter.
11. Measure and record seawater pH.
12. Weigh and record FW of biomass removed from the reactor.
13. Perform biomass extraction.
14. Analyze seawater and biomass extraction samples by HPLC analysis.

Equilibrium Partitioning of PAH between Acrosiphonia coalita and Seawater

Experiment Procedure:

1. Sterilize seawater by autoclaving. Allow 24 hours for the seawater to cool and equilibrate prior to use.
2. Prepare 20 mL glass vials
    a. Passivate the glass surfaces of the vials by placing the vials in an aqueous bath of 20% nitric acid. Allow the vials to remain in the acid for a minimum of 24 hour.
    b. Remove the vials from the acid and rinse several times with deionized water to remove any residual acid.
    c. Rinse the inner surfaces of the vials with acetone to remove any organic soluble compounds. Dispose of the acetone wash in the organic waste container, and allow sufficient time for the vials to dry completely. Note: Acetone remaining in the vials will volatilize. The vials should be left in the chemical fume hood until completely dry.
3. Cap the vials with Teflon-lined caps.
4. Sterilize the vials by autoclaving for approximately 30 minutes.
5. Prepare culture medium.
    a. If not in stock, prepare and sterilize 50 X PES nutrient solution.
    b. Add 20 mL ± 0.5 mL of 50 X PES nutrient solution per 1 L of seawater.
    c. Transfer culture medium to sterile 1 L medium bottles.
6. Add biomass to the vials.
    a. Biomass used for experimental purposes should be obtained 25 to 35 days after subculture.
    b. Transfer the culture flasks from the incubator to the laminar flow hood, and separate the biomass from the culture medium by filtration with filter paper and a filter funnel under vacuum.

c. Remove excess liquid from the biomass by gently pressing between sterile paper towels.
d. Weigh the appropriate amount of biomass directly into the vials. Record the exact mass added to each vial.
e. For PAH equilibrium partitioning between seawater and heat-killed *A. coalita* tissue, heat-kill the alga at this point. Refer to protocol of heat-killing *Acrosiphonia coalita* tissue cultures.

7. Add PAH to seawater medium.
    a. Prepare PAH stock solution by dissolving PAH compound in 100 % pure ethanol to create a solution with a concentration of approximately 10 mg PAH/mL.
    b. Calculate the amount of stock solution required to achieve the desired initial PAH concentration in the seawater.
    c. Add the calculated amount of stock solution using a mechanical pipette.
8. Add PAH medium to the vials. Note: Initial PAH concentrations in the seawater are varied from vial to vial. Solutions of various concentrations can be prepared by adding different amounts of stock solution or by preparing one high PAH concentration seawater solution and diluting that solution with fresh medium.

Experiment Conditions:

1. Sealed vials are place on an orbital shaker and secured in place.
2. The vials are mixed at 162 rpm.
3. Experiments are conducted in incubators at 12.0 ºC.
4. Light intensity is 80-100 $\mu E/m^2$-s on a 16 hour light/8 hour dark photoperiod.
5. Experiment period is approximately 120 hours

Sampling:

1. Remove vials from the orbital shaker.
2. Take a 0.5 mL seawater sample from each vial and transfer to 4 mL glass vials. Dilute each sample with 2 mL acetonitrile.
3. Separate the biomass from the seawater by filtering through a nylon mesh filter.
4. Measure and record the pH of the filtered seawater.
5. Measure and record the final biomass weight in each vial.
6. Perform biomass extraction.
7. Analyze seawater samples and biomass extraction samples by HPLC analysis.

Heat Treatment of Acrosiphonia coalita Tissue Cultures

1. Separate the biomass to be killed from the culture medium by filtration using a filter funnel and filter paper under vacuum.
2. Remove excess liquid from the biomass by pressing between sterile paper towels.
3. Measure and record the FW of the biomass prior to heat treatment.
4. Transfer the biomass to an autoclave safe container.
5. Heat-kill the biomass by autoclaving for 20 minutes at 120 ºC at 20 psi on the “dry” cycle.

Experiment Procedure:

Stage 1 Biomass Loading:

1. Sterilize seawater by autoclaving. Allow 24 hours for the seawater to cool and equilibrate prior to use.
2. Prepare the bioreactor vessel.
    a. Passivate the inner glass surface of the reactor by filling the reactor with an aqueous solution of 20% nitric acid. Allow the acid to remain in the reactor for a minimum of 24 hour.
    b. Drain the acid from the reactor and rinse several times with deionized water to remove any residual acid.
    c. Rinse the inner surface of the reactor with acetone to remove any organic soluble compounds. Dispose of the acetone wash in the organic waste container, and allow sufficient time for the reactor to dry completely. Note: Acetone remaining in the reactor will volatilize. The reactor should be left in the chemical fume hood until completely dry.
3. Cap the reactor with a solid lid to seal the vessel. Note: Time course samples are not taken for this type of experiment, so a sample port is not needed.
4. Sterilize the reactor by autoclaving for approximately 30 minutes.
5. Prepare culture medium.
    a. If not in stock, prepare and sterilize 50 X PES nutrient solution.
    b. Add 20 mL ± 0.5 mL of 50 X PES nutrient solution per 1 L of seawater.
    c. Transfer culture medium directly to sterilize reactor.
6. Add biomass to the reactor.
    a. Biomass used for experimental purposes should be obtained 25 to 35 days after subculture.

   b. Transfer the culture flasks from the incubator to the laminar flow hood, and separate the biomass from the culture medium by filtration using a filter funnel and filter paper under vacuum.
   c. Remove excess liquid from the biomass by gently pressing between sterile paper towels.
   d. Weigh approximately 1.0 to 1.5 g FW biomass. Record the exact mass.
   e. Transfer the biomass to the reactor.
7. Allow 24 hours for the biomass to adapt to the reactor vessel.
8. Add PAH
   a. Prepare PAH stock solution by dissolving PAH compound in 100 % pure ethanol to create a solution with a concentration of approximately 10 mg PAH/mL.
   b. Calculate the amount of stock solution required to achieve the desired initial PAH concentration in the seawater.
   c. Add the calculated amount of stock solution using a mechanical pipette.
9. Allow 48 hours for PAH uptake to occur.
10. At the end of 48 hours, separate the biomass from the seawater by filtration with a filter funnel and filter paper under vacuum.
11. Remove excess liquid from the biomass by gently pressing between sterile paper towels.
12. Measure and record the FW of the biomass
13. Sample the biomass, and perform a biomass extraction on the sample.

Experiment Procedure Stage 2: Desorption

1. Prepare 20 mL glass vials
   a. Passivate the glass surfaces of the vials by placing the vials in an aqueous solution of 20% nitric acid. Allow the vials to remain in the acid for a minimum of 24 hour.
   b. Remove the vials from the acid and rinse several times with deionized water to remove any residual acid.

   c. Rinse the inner surfaces of the vials with acetone to remove any organic soluble compounds. Dispose of the acetone wash in the organic waste container, and allow sufficient time for the vials to dry completely. Note: Acetone remaining in the vials will volatilize. The vials should be left in the chemical fume hood until completely dry.
2. Cap the vials with Teflon-lined caps.
3. Sterilize the vials by autoclaving for approximately 30 minutes.
4. Prepare culture medium.
   a. If not in stock, prepare and sterilize 50 X PES nutrient solution.
   b. Add 20 mL ± 0.5 mL of 50 X PES nutrient solution per 1 L of seawater.
   c. Transfer culture medium to sterile 1 L medium bottles.
5. Add biomass to the vials.
   a. Using the biomass loaded in stage 1, weigh the appropriate amount of biomass directly to each vial.
   b. Record the exact amount added
   c. Note: To vary the total amount of PAH added to each vial, the amount of biomass is varied from vial to vial.
6. Add 20 mL fresh medium to each vial.
7. Seal the vials and secure the vials in place on an orbital shaker.
8. Mix the vials at 162 rpm for a period of 120 hours to allow equilibrium to be achieved.

Sampling:

1. Remove vials from the orbital shaker.
2. Take a 0.5 mL seawater sample from each vial and transfer to 4 mL glass vials. Dilute each sample with 2 mL acetonitrile.
3. Separate the biomass from the seawater by filtration using a nylon mesh filter.
4. Measure and record the seawater pH.
5. Measure and record the final biomass weight in each vial.

6. Perform biomass extraction.
7. Analyze seawater samples and biomass extraction samples by HPLC analysis.

Experiment Conditions:

1. The experiment is conducted at 12.0 ºC.
2. Light intensity is 80-100 μE/m^2-s on a 16 hour light/8 hour dark photoperiod.
3. Stage 1 duration is 48 hours
4. Stage 1 reactor is well mixed using a stir bar and stir plate
5. Stage 2 duration is approximately 120 hours
6. Stage 2 vials are well mixed using an orbital shaker at 162 rpm.

Biomass Extraction

1. Measure the fresh weight of the biomass sample to be extracted.
2. Transfer the sample to a 4 mL vial with a Teflon-lined cap.
3. Add HPLC grade acetonitrile to the vial. The acetonitrile to biomass ratio should be approximately 1 mL per 20 mg FW.
4. Allow biomass samples to remain in acetonitrile for a minimum of 36 hours.
5. During the 36 hour extraction period, sonicate the vials for a minimum of 90 minutes.
6. After the extraction period, separate the biomass from the solvent by filtering through a 0.45 μm nylon syringe filter.
7. Collect the biomass for later analysis.
8. Collect the filtrate for later analysis.

Solid Phase Extraction for Concentrating Metabolites for GC-MS Analysis

Procedure

1. Obtain approximately 200 mL of the aqueous sample containing the metabolites to be concentrated.
2. Filter the sample through a 0.2 μm PES or cellulose acetate bottle top filter to remove any solid particles or biomass.
3. Obtain a 6 mL Supelco supelclean LC-18 SPE column.
4. Use a ring stand and a clamp to secure the column in an upright position.
5. Obtain a filter flask fitted with a two-hole rubber stopper.
6. Fit glass tubing through the two holes in the stopper.
7. Attach one of the pieces of glass tubing to a short length of rubber tubing, and connect the other end of the rubber tubing to the end of the SPE column.
8. Attach a vacuum line to the filter flask.
9. To achieve flow through the column for column washes and sample loading:
    a. Turn on the vacuum source.
    b. Slowly add liquid to the top of the column.
    c. Manually control the strength of the vacuum by manipulating the opening of the second glass tube.
    d. Flow through the column should be at a steady drip.
    e. Do not allow the column to go completely dry. Stop each step when the liquid level in the column is just above the top column frit.
10. Rinse the column with approximately 10 mL of DI water.
11. Load approximately 200 mL of the filtered sample onto the column.
12. Once the entire sample is loaded onto the column, rinse the column with 8 mL of DI water to remove any salts.
13. After the DI wash, disconnect the SPE column from the vacuum system.
14. For elution, flow through the column is achieved by gravitational force only.
15. Add 0.5 mL of acetonitrile to the column. Collect the effluent.

16. Add 3 mL of acetonitrile to the column. Collect the effluent. This sample is used for analysis.
17. Derivatize the sample as described in the *Derivatization of PAH Metabolite Samples for GC-MS Analysis* protocol and analyze by GC-MS.

Derivatization of PAH Metabolite Samples for GC-MS Analysis

Reagents:

1. Derivatization reagent: N-Methyl-N-trimethylsilyltriflouroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS)
2. Anhydrous sodium sulfate
3. Sample to be derivatized dissolved in acetonitrile

Procedure

1. Add 0.5 mL of sample to a 4 mL vial.
2. Dry the sample over 0.1 g anhydrous sodium sulfate.
    a. Weigh the required amount of sodium sulfate and transfer to the vial.
    b. Mix the contents of the vial well by vortexing.
    c. Allow the mixture to sit for a minimum of 24 hours.
3. Reduce the volume in the vial and remove any remaining water by blowing down the sample under nitrogen to a final volume of approximately 0.1 mL. Do not blow completely dry.
4. Add 0.125 mL MSTFA + 1% TMCS to the vial containing the sample. Note: This reagent is moisture sensitive. It is stored at 4 ºC. The reagent should be brought to room temperature prior to use and precautions should be taken to maintain conditions that are as dry as possible.
5. Cap the vial, mix well by vortexing, and let stand for a minimum of 15 minutes.
6. Add approximately 0.275 mL of ethyl acetate to the vial.
7. Filter the sample using a 0.45 µm nylon syringe filter.
8. Analyze the sample by GC-MS.

Experiment AC205: Uptake of phenanthrene: control experiment in 2 L Belco bioreactor

Table B.1. Uptake of phenanthrene: control experiment conditions

Run Description	
Run ID #:	AC205
Date Started:	07/26/06
Time Started:	15:20
Date Ended:	08/01/06
Time Ended:	12:05
Experiment Duration:	140.8 hr
Culture Loading	
Culture:	NA
Cell Line I.D.:	NA
Age of Inoculum:	NA
Initial Biomass Weight:	0
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	2 L
Initial pH Medium:	NA
Initial Biomass Density:	0
Test Parameters: Start-up Summary	
System Location:	VWR #2 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	15 ºC
Incident Light Intensity:	0, wrapped in tin foil
Light Distance From Vessel Surface:	NA
Photoperiod :	NA
Dark Period:	NA
Reactor Type:	2 L Belco reactor
Working Liquid Volume	2.0 L
Mixing:	Stir plate/stir bar
Mixer Speed (rpm):	150
PAH:	Phenanthrene
PAH Stock Prep.:	19.9 mg phen. 2 mL EtOH
PAH Stock Conc.:	9.95 mg/mL
Vol. PAH Stock:	120.6 µL
Calculated Initial PAH Conc.:	0.6 mg/L
Measured Initial PAH Conc.:	0.45 mg/L

Table B.1. Continued

Shut-down Summary	
Final Weight Biomass:	NA
Final Medium pH:	NA
Total Volume Medium:	NA
Final Biomass Density:	NA
Temperature Readout:	12.0 ºC
Final Volume in Reactor:	1875.2 mL
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00124 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm
Dilution Factor (Liquid Samples):	1:4, 0.5 mL diluted w/ 2 mL ACN

Table B.2. Uptake of phenanthrene: control experiment raw data

HPLC Data: Liquid Samples											
Sample Name	Date Sampled	Sample Time	Elapsed Time (hr)	Temp. (ºC)	Vol. of Sample (mL)	HPLC file	Injn. #	Date of HPLC Analysis	Peak Area	Reten-tion Time	Phen. Conc. Liquid (mg/L)
205-1	7/26/06	15:20	0.0	15.0	11.0	205p2.ASC	1189	8/1/06	72.70	8.533	0.4507
205-1	7/26/06	15:20	0.0	15.0	11.0	205p11.ASC	1198	8/1/06	72.30	8.566	0.4483
205-2	7/27/06	10:00	18.7	12.4	NA	205p4.ASC	1191	8/1/06	70.55	8.533	0.4374
205-2	7/27/06	10:00	18.7	12.4	NA	205p10.ASC	1197	8/1/06	69.60	8.516	0.4315
205-3	7/28/06	11:05	43.8	12.0	16.0	205p5.ASC	1192	8/1/06	58.75	8.516	0.3643
205-3	7/28/06	11:05	43.8	12.0	16.0	205p9.ASC	1196	8/1/06	52.65	8.433	0.3264
205-4	7/31/06	10:00	114.7	12.0	14.9	205p6.ASC	1193	8/1/06	42.10	8.583	0.2610
205-4	7/31/06	10:00	114.7	12.0	14.9	205p8.ASC	1195	8/1/06	42.95	8.500	0.2663
205-5	8/1/06	12:05	140.8	12.0	20.5	205p3.ASC	1190	8/1/06	42.20	8.500	0.2616
205-5	8/1/06	12:05	140.8	12.0	20.5	205p7.ASC	1194	8/1/06	38.50	8.533	0.2387

Table B.3. Uptake of phenanthrene: control experiment mass balance

Mass Phenanthrene Added to Reactor (mg)	Mass Phenanthrene Removed in Samples (mg)	Final Mass Phenanthrene in Liquid (mg)	Mass Unaccounted For (mg)
0.901	0.039	0.448	0.415
Mass Phenanthrene Added to Reactor (%)	**Mass Phenanthrene Removed in Samples (%)**	**Final Mass Phenanthrene in Liquid (%)**	**Mass Unaccounted For (%)**
100.0	4.3	49.7	46.0

Table B.4. Uptake of phenanthrene: Trial 1 experiment conditions

Run Description	
Run ID #:	AC206
Date Started:	08/03/06
Time Started:	10:32
Date Ended:	08/08/06
Time Ended:	14:45
Experiment Duration:	124.2 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-24
Age of Inoculum:	16 days
Initial Biomass Weight:	2.008 g FW
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	2.0 L
Initial pH Medium:	8.320
Initial Biomass Density:	1 g FW/L
Date Biomass Added:	08/02/06
Time Biomass Added:	15:55
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00128 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 0.5 mL diluted w/ 2 mL acetonitrile

Table B.4. Continued

Test Parameters: Start-up Summary	
System Location:	VWR #2 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	12.8 ºC
Incident Light Intensity (Center)	200 ($\mu E/m^2$-s):
Incident Light Intensity (Right)	250 ($\mu E/m^2$-s):
Incident Light Intensity (Left)	300 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	4 inches on each side
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	2 L Belco reactor
Working Liquid Volume	2.0 L
Mixing:	Stir plate/stir bar
Mixer Speed (rpm):	150
PAH:	Phenanthrene
PAH Stock Prep.:	21.3 mg phen. 2 mL EtOH
PAH Stock Conc.:	10.65 mg/mL
Vol. PAH Stock:	112.7 µL
Calculated Initial PAH Conc.:	0.6 mg/L
Measured Initial PAH Conc.:	0.62 mg/L
Date PAH Added:	08/03/06
Time PAH Added:	10:32
Shut-down Summary	
Final Weight Biomass:	2.800 g FW
Final Medium pH:	10.36
Total Volume Medium:	1688 mL
Final Biomass Density:	1.66 g FW/L
Temperature Readout:	14.9 ºC
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extract Volume (mL):	
Samples 1-12	1.0 mL
Sample 13	5.0 mL
Time Extraction Started:	15:45
Date Extraction Started:	08/08/06
Time Extraction Ended:	NA
Date Extraction Ended:	08/09/06
Total Extraction Time:	NA
Sonication:	10:35 08/09/06 to 12:57 08/09/06

Table B.5. Uptake of phenanthrene: Trial 1 liquid phase raw data

HPLC Data: Liquid Samples											
Sample Name	Date Sampled	Sample Time	Time (hr)	Temp (ºC)	Liquid pH	HPLC file	Injn. #	Date of HPLC Analysis	Peak Area	Reten-tion Time	Phen. Conc. (mg/L)
206-0-1	8/3/2006	10:13	0.0	12.9	9.330	206p10.ASC	1207	8/7/2006	0.00	NA	0.0000
206-0-2	8/3/2006	10:13	0.0	12.9	9.330	206p11.ASC	1208	8/7/2006	0.00	NA	0.0000
206-1-1	8/3/2006	10:35	0.1	14.5	9.640	206p12.ASC	1209	8/7/2006	96.55	8.500	0.6179
206-1-2	8/3/2006	10:35	0.1	14.5	9.640	206p13.ASC	1210	8/7/2006	94.30	8.500	0.6035
206-2-1	8/3/2006	11:32	1.0	14.8	9.820	206p14.ASC	1211	8/7/2006	50.85	8.466	0.3254
206-2-2	8/3/2006	11:32	1.0	14.8	9.820	206p15.ASC	1212	8/7/2006	48.70	8.466	0.3117
206-3-1	8/3/2006	12:32	2.0	14.8	9.980	206p20.ASC	1217	8/8/2006	38.65	8.533	0.2474
206-3-2	8/3/2006	12:32	2.0	14.8	9.980	206p21.ASC	1218	8/8/2006	38.90	8.533	0.2490
206-4-1	8/3/2006	14:28	3.9	14.6	10.150	206p22.ASC	1219	8/8/2006	39.05	8.566	0.2499
206-4-2	8/3/2006	14:28	3.9	14.6	10.150	206p23.ASC	1220	8/8/2006	33.65	8.550	0.2154
206-5-1	8/3/2006	16:33	6.0	14.7	10.200	206p24.ASC	1221	8/8/2006	35.20	8.566	0.2253
206-5-2	8/3/2006	16:33	6.0	14.7	10.200	206p25.ASC	1222	8/8/2006	35.85	8.566	0.2294
206-6-1	8/3/2006	19:11	8.7	15.2	10.210	206p26.ASC	1223	8/8/2006	39.20	8.550	0.2509
206-6-2	8/3/2006	19:11	8.7	15.2	10.210	206p27.ASC	1224	8/8/2006	42.30	8.550	0.2707
206-7-1	8/3/2006	22:00	11.5	17.8	10.170	206p28.ASC	1225	8/8/2006	40.10	8.583	0.2566
206-7-1	8/3/2006	22:00	11.5	17.8	10.170	206p43.ASC	1240	8/8/2006	40.30	8.500	0.2579
206-7-2	8/3/2006	22:00	11.5	17.8	10.170	206p29.ASC	1226	8/8/2006	49.60	8.533	0.3174
206-7-2	8/3/2006	22:00	11.5	17.8	10.170	206p44.ASC	1241	8/8/2006	42.05	8.433	0.2691
206-8-1	8/4/2006	10:00	23.5	12.6	9.880	206p30.ASC	1227	8/8/2006	40.60	8.516	0.2598
206-8-1	8/4/2006	10:00	23.5	12.6	9.880	206p45.ASC	1242	8/8/2006	47.00	8.500	0.3008
206-8-2	8/4/2006	10:00	23.5	12.6	10.047	206p31.ASC	1228	8/8/2006	50.20	8.533	0.3213
206-8-2	8/4/2006	10:00	23.5	12.6	10.047	206p46.ASC	1243	8/8/2006	46.70	8.450	0.2989
206-9-1	8/5/2006	13:19	50.8	12.0	10.019	206p32.ASC	1229	8/8/2006	33.60	8.533	0.2150
206-9-2	8/5/2006	13:19	50.8	12.0	10.201	206p33.ASC	1230	8/8/2006	34.30	8.550	0.2195

Table B.5. Continued

HPLC Data: Liquid Samples

Sample Name	Date Sampled	Sample Time	Time (hr)	Temp (ºC)	Liquid pH	HPLC file	Injn. #	Date of HPLC Analysis	Peak Area	Reten-tion Time	Phen. Conc. (mg/L)
206-10-1	8/6/2006	11:45	73.2	12.0	10.050	206p34.ASC	1231	8/8/2006	34.30	8.566	0.2195
206-10-2	8/6/2006	11:45	73.2	12.0	10.203	206p35.ASC	1232	8/8/2006	34.20	8.600	0.2189
206-11-1	8/7/06	10:33	96.0	13.0	10.054	206p36.ASC	1233	8/8/2006	34.60	8.600	0.2214
206-11-2	8/7/06	10:33	96.0	13.0	10.092	206p37.ASC	1234	8/8/2006	35.00	8.550	0.2240
206-12-1	8/8/06	10:50	120.3	15.1	10.016	206p38.ASC	1235	8/8/2006	29.40	8.600	0.1882
206-12-2	8/8/06	10:50	120.3	15.1	10.151	206p39.ASC	1236	8/8/2006	47.60	8.533	0.3046
206-12-2	8/8/06	10:50	120.3	15.1	10.151	206p40.ASC	1237	8/8/2006	30.10	8.533	0.1926
206-13-1	8/8/06	14:45	124.2	14.9	10.360	206p41.ASC	1238	8/8/2006	29.50	8.500	0.1888
206-13-2	8/8/06	14:45	124.2	14.9	10.360	206p42.ASC	1239	8/8/2006	42.80	8.466	0.2739
206-13-2	8/8/06	14:45	124.2	14.9	10.360	206p47.ASC	1244	8/8/2006	31.40	8.483	0.2010

Table B.6. Uptake of phenanthrene: Trial 1 biomass extraction raw data

HPLC Data: Biomass Extraction Samples

Sample Name	Date Sampled	Sample Time	Time (hr)	HPLC file	Injn. #	Date of HPLC Analysis	Peak Area	Retention Time	Phen. in Extract (mg)	Bio-mass Weight (mg FW)	Phen. Conc. (mg/g FW)
206-0	8/3/06	10:13	0.0	206p48.ASC	1274	8/10/06	7.90	8.350	0.0000	43.0	0.00024
206-1	8/3/06	10:35	0.1	206p49.ASC	1275	8/10/06	296.40	8.333	0.0004	34.5	0.01100
206-2	8/3/06	11:32	1.0	206p51.ASC	1277	8/10/06	764.55	8.383	0.0010	22.1	0.04428
206-3	8/3/06	12:32	2.0	206p52.ASC	1278	8/10/06	2745.00	8.400	0.0035	41.3	0.08508
206-4	8/3/06	14:28	3.9	206p53.ASC	1279	8/10/06	1304.60	8.350	0.0017	23.7	0.07046
206-5	8/3/06	16:33	6.0	206p54.ASC	1280	8/10/06	1565.50	8.400	0.0020	31.9	0.06282
206-6	8/3/06	19:11	8.7	206p55.ASC	1281	8/10/06	795.00	8.383	0.0010	20.7	0.04916
206-7	8/3/06	22:00	11.5	206p56.ASC	1282	8/10/06	1454.50	8.350	0.0019	36.8	0.05059
206-8-1	8/4/06	10:00	23.5	206p57.ASC	1283	8/10/06	891.40	8.333	0.0011	21.0	0.05433
206-8-2	8/4/06	10:00	23.5	206p58.ASC	1284	8/10/06	2526.15	8.366	0.0032	43.1	0.07502
206-9-1	8/5/06	13:19	50.8	206p59.ASC	1285	8/10/06	719.75	8.333	0.0009	15.5	0.05944
206-9-2	8/5/06	13:19	50.8	206p60.ASC	1286	8/10/06	836.40	8.316	0.0011	23.7	0.04517
206-10-1	8/6/06	11:45	73.2	206p61.ASC	1287	8/10/06	1182.60	8.333	0.0015	34.7	0.04362
206-10-2	8/6/06	11:45	73.2	206p62.ASC	1288	8/10/06	2897.05	8.366	0.0037	60.5	0.06129
206-11-1	8/7/06	10:33	96.0	206p63.ASC	1289	8/10/06	1022.40	8.366	0.0013	36.0	0.03635
206-11-2	8/7/06	10:33	96.0	206p64.ASC	1290	8/10/06	752.60	8.350	0.0010	25.9	0.03719
206-12-1	8/8/06	10:50	120.3	206p50.ASC	1276	8/10/06	802.10	8.400	0.0010	36.0	0.02852
206-12-2	8/8/06	10:50	120.3	206p65.ASC	1291	8/10/06	883.20	8.416	0.0011	35.9	0.03149
206-13-1	8/8/06	14:45	124.2	206p66.ASC	1292	8/10/06	772.30	8.366	0.0049	124.6	0.03967
206-13-2	8/8/06	14:45	124.2	206p67.ASC	1293	8/10/06	1259.00	8.400	0.0081	191.2	0.04214

Table B.7. Uptake of phenanthrene: Trial 1 data summary

Averages			
Elapsed Time (hr)	Phenanthrene Concentration in Liquid (mg/L)	Phenanthrene Concentration in Biomass (mg /g FW)	Liquid pH
0.0	0.0000	0.0002	9.330
0.1	0.6107	0.0110	9.640
1.0	0.3186	0.0443	9.820
2.0	0.2482	0.0851	9.980
3.9	0.2326	0.0705	10.150
6.0	0.2274	0.0628	10.200
8.7	0.2608	0.0492	10.210
11.5	0.2753	0.0506	10.170
23.5	0.2952	0.0647	9.964
50.8	0.2173	0.0523	10.110
73.2	0.2192	0.0525	10.127
96.0	0.2227	0.0368	10.073
120.3	0.2285	0.0300	10.084
124.2	0.2212	0.0409	10.360

Table B.8. Uptake of phenanthrene: Trial 1 mass balance

Sample Name	Total Liquid Volume Sampled (mL)	Liquid Phase Phen. Conc. (mg/L)	Mass Phen. Removed in Liquid Sample (µg)	Phen. Removed in Biomass Samples (µg)
206-0	21.5	0.0000	0.00	0.01
206-1	16.7	0.6107	10.20	0.38
206-2	11.1	0.3186	3.54	0.98
206-3	19.0	0.2482	4.72	3.51
206-4	15.5	0.2326	3.61	1.67
206-5	14.8	0.2274	3.36	2.00
206-6	15.0	0.2608	3.91	1.02
206-7	15.5	0.2753	4.27	1.86
206-8-1	11.4	0.2803	3.20	1.14
206-8-2	20.0	0.3101	6.20	3.23
206-9-1	8.5	0.2150	1.83	0.92
206-9-2	13.0	0.2195	2.85	1.07
206-10-1	16.5	0.2195	3.62	1.51
206-10-2	21.3	0.2189	4.66	3.71
206-11-1	23.5	0.2214	5.20	1.31
206-11-2	19.3	0.2240	4.32	0.96
206-12-1	21.9	0.1882	4.12	1.03
206-12-2	16.8	0.2486	4.18	1.13
206-Final-1	0.5	0.1888	0.09	4.94
206-Final-2	0.5	0.2374	0.12	8.06

Table B.8. Continued

Mass Phenanthrene Added to Reactor (μg)	Mass Phenanthrene Removed in Samples (μg)	Final Mass Phenanthrene in Liquid (μg)	Final Mass Phenanthrene in Biomass (μg)	Mass Unaccounted For (μg)
1235.8	114.5	373.4	101.6	646.3
Mass Phenanthrene Added to Reactor (%)	Mass Phenanthrene Removed in Samples (%)	Final Mass Phenanthrene in Liquid (%)	Final Mass Phenanthrene in Biomass (%)	Mass Unaccounted For (%)
100.0	9.3	30.2	8.2	52.3

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Table B.9. Uptake of phenanthrene: Trial 2 experiment conditions

Run Description	
Run ID #:	AC207
Date Started:	08/10/06
Time Started:	10:30
Date Ended:	08/15/06
Time Ended:	10:00
Experiment Duration:	119.5 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-24
Age of Inoculum:	29 days
Initial Biomass Weight:	3.003 g FW
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	2.0 L
Initial pH Medium:	8.490
Initial Biomass Density:	1.5 g FW/L
Date Biomass Added:	08/09/06
Time Biomass Added:	15:45
Shut-down Summary	
Final Weight Biomass:	3.305 g FW
Final Medium pH:	9.93
Total Volume Medium:	1600 mL
Final Biomass Density:	2.07 g FW/L
Temperature Readout:	12.3 ºC
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00128 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile

Table B.9. Continued

Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extract Volume (mL):	1.0
Test Parameters: Start-up Summary	
System Location:	VWR #2 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	12.0 ºC
Incident Light Intensity (Center)	200 (μE/m^2-s):
Incident Light Intensity (Right)	250 (μE/m^2-s):
Incident Light Intensity (Left)	300 (μE/m^2-s):
Light Distance From Vessel Surface:	4 inches on each side
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	2 L Belco reactor
Working Volume	2.0 L
Mixing:	Stir plate/stir bar
Mixer Speed (rpm):	150
PAH:	Phenanthrene
PAH Stock Prep.:	21.2 mg phen. 2 mL EtOH
PAH Stock Conc.:	10.6 mg/mL
Vol. PAH Stock:	113.2 μL
Calculated Initial PAH Conc.:	0.6 mg/L
Measured Initial PAH Conc.:	0.6134 mg/L
Date PAH Added:	08/10/06
Time PAH Added:	10:30

Table B.10. Uptake of phenanthrene: Trial 2 liquid phase raw data

Sample Name	Date Sampled	Sample Time	Time (hr)	Temp. (ºC)	Liquid pH	HPLC file	Injn. #	Date of Analysis	Peak Area	Retention Time	Phen. Conc. (mg/L)
207-0-1	8/10/06	10:10	0.0	13.1	9.215	207p2.ASC	1320	8/18/06	0.00	NA	0.0000
207-0-2	8/10/06	10:10	0.0	13.1	9.215	207p3.ASC	1321	8/18/06	0.00	NA	0.0000
207-1-1	8/10/06	10:31	0.0	13.8	9.230	207p4.ASC	1322	8/18/06	83.50	8.550	0.5344
207-1-2	8/10/06	10:31	0.0	13.8	9.230	207p5.ASC	1323	8/18/06	99.60	8.533	0.6374
207-2-1	8/10/06	11:31	1.0	13.7	9.300	207p6.ASC	1324	8/18/06	50.50	8.550	0.3232
207-2-2	8/10/06	11:31	1.0	13.7	9.300	207p7.ASC	1325	8/18/06	51.40	8.550	0.3290
207-3-1	8/10/06	12:30	2.0	13.6	9.481	207p8.ASC	1326	8/18/06	43.25	8.516	0.2768
207-3-2	8/10/06	12:30	2.0	13.6	9.481	207p9.ASC	1327	8/18/06	30.15	8.583	0.1930
207-4-1	8/10/06	14:30	4.0	13.0	9.782	207p10.ASC	1328	8/18/06	33.00	8.483	0.2112
207-4-2	8/10/06	14:30	4.0	13.0	9.782	207p11.ASC	1329	8/18/06	37.20	8.450	0.2381
207-4-2	8/10/06	14:30	4.0	13.0	9.782	207p12.ASC	1330	8/18/06	39.65	8.483	0.2538
207-5-1	8/10/06	16:30	6.0	13.6	9.986	207p13.ASC	1331	8/18/06	32.40	8.450	0.2074
207-5-2	8/10/06	16:30	6.0	13.6	9.986	207p14.ASC	1332	8/18/06	28.35	8.466	0.1814
207-6-1	8/10/06	19:30	9.0	14.3	10.019	207p15.ASC	1333	8/18/06	36.10	8.466	0.2310
207-6-2	8/10/06	19:30	9.0	14.3	10.019	207p16.ASC	1334	8/18/06	49.95	8.483	0.3197
207-7-1	8/10/06	21:50	11.4	16.3	10.012	207p17.ASC	1335	8/18/06	38.35	8.483	0.2454
207-7-2	8/10/06	21:50	11.4	16.3	10.012	207p18.ASC	1336	8/18/06	41.00	8.483	0.2624
207-8-1	8/11/06	10:12	23.7	12.1	9.860	207p19.ASC	1337	8/18/06	32.70	8.466	0.2093
207-8-2	8/11/06	10:12	23.7	12.1	9.874	207p20.ASC	1338	8/18/06	37.30	8.500	0.2387
207-9-1	8/12/06	11:02	48.5	12.2	9.945	207p21.ASC	1339	8/18/06	39.20	8.516	0.2509
207-9-2	8/12/06	11:02	48.5	12.2	9.919	207p22.ASC	1340	8/18/06	33.25	8.466	0.2128
207-10-1	8/13/06	11:56	73.4	12.6	9.881	207p29.ASC	1341	8/21/06	35.10	8.466	0.2246
207-10-2	8/13/06	11:56	73.4	12.6	10.091	207p30.ASC	1342	8/21/06	21.00	8.400	0.1344
207-11-1	8/14/06	12:15	97.7	12.0	9.876	207p31.ASC	1343	8/21/06	28.80	8.516	0.1843
207-11-2	8/14/06	12:15	97.7	12.0	9.872	207p32.ASC	1344	8/21/06	30.10	8.483	0.1926
207-12-1	8/15/2006	10:00	119.5	12.0	9.775	207p33.ASC	1345	8/21/2006	26.80	8.516	0.1715
207-12-2	8/15/2006	10:00	119.5	12.0	9.883	207p34.ASC	1346	8/21/2006	32.10	8.516	0.2054

Table B.11. Uptake of phenanthrene: Trial 2 biomass extraction raw data

HPLC Data: Biomass Extraction Samples											
Sample Name	Date Sampled	Sample Time	Time (hr)	HPLC file	Injn. #	Date of HPLC	Peak Area	Reten-tion Time	Phen. in Sample (mg)	Bio-mass Mass (mg FW)	Phen. Conc. (mg/ g FW)
207-0	8/10/06	10:10	0.00	207p35.ASC	1347	8/22/06	0.00	NA	0.00000	58.6	0.0000
207-1	8/10/06	10:31	0.02	207p36.ASC	1348	8/22/06	1398.00	8.450	0.00179	48.9	0.0366
207-2	8/10/06	11:31	1.02	207p37.ASC	1349	8/22/06	754.30	8.416	0.00097	32.8	0.0294
207-3	8/10/06	12:30	2.00	207p38.ASC	1350	8/22/06	3742.60	8.433	0.00479	47.5	0.1009
207-4	8/10/06	14:30	4.00	207p39.ASC	1351	8/22/06	1232.35	8.416	0.00158	36.7	0.0430
207-5	8/10/06	16:30	6.00	207p40.ASC	1352	8/22/06	6436.10	8.433	0.00824	54.6	0.1509
207-6	8/10/06	19:30	9.00	207p41.ASC	1353	8/22/06	3389.10	8.416	0.00434	45.0	0.0964
207-7	8/10/06	21:50	11.40	207p42.ASC	1354	8/22/06	2770.50	8.416	0.00355	46.5	0.0763
207-8-1	8/11/06	10:12	23.70	207p43.ASC	1355	8/22/06	3745.20	8.400	0.00479	49.5	0.0968
207-8-2	8/11/06	10:12	23.70	207p44.ASC	1356	8/22/06	2343.35	8.416	0.00300	44.5	0.0674
207-9-1	8/12/06	11:02	48.53	207p45.ASC	1357	8/22/06	2757.60	8.366	0.00353	65.3	0.0541
207-9-2	8/12/06	11:02	48.53	207p46.ASC	1358	8/22/06	3950.35	8.400	0.00506	48.9	0.1034
207-10-1	8/13/06	11:56	73.43	207p47.ASC	1359	8/22/06	1382.90	8.366	0.00177	43.9	0.0403
207-10-2	8/13/06	11:56	73.43	207p48.ASC	1360	8/22/06	967.05	8.383	0.00124	38.4	0.0322
207-11-1	8/14/06	12:15	97.75	207p49.ASC	1361	8/22/06	2896.00	8.433	0.00371	64.1	0.0578
207-11-2	8/14/06	12:15	97.75	207p50.ASC	1362	8/22/06	980.40	8.383	0.00125	39.3	0.0319
207-12-1	8/15/06	10:00	119.50	207p51.ASC	1363	8/22/06	2080.80	8.400	0.00266	58.3	0.0457
207-12-2	8/15/06	10:00	119.50	207p52.ASC	1364	8/22/06	1047.80	8.400	0.00134	45.2	0.0297

Table B.12. Uptake of phenanthrene: Trial 2 data summary

Averages			
Elapsed Time (hr)	Phenanthrene Concentration in Liquid (mg/L)	Phenanthrene Concentration in Biomass (mg /g FW)	Liquid pH
0	0	0.0000	9.215
0.02	0.5859	0.0366	9.230
1.0	0.3261	0.0294	9.300
2.0	0.2349	0.1009	9.481
4.0	0.2343	0.0430	9.782
6.0	0.1944	0.1509	9.986
9.0	0.2754	0.0964	10.019
11.4	0.2539	0.0763	10.012
23.7	0.2240	0.0821	9.867
48.5	0.2318	0.0787	9.932
73.4	0.1795	0.0363	9.986
97.7	0.1885	0.0449	9.874
119.5	0.1885	0.0377	9.829

Table B.13. Uptake of phenanthrene: Trial 2 mass balance

Sample Name	Total Liquid Volume Sampled (mL)	Liquid Phase Phenanthrene Concentration (mg/L)	Total Mass Phenanthrene Removed in Liquid Sample (μg)	Phenanthrene Removed in Biomass Samples (μg)
207-0	28.2	0.0000	0	0
207-1	21.5	0.5859	12.6	1.789
207-2	16.5	0.3261	5.38	0.966
207-3	22.0	0.2349	5.17	4.790
207-4	20.7	0.2343	4.85	1.577
207-5	28.8	0.1944	5.60	8.238
207-6	21.3	0.2754	5.87	4.338
207-7	21.6	0.2539	5.48	3.546
207-8-1	22.0	0.2240	4.93	4.794
207-8-2	20.4	0.2240	4.57	2.999
207-9-1	25.5	0.2318	5.91	3.530
207-9-2	22.8	0.2318	5.29	5.056
207-10-1	15.3	0.1795	2.75	1.770
207-10-2	19.9	0.1795	3.57	1.238
207-11-1	25.0	0.1885	4.712	3.707
207-11-2	20.5	0.1885	3.86	1.255
207-12-1	21.4	0.1885	4.03	2.663
207-12-2	NA	0.1885	NA	1.341

Total Mass Phenanthrene Added to Reactor (μg)	Total Mass Phenanthrene Removed in Samples (μg)	Final Mass Phenanthrene in Liquid (μg)	Final Mass Phenanthrene in Biomass (μg)	Mass Unaccounted For (μg)
1171.8	138.2	301.6	124.5	607.6

Total Mass Phenanthrene Added to Reactor (%)	Total Mass Phenanthrene Removed in Samples (%)	Final Mass Phenanthrene in Liquid (%)	Final Mass Phenanthrene in Biomass (%)	Mass Unaccounted For (%)
100.0	11.8	25.7	10.6	51.8

Table B.14. Uptake of phenanthrene: control experiment conditions

Run Description	
Run ID #:	AC208
Date Started:	08/21/06
Time Started:	15:21
Date Ended:	08/30/06
Time Ended:	12:00
Experiment Duration:	212.7 HR
Culture Loading	
Culture:	NA
Cell Line I.D.:	NA
Age of Inoculum:	NA
Initial Biomass Weight:	0
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	2.0 L
Initial pH Medium:	NA
Initial Biomass Density:	0
Shut-down Summary	
Final Weight Biomass:	NA
Final Medium pH:	8.373
Total Volume Medium:	NA
Final Biomass Density:	NA
Temperature Readout:	12.5 ºC
Final Reactor Volume	1877.6
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00124
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile

Table B.14. Continued

Test Parameters: Start-up Summary	
System Location:	VWR #2 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	12.0 ºC
Incident Light Intensity:	150 at reactor surface 75 at reactor center
Light Distance From Vessel Surface:	NA
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	2 L Bottle Reactor
Reactor ID:	NA
Mixing:	Stir plate/stir bar
Mixer Speed (rpm):	150
PAH:	Phenanthrene
PAH Stock Prep.:	20.0 mg phen. 2 mL EtOH
PAH Stock Conc.:	10.0 mg/mL
Vol. PAH Stock:	120.0 µL
Calculated Initial PAH Conc.:	0.6 mg/L
Measured Initial PAH Conc.:	0.548 mg/L

Table B.15. Uptake of phenanthrene: control experiment raw data

HPLC Data: Liquid Samples												
Sample Name	Date Sampled	Sample Time	Time (hr)	Temp. (ºC)	Volume Sampled (mL)	Liquid pH	HPLC file	Injn. #	Date of HPLC Analysis	Peak Area	Retention Time	Phen. Conc. in Liquid (mg/L)
208-1	8/21/06	15:25	0.1	14.7	14.7	8.344	208p2.ASC	1365	8/30/06	88.40	8.450	0.5481
208-2	8/21/06	15:36	0.3	15.6	18.4	8.452	208p3.ASC	1366	8/30/06	85.70	8.466	0.5313
208-3	8/22/06	16:40	25.3	12.0	16.1	8.374	208p4.ASC	1367	8/30/06	101.85	8.483	0.6315
208-4	8/23/06	16:50	49.5	12.0	19.5	8.356	208p5.ASC	1368	8/30/06	91.75	8.500	0.5689
208-5	8/25/06	13:35	94.2	12.0	18.5	8.354	208p6.ASC	1369	8/30/06	83.40	8.500	0.5171
208-6	8/28/06	14:00	166.7	13.1	14.4	8.322	208p7.ASC	1370	8/30/06	81.25	8.500	0.5038
208-7	8/30/06	12:00	212.7	12.5	20.8	8.373	208p8.ASC	1371	8/30/06	81.00	8.516	0.5022

Table B.16. Uptake of phenanthrene: control experiment mass balance

Total Mass Phenanthrene Added to Reactor (mg)	Total Mass Phenanthrene Removed in Samples (mg)	Final Mass Phenanthrene in Liquid (mg)	Mass Unaccounted For (mg)
1.10	0.07	0.94	0.09

Total Mass Phenanthrene Added to Reactor (%)	Total Mass Phenanthrene Removed in Samples (%)	Final Mass Phenanthrene in Liquid (%)	Mass Unaccounted For (%)
100.00	6.05	86.02	7.93

Table B.17. Uptake of phenanthrene: Trial 3 experiment conditions

Run Description	
Run ID #:	AC222
Date Started:	03/28/07
Time Started:	14:30
Date Ended:	03/31/07
Time Ended:	14:00
Experiment Duration:	71.5 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-35
Age of Inoculum:	36 days
Initial Biomass Weight:	1.0002 g FW
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	1.0 L
Initial Biomass Density:	1.00 g FW/L
Date Biomass Added:	03/28/07
Time Biomass Added:	14:30
Test Parameters: Start-up Summary	
System Location:	VWR #2
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	13.1 ºC
Incident Light Intensity	100 ($\mu E/m^2$-s):
Light distance from Reactor	4 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	1 L Bottle Reactors
Working Liquid Volume:	1.0 L
Mixing:	Stir plate/stir bar
PAH:	Phenanthrene
PAH Stock Conc.:	10.45 mg/mL
Volume Stock added to Reactor	57.4 µL
Initial medium pH	8.425
Calculated Initial PAH Conc.:	0.6 mg/L
Measured Initial PAH Conc.:	0.603 mg/L

Table B.17. Continued

Shut-down Summary	
Final Weight Biomass	0.4615 g FW
Final Liquid Volume	457 mL
Final Medium pH:	9.62
Temperature Setpoint:	12.0 ºC
Temperature Readout:	12.0 ºC
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00124 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extract Volume (mL):	2.0
End Point Extraction Volume (mL)	5.0
Sonication:	12:30 04/02/07 to 14:00 04/02/07

Table B.18. Uptake of phenanthrene: Trial 3 liquid phase raw data

HPLC Data: Liquid Samples											
Sample Name	Date Sampled	Sample Time	Time (hr)	Temp. (ºC)	Liquid pH	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Retention Time	Phen. Conc. in Liquid (mg/L)
222-1	3/28/07	14:20	0.0	12.0	8.425	222p2.ASC	2212	3/30/07	97.20	8.450	0.6026
222-2	3/28/07	15:30	1.0	12.0	8.657	222p3.ASC	2213	3/30/07	46.90	8.366	0.2908
222-3	3/28/07	16:30	2.0	12.0	8.767	222p4.ASC	2214	3/30/07	44.65	8.400	0.2768
222-4	3/28/07	18:40	4.2	12.0	8.990	222p5.ASC	2215	3/30/07	51.30	8.400	0.3181
222-5	3/28/07	21:30	7.0	12.0	9.237	222p6.ASC	2216	3/30/07	46.15	8.416	0.2861
222-6	3/29/07	1:20	10.8	12.0	9.101	222p7.ASC	2217	3/30/07	61.90	8.400	0.3838
222-7	3/29/07	5:15	14.8	12.0	8.894	222p8.ASC	2218	3/30/07	63.05	8.450	0.3909
222-8	3/29/07	10:30	20.0	12.0	9.019	222p9.ASC	2219	3/30/07	63.65	8.433	0.3946
222-9	3/29/07	14:00	23.5	12.0	9.114	222p10.ASC	2220	3/30/07	52.85	8.433	0.3277
222-10	3/29/07	17:30	27.0	12.0	9.299	222p11.ASC	2221	3/30/07	61.20	8.433	0.3794
222-11	3/29/07	21:40	31.2	12.0	9.430	222p12.ASC	2222	3/30/07	70.10	8.416	0.4346
222-12	3/30/07	1:30	35.0	12.0	9.247	222p13.ASC	2223	3/30/07	68.00	8.433	0.4216
222-13	3/30/07	5:55	39.4	12.0	9.009	222p14.ASC	2224	4/2/07	71.25	8.350	0.4418
222-14	3/30/07	10:55	44.4	12.0	9.096	222p15.ASC	2225	4/2/07	78.85	8.350	0.4889
222-15	3/30/07	14:30	48.0	12.0	9.259	222p17.ASC	2227	4/2/07	66.70	8.316	0.4135
222-16	3/30/07	18:00	51.5	12.0	9.461	222p19.ASC	2229	4/2/07	70.55	8.316	0.4374
222-17	3/30/07	21:30	55.0	12.0	9.659	222p21.ASC	2231	4/2/07	79.90	8.366	0.4954
222-18	3/31/07	1:50	59.3	12.0	9.365	222p23.ASC	2233	4/2/07	72.50	8.400	0.4495
222-19	3/31/07	6:50	64.3	12.0	9.193	222p25.ASC	2235	4/2/07	79.90	8.400	0.4954
222-20	3/31/07	10:30	68.0	12.0	9.336	222p27.ASC	2237	4/2/07	73.85	8.383	0.4579
222-21	3/31/07	14:00	71.5	12.0	9.620	222p29.ASC	2239	4/2/07	64.20	8.183	0.3980

Table B.19. Uptake of phenanthrene: Trial 3 biomass extraction raw data

HPLC Data: Biomass Extraction Samples

Sample Name	Date Sampled	Sample Time	Time (hr)	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Reten-tion Time	Phen. in Sample (mg)	Biomass Mass (mg FW)	Phen. Conc. (mg/g FW)
222-1	3/28/07	14:20	0.0	222p42.ASC	2262	4/3/07	0.00	NA	0.0000	22.4	0.0000
222-2	3/28/07	15:30	1.0	222p43.ASC	2263	4/3/07	832.80	8.233	0.0021	15.2	0.1359
222-3	3/28/07	16:30	2.0	222p44.ASC	2264	4/3/07	1033.70	8.300	0.0026	14.4	0.1780
222-4	3/28/07	18:40	4.2	222p45.ASC	2265	4/3/07	720.10	8.183	0.0018	12.9	0.1384
222-5	3/28/07	21:30	7.0	222p46.ASC	2266	4/4/07	1055.50	8.233	0.0026	15.3	0.1711
222-6	3/29/07	1:20	10.8	222p47.ASC	2267	4/4/07	1140.30	8.083	0.0028	16.7	0.1693
222-7	3/29/07	5:15	14.8	222p48.ASC	2268	4/4/07	1076.80	8.050	0.0027	21.5	0.1242
222-8	3/29/07	10:30	20.0	222p49.ASC	2269	4/4/07	1593.90	8.200	0.0040	25.2	0.1569
222-9	3/29/07	14:00	23.5	222p50.ASC	2270	4/4/07	1111.60	8.183	0.0028	16.2	0.1702
222-10	3/29/07	17:30	27.0	222p51.ASC	2271	4/4/07	1099.35	8.083	0.0027	16.3	0.1673
222-11	3/29/07	21:40	31.2	222p52.ASC	2272	4/4/07	720.55	8.216	0.0018	11.4	0.1568
222-12	3/30/07	1:30	35.0	222p53.ASC	2273	4/4/07	1196.90	8.133	0.0030	17.4	0.1706
222-13	3/30/07	5:55	39.4	222p54.ASC	2274	4/4/07	1716.60	8.116	0.0043	24.4	0.1745
222-14	3/30/07	10:55	44.4	222p55.ASC	2275	4/4/07	1305.70	8.116	0.0032	18.4	0.1760
222-15	3/30/07	14:30	48.0	222p56.ASC	2276	4/4/07	1251.00	7.700	0.0031	15.9	0.1951
222-16	3/30/07	18:00	51.5	222p57.ASC	2277	4/4/07	1317.25	8.150	0.0033	16.1	0.2029
222-17	3/30/07	21:30	55.0	222p58.ASC	2278	4/4/07	1845.90	8.333	0.0046	21.6	0.2119
222-18	3/31/07	1:50	59.3	222p59.ASC	2279	4/4/07	1547.80	8.233	0.0038	17.9	0.2144
222-19	3/31/07	6:50	64.3	222p60.ASC	2280	4/4/07	2038.00	8.350	0.0051	23.1	0.2188
222-20	3/31/07	10:30	68.0	222p61.ASC	2281	4/4/07	1413.30	8.316	0.0035	16.2	0.2164
222-21	3/31/07	14:00	71.5	222p62.ASC	2282	4/4/07	1852.00	8.233	0.0046	20.1	0.2285
222-22	3/31/07	14:00	75.0	222p56.ASC	2293	4/4/07	23049.3	8.316	0.1429	429.2	0.3330

Table B.20. Uptake of phenanthrene: Trial 3 mass balance

Sample Name	Total Liquid Volume Sampled (mL)	Liquid Phase Phenanthrene Concentration (mg/L)	Total Mass Phenanthrene Removed in Liquid Sample (mg)	Phenanthrene Removed in Biomass Samples (mg)
222-1	0.5	0.6026	0.00030	0.00000
222-2	25.9	0.2908	0.00753	0.00207
222-3	23.5	0.2768	0.00651	0.00256
222-4	25.8	0.3181	0.00821	0.00179
222-5	29.7	0.2861	0.00850	0.00262
222-6	28.1	0.3838	0.01078	0.00283
222-7	30.7	0.3909	0.01200	0.00267
222-8	35.6	0.3946	0.01405	0.00395
222-9	25.9	0.3277	0.00849	0.00276
222-10	28.5	0.3794	0.01081	0.00273
222-11	21.3	0.4346	0.00926	0.00179
222-12	29.5	0.4216	0.01244	0.00297
222-13	32.8	0.4418	0.01449	0.00426
222-14	27.3	0.4889	0.01335	0.00324
222-15	20.2	0.4135	0.00835	0.00310
222-16	21.4	0.4374	0.00936	0.00327
222-17	31.5	0.4954	0.01560	0.00458
222-18	28.6	0.4495	0.01286	0.00384
222-19	29.5	0.4954	0.01461	0.00505
222-20	26.0	0.4579	0.01190	0.00350
222-21	0.5	0.3980	0.00020	0.00459

Total Mass Phenanthrene Added to Reactor (mg)	Total Mass Phenanthrene Removed in Samples (mg)	Final Mass Phenanthrene in Liquid (mg)	Final Mass Phenanthrene in Biomass (mg)	Mass Unaccounted For (mg)
0.6026	0.2738	0.1819	0.1055	0.0415

Phenanthrene Added to Reactor (%)	Phenanthrene Removed in Samples (%)	Phenanthrene in Liquid (%)	Phenanthrene in Biomass (%)	Unaccounted For (%)
100.0	45.4	30.2	17.5	6.9

Table B.21. Uptake of naphthalene: experiment conditions

Run Description	
Run ID #:	AC212
Date Started:	11/02/06
Time Started:	11:00
Date Ended:	11/07/06
Time Ended:	11:30
Experiment Duration:	120.5 hr
Culture Loading (Reactor #1)	
Culture:	NA
Cell Line I.D.:	NA
Age of Inoculum:	NA
Initial Biomass Weight:	NA
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	1.0 L
Initial Biomass Density:	NA
Date Biomass Added:	NA
Time Biomass Added:	NA
Culture Loading (Reactor #2)	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-27
Age of Inoculum:	36 days
Initial Biomass Weight:	1.25 g FW
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	1 L
Initial Biomass Density:	1.25 g FW/L
Date Biomass Added:	11/02/06
Time Biomass Added:	11:00

Table B.21. Continued

Test Parameters: Start-up Summary	
System Location:	Precision Science #1
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	13.7 ºC/12.4 ºC
Incident Light Intensity (Reactor 1)	130 (μE/m^2-s):
Incident Light Intensity (Reactor 2)	130 (μE/m^2-s):
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	1 L Bottle Reactors
Working Liquid Volume	1.0 L
Mixing:	Stir plate/stir bar
PAH:	Naphthalene
PAH Stock Prep.:	
Reactor 1:	20.5 mg naphthalene dissolved in 1 L medium
Reactor 2:	21.4 mg naphthalene dissolved in 1 L medium
Naphthalene is added to reactors and the solution is mixed for 48 hours. Then the solution is filtered through a 0.2 μm filter to remove any undissolved naphthalnene.	
Measured Initial PAH Conc.:	
Reactor 1:	13.92 mg/L
Reactor 2:	14.81 mg/L
Date PAH Added:	10/31/06
Shut-down Summary	
Final Weight Biomass (Reactor 2):	0.9707 g FW
Final Biomass Density (Reactor 2):	1.28 g FW/L
Final Medium pH:	
Reactor 1:	8.508
Reactor 2:	7.743
Final Volume Medium (mL):	
Reactor 1:	805
Reactor 2:	760
Temperature Readout:	16.4 ºC/14.3 ºC

Table B.21. Continued

HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.0193 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	254 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extract Volume (mL):	
Samples 212-2-2, 212-2-20	3.0
All other samples	1.0
Sonication:	12:00 11/08/06 to 13:30 11/08/06

Table B.22. Uptake of naphthalene: liquid phase raw data

HPLC Data: Liquid Samples Reactor 1 (Control)

Sample Name	Date Sampled	Sample Time	Time (hr)	Temp. (ºC)	Vol. (mL)	Liquid pH	HPLC file	Injn #	Date of Analysis	Peak Area	Reten-tion Time	Nap. Conc. (mg/L)
212-1-1	11/02/06	10:40	0.0	13.6/12.4	0.5	NA	212p14	1634	11/08/06	144.30	5.650	13.925
212-1-3	11/02/06	12:00	1.0	15.0/13.2	21.2	8.472	212p15	1635	11/08/06	144.95	5.633	13.988
212-1-5	11/02/06	13:30	2.5	17.3/14.7	25.7	8.496	212p16	1636	11/08/06	144.60	5.633	13.954
212-1-7	11/02/06	16:00	5.0	17.9/15.1	16.4	8.424	212p17	1637	11/08/06	140.75	5.666	13.582
212-1-9	11/02/06	19:00	8.0	18.6/15.4	23.6	8.439	212p18	1638	11/08/06	145.20	5.650	14.012
212-1-11	11/03/06	12:00	25.0	16.8/14.4	17.8	8.441	212p19	1639	11/08/06	147.25	5.650	14.210
212-1-13	11/04/06	10:30	47.5	16.9/14.9	20.3	8.467	212p20	1640	11/08/06	148.10	5.650	14.292
212-1-15	11/05/06	11:50	72.8	17.1/14.9	28.7	8.474	212p21	1641	11/08/06	153.05	5.633	14.769
212-1-17	11/06/06	10:00	95.0	16.6/14.4	23.6	8.492	212p22	1642	11/09/06	150.20	5.616	14.494
212-1-19	11/07/06	11:30	120.5	16.6/14.3	0.5	8.508	212p23	1643	11/09/06	147.90	5.616	14.272

HPLC Data: Liquid Samples Reactor 2

Sample Name	Date Sampled	Sample Time	Time (hr)	Temp. (ºC)	Vol. (mL)	Liquid pH	HPLC file	Injn #	Date of Analysis	Peak Area	Reten-tion Time	Nap. Conc. (mg/L)
212-2-2	11/02/06	10:40	0.0	13.6/12.4	0.5	NA	212p24	1644	11/09/06	153.50	5.633	14.81
212-2-4	11/02/06	12:00	1.0	15.0/13.2	34.1	8.509	212p25	1645	11/09/06	125.40	5.616	12.10
212-2-6	11/02/06	13:30	2.5	17.3/14.7	32.4	8.598	212p26	1646	11/09/06	128.00	5.633	12.35
212-2-8	11/02/06	16:00	5.0	17.9/15.1	27.3	8.689	212p27	1647	11/09/06	127.30	5.650	12.28
212-2-10	11/02/06	19:00	8.0	18.6/15.4	27.2	8.791	212p28	1648	11/09/06	120.60	5.616	11.64
212-2-12	11/03/06	12:00	25.0	16.8/14.4	30.3	8.668	212p29	1649	11/09/06	126.25	5.633	12.18
212-2-14	11/04/06	10:30	47.5	16.9/14.9	28.8	8.395	212p30	1650	11/09/06	121.70	5.633	11.74
212-2-16	11/05/06	11:50	72.8	17.1/14.9	36.5	8.004	212p31	1651	11/09/06	125.90	5.633	12.15
212-2-18	11/06/06	10:00	95.0	16.6/14.4	31.8	7.859	212p32	1652	11/09/06	121.60	5.633	11.73
212-2-20	11/07/06	11:30	120.5	16.6/14.3	0.5	7.743	212p33	1653	11/09/06	124.30	5.616	11.99

Table B.23. Uptake of naphthalene: biomass extraction raw data

HPLC Data: Biomass Extraction Samples											
Sample Name	Date Sampled	Sample Time	Time (hr)	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time	Nap. in Sample (mg)	Bio-mass Weight (mg FW)	Nap. Conc. (mg/ g FW)
212-2-2	11/02/06	11:00	0.0	212p34	1654	11/09/06	0.00	NA	0.0000	136.1	0.0000
212-2-4	11/02/06	12:00	1.0	212p35	1655	11/09/06	535.65	5.516	0.0103	20.6	0.5018
212-2-6	11/02/06	13:30	2.5	212p36	1656	11/09/06	416.80	5.550	0.0080	18.5	0.4348
212-2-8	11/02/06	16:00	5.0	212p37	1657	11/09/06	431.40	5.550	0.0083	13.9	0.5990
212-2-10	11/02/06	19:00	8.0	212p38	1658	11/09/06	1790.00	5.516	0.0345	22.2	1.5562
212-2-12	11/03/06	12:00	25.0	212p39	1659	11/09/06	860.15	5.533	0.0166	22.5	0.7378
212-2-14	11/04/06	10:30	47.5	212p40	1660	11/09/06	1006.10	5.500	0.0194	25.6	0.7585
212-2-16	11/05/06	11:50	72.8	212p41	1661	11/09/06	1352.90	5.450	0.0261	29.6	0.8821
212-2-18	11/06/06	10:00	95.0	212p42	1662	11/09/06	1232.10	5.550	0.0238	28.1	0.8462
212-2-20	11/07/06	11:30	120.5	212p43	1663	11/09/06	916.30	5.533	0.0531	100.2	0.5295

Table B.24. Uptake of naphthalene: mass balance

Reactor 1: Control				
Sample Name	Total Liquid Volume Sampled (mL)	Liquid Phase Naphthalene Concentration (mg/L)	Total Mass Naphthalene Removed in Liquid Sample (mg)	Naphthalene Removed in Biomass Samples (mg)
212-1-1	0.5	13.92	0.0070	0
212-1-3	21.2	13.99	0.2965	0
212-1-5	25.7	13.95	0.3586	0
212-1-7	16.4	13.58	0.2228	0
212-1-9	23.6	14.01	0.3307	0
212-1-11	17.8	14.21	0.2529	0
212-1-13	20.3	14.29	0.2901	0
212-1-15	28.7	14.77	0.4239	0
212-1-17	23.6	14.49	0.3421	0
212-1-19	0.5	14.27	0.0071	0

Reactor 1: Control				
Total Mass Naphthalene Added to Reactor (mg)	Total Mass Naphthalene Removed in Samples (mg)	Final Mass Naphthalene in Liquid (mg)	Final Mass Naphthalene in Biomass (mg)	Mass Unaccounted For (mg)
13.92	2.53	11.49	NA	-0.10
Naphthalene Added to Reactor	Naphthalene Removed in Samples (% initial)	Naphthalene in Liquid (% initial)	Naphthalene in Biomass (% initial)	Unaccounted For (% initial)
100	18.2	82.5	NA	-0.73

Reactor 2: With Biomass				
Sample Name	Total Liquid Volume Sampled (mL)	Liquid Phase Naphthalene Concentration (mg/L)	Total Mass Naphthalene Removed in Liquid Sample (mg)	Naphthalene Removed in Biomass Samples (mg)
212-2-2	0.5	14.81	0.0074	0.0000
212-2-4	34.1	12.10	0.4126	0.0103
212-2-6	32.4	12.35	0.4002	0.0080
212-2-8	27.3	12.28	0.3354	0.0083
212-2-10	27.2	11.64	0.3166	0.0345
212-2-12	30.3	12.18	0.3691	0.0166
212-2-14	28.8	11.74	0.3382	0.0194
212-2-16	36.5	12.15	0.4435	0.0261
212-2-18	31.8	11.73	0.3732	0.0238
212-2-20	0.5	11.99	0.0060	0.0531

Reactor 2: With Biomass				
Total Mass Naphthalene Added to Reactor (mg)	Total Mass Naphthalene Removed in Samples (mg)	Final Mass Naphthalene in Liquid (mg)	Final Mass Naphthalene in Biomass (mg)	Mass Unaccounted For (mg)
14.81	3.20	9.12	0.68	1.81
Naphthalene Added to Reactor (% initial)	Naphthalene Removed in Samples (% initial)	Naphthalene in Liquid (% initial)	Naphthalene in Biomass (% initial)	Unaccounted For (% initial)
100	21.62304691	61.6	4.6	12.2

Table B.25. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 1 experiment conditions

Run Description	
Run ID #:	AC215
Date Started:	01/04/07
Time Started:	17:00
Date Ended:	01/09/07
Time Ended:	10:00
Experiment Duration:	113 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-31
Age of Inoculum:	30 days
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	20 mL per vial
Initial Biomass Density:	~1 g FW/L
Initial Biomass Weight:	~20 mg FW
Control Biomass Density:	0 g FW/L
Control Biomass Weight:	0 mg FW
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00124 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extract Volume:	3.0 mL added to all biomass samples
Date Extraction Started:	01/09/07
Date Extraction Ended:	1/16/2007
Sonication:	14:00-16:00 01/09/07
FW to dry cell mass conversion	0.358

Solution Preparation	
PAH:	Phenanthrene
PAH Stock Prep.:	40.0 mg phen. in 4 mL EtOH
PAH Stock Conc.:	10.0 mg/mL
Vol. PAH Stock:	60 μL added to 1 L soln.
Calculated Initial PAH Conc.:	0.6 mg/L
pH 0.6 mg/L Phen. Soln.:	8.707
pH 0 mg/L Phen. Soln.:	8.868

Solution Dilution:		
Solution Concentration (mg/L)	Volume 0.6 mg/L Soln.	Volume 0 mg/L Soln.
0	0.0	100.0
0.1	16.7	83.3
0.2	33.3	66.7
0.3	50.0	50.0
0.4	66.7	33.3
0.5	83.3	16.7
0.6	100.0	0.0

Solution Concentrations:	
Calculated Soln. Conc. (mg/L)	Measured Soln. Conc. (mg/L)
0	0.0000
0.1	0.0907
0.2	0.1807
0.3	0.2542
0.4	0.3398
0.5	0.4304
0.6	0.5118

Test Parameters: Start-up Summary	
System Location:	Prec. Science #1 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	13.2 ºC/12.3 ºC
Incident Light Intensity	~90 (μE/m^2-s)
Light Distance From Vessel Surface:	1.5 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	20 mL Vials w/ Teflon Lined Caps
Liquid Volume	20 mL per vial
Mixing:	Orbital Shaker
Mixer Speed (rpm):	155

Table B.26. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 1 vial summary

Vial #	Initial Biomass Weight (mg FW)	Initial Phen. Conc. (mg/L)	Final Biomass Weight (mg FW)	Final pH	(X_f/X_0)
1	0	0	0	8.707	NA
2	0	0	0	8.729	NA
3	0	0.1	0	8.334	NA
4	0	0.1	0	8.324	NA
5	0	0.2	0	8.667	NA
6	0	0.2	0	8.704	NA
7	0	0.3	0	8.648	NA
8	0	0.3	0	8.663	NA
9	0	0.4	0	8.506	NA
10	0	0.4	0	8.516	NA
11	0	0.5	0	7.301	NA
12	0	0.5	0	8.445	NA
13	0	0.6	0	8.657	NA
14	0	0.6	0	7.196	NA
15	20	0	26.2	9.603	1.31
16	20.3	0	30.7	9.945	1.51
17	20.5	0	29.5	9.965	1.44
18	20.1	0.1	28.4	10.027	1.41
19	20.1	0.1	30.8	9.987	1.53
20	20.3	0.1	26.4	9.203	1.30
21	20	0.2	34.1	9.243	1.71
22	20.6	0.2	33.2	9.897	1.61
23	20.1	0.2	30.0	9.828	1.49
24	20.4	0.3	29.5	9.946	1.45
25	20.4	0.3	29.6	8.788	1.45
26	20.3	0.3	30.0	9.818	1.48
27	21.4	0.4	30.1	9.601	1.41
28	21.7	0.4	31.5	9.799	1.45
29	20.6	0.4	27.6	8.537	1.34
30	21	0.5	27.7	9.860	1.32
31	21.8	0.5	30.4	9.887	1.39
32	20.2	0.5	26.3	8.799	1.30
33	20.5	0.6	31.3	9.013	1.53
34	20.7	0.6	33.6	9.713	1.62
35	21.4	0.6	33.5	8.493	1.57

Table B.27. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 1 liquid phase raw data

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Phen. Conc. (mg/L)
0 mg/L Stock 1	1/4/07	215p2.ASC	1833	1/6/07	0.00	NA	0.0000
0 mg/L Stock 2	1/4/07	215p3.ASC	1834	1/6/07	0.00	NA	0.0000
0.1 mg/L Stock 1	1/4/07	215p4.ASC	1835	1/6/07	14.80	8.666	0.0918
0.1 mg/L Stock 2	1/4/07	215p5.ASC	1836	1/6/07	14.45	8.683	0.0896
0.2 mg/L Stock 1	1/4/07	215p6.ASC	1837	1/6/07	28.40	8.666	0.1761
0.3 mg/L Stock 2	1/4/07	215p7.ASC	1838	1/6/07	29.90	8.666	0.1854
0.4 mg/L Stock 1	1/4/07	215p8.ASC	1839	1/6/07	42.00	8.616	0.2604
0.2 mg/L Stock 2	1/4/07	215p9.ASC	1840	1/6/07	40.00	8.633	0.2480
0.3 mg/L Stock 3	1/4/07	215p10.ASC	1841	1/6/07	55.00	8.533	0.3410
0.4 mg/L Stock 4	1/4/07	215p11.ASC	1842	1/6/07	54.60	8.633	0.3385
0.5 mg/L Stock 1	1/4/07	215p12.ASC	1843	1/6/07	65.30	8.633	0.4049
0.6 mg/L Stock 2	1/4/07	215p13.ASC	1844	1/6/07	73.55	8.616	0.4560
0.6 mg/L Stock 1	1/4/07	215p14.ASC	1845	1/6/07	86.30	8.633	0.5351
0.6 mg/L Stock 2	1/4/07	215p15.ASC	1846	1/6/07	78.80	8.616	0.4886
215-1	1/9/07	215p25.ASC	1856	1/9/07	0.00	NA	0.0000
215-2	1/9/07	215p26.ASC	1857	1/9/07	0.00	NA	0.0000
215-3	1/9/07	215p27.ASC	1858	1/9/07	15.40	8.333	0.0955
215-4	1/9/07	215p28.ASC	1859	1/9/07	10.80	8.316	0.0670
215-5	1/9/07	215p29.ASC	1860	1/9/07	23.00	8.466	0.1426
215-6	1/9/07	215p30.ASC	1861	1/9/07	23.80	8.383	0.1476
215-7	1/9/07	215p31.ASC	1862	1/9/07	33.40	8.383	0.2071
215-8	1/9/07	215p32.ASC	1863	1/9/07	13.80	8.850	0.0856
215-9	1/9/07	215p33.ASC	1864	1/9/07	40.10	8.350	0.2486

Table B.27. Continued

HPLC Data: Liquid Samples							
Sample Name	Date Samp-led	HPLC file	Injn #	Date of Analysis	Peak Area	Reten-tion Time (min)	Phen. Conc. (mg/L)
215-10	1/9/07	215p34.ASC	1865	1/9/07	53.20	8.433	0.3298
215-11	1/9/07	215p41.ASC	1873	1/10/07	56.70	8.500	0.3515
215-12	1/9/07	215p42.ASC	1874	1/10/07	55.10	8.516	0.3416
215-13	1/9/07	215p43.ASC	1875	1/10/07	70.90	8.483	0.4396
215-14	1/9/07	215p44.ASC	1876	1/10/07	68.40	8.466	0.4241
215-15	1/9/07	215p45.ASC	1877	1/10/07	0.00	NA	0.0000
215-16	1/9/07	215p46.ASC	1878	1/10/07	5.45	8.516	0.0338
215-17	1/9/07	215p47.ASC	1879	1/10/07	0.00	NA	0.0000
215-18	1/9/07	215p48.ASC	1880	1/10/07	6.00	8.433	0.0372
215-19	1/9/07	215p49.ASC	1881	1/10/07	0.00	NA	0.0000
215-20	1/9/07	215p50.ASC	1882	1/10/07	6.10	8.400	0.0378
215-21	1/9/07	215p51.ASC	1883	1/10/07	9.80	8.450	0.0608
215-22	1/9/07	215p52.ASC	1884	1/10/07	8.25	8.483	0.0512
215-23	1/9/07	215p53.ASC	1885	1/10/07	8.10	8.450	0.0502
215-24	1/9/07	215p54.ASC	1886	1/10/07	14.80	8.466	0.0918
215-25	1/9/07	215p55.ASC	1887	1/10/07	19.10	8.333	0.1184
215-26	1/9/07	215p56.ASC	1888	1/10/07	21.20	8.450	0.1314
215-27	1/9/07	215p57.ASC	1889	1/10/07	21.40	8.433	0.1327
215-28	1/9/07	215p58.ASC	1890	1/10/07	22.10	8.383	0.1370
215-29	1/9/07	215p59.ASC	1891	1/10/07	51.40	8.466	0.3187
215-30	1/9/07	215p60.ASC	1892	1/10/07	28.30	8.450	0.1755
215-31	1/9/07	215p61.ASC	1893	1/10/07	34.60	8.450	0.2145
215-32	1/9/07	215p62.ASC	1894	1/10/07	32.80	8.450	0.2034
215-33	1/9/07	215p63.ASC	1895	1/10/07	34.55	8.450	0.2142
215-34	1/9/07	215p64.ASC	1896	1/10/07	35.95	8.383	0.2229
215-35	1/9/07	215p65.ASC	1897	1/10/07	59.85	8.450	0.3711

Table B.28. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 1 biomass extraction raw data

HPLC Data: Biomass Extraction Samples											
Sample Name	Date Sampled	HPLC file	Injn #	Date Analyzed	Peak Area	Reten-tion Time (min)	Mass Phen. extracted (mg)	Final FW Biomass (mg)	Phen. Conc. (mg/g FW)	Estimated DW (mg)	Phen. Conc. (mg/g DW)
215-15	1/9/07	215p66	1932	1/17/07	0.00	NA	0.00000	26.2	0.00000	9.4	0.0000
215-16	1/9/07	215p67	1933	1/17/07	0.00	NA	0.00000	30.7	0.00000	11.0	0.0000
215-17	1/9/07	215p68	1934	1/17/07	0.00	NA	0.00000	29.5	0.00000	10.6	0.0000
215-18	1/9/07	215p69	1935	1/17/07	60.20	8.400	0.00022	28.4	0.00789	10.2	0.0220
215-19	1/9/07	215p70	1936	1/17/07	71.70	8.383	0.00027	30.8	0.00866	11.0	0.0242
215-20	1/9/07	215p71	1937	1/17/07	70.00	8.433	0.00026	26.4	0.00986	9.5	0.0276
215-21	1/9/07	215p72	1938	1/17/07	195.75	8.416	0.00073	34.1	0.02135	12.2	0.0596
215-22	1/9/07	215p73	1939	1/17/07	151.80	8.400	0.00056	33.2	0.01701	11.9	0.0475
215-23	1/9/07	215p74	1940	1/17/07	141.80	8.333	0.00053	30.0	0.01758	10.7	0.0491
215-24	1/9/07	215p75	1941	1/17/07	211.40	8.400	0.00079	29.5	0.02666	10.6	0.0745
215-25	1/9/07	215p76	1942	1/17/07	193.25	8.366	0.00072	29.6	0.02429	10.6	0.0678
215-26	1/9/07	215p77	1943	1/17/07	160.90	8.400	0.00060	30.0	0.01995	10.7	0.0557
215-27	1/9/07	215p78	1944	1/17/07	301.80	8.400	0.00112	30.1	0.03730	10.8	0.1042
215-28	1/9/07	215p79	1945	1/17/07	299.60	8.383	0.00111	31.5	0.03538	11.3	0.0988
215-29	1/9/07	215p80	1946	1/17/07	262.80	8.366	0.00098	27.6	0.03542	9.9	0.0989
215-30	1/9/07	215p81	1947	1/17/07	284.10	8.400	0.00106	27.7	0.03815	9.9	0.1066
215-31	1/9/07	215p82	1948	1/17/07	403.00	8.400	0.00150	30.4	0.04931	10.9	0.1377
215-32	1/9/07	215p83	1949	1/17/07	355.60	8.366	0.00132	26.3	0.05030	9.4	0.1405
215-33	1/9/07	215p84	1950	1/17/07	445.90	8.366	0.00166	31.3	0.05300	11.2	0.1480
215-34	1/9/07	215p85	1951	1/17/07	340.80	8.366	0.00127	33.6	0.03773	12.0	0.1054
215-35	1/9/07	215p86	1952	1/17/07	497.90	8.316	0.00185	33.5	0.05529	12.0	0.1544

Table B.29. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 1 three point averages for 113 partitioning

Initial Phenanthrene Concentration in Liquid (mg/L)	Final Phenanthrene Concentration in Liquid (mg/L)	Final Phenanthrene Concentration in Biomass (mg/g FW)	Final Phenanthrene Concentration in Biomass (mg/g DW)
0.0000	0.0113	0.00000	0.0000
0.0907	0.0250	0.00880	0.0246
0.1807	0.0540	0.01865	0.0521
0.2542	0.1139	0.02363	0.0660
0.3398	0.1961	0.03603	0.1007
0.4304	0.1978	0.04592	0.1283
0.5118	0.2694	0.04867	0.1360

Table B.30. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 1 mass balance

Phenanthrene Mass Balance for Vials with Biomass								
Initial Phen. Conc. (mg/L)	Vial	Total Mass (mg)	Mass in Liquid (mg)	In Liquid (%)	Mass in Biomass (mg)	In Biomass (%)	Mass Unaccounted For (mg)	Unaccounted For (%)
0.0000	15	0.0000	0.0000	NA	0.00000	NA	NA	NA
0.0000	16	0.0000	0.0007	NA	0.00000	NA	NA	NA
0.0000	17	0.0000	0.0000	NA	0.00000	NA	NA	NA
0.0907	18	0.0018	0.0007	41.0	0.00022	12.3	0.00085	46.6
0.0907	19	0.0018	0.0000	0.0	0.00027	14.7	0.00155	85.3
0.0907	20	0.0018	0.0008	41.7	0.00026	14.4	0.00080	43.9
0.1807	21	0.0036	0.0012	33.6	0.00073	20.1	0.00167	46.2
0.1807	22	0.0036	0.0010	28.3	0.00056	15.6	0.00203	56.1
0.1807	23	0.0036	0.0010	27.8	0.00053	14.6	0.00208	57.6
0.2542	24	0.0051	0.0018	36.1	0.00079	15.5	0.00246	48.4
0.2542	25	0.0051	0.0024	46.6	0.00072	14.1	0.00200	39.3
0.2542	26	0.0051	0.0026	51.7	0.00060	11.8	0.00186	36.5
0.3398	27	0.0068	0.0027	39.1	0.00112	16.5	0.00302	44.4
0.3398	28	0.0068	0.0027	40.3	0.00111	16.4	0.00294	43.3
0.3398	29	0.0068	0.0064	93.8	0.00098	14.4	-0.00056	-8.2
0.4304	30	0.0086	0.0035	40.8	0.00106	12.3	0.00404	47.0
0.4304	31	0.0086	0.0043	49.8	0.00150	17.4	0.00282	32.7
0.4304	32	0.0086	0.0041	47.2	0.00132	15.4	0.00322	37.4
0.5118	33	0.0102	0.0043	41.9	0.00166	16.2	0.00429	41.9
0.5118	34	0.0102	0.0045	43.5	0.00127	12.4	0.00451	44.1
0.5118	35	0.0102	0.0074	72.5	0.00185	18.1	0.00096	9.4

Table B.30. Continued

Phenanthrene Mass Balance for Control Vials (No Biomass)								
Initial Phen. Conc. (mg/L)	Vial	Total Mass	Mass in Liquid (mg)	In Liquid (% initial)	Mass in Biomass (mg)	In Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.0000	1	0.0000	0.0000	NA	0	0	NA	NA
0.0000	2	0.0000	0.0000	NA	0	0	NA	NA
0.0907	3	0.0018	0.0019	105.3	0	0	-0.00010	-5.3
0.0907	4	0.0018	0.0013	73.8	0	0	0.00047	26.2
0.1807	5	0.0036	0.0029	78.9	0	0	0.00076	21.1
0.1807	6	0.0036	0.0030	81.6	0	0	0.00066	18.4
0.2542	7	0.0051	0.0041	81.5	0	0	0.00094	18.5
0.2542	8	0.0051	0.0017	33.7	0	0	0.00337	66.3
0.3398	9	0.0068	0.0050	73.2	0	0	0.00182	26.8
0.3398	10	0.0068	0.0066	97.1	0	0	0.00020	2.9
0.4304	11	0.0086	0.0070	81.7	0	0	0.00158	18.3
0.4304	12	0.0086	0.0068	79.4	0	0	0.00178	20.6
0.5118	13	0.0102	0.0088	85.9	0	0	0.00144	14.1
0.5118	14	0.0102	0.0085	82.9	0	0	0.00175	17.1

Table B.30. Continued

Average Phenanthrene Mass Balance for Vials with Biomass								
Initial Phen. Conc. (mg/L)	Vial	Total Mass	Mass in Liquid (mg)	In Liquid (% initial)	Mass in Biomass (mg)	In Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.0000	15,16,17	0.0000	0.0002	NA	NA	NA	NA	NA
0.0907	17,19,20	0.0018	0.0005	27.6	0.0003	13.8	0.0011	58.6
0.1807	21,22,23	0.0036	0.0011	29.9	0.0006	16.8	0.0019	53.3
0.2542	24,25,26	0.0051	0.0023	44.8	0.0007	13.8	0.0021	41.4
0.3398	27,28,29	0.0068	0.0039	57.7	0.0011	15.8	0.0018	26.5
0.4304	30,31,32	0.0086	0.0040	45.9	0.0013	15.0	0.0034	39.0
0.5118	33,34,35	0.0102	0.0054	52.6	0.0016	15.6	0.0033	31.8

Average Phenanthrene Mass Balance for Control Vials (No Biomass)								
Initial Phen. Conc. (mg/L)	Vial	Total Mass	Mass in Liquid (mg)	In Liquid (% initial)	Mass in Biomass (mg)	In Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.0000	1,2	0.0000	0.0000	NA	0	0	NA	NA
0.0907	3,4	0.0018	0.0016	89.6	0	0	0.0002	10.4
0.1807	5,6	0.0036	0.0029	80.3	0	0	0.0007	19.7
0.2542	7,8	0.0051	0.0029	57.6	0	0	0.0022	42.4
0.3398	9,10	0.0068	0.0058	85.1	0	0	0.0010	14.9
0.4304	11,12	0.0086	0.0069	80.5	0	0	0.0017	19.5
0.5118	13,14	0.0102	0.0086	84.4	0	0	0.0016	15.6

Table B.31. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 2 experiment conditions

Run Description	
Run ID #:	AC220
Date Started:	03/08/07
Time Started:	17:05
Date Ended:	03/13/07
Time Ended:	11:00
Experiment Duration:	113.9 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-35
Age of Inoculum:	17 days
Initial Biomass Density:	~1.0 g FW/L
Solution Prep.	
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Phenanthrene Solution Prep.:	
PAH:	Phenanthrene
PAH Stock Prep.:	21.7 mg naphthalene dissolved in 2 mL Ethanol
PAH Stock Conc.:	10.85 mg/mL
Vol. PAH Stock:	32.3 µL added to 500 mL medium to give 0.7 mg/L soln.

Seven solution of various phenanthrene concentrations are prepared by diluting the 0.7 mg/L phenanthrene solution with medium not containing phenanthrene.

Solution	Vol. Non-Phen. Soln. (mL)	Vol. phen. Soln. (mL)
1	85.7	14.3
2	71.4	28.6
3	57.1	42.9
4	42.9	57.1
5	28.6	71.4
6	14.3	85.7
7	0	100

Table B.31. Continued

Solution Prep:	
pH Phenanthrene soln.	8.412
pH Non-Phenanthrene soln.	8.351
Calculated Initial Concentration of Phenanthrene (mg/L)	
Solution 1:	0.1
Solution 2:	0.2
Solution 3:	0.3
Solution 4:	0.4
Solution 5:	0.5
Solution 6:	0.6
Solution 7:	0.7
Measured Initial Concentration of Phenanthrene (mg/L)	
Solution 1:	0.114
Solution 2:	0.144
Solution 3:	0.313
Solution 4:	0.404
Solution 5:	0.512
Solution 6:	0.625
Solution 7:	0.899
Test Parameters: Start-up Summary	
System Location:	Prec. Science #1 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	12.9 ºC/12.3 ºC
Incident Light Intensity	130-150 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	1.5 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	20 mL Vials w/ Teflon Lined Caps
Mixing:	Orbital Shaker
Mixer Speed (rpm):	162
Medium Volume:	20 mL per vial
Shut-Down Temp	16.8 ºC/14.8 ºC
FW to DW Conversion	0.358

Table B.31. Continued

Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extraction 1 start:	1/13/2007 0:00
Extraction 1 end:	03/15/07
Sonicated from 11:30-13:00 on 03/14/07	
Extraction Volume (mL):	3.0
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00124 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile

Table B.32. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 2 vial summary

Vial #	Initial Biomass Weight Measured (mg FW)	Initial Phenanthrene Concentration (mg/L)	Final Biomass Weight Measured (mg FW)	Final pH	X_f/X_0
1	23.6	0.114	28.6	9.429	1.21
2	19.6	0.114	25.1	9.440	1.28
3	19.9	0.144	27.0	9.245	1.36
4	22.2	0.144	30.8	9.262	1.39
5	19.8	0.313	26.7	9.098	1.35
6	22.0	0.313	25.5	9.220	1.16
7	22.6	0.404	30.4	9.123	1.35
8	20.2	0.404	25.8	9.130	1.28
9	19.2	0.512	23.8	9.014	1.24
10	19.4	0.512	24.8	9.037	1.28
11	21.5	0.625	26.3	9.009	1.22
12	20.5	0.625	22.7	9.022	1.11
13	21.8	0.899	21.6	8.918	0.99
14	23.1	0.899	26.3	8.982	1.14

Table B.33. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 2 liquid phase raw data

HPLC Data: Liquid Samples

Sample Name	Date Sampled	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Retention Time (min)	Phenanthrene Concentration in Liquid (mg/L)
0.1 mg/L Stock 1	03/13/07	220p2.ASC	2120	03/14/07	16.20	8.316	0.1004
0.1 mg/L Stock 2	03/13/07	220p3.ASC	2121	03/14/07	20.55	8.283	0.1274
0.2 mg/L Stock 1	03/13/07	220p4.ASC	2122	03/14/07	34.75	8.333	0.2155
0.3 mg/L Stock 2	03/13/07	220p5.ASC	2123	03/14/07	11.65	8.816	0.0722
0.4 mg/L Stock 1	03/13/07	220p6.ASC	2124	03/14/07	49.25	8.333	0.3054
0.2 mg/L Stock 2	03/13/07	220p7.ASC	2125	03/14/07	51.70	8.316	0.3205
0.3 mg/L Stock 1	03/13/07	220p8.ASC	2126	03/14/07	60.00	8.350	0.3720
0.4 mg/L Stock 2	03/13/07	220p9.ASC	2127	03/14/07	70.40	8.350	0.4365
0.5 mg/L Stock 1	03/13/07	220p10.ASC	2128	03/14/07	84.05	8.350	0.5211
0.6 mg/L Stock 2	03/13/07	220p11.ASC	2129	03/14/07	81.00	8.350	0.5022
0.7 mg/L Stock 1	03/13/07	220p12.ASC	2130	03/14/07	99.20	8.333	0.6150
0.5 mg/L Stock 2	03/13/07	220p13.ASC	2131	03/14/07	102.50	8.366	0.6355
0.6 mg/L Stock 1	03/13/07	220p14.ASC	2132	03/14/07	175.95	8.366	1.0909
0.7 mg/L Stock 2	03/13/07	220p15.ASC	2133	03/14/07	114.10	8.333	0.7074
220-1	03/13/07	220p16.ASC	2134	03/14/07	5.20	8.300	0.0322
220-2	03/13/07	220p17.ASC	2135	03/14/07	12.35	8.366	0.0766
220-3	03/13/07	220p18.ASC	2136	03/14/07	13.00	8.300	0.0806
220-4	03/13/07	220p19.ASC	2137	03/14/07	26.40	8.316	0.1637
220-5	03/13/07	220p20.ASC	2138	03/14/07	18.55	7.850	0.1150
220-6	03/13/07	220p21.ASC	2139	03/15/07	23.10	8.316	0.1432
220-7	03/13/07	220p22.ASC	2140	03/15/07	24.05	8.300	0.1491
220-8	03/13/07	220p23.ASC	2141	03/15/07	26.40	8.250	0.1637
220-9	03/13/07	220p24.ASC	2142	03/15/07	34.40	8.266	0.2133

Table B.33. Continued

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Retention Time (min)	Phenanthrene Concentration in Liquid (mg/L)
220-10	03/13/07	220p25.ASC	2143	03/15/07	33.85	8.316	0.2099
220-11	03/13/07	220p26.ASC	2144	03/15/07	45.50	8.350	0.2821
220-12	03/13/07	220p27.ASC	2145	03/15/07	56.60	8.366	0.3509
220-13	03/13/07	220p28.ASC	2146	03/15/07	60.35	8.350	0.3742
220-14	03/13/07	220p29.ASC	2147	03/15/07	58.10	8.333	0.3602

Table B.34. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 2 averages for partitioning

Vials	Initial Phenanthrene Concentration in Liquid (mg/L)	Final Phenanthrene Concentration in Liquid (mg/L)	Final Phenanthrene Concentration in Biomass (mg/g FW)	Final Phenanthrene Concentration in Biomass (mg/g DW)
1,2	0.1139	0.0544	0.00599	0.01674
3,4	0.1438	0.1221	0.01898	0.05301
5,6	0.3129	0.1291	0.02545	0.07109
7,8	0.4042	0.1564	0.02976	0.08313
9,10	0.5117	0.2116	0.04159	0.11616
11,12	0.6253	0.3165	0.04562	0.12743
13,14	0.8992	0.3672	0.05611	0.15673

Table B.35. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 2 biomass extraction raw data

HPLC Data: Biomass Extraction											
Sample Name	Date Sampled	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Reten-tion Time (min)	Mass Phen. (mg)	FW Bio-mass (mg)	Phen. Conc. in Biomass (mg/g FW)	DW (mg)	Phen. Conc. (mg/g DW)
220-1	03/13/07	220p45.ASC	2162	03/16/07	46.90	8.13	0.00017	28.6	0.00610	10.2	0.0170
220-2	03/13/07	220p46.ASC	2163	03/16/07	39.70	8.10	0.00015	25.1	0.00588	9.0	0.0164
220-3	03/13/07	220p47.ASC	2164	03/16/07	153.00	8.18	0.00057	27.0	0.02108	9.7	0.0589
220-4	03/13/07	220p48.ASC	2165	03/16/07	139.70	8.17	0.00052	30.8	0.01687	11.0	0.0471
220-5	03/13/07	220p49.ASC	2166	03/16/07	191.30	8.20	0.00071	26.7	0.02665	9.6	0.0744
220-6	03/13/07	220p50.ASC	2167	03/16/07	166.20	8.18	0.00062	25.5	0.02425	9.1	0.0677
220-7	03/13/07	220p51.ASC	2168	03/16/07	282.45	8.18	0.00105	30.4	0.03456	10.9	0.0965
220-8	03/13/07	220p52.ASC	2169	03/16/07	173.10	8.20	0.00064	25.8	0.02496	9.2	0.0697
220-9	03/13/07	220p53.ASC	2170	03/16/07	262.35	8.18	0.00098	23.8	0.04101	8.5	0.1145
220-10	03/13/07	220p54.ASC	2171	03/16/07	281.10	8.22	0.00105	24.8	0.04217	8.9	0.1178
220-11	03/13/07	220p55.ASC	2172	03/16/07	268.30	8.20	0.00100	26.3	0.03795	9.4	0.1060
220-12	03/13/07	220p56.ASC	2173	03/16/07	325.20	8.20	0.00121	22.7	0.05329	8.1	0.1489
220-13	03/13/07	220p57.ASC	2174	03/16/07	284.55	8.17	0.00106	21.6	0.04901	7.7	0.1369
220-14	03/13/07	220p58.ASC	2175	03/16/07	446.90	8.20	0.00166	26.3	0.06321	9.4	0.1766

Table B.36. Equilibrium partitioning of phenanthrene between seawater and living *A. coalita* tissue: Trial 2 mass balance

Phenanthrene Mass Balance								
Initial Phen. Conc. (mg/L)	Vial	Total Mass (mg)	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (mg) (% initial)
0.1139	1	0.0023	0.0006	28.3	0.00017	7.7	0.00146	64.0
0.1139	2	0.0023	0.0015	67.2	0.00015	6.5	0.00060	26.3
0.1438	3	0.0029	0.0016	56.0	0.00057	19.8	0.00070	24.2
0.1438	4	0.0029	0.0033	113.8	0.00052	18.1	-0.00092	-31.9
0.3129	5	0.0063	0.0023	36.8	0.00071	11.4	0.00325	51.9
0.3129	6	0.0063	0.0029	45.8	0.00062	9.9	0.00278	44.4
0.4042	7	0.0081	0.0030	36.9	0.00105	13.0	0.00405	50.1
0.4042	8	0.0081	0.0033	40.5	0.00064	8.0	0.00417	51.5
0.5117	9	0.0102	0.0043	41.7	0.00098	9.5	0.00499	48.8
0.5117	10	0.0102	0.0042	41.0	0.00105	10.2	0.00499	48.8
0.6253	11	0.0125	0.0056	45.1	0.00100	8.0	0.00587	46.9
0.6253	12	0.0125	0.0070	56.1	0.00121	9.7	0.00428	34.2
0.8992	13	0.0180	0.0075	41.6	0.00106	5.9	0.00944	52.5
0.8992	14	0.0180	0.0072	40.1	0.00166	9.2	0.00912	50.7

Note: for experimental conditions see table B.31.

Table B.37. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 1 vial summary

Vial #	Initial Biomass Weight Measured (mg FW)	Initial Phenanthrene Concentration (mg/L)	Final Biomass Weight Measured (mg FW)	Final pH
15	22.0	0.114	18.3	8.304
16	18.7	0.114	15.8	8.274
17	18.3	0.144	14.8	8.296
18	23.0	0.144	20.6	8.272
19	20.5	0.313	15.3	8.279
20	20.3	0.313	18.6	8.284
21	23.9	0.404	19.2	8.272
22	20.9	0.404	19.5	8.264
23	21.1	0.512	19.5	8.242
24	20.0	0.512	16.8	8.251
25	21.6	0.625	20.4	8.256
26	21.2	0.625	18.0	8.281
27	23.6	0.899	20.5	8.229
28	20.4	0.899	17.6	8.284

Table B.38. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 1 liquid phase raw data 126

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Phen. Conc. in Liquid (mg/L)
0.1 mg/L Stock 1	03/13/07	220p2.ASC	2120	03/14/07	16.20	8.316	0.1004
0.1 mg/L Stock 2	03/13/07	220p3.ASC	2121	03/14/07	20.55	8.283	0.1274
0.2 mg/L Stock 1	03/13/07	220p4.ASC	2122	03/14/07	34.75	8.333	0.2155
0.2 mg/L Stock 2	03/13/07	220p5.ASC	2123	03/14/07	11.65	8.816	0.0722
0.3 mg/L Stock 1	03/13/07	220p6.ASC	2124	03/14/07	49.25	8.333	0.3054
0.3 mg/L Stock 2	03/13/07	220p7.ASC	2125	03/14/07	51.70	8.316	0.3205
0.4 mg/L Stock 1	03/13/07	220p8.ASC	2126	03/14/07	60.00	8.350	0.3720
0.4 mg/L Stock 2	03/13/07	220p9.ASC	2127	03/14/07	70.40	8.350	0.4365
0.5 mg/L Stock 1	03/13/07	220p10.ASC	2128	03/14/07	84.05	8.350	0.5211
0.5 mg/L Stock 2	03/13/07	220p11.ASC	2129	03/14/07	81.00	8.350	0.5022
0.6 mg/L Stock 1	03/13/07	220p12.ASC	2130	03/14/07	99.20	8.333	0.6150
0.6 mg/L Stock 2	03/13/07	220p13.ASC	2131	03/14/07	102.50	8.366	0.6355
0.7 mg/L Stock 1	03/13/07	220p14.ASC	2132	03/14/07	175.95	8.366	1.0909
0.7 mg/L Stock 2	03/13/07	220p15.ASC	2133	03/14/07	114.10	8.333	0.7074
220-15	03/13/07	220p30.ASC	2148	03/15/07	0.00	NA	0.0000
220-16	03/13/07	220p31.ASC	2149	03/15/07	0.00	NA	0.0000
220-17	03/13/07	220p32.ASC	2150	03/15/07	5.20	8.366	0.0322
220-18	03/13/07	220p33.ASC	2151	03/15/07	14.05	8.333	0.0871
220-19	03/13/07	220p34.ASC	2152	03/15/07	8.60	8.333	0.0533
220-20	03/13/07	220p35.ASC	2153	03/15/07	7.00	8.316	0.0434
220-21	03/13/07	220p36.ASC	2154	03/15/07	9.60	8.333	0.0595
220-22	03/13/07	220p37.ASC	2155	03/15/07	17.30	8.316	0.1073
220-23	03/13/07	220p38.ASC	2156	03/15/07	12.20	8.283	0.0756
220-24	03/13/07	220p39.ASC	2157	03/16/07	15.30	8.300	0.0949

Table B.38. Continued

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Phen. Conc. in Liquid (mg/L)
220-25	03/13/07	220p40.ASC	2158	03/16/07	14.10	8.300	0.0874
220-26	03/13/07	220p41.ASC	2159	03/16/07	15.25	8.266	0.0946
220-27	03/13/07	220p42.ASC	2160	03/16/07	20.30	8.250	0.1259
220-28	03/13/07	220p43.ASC	2161	03/16/07	17.80	8.316	0.1104

Table B.39. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 1 averages for partitioning

Vials	Initial Phenanthrene Concentration in Liquid (mg/L)	Final Phenanthrene Concentration in Liquid (mg/L)	Final Phenanthrene Concentration in Biomass (mg/g DW)
15,16	0.1139	0.0000	0.0248
17,18	0.1438	0.0597	0.0847
19,20	0.3129	0.0484	0.0986
21,22	0.4042	0.0834	0.1117
23,24	0.5117	0.0853	0.1974
25,26	0.6253	0.0910	0.2017
27,28	0.8992	0.1259	0.2115

Table B.40. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 1 biomass extraction raw data

HPLC Data: Biomass Extraction

Sample Name	Date Sampled	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Retention Time (min)	Mass Phen. (mg)	FW Bio-mass (mg)	Phen. Conc. (mg/g FW)	Dry Weight (mg)	Phen. Conc. (mg/g Dry Mass)
220-15	03/13/07	220p59.ASC	2176	03/16/07	50.25	8.17	0.00019	18.3	0.01021	7.9	0.0237
220-16	03/13/07	220p60.ASC	2177	03/16/07	46.70	8.20	0.00017	15.8	0.01100	6.7	0.0259
220-17	03/13/07	220p61.ASC	2178	03/19/07	140.25	8.20	0.00052	14.8	0.03525	6.6	0.0796
220-18	03/13/07	220p62.ASC	2179	03/19/07	198.80	8.22	0.00074	20.6	0.03590	8.2	0.0898
220-19	03/13/07	220p63.ASC	2180	03/19/07	232.10	8.18	0.00086	15.3	0.05643	7.3	0.1176
220-20	03/13/07	220p64.ASC	2181	03/19/07	155.30	8.22	0.00058	18.6	0.03106	7.3	0.0795
220-21	03/13/07	220p65.ASC	2182	03/19/07	189.70	8.18	0.00071	19.2	0.03675	8.6	0.0825
220-22	03/13/07	220p66.ASC	2183	03/19/07	283.35	8.25	0.00105	19.5	0.05405	7.5	0.1409
220-23	03/13/07	220p67.ASC	2184	03/19/07	439.00	8.22	0.00163	19.5	0.08375	7.6	0.2162
220-24	03/13/07	220p68.ASC	2185	03/19/07	343.80	8.27	0.00128	16.8	0.07613	7.2	0.1786
220-25	03/13/07	220p69.ASC	2186	03/19/07	427.80	8.27	0.00159	20.4	0.07801	7.7	0.2058
220-26	03/13/07	220p70.ASC	2187	03/19/07	402.95	8.25	0.00150	18.0	0.08328	7.6	0.1975
220-27	03/13/07	220p71.ASC	2188	03/19/07	378.20	8.28	0.00141	20.5	0.06863	8.4	0.1665
220-28	03/13/07	220p72.ASC	2189	03/19/07	503.40	8.22	0.00187	17.6	0.10640	7.3	0.2564

Table B.41. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 1 mass balance

Phenanthrene Mass Balance								
Initial Phen. Conc. (mg/L)	Vial	Total Mass (mg)	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.1139	15	0.0023	0.0000	0.0	0.00019	8.2	0.00209	91.8
0.1139	16	0.0023	0.0000	0.0	0.00017	7.6	0.00210	92.4
0.1438	17	0.0029	0.0006	22.4	0.00052	18.1	0.00171	59.5
0.1438	18	0.0029	0.0017	60.6	0.00074	25.7	0.00040	13.7
0.3129	19	0.0063	0.0011	17.0	0.00086	13.8	0.00433	69.2
0.3129	20	0.0063	0.0009	13.9	0.00058	9.2	0.00481	76.9
0.4042	21	0.0081	0.0012	14.7	0.00071	8.7	0.00619	76.5
0.4042	22	0.0081	0.0021	26.5	0.00105	13.0	0.00489	60.4
0.5117	23	0.0102	0.0015	14.8	0.00163	16.0	0.00709	69.3
0.5117	24	0.0102	0.0019	18.5	0.00128	12.5	0.00706	69.0
0.6253	25	0.0125	0.0017	14.0	0.00159	12.7	0.00917	73.3
0.6253	26	0.0125	0.0019	15.1	0.00150	12.0	0.00912	72.9
0.8992	27	0.0180	0.0025	14.0	0.00141	7.8	0.01406	78.2
0.8992	28	0.0180	0.0022	12.3	0.00187	10.4	0.01390	77.3

Table B.42. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 2 experiment conditions

Run Description	
Run ID #:	AC221
Date Started:	03/28/07
Time Started:	17:00
Date Ended:	04/02/07
Time Ended:	12:45
Experiment Duration:	115.8 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-36
Age of Inoculum:	29 days
Test Parameters: Start-up Summary	
System Location:	Prec. Science #1 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	15.4 ºC/13.7 ºC
Incident Light Intensity	80 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	3.25 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	20 mL Vials w/ Teflon Lined Caps
Mixing:	Orbital Shaker
Mixer Speed (rpm):	162
Liquid Volume:	20 mL per vial
FW to DW Conversion	0.358

Table B.42. Continued

Solution Prep.:	
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
PAH:	Phenanthrene
PAH Stock Prep.:	20.9 mg phenanthrene dissolved in 2 mL Ethanol
PAH Stock Conc.:	10.45 mg/mL
pH Phenanthrene soln.	8.367
pH Non-Phenanthrene soln.	8.429
Vol. PAH Stock:	33.5 µL added to 500 mL medium to give 0.7 mg/L soln.

Seven solution of various phenanthrene concentrations are prepared by diluting the 0.7 mg/L phenanthrene solution with medium not containing phenanthrene.

Solution	Vol. Non-Phen. Soln. (mL)	Vol. phen. Soln. (mL)
1	85.7	14.3
2	71.4	28.6
3	57.1	42.9
4	42.9	57.1
5	28.6	71.4
6	14.3	85.7
7	0	100

Solution	Calculated Initial Concentration of Phenanthrene (mg/L)	Measured Initial Concentration of Phenanthrene (mg/L)
Solution 1:	0.1	0.083
Solution 2:	0.2	0.264
Solution 3:	0.3	0.357
Solution 4:	0.4	0.387
Solution 5:	0.5	0.493
Solution 6:	0.6	0.610
Solution 7:	0.7	0.700

Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extraction 1 start:	4/2/2007 0:00
Extraction 1 end:	04/04/07
Sonicated from 08:30-09:30 on 04/04/07	
Extraction Volume (mL):	3.0

Table B.42. Continued

HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00124 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile

Table B.43. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 2 vial summary

Vial #	Initial Biomass Weight Measured (mg FW)	Initial PAH Concentration (mg/L)	Final Biomass Weight Measured (mg FW)	Final pH
15	19.2	0.083	11.9	8.266
16	19.2	0.083	13.8	8.400
17	20.0	0.264	15.0	8.330
18	19.2	0.264	14.0	8.066
19	20.3	0.357	13.0	8.382
20	19.2	0.357	14.0	8.407
21	21.3	0.387	14.9	8.392
22	20.2	0.387	15.0	8.430
23	19.5	0.493	14.1	7.249
24	22.5	0.493	15.7	8.410
25	21.2	0.610	13.9	8.382
26	19.2	0.610	14.3	8.393
27	19.2	0.700	13.0	7.242
28	19.6	0.700	16.8	8.296

Table B.44. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 2 liquid phase raw data 133

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Phen. Conc. (mg/L)
0.1 mg/L Stock 1	03/28/07	221p2.ASC	2198	03/29/07	17.00	8.400	0.1054
0.1 mg/L Stock 2	03/28/07	221p3.ASC	2199	03/29/07	9.85	8.383	0.0611
0.2 mg/L Stock 1	03/28/07	221p4.ASC	2200	03/29/07	37.60	8.316	0.2331
0.2 mg/L Stock 2	03/28/07	221p5.ASC	2201	03/29/07	47.40	8.400	0.2939
0.3 mg/L Stock 1	03/28/07	221p6.ASC	2202	03/29/07	52.60	8.383	0.3261
0.3 mg/L Stock 2	03/28/07	221p7.ASC	2203	03/29/07	62.60	8.383	0.3881
0.4 mg/L Stock 1	03/28/07	221p8.ASC	2204	03/29/07	62.55	8.400	0.3878
0.4 mg/L Stock 2	03/28/07	221p9.ASC	2205	03/29/07	62.15	8.416	0.3853
0.5 mg/L Stock 1	03/28/07	221p10.ASC	2206	03/29/07	80.40	8.400	0.4985
0.5 mg/L Stock 2	03/28/07	221p11.ASC	2207	03/29/07	78.75	8.400	0.4883
0.6 mg/L Stock 1	03/28/07	221p12.ASC	2208	03/29/07	99.40	8.433	0.6163
0.6 mg/L Stock 2	03/28/07	221p13.ASC	2209	03/29/07	97.25	7.916	0.6030
0.7 mg/L Stock 1	03/28/07	221p14.ASC	2210	03/30/07	116.20	8.416	0.7204
0.7 mg/L Stock 2	03/28/07	221p15.ASC	2211	03/30/07	109.70	8.366	0.6801
221-15	04/02/07	22p99.ASC	2336	04/05/07	0.00	NA	0.0000
221-16	04/02/07	22p100.ASC	2337	04/05/07	8.70	8.116	0.0539
221-17	04/02/07	22p101.ASC	2338	04/05/07	9.30	8.316	0.0577
221-18	04/02/07	22p102.ASC	2339	04/05/07	6.65	8.316	0.0412
221-19	04/02/07	22p103.ASC	2340	04/05/07	11.20	8.333	0.0694
221-20	04/02/07	22p104.ASC	2341	04/05/07	12.30	8.183	0.0763
221-21	04/02/07	22p105.ASC	2342	04/05/07	15.05	8.383	0.0933
221-22	04/02/07	22p106.ASC	2343	04/05/07	14.25	8.316	0.0884
221-23	04/02/07	22p107.ASC	2344	04/05/07	20.40	8.366	0.1265
221-24	04/02/07	22p108.ASC	2345	04/05/07	14.45	8.333	0.0896

Table B.44. Continued

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Phen. Conc. (mg/L)
221-25	04/02/07	22p109.ASC	2346	04/05/07	21.80	8.383	0.1352
221-26	04/02/07	22p110.ASC	2347	04/05/07	22.75	8.316	0.1411
221-27	04/02/07	22p111.ASC	2348	04/05/07	30.75	8.383	0.1907
221-28	04/02/07	22p112.ASC	2349	04/05/07	26.70	8.300	0.1655

Table B.45. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 2 averages for partitioning

Vials	Initial Phenanthrene Concentration in Liquid (mg/L)	Final Phenanthrene Concentration in Liquid (mg/L)	Phenanthrene Concentration in Biomass (mg/g DW)
15,16	0.0832	0.0270	0.0501
17,18	0.2635	0.0494	0.0742
19,20	0.3571	0.0729	0.1498
21,22	0.3866	0.0908	0.1829
23,24	0.4934	0.1080	0.2293
25,26	0.6096	0.1381	0.2506
27,28	0.7003	0.1907	0.3642

Table B.46. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 2 biomass extraction raw data

HPLC Data: Biomass Extraction									
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Mass Phen. Extracted (mg)	DW Biomass (mg)	Phen.Conc. (mg/g Dry Mass)
221-15	04/02/07	22p71.ASC	2308	04/05/07	78.85	8.266	0.00029	6.9	0.0427
221-16	04/02/07	22p72.ASC	2309	04/05/07	106.40	8.183	0.00040	6.9	0.0576
221-17	04/02/07	22p73.ASC	2310	04/05/07	174.90	8.166	0.00065	7.2	0.0909
221-18	04/02/07	22p74.ASC	2311	04/05/07	106.35	8.250	0.00040	6.9	0.0576
221-19	04/02/07	22p75.ASC	2312	04/05/07	301.80	8.166	0.00112	7.3	0.1545
221-20	04/02/07	22p76.ASC	2313	04/05/07	268.30	8.183	0.00100	6.9	0.1452
221-21	04/02/07	22p77.ASC	2314	04/05/07	441.70	8.166	0.00164	7.6	0.2155
221-22	04/02/07	22p78.ASC	2315	04/05/07	292.30	8.250	0.00109	7.2	0.1504
221-23	04/02/07	22p79.ASC	2316	04/05/07	595.85	8.250	0.00222	7.0	0.3175
221-24	04/02/07	22p80.ASC	2317	04/05/07	305.35	8.300	0.00114	8.1	0.1410
221-25	04/02/07	22p81.ASC	2318	04/05/07	656.65	8.266	0.00244	7.6	0.3219
221-26	04/02/07	22p82.ASC	2319	04/05/07	331.30	8.283	0.00123	6.9	0.1793
221-27	04/02/07	22p83.ASC	2320	04/05/07	767.50	8.250	0.00286	6.9	0.4154
221-28	04/02/07	22p84.ASC	2321	04/05/07	590.40	8.266	0.00220	7.0	0.3130

Table B.47. Equilibrium partitioning of phenanthrene between seawater and heat-killed *A. coalita* tissue: Trial 2 mass balance

Phenanthrene Mass Balance								
Initial Phen. Conc. (mg/L)	Vial	Total Mass (mg)	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.0832	15	0.0017	0.0000	0.0	0.00029	17.6	0.00137	82.4
0.0832	16	0.0017	0.0011	64.8	0.00040	23.8	0.00019	11.4
0.2635	17	0.0053	0.0012	21.9	0.00065	12.3	0.00347	65.8
0.2635	18	0.0053	0.0008	15.6	0.00040	7.5	0.00405	76.8
0.3571	19	0.0071	0.0014	19.4	0.00112	15.7	0.00463	64.8
0.3571	20	0.0071	0.0015	21.4	0.00100	14.0	0.00462	64.7
0.3866	21	0.0077	0.0019	24.1	0.00164	21.3	0.00422	54.6
0.3866	22	0.0077	0.0018	22.9	0.00109	14.1	0.00488	63.1
0.4934	23	0.0099	0.0025	25.6	0.00222	22.5	0.00512	51.9
0.4934	24	0.0099	0.0018	18.2	0.00114	11.5	0.00694	70.3
0.6096	25	0.0122	0.0027	22.2	0.00244	20.0	0.00705	57.8
0.6096	26	0.0122	0.0028	23.1	0.00123	10.1	0.00814	66.8
0.7003	27	0.0140	0.0038	27.2	0.00286	20.4	0.00734	52.4
0.7003	28	0.0140	0.0033	23.6	0.00220	15.7	0.00850	60.7

Table B.48. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 1 experiment conditions

Run Description	
Run ID #:	AC218
Date Started:	01/23/07
Time Started:	17:45
Date Ended:	01/26/07
Time Ended:	10:30
Experiment Duration:	64.75 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-32
Age of Inoculum:	32 days
Test Parameters: Start-up Summary	
System Location:	Prec. Science #1 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	20.7 ºC/17.3 ºC
Incident Light Intensity	~90 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	1.5 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	20 mL Vials w/ Teflon Lined Caps
Mixing:	Orbital Shaker
Mixer Speed (rpm):	155
Medium Volume:	20 mL per vial
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.000853 Area/0.1 µg injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	214 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile

Table B.48. Continued

Solution Prep.:	
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
PAH:	Naphthalene
PAH Stock Prep.:	45.3 mg naphthalene dissolved in 3.0 mL EtOH
PAH Stock Conc.:	15.1 mg/mL
Vol. PAH Stock:	33.1 μL added to 500 mL medium to give 1.0 mg/L soln.

Five solution of various naphthalene concentrations are prepared by diluting the 1.0 mg/L naphthalene solution with medium not containing naphthalene.

Solution	Vol. Non-Nap. Soln. (mL)	Vol. Nap. Soln. (mL)
1	80	20
2	60	40
3	40	60
4	20	80
5	0	100

Solution	Calculated Initial Concentration of Naphthalene (mg/L)	Measured Initial Concentration of Naphthalene (mg/L)
1	0.2	0.143
2	0.4	0.295
3	0.6	0.470
4	0.8	0.564
5	1.0	0.784

Solution	pH
Solution 1:	8.543
Solution 2:	8.592
Solution 3:	8.604
Solution 4:	8.628
Solution 5:	8.667

Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extraction 1 start:	1/26/2007 11:30
Extraction 1 end:	02/01/07
Sonicated from 13:50-15:45 on 01/29/07	
Extraction Volume (mL):	2.0
FW to dry mass Conversion	0.358

Table B.49. Equilibrium partitioning of naphthalene between seawater and living *A.*139 *coalita* tissue: Trial 1 vial summary

Vial #	Initial Biomass Weight Measured (mg FW)	Initial Naphthalene Concentration (mg/L)	Final Biomass Weight Measured (mg FW)	Final pH	X_f/X_0
1	21.0	0.143	20.0	9.152	0.95
2	20.6	0.143	23.0	9.745	1.12
3	20.3	0.295	23.2	9.926	1.14
4	20.2	0.295	23.7	9.992	1.17
5	22.3	0.470	27.0	10.034	1.21
6	21.6	0.470	23.6	10.039	1.09
7	22.9	0.564	29.8	10.065	1.30
8	23.2	0.564	28.7	10.117	1.24
9	21.0	0.784	26.3	10.110	1.25
10	21.3	0.784	27.4	10.066	1.29
11	0.0	0.143	0.0	8.670	NA
12	0.0	0.143	0.0	8.641	NA
13	0.0	0.295	0.0	8.623	NA
14	0.0	0.295	0.0	8.623	NA
15	0.0	0.470	0.0	7.901	NA
16	0.0	0.470	0.0	8.111	NA
17	0.0	0.564	0.0	8.623	NA
18	0.0	0.564	0.0	8.629	NA
19	0.0	0.784	0.0	8.466	NA
20	0.0	0.784	0.0	8.646	NA

Table B.50. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 1 averages for partitioning

Vials	Initial Naphthalene Concentration in Liquid (mg/L)	Final Naphthalene Concentration in Liquid (mg/L)	Final Naphthalene Concentration in Biomass (mg/g FW)	Naphthalene Concentration in Biomass (mg/g dry mass)
1,2	0.1426	0.1664	0.00561	0.016
3,4	0.2947	0.2793	0.01131	0.032
5,6	0.4696	0.3779	0.02160	0.060
7,8	0.5639	0.4721	0.02027	0.057
9,10	0.7839	0.6506	0.02834	0.079

Table B.51. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 1 liquid phase raw data 140

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Retention Time (min)	Naphthalene Concentration (mg/L)
0.2 mg/L Stock 1	01/26/07	218p10.ASC	2053	01/31/07	34.90	5.616	0.1488
0.2 mg/L Stock 2	01/26/07	218p11.ASC	2054	01/31/07	31.95	5.500	0.1363
0.4 mg/L Stock 1	01/26/07	218p12.ASC	2055	01/31/07	73.70	5.633	0.3143
0.4 mg/L Stock 2	01/26/07	218p13.ASC	2056	01/31/07	64.50	5.500	0.2751
0.6 mg/L Stock 1	01/26/07	218p14.ASC	2057	01/31/07	101.00	5.633	0.4308
0.6 mg/L Stock 2	01/26/07	218p15.ASC	2058	01/31/07	119.20	5.583	0.5084
0.8 mg/L Stock 1	01/26/07	218p16.ASC	2059	01/31/07	132.60	5.600	0.5655
0.8 mg/L Stock 2	01/26/07	218p17.ASC	2060	01/31/07	131.85	5.600	0.5623
1.0 mg/L Stock 1	01/26/07	218p18.ASC	2061	01/31/07	196.40	5.516	0.8376
1.0 mg/L Stock 2	01/26/07	218p19.ASC	2062	01/31/07	171.20	5.583	0.7302
218-1	01/26/07	218p20.ASC	2063	01/31/07	28.45	5.583	0.1213
218-2	01/26/07	218p21.ASC	2064	02/01/07	49.60	5.666	0.2115
218-3	01/26/07	218p22.ASC	2065	02/01/07	67.60	5.633	0.2883
218-4	01/26/07	218p23.ASC	2066	02/01/07	63.35	5.633	0.2702
218-5	01/26/07	218p24.ASC	2067	02/01/07	87.30	5.633	0.3723
218-6	01/26/07	218p25.ASC	2068	02/01/07	89.90	5.650	0.3834
218-7	01/26/07	218p26.ASC	2069	02/01/07	109.20	5.633	0.4657
218-8	01/26/07	218p27.ASC	2070	02/01/07	112.20	5.633	0.4785
218-9	01/26/07	218p28.ASC	2071	02/01/07	140.80	5.433	0.6005
218-10	01/26/07	218p29.ASC	2072	02/01/07	164.30	5.633	0.7007
218-11	01/26/07	218p40.ASC	2083	02/05/07	29.05	5.566	0.1239
218-12	01/26/07	218p41.ASC	2084	02/05/07	45.60	5.516	0.1945
218-13	01/26/07	218p42.ASC	2085	02/05/07	62.40	5.616	0.2661
218-14	01/26/07	218p43.ASC	2086	02/05/07	93.40	5.600	0.3984
218-15	01/26/07	218p44.ASC	2087	02/05/07	100.10	4.783	0.4269

Table B.51. Continued

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Retention Time (min)	Naphthalene Concentration (mg/L)
218-16	01/26/07	218p45.ASC	2088	02/05/07	94.60	5.616	0.4035
218-17	01/26/07	218p46.ASC	2089	02/05/07	133.90	5.616	0.5711
218-18	01/26/07	218p47.ASC	2090	02/05/07	117.60	5.650	0.5016
218-19	01/26/07	218p48.ASC	2091	02/05/07	164.65	5.616	0.7022
218-20	01/26/07	218p49.ASC	2092	02/05/07	165.70	5.616	0.7067

Table B.52. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 1 biomass extraction raw data

HPLC Data: Biomass Extraction											
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Reten-tion Time (min)	Mass Nap. Extracted (mg)	FW Bio-mass (mg)	Nap. Conc. (mg/g FW)	Biomass Weight (mg DW)	Nap. Conc. (mg/g Dry Mass)
218-1	01/26/07	218p30.ASC	2073	02/01/07	66.70	5.50	0.00011	20.0	0.00569	7.2	0.016
218-2	01/26/07	218p31.ASC	2074	02/01/07	74.60	5.48	0.00013	23.0	0.00553	8.2	0.015
218-3	01/26/07	218p32.ASC	2075	02/01/07	153.50	5.52	0.00026	23.2	0.01129	8.3	0.032
218-4	01/26/07	218p33.ASC	2076	02/01/07	157.55	5.48	0.00027	23.7	0.01134	8.5	0.032
218-5	01/26/07	218p34.ASC	2077	02/01/07	350.50	5.47	0.00060	27.0	0.02215	9.7	0.062
218-6	01/26/07	218p35.ASC	2078	02/01/07	291.20	5.50	0.00050	23.6	0.02105	8.4	0.059
218-7	01/26/07	218p36.ASC	2079	02/01/07	347.80	5.52	0.00059	29.8	0.01991	10.7	0.056
218-8	01/26/07	218p37.ASC	2080	02/01/07	347.20	5.52	0.00059	28.7	0.02064	10.3	0.058
218-9	01/26/07	218p38.ASC	2081	02/01/07	413.00	5.45	0.00070	26.3	0.02679	9.4	0.075
218-10	01/26/07	218p39.ASC	2082	02/01/07	480.10	5.52	0.00082	27.4	0.02989	9.8	0.083

Table B.53. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 1 mass balance

Naphthalene Mass Balance for Vials with Biomass								
Initial Naphthalene Conc. (mg/L)	Vial	Total Mass (mg)	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.1426	1	0.0029	0.0024	85.1	0.00011	4.0	0.00031	10.9
0.1426	2	0.0029	0.0042	148.4	0.00013	4.5	-0.00151	-52.9
0.2947	3	0.0059	0.0058	97.8	0.00026	4.4	-0.00013	-2.3
0.2947	4	0.0059	0.0054	91.7	0.00027	4.6	0.00022	3.8
0.4696	5	0.0094	0.0074	79.3	0.00060	6.4	0.00135	14.3
0.4696	6	0.0094	0.0077	81.7	0.00050	5.3	0.00123	13.1
0.5639	7	0.0113	0.0093	82.6	0.00059	5.3	0.00137	12.2
0.5639	8	0.0113	0.0096	84.9	0.00059	5.3	0.00112	9.9
0.7839	9	0.0157	0.0120	76.6	0.00070	4.5	0.00296	18.9
0.7839	10	0.0157	0.0140	89.4	0.00082	5.2	0.00084	5.4
Average Naphthalene Mass Balance for Vials with Biomass								
Initial Naphthalene Conc. (mg/L)	Vial	Total Mass (mg)	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.1426	1,2	0.0029	0.0033	116.7539	0.0001	4.2274	-0.0006	-20.9813
0.2947	3,4	0.0059	0.0056	94.7540	0.0003	4.5014	0.0000	0.7446
0.4696	5,6	0.0094	0.0076	80.4723	0.0005	5.8283	0.0013	13.6994
0.5639	7,8	0.0113	0.0094	83.7209	0.0006	5.2562	0.0012	11.0229
0.7839	9,10	0.0157	0.0130	82.9978	0.0008	4.8591	0.0019	12.1431

Table B.53. Continued

Naphthalene Mass Balance for Control Vials (No Biomass)								
Initial Naphthalene Conc. (mg/L)	Vial	Total Mass (mg)	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.1426	11	0.0029	0.0025	86.9	0.0	0.0	0.00037	13.1
0.1426	12	0.0029	0.0039	136.4	0.0	0.0	-0.00104	-36.4
0.2947	13	0.0059	0.0053	90.3	0.0	0.0	0.00057	9.7
0.2947	14	0.0059	0.0080	135.2	0.0	0.0	-0.00207	-35.2
0.4696	15	0.0094	0.0085	90.9	0.0	0.0	0.00085	9.1
0.4696	16	0.0094	0.0081	85.9	0.0	0.0	0.00132	14.1
0.5639	17	0.0113	0.0114	101.3	0.0	0.0	-0.00014	-1.3
0.5639	18	0.0113	0.0100	88.9	0.0	0.0	0.00125	11.1
0.7839	19	0.0157	0.0140	89.6	0.0	0.0	0.00163	10.4
0.7839	20	0.0157	0.0141	90.2	0.0	0.0	0.00154	9.8
Average Naphthalene Mass Balance for Control Vials (No Biomass)								
Initial Naphthalene Conc. (mg/L)	Vial	Total Mass (mg)	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.1426	11,12	0.0029	0.0032	NA	0.0	0.0	-0.0002	-13.4
0.2947	13,14	0.0059	0.0066	112.7	0.0	0.0	-0.0008	-12.7
0.4696	15,16	0.0094	0.0083	88.4	0.0	0.0	0.0011	11.6
0.5639	17,18	0.0113	0.0107	95.1	0.0	0.0	0.0006	4.9
0.7839	19,20	0.0157	0.0141	89.9	0.0	0.0	0.0016	10.1

Table B.54. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 2 experiment conditions

Run Description	
Run ID #:	AC223
Date Started:	04/13/07
Time Started:	17:30
Date Ended:	04/18/07
Time Ended:	11:30
Experiment Duration:	114 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-37
Age of Inoculum:	22 days
Test Parameters: Start-up Summary	
System Location:	Prec. Science #1 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	16.2 ºC
Incident Light Intensity	50 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	3.25 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	20 mL Vials w/ Teflon Lined Caps
Mixing:	Orbital Shaker
Mixer Speed (rpm):	162
Liquid Volume:	20 mL per vial
FW to DW Conversion	0.358
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extraction 1 start:	4/18/2007 0:00
Extraction 1 end:	04/24/07
Sonicated from 10:30-12:00 04/23/07	
Extraction Volume (mL):	3.0

Table B.54. Continued

Solution Prep.:	
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
PAH:	Naphthalene
PAH Stock Prep.:	23.0 mg nap. In 2 mL Ethanol
PAH Stock Conc.:	11.51 mg/mL
Vol. PAH Stock:	43.5 μL added to 500 mL medium to give 1.0 mg/L soln.
pH Naphthalenesoln.	8.573
pH Non-Naphthalenesoln.	8.592

Five solution of various Naphthalene concentrations are prepared by diluting the 1.0 mg/L Naphthalene solution with medium not containing Naphthalene.

Solution	Vol. Non-Nap. Soln. (mL)	Vol. Nap. Soln. (mL)
1	80	20
2	60	40
3	40	60
4	20	80
5	0	100

Solution	Calculated Initial Concentration of Naphthalene(mg/L)	Measured Initial Concentration of Naphthalene(mg/L)
1	0.2	0.170
2	0.4	0.363
3	0.6	0.517
4	0.8	0.719
5	1.0	0.944

HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 μL
Standard Method:	External Calibration Curve
Response Factor:	0.000853 area/0.1 μg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	214 nm
Column:	Waters spherisorb ODS2, 5 μm particle size
Dilution Factor (Liquid Samples):	1:4, 500 μL diluted w/ 2 mL acetonitrile

Table B.55. Equilibrium partitioning of naphthalene between seawater and living *A.*146 *coalita* tissue: Trial 2 vial summary

Vial #	Initial Biomass Weight Measured (mg FW)	Initial PAH Concentration (mg/L)	Final Biomass Weight Measured (mg FW)	Final pH	X_f/X_0
1	20.6	0.170	29.3	9.104	1.42
2	20.3	0.170	27.5	9.027	1.35
3	20.6	0.363	28.6	9.982	1.39
4	20.5	0.363	27.9	9.933	1.36
5	21.2	0.517	29.7	9.948	1.40
6	20.2	0.517	30.6	10.057	1.51
7	22.8	0.719	29.2	8.932	1.28
8	23.4	0.719	27.8	9.131	1.19
9	21.2	0.944	24.6	7.940	1.16
10	22.4	0.944	27.7	8.530	1.24

Table B.56. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 2 averages for partitioning

Vials	Final Liquid Naphthalene Concentration (mg/L)	Naphthalene Concentration in Biomass (mg/g Dry Mass)	Naphthalene Concentration in Biomass (mg/g FW)
1,2	0.1464	0.0215	0.0077
3,4	0.3022	0.0451	0.0162
5,6	0.4428	0.0592	0.0212
7,8	0.5427	0.1099	0.0393
9,10	0.7648	0.1005	0.0360

Table B.57. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 2 liquid phase raw data

HPLC Data: Liquid Samples

Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Naphthalene Concentration (mg/L)
0.2 mg/L Stock 1	04/13/07	223p4.ASC	2350	04/23/07	40.10	5.583	0.1710
0.2 mg/L Stock 2	04/13/07	223p54.ASC	2400	04/24/07	39.40	5.600	0.1680
0.4 mg/L Stock 1	04/13/07	223p6.ASC	2352	04/23/07	81.00	5.583	0.3455
0.4 mg/L Stock 2	04/13/07	223p7.ASC	2353	04/23/07	89.30	5.600	0.3809
0.6 mg/L Stock 1	04/13/07	223p8.ASC	2354	04/23/07	121.60	5.583	0.5186
0.6 mg/L Stock 2	04/13/07	223p9.ASC	2355	04/23/07	121.00	5.633	0.5161
0.8 mg/L Stock 1	04/13/07	223p10.ASC	2356	04/23/07	172.30	5.616	0.7349
0.8 mg/L Stock 2	04/13/07	223p11.ASC	2357	04/23/07	164.80	5.616	0.7029
1.0 mg/L Stock 1	04/13/07	223p12.ASC	2358	04/23/07	220.15	5.600	0.9389
1.0 mg/L Stock 2	04/13/07	223p13.ASC	2359	04/23/07	222.50	5.583	0.9490
223-1	04/18/07	223p14.ASC	2360	04/23/07	33.35	5.633	0.1422
223-2	04/18/07	223p15.ASC	2361	04/23/07	35.30	5.600	0.1506
223-3	04/18/07	223p16.ASC	2362	04/23/07	69.45	5.516	0.2962
223-4	04/18/07	223p17.ASC	2363	04/23/07	72.25	5.566	0.3081
223-5	04/18/07	223p18.ASC	2364	04/23/07	100.95	5.633	0.4306
223-6	04/18/07	223p19.ASC	2365	04/23/07	106.70	5.600	0.4551
223-7	04/18/07	223p20.ASC	2366	04/23/07	134.70	5.616	0.5745
223-8	04/18/07	223p21.ASC	2367	04/23/07	119.80	5.616	0.5109
223-9	04/18/07	223p22.ASC	2368	04/23/07	181.45	5.600	0.7739
223-10	04/18/07	223p23.ASC	2369	04/23/07	177.20	5.616	0.7558

Table B.58. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 2 biomass extraction raw data

HPLC Data: Biomass Extraction											
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Reten-tion Time (min)	Mass Nap. Extracted (mg)	FW Biomass (mg)	Nap. Conc. (mg/g FW)	DW Biomass (mg)	Nap. Conc. (mg/g Dry Mass)
223-1	04/18/07	223p34.ASC	2380	04/23/07	95.15	5.500	0.00024	29.3	0.00831	10.5	0.0232
223-2	04/18/07	223p35.ASC	2381	04/23/07	75.75	5.516	0.00019	27.5	0.00705	9.8	0.0197
223-3	04/18/07	223p36.ASC	2382	04/23/07	163.00	5.533	0.00042	28.6	0.01458	10.2	0.0407
223-4	04/18/07	223p37.ASC	2383	04/23/07	193.20	5.533	0.00049	27.9	0.01772	10.0	0.0495
223-5	04/18/07	223p38.ASC	2384	04/24/07	268.70	5.533	0.00069	29.7	0.02315	10.6	0.0647
223-6	04/18/07	223p39.ASC	2385	04/24/07	230.00	5.483	0.00059	30.6	0.01923	11.0	0.0537
223-7	04/18/07	223p40.ASC	2386	04/24/07	402.90	5.500	0.00103	29.2	0.03531	10.5	0.0986
223-8	04/18/07	223p41.ASC	2387	04/24/07	470.90	5.466	0.00121	27.8	0.04335	10.0	0.1211
223-9	04/18/07	223p42.ASC	2388	04/24/07	360.90	5.500	0.00092	24.6	0.03754	8.8	0.1049
223-10	04/18/07	223p58.ASC	2404	04/24/07	372.50	5.516	0.00095	27.7	0.03441	9.9	0.0961

Table B.59. Equilibrium partitioning of naphthalene between seawater and living *A. coalita* tissue: Trial 2 mass balance

Initial Naphthalene Conc. (mg/L)	Vial	Total Mass	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.1695	1	0.00339	0.00284	83.9	0.00024	7.18	0.00030	8.92
0.1695	2	0.00339	0.00301	88.8	0.00019	5.72	0.00019	5.48
0.3632	3	0.00726	0.00592	81.6	0.00042	5.74	0.00092	12.70
0.3632	4	0.00726	0.00616	84.9	0.00049	6.81	0.00061	8.34
0.5173	5	0.01035	0.00861	83.2	0.00069	6.65	0.00105	10.13
0.5173	6	0.01035	0.00910	88.0	0.00059	5.69	0.00066	6.35
0.7189	7	0.01438	0.01149	79.9	0.00103	7.17	0.00186	12.91
0.7189	8	0.01438	0.01022	71.1	0.00121	8.38	0.00295	20.54
0.9440	9	0.01888	0.01548	82.0	0.00092	4.89	0.00248	13.12
0.9440	10	0.01888	0.01512	80.1	0.00095	5.05	0.00281	14.89

Table B.60. Equilibrium partitioning of high concentration naphthalene between seawater and living *A. coalita* tissue: experiment conditions

Run Description	
Run ID #:	AC214
Date Started:	12/13/06
Time Started:	18:00
Date Ended:	12/18/06
Time Ended:	12:00
Experiment Duration:	114 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-30
Age of Inoculum:	22 days
Test Parameters: Start-up Summary	
System Location:	Prec. Science #1 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	12.4 ºC/12.5 ºC
Incident Light Intensity	~90 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	1.5 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	20 mL Vials w/ Teflon Lined Caps
Mixing:	Orbital Shaker
Liquid Volume	20 mL per vial
Mixer Speed (rpm):	155
Medium Volume:	20 mL per vial
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.0196 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	254 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile
Fresh Weigh to Dry mass Conversion	0.358

Table B.60. Continued

Solution Prep.:	
PAH:	Naphthalene
PAH Stock Prep.:	21.3 mg naphthalene added to 1.0 L medium
Naphthalene is added and then the solution is mixed for 8 days. The solution is then filtered through a 0.2 μm filter to remove any undissolved naphthalene. Ethanol bridge is not used.	
Measured Initial PAH Conc.:	15.93 mg/L
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
PAH:	NA
PAH Stock Prep.:	NA
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave

Five solution of various naphthalene concentrations are prepared by diluting the 15.93 mg/L naphthalene solution with non-naphthalene solution.

Solution	pH
Solution 1:	8.538
Solution 2:	8.526
Solution 3:	8.545
Solution 4:	8.542
Solution 5:	8.576

Solution	Vol. Non-Nap. Soln. (mL)	Vol. Nap. Soln. (mL)
1	0	100
2	25	75
3	50	50
4	75	25
5	100	0

Solution	Calculated Initial Concentration of Naphthalene (mg/L)	Measured Initial Concentration of Naphthalene (mg/L
Solution 1:	15.93	15.93
Solution 2:	11.95	11.05
Solution 3:	7.97	7.39
Solution 4:	3.98	3.64
Solution 5:	0.00	0

Table B.60. Continued

Biomass Extractions	
Extraction Solvent:	Acetonitrile
Extraction 1 start:	12/18/2006 14:00:00 PM
Extraction 1 end:	12/19/06
Sonicated for ~3 hrs	
Extraction 2 start:	12/19/06
Extraction 2 end:	12/21/06
Sonicated for ~60 minutes	
Extraction 3 start:	12/21/06
Extraction 3 end:	01/03/07
Extract Volume Added (mL):	
Extraction 1:	4.0
Extraction 2:	3.5
Extraction 3:	3.0
Extract Volume Sampled (mL):	
Extraction 1:	3.9
Extraction 2:	3.5
Extraction 3:	3.0

Table B.61. Equilibrium partitioning of high concentration naphthalene between seawater and living *A. coalita* tissue: vial summary

Vial	Initial Naphthalene Concentration (mg/L)	Measured Initial Biomass Weight (mg)	Measured Final Biomass Weight (mg)	pH	X_f/X_0
1	0	0.0	0.0	8.734	NA
2	0	0.0	0.0	8.711	NA
3	0	58.9	78.5	10.012	1.33
4	0	59.4	84.0	10.093	1.41
5	3.64	0.0	0.0	8.761	NA
6	3.64	0.0	0.0	8.743	NA
7	3.64	59.4	91.3	10.125	1.54
8	3.64	59.4	91.9	10.125	1.55
9	7.39	0.0	0.0	8.780	NA
10	7.39	0.0	0.0	8.756	NA
11	7.39	59.3	76.9	9.039	1.30
12	7.39	59.5	84.5	10.142	1.42
13	11.05	0.0	0.0	8.814	NA
14	11.05	0.0	0.0	8.776	NA
15	11.05	60.6	77.7	9.133	1.28
16	11.05	60.0	69.5	9.120	1.16
17	15.93	0.0	0.0	8.790	NA
18	15.93	0.0	0.0	8.790	NA
19	15.93	60.7	65.8	8.261	1.08
20	15.93	59.3	66.9	8.280	1.13

Table B.62. Equilibrium partitioning of high concentration naphthalene between seawater and living *A. coalita* tissue: liquid phase raw data

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Reten-tion Time (min)	Nap. Conc (mg/ L)
0% - 1	12/18/06	214p2	1765	12/19/06	0.00	NA	0.00
0% - 2	12/18/06	214p3	1766	12/19/06	0.00	NA	0.00
25% - 1	12/18/06	214p4	1767	12/19/06	43.10	5.700	4.22
25% - 2	12/18/06	214p5	1768	12/19/06	31.20	5.700	3.06
50% - 1	12/18/06	214p6	1769	12/19/06	78.50	5.716	7.69
50% - 2	12/18/06	214p7	1770	12/19/06	72.30	5.716	7.09
75% - 1	12/18/06	214p8	1771	12/19/06	116.00	5.716	11.37
75% - 2	12/18/06	214p9	1772	12/19/06	109.50	5.716	10.73
100% - 1	12/18/06	214p10	1773	12/19/06	161.70	5.700	15.85
100% - 2	12/18/06	214p11	1774	12/19/06	163.40	5.716	16.01
214-1	12/18/06	214p20	1783	12/19/06	0.00	NA	0.00
214-2	12/18/06	214p21	1784	12/19/06	0.00	NA	0.00
214-3	12/18/06	214p22	1785	12/19/06	0.00	NA	0.00
214-4	12/18/06	214p23	1786	12/19/06	0.00	NA	0.00
214-5	12/18/06	214p24	1787	12/19/06	31.00	5.683	3.04
214-6	12/18/06	214p25	1788	12/19/06	30.20	5.666	2.96
214-7	12/18/06	214p26	1789	12/19/06	23.30	5.700	2.28
214-8	12/18/06	214p27	1790	12/19/06	23.40	5.683	2.29
214-9	12/18/06	214p28	1791	12/19/06	71.00	5.666	6.96
214-10	12/18/06	214p29	1792	12/19/06	67.40	5.700	6.61
214-11	12/18/06	214p30	1793	12/19/06	53.80	5.700	5.27
214-12	12/18/06	214p31	1794	12/19/06	49.40	5.650	4.84
214-13	12/18/06	214p32	1795	12/19/06	104.05	5.700	10.20
214-14	12/18/06	214p33	1796	12/19/06	104.25	5.633	10.22
214-15	12/18/06	214p34	1797	12/19/06	77.75	5.683	7.62
214-16	12/18/06	214p35	1798	12/19/06	74.85	5.683	7.34
214-17	12/18/06	214p36	1799	12/19/06	149.15	5.650	14.62
214-18	12/18/06	214p37	1800	12/19/06	148.30	5.616	14.53
214-19	12/18/06	214p38	1801	12/19/06	110.70	5.683	10.85
214-20	12/18/06	214p39	1802	12/19/06	109.90	5.683	10.77

Table B.63. Equilibrium partitioning of high concentration naphthalene between seawater and living *A. coalita* tissue: biomass extraction raw data

HPLC Data: Biomass Extraction 1							
Sample Name	Date Sampled	HPLC file	Injection #	Date of Analysis	Peak Area	Retention Time (min)	Naphthalene Extracted (mg)
213-3-EX1	12/19/06	214p40.ASC	1803	12/20/06	0.00	NA	0.000000
213-4-EX1	12/19/06	214p41.ASC	1804	12/20/06	0.00	NA	0.000000
213-7-EX1	12/19/06	214p42.ASC	1805	12/20/06	76.00	5.600	0.005809
213-8-EX1	12/19/06	214p43.ASC	1806	12/20/06	76.15	5.616	0.005821
213-11-EX1	12/19/06	214p44.ASC	1807	12/20/06	137.10	5.616	0.010480
213-12-EX1	12/19/06	214p45.ASC	1808	12/20/06	154.90	5.616	0.011841
213-15-EX1	12/19/06	214p46.ASC	1809	12/20/06	256.80	5.600	0.019630
213-16-EX1	12/19/06	214p47.ASC	1810	12/20/06	190.05	5.583	0.014527
213-19-EX1	12/19/06	214p48.ASC	1811	12/20/06	394.10	5.583	0.030125
213-20-EX1	12/19/06	214p49.ASC	1812	12/20/06	400.00	5.550	0.030576
HPLC Data: Biomass Extraction 2							
Sample Name	Date Sampled	HPLC file	Injection #	Date of Analysis	Peak Area	Retention Time (min)	Napthalene Extracted (mg)
213-3-EX2	12/21/06	214p50.ASC	1813	12/21/06	0.00	NA	0.00000
213-4-EX2	12/21/06	214p51.ASC	1814	12/21/06	0.00	NA	0.00000
213-7-EX2	12/21/06	214p52.ASC	1815	12/21/06	5.80	5.483	0.00040
213-8-EX2	12/21/06	214p53.ASC	1816	12/21/06	27.20	5.533	0.00187
213-11-EX2	12/21/06	214p54.ASC	1817	12/21/06	50.10	5.550	0.00344
213-12-EX2	12/21/06	214p55.ASC	1818	12/21/06	50.70	5.533	0.00348
213-15-EX2	12/21/06	214p56.ASC	1819	12/21/06	43.45	5.533	0.00298
213-16-EX2	12/21/06	214p57.ASC	1820	12/21/06	76.40	5.533	0.00524
213-19-EX2	12/21/06	214p58.ASC	1821	12/21/06	45.00	5.533	0.00309
213-20-EX2	12/21/06	214p59.ASC	1822	12/21/06	74.75	5.483	0.00513

Table B.63. Continued

HPLC Data: Biomass Extraction 3							
Sample Name	Date Sampled	HPLC file	Injection #	Date of Analysis	Peak Area	Retention Time (min)	Napthalene Extracted (mg)
213-3-EX3	01/03/07	214p60.ASC	1823	01/03/07	0.00	NA	0.00000
213-4-EX3	01/03/07	214p61.ASC	1824	01/03/07	0.00	NA	0.00000
213-7-EX3	01/03/07	214p62.ASC	1825	01/03/07	26.70	5.516	0.00157
213-8-EX3	01/03/07	214p63.ASC	1826	01/03/07	0.00	NA	0.00000
213-11-EX3	01/03/07	214p64.ASC	1827	01/03/07	21.20	5.483	0.00125
213-12-EX3	01/03/07	214p65.ASC	1828	01/03/07	33.10	5.500	0.00195
213-15-EX3	01/03/07	214p66.ASC	1829	01/03/07	37.90	5.500	0.00223
213-16-EX3	01/03/07	214p67.ASC	1830	01/03/07	42.50	5.516	0.00250
213-19-EX3	01/03/07	214p68.ASC	1831	01/03/07	27.60	5.516	0.00162
213-20-EX3	01/03/07	214p69.ASC	1832	01/03/07	45.70	5.483	0.00269

Table B.64. Equilibrium partitioning of high concentration naphthalene between seawater and living *A. coalita* tissue: experiment 157 summary

Vial	Final FW Biomass (mg)	Dry Mass (mg)	Initial Naphthalene Concentration in Liquid (mg/L)	Final Naphthalene Concentration in Liquid (mg/L)	Mass Naphthalene Extracted from Biomass (mg)	Concentration Naphthalene in Biomass (mg/g FW)	Concentration Naphthalene in Biomass (mg/g dry mass)
3	78.5	28.1	0.0	0.0	0.00000	0.00	0.0000
4	84.0	30.1	0.0	0.0	0.00000	0.00	0.0000
7	91.3	32.7	3.6	2.3	0.00778	0.09	0.2379
8	91.9	32.9	3.6	2.3	0.00769	0.08	0.2336
11	76.9	27.5	7.4	5.3	0.01516	0.20	0.5508
12	84.5	30.3	7.4	4.8	0.01726	0.20	0.5707
15	77.7	27.8	11.0	7.6	0.02484	0.32	0.8930
16	69.5	24.9	11.0	7.3	0.02227	0.32	0.8950
19	65.8	23.6	15.9	10.8	0.03483	0.53	1.4788
20	66.9	24.0	15.9	10.8	0.03839	0.57	1.6030

Table B.65. Equilibrium partitioning of high concentration naphthalene between seawater and living *A. coalita* tissue: mass balance

Naphthalene Mass Balance for Vials with Biomass								
Initial Naphthalene Conc. (mg/L)	Vial	Total Mass	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.0	3	0.0000	0.0000	NA	0.0000	NA	NA	NA
0.0	4	0.0000	0.0000	NA	0.0000	NA	NA	NA
3.6	7	0.0728	0.0457	62.7	0.0078	10.7	0.0194	26.6
3.6	8	0.0728	0.0459	63.0	0.0077	10.6	0.0193	26.5
7.4	11	0.1478	0.1054	71.4	0.0152	10.3	0.0272	18.4
7.4	12	0.1478	0.0968	65.5	0.0173	11.7	0.0337	22.8
11.0	15	0.2210	0.1524	69.0	0.0248	11.2	0.0438	19.8
11.0	16	0.2210	0.1467	66.4	0.0223	10.1	0.0520	23.5
15.9	19	0.3186	0.2170	68.1	0.0348	10.9	0.0668	21.0
15.9	20	0.3186	0.2154	67.6	0.0384	12.0	0.0648	20.3
Averages Mass Balance for Vials with Biomass								
Initial Naphthalene Conc. (mg/L)	Vial	Total Mass	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.0	3,4	0.0000	0.0000	NA	0.0000	NA	0.0000	NA
3.6	7,8	0.0728	0.0458	62.9	0.0077	10.6	0.0193	26.5
7.4	11,12	0.1478	0.1011	68.4	0.0162	11.0	0.0304	20.6
11.0	15,16	0.2210	0.1495	67.7	0.0236	10.7	0.0479	21.7
15.9	19,20	0.3186	0.2162	67.9	0.0366	11.5	0.0658	20.7

Table B.65. Continued

Naphthalene Mass Balance for Control Vials (No Biomass)								
Initial Naphthalene Conc. (mg/L)	Vial	Total Mass	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.0	1	0.0000	0.0000	NA	0.0	0.0	0.0000	NA
0.0	2	0.0000	0.0000	NA	0.0	0.0	0.0000	NA
3.6	5	0.0728	0.0608	83.4	0.0	0.0	0.0121	16.6
3.6	6	0.0728	0.0592	81.3	0.0	0.0	0.0136	18.7
7.4	9	0.1478	0.1392	94.2	0.0	0.0	0.0086	5.8
7.4	10	0.1478	0.1321	89.4	0.0	0.0	0.0157	10.6
11.0	13	0.2210	0.2039	92.3	0.0	0.0	0.0171	7.7
11.0	14	0.2210	0.2043	92.5	0.0	0.0	0.0167	7.5
15.9	17	0.3186	0.2923	91.8	0.0	0.0	0.0263	8.2
15.9	18	0.3186	0.2907	91.2	0.0	0.0	0.0279	8.8

Average Mass Balance for Control Vials (No Biomass)								
Initial Naphthalene Conc. (mg/L)	Vial	Total Mass	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.0	1,2	0.0000	0.0000	NA	0.0	0.0	0.0000	NA
3.6	5,6	0.0728	0.0600	82.4	0.0	0.0	0.0128	17.6
7.4	9,10	0.1478	0.1356	91.8	0.0	0.0	0.0122	8.2
11.0	13,14	0.2210	0.2041	92.4	0.0	0.0	0.0169	7.6
15.9	17,18	0.3186	0.2915	91.5	0.0	0.0	0.0271	8.5

Table B.66. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 1 experiment conditions

Run Description	
Run ID #:	AC221
Date Started:	03/28/07
Time Started:	17:00
Date Ended:	04/02/07
Time Ended:	12:45
Experiment Duration:	115.8 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-36
Age of Inoculum:	29 days
Test Parameters: Start-up Summary	
System Location:	Prec. Science #1 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	15.4 ºC/13.7 ºC
Incident Light Intensity	80 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	3.25 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type:	20 mL Vials w/ Teflon Lined Caps
Mixing:	Orbital Shaker
Mixer Speed (rpm):	162
Liquid Volume:	20 mL per vial
FW to DW Conversion	0.358
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extraction 1 start:	4/2/2007 0:00
Extraction 1 end:	04/04/07
Sonicated from 08:30-09:30 on 04/04/07	
Extraction Volume (mL):	3.0

Solution Prep.:	
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
pH Naphthalene soln.	8.548
pH Non-Naphthalene soln.	8.429
PAH:	Naphthalene
PAH Stock Prep.:	22.2 mg naphthalene dissolved in 2.0 mL Ethanol
PAH Stock Conc.:	11.1 mg/mL
Vol. PAH Stock:	45.0 µL added to 500 mL medium to give 1.0 mg/L soln.

Five solutions of various Naphthalene concentrations are prepared by diluting the 1.0 mg/L Naphthalene solution with medium not containing naphthalene.

Solution	Vol. Non-Nap. Soln. (mL)	Vol. Nap. Soln. (mL)
1	80	20
2	60	40
3	40	60
4	20	80
5	0	100

Solution	Calculated Initial Concentration of Naphthalene (mg/L)	Measured Initial Concentration of Naphthalene(mg/L)
Solution 1:	0.2	0.216
Solution 2:	0.4	0.368
Solution 3:	0.6	0.604
Solution 4:	0.8	0.708
Solution 5:	1.0	0.929

HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.000853 area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	214 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile

Table B.67. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 1 vial summary

Vial #	Initial Biomass Weight Measured (mg FW)	Initial PAH Concentration (mg/L)	Biomass Weight Measured (mg FW)	Final pH
29	21.0	0.216	16.0	8.388
30	20.2	0.216	15.6	8.407
31	22.1	0.368	14.9	8.392
32	19.5	0.368	15.4	8.378
33	19.4	0.604	15.3	8.372
34	20.2	0.604	14.4	8.422
35	20.9	0.708	14.0	8.377
36	21.6	0.708	14.5	8.404
37	21.1	0.929	13.5	8.392
38	21.3	0.929	17.4	8.378

Table B.68. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 1 liquid phase raw data

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Naphthalene Concentration (mg/L)
0.2 mg/L Stock 1	03/28/07	221p16.ASC	2242	03/30/07	55.70	5.633	0.2376
0.2 mg/L Stock 2	03/28/07	221p17.ASC	2243	03/30/07	45.60	5.500	0.1945
0.4 mg/L Stock 1	03/28/07	221p18.ASC	2244	03/30/07	87.30	5.616	0.3723
0.4 mg/L Stock 2	03/28/07	221p19.ASC	2245	03/30/07	85.50	5.616	0.3647
0.6 mg/L Stock 1	03/28/07	221p20.ASC	2246	03/30/07	140.85	5.633	0.6007
0.6 mg/L Stock 2	03/28/07	221p21.ASC	2247	03/30/07	142.40	5.616	0.6073
0.8 mg/L Stock 1	03/28/07	221p22.ASC	2248	03/30/07	173.20	5.616	0.7387
0.8 mg/L Stock 2	03/28/07	221p23.ASC	2249	03/30/07	158.90	5.616	0.6777
1.0 mg/L Stock 1	03/28/07	221p24.ASC	2250	03/30/07	217.80	5.650	0.9289
1.0 mg/L Stock 2	03/28/07	221p25.ASC	2251	03/30/07	217.70	5.583	0.9285
221-29	04/02/07	222p32.ASC	2252	04/04/07	34.00	5.616	0.1450
221-30	04/02/07	222p33.ASC	2253	04/04/07	37.00	5.616	0.1578
221-31	04/02/07	222p34.ASC	2254	04/04/07	106.70	5.633	0.4551
221-32	04/02/07	222p35.ASC	2255	04/04/07	73.10	5.650	0.3118
221-33	04/02/07	222p36.ASC	2256	04/04/07	105.50	5.600	0.4500
221-34	04/02/07	222p37.ASC	2257	04/04/07	98.10	5.633	0.4184
221-35	04/02/07	222p38.ASC	2258	04/04/07	138.25	5.650	0.5896
221-36	04/02/07	222p39.ASC	2259	04/04/07	137.95	5.650	0.5884
221-37	04/02/07	222p40.ASC	2260	04/04/07	173.30	5.600	0.7391
221-38	04/02/07	222p41.ASC	2261	04/04/07	179.90	5.650	0.7673

Table B.69. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 1 biomass extraction 164 raw data

Sample Name	Date Sampled	HPLC file	Injection #	Date of Analysis	Peak Area	Reten-tion Time (min)	Mass Nap. Extracted (mg)	DW Bio-mass (mg)	Naphthalene Concentration (mg/g Dry Mass)
221-29	04/02/07	222p46.ASC	2283	04/04/07	62.65	5.483	0.00016	7.5	0.0213
221-30	04/02/08	222p47.ASC	2284	04/04/07	76.20	5.550	0.00019	7.2	0.0270
221-31	04/02/09	222p48.ASC	2285	04/04/07	104.20	5.516	0.00027	7.9	0.0337
221-32	04/02/10	222p49.ASC	2286	04/04/07	104.35	5.500	0.00027	7.0	0.0383
221-33	04/02/11	222p50.ASC	2287	04/04/07	171.30	5.516	0.00044	6.9	0.0631
221-34	04/02/12	222p51.ASC	2288	04/04/07	179.60	5.533	0.00046	7.2	0.0636
221-35	04/02/13	222p52.ASC	2289	04/04/07	204.10	5.516	0.00052	7.5	0.0698
221-36	04/02/14	222p53.ASC	2290	04/04/07	264.75	5.516	0.00068	7.7	0.0876
221-37	04/02/15	222p54.ASC	2291	04/04/07	315.00	5.550	0.00081	7.6	0.1067
221-38	04/02/16	222p55.ASC	2292	04/04/07	346.45	5.533	0.00089	7.6	0.1163

Table B.70. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 1 averages for partitioning

Vials	Liquid Naphthalene Concentration (mg/L)	Naphthalene Concentration in Biomass (mg/g Dry Mass)
29, 30	0.1514	0.0241
31,32	0.3834	0.0360
33,34	0.4342	0.0633
35,36	0.5890	0.0787
37,38	0.7532	0.1115

Table B.71. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 1 mass balance

Initial Naphthalene Conc. (mg/L)	Vial	Total Mass	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.216	29	0.00432	0.00290	67.1	0.00016	3.71	0.00126	29.2
0.216	30	0.00432	0.00316	73.1	0.00019	4.51	0.00097	22.4
0.368	31	0.00737	0.00910	123.5	0.00027	3.62	-0.00200	-27.1
0.368	32	0.00737	0.00624	84.6	0.00027	3.62	0.00087	11.8
0.604	33	0.01208	0.00900	74.5	0.00044	3.63	0.00264	21.9
0.604	34	0.01208	0.00837	69.3	0.00046	3.80	0.00325	26.9
0.708	35	0.01416	0.01179	83.3	0.00052	3.69	0.00185	13.1
0.708	36	0.01416	0.01177	83.1	0.00068	4.78	0.00172	12.1
0.929	37	0.01857	0.01478	79.6	0.00081	4.34	0.00299	16.1
0.929	38	0.01857	0.01535	82.6	0.00089	4.77	0.00234	12.6

Note: for experiment conditions see table B.55

Table B.72. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 2 vial summary

Vial #	Initial Biomass Weight Measured (mg FW)	Initial PAH Concentration (mg/L)	Final Biomass Weight Measured (mg FW)	Final pH
11	20.9	0.170	16.3	8.341
12	21.2	0.170	15.3	8.350
13	22.9	0.363	15.5	8.318
14	20.5	0.363	15.5	8.343
15	20.5	0.517	15.6	8.320
16	20.6	0.517	14.2	8.336
17	20.4	0.719	17.9	8.360
18	22.1	0.719	16.4	8.363
19	24.5	0.944	14.8	8.360
20	22.0	0.944	14.3	8.380

Table B.73. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 2 liquid phase raw data

HPLC Data: Liquid Samples

Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Nap. Conc. (mg/L)
0.2 mg/L - 1	04/13/07	223p4.ASC	2350	04/23/07	40.10	5.583	0.1710
0.2 mg/L - 2	04/13/07	223p54.ASC	2400	04/24/07	39.40	5.600	0.1680
0.4 mg/L - 1	04/13/07	223p6.ASC	2352	04/23/07	81.00	5.583	0.3455
0.4 mg/L - 2	04/13/07	223p7.ASC	2353	04/23/07	89.30	5.600	0.3809
0.6 mg/L - 1	04/13/07	223p8.ASC	2354	04/23/07	121.60	5.583	0.5186
0.6 mg/L - 2	04/13/07	223p9.ASC	2355	04/23/07	121.00	5.633	0.5161
0.8 mg/L - 1	04/13/07	223p10.ASC	2356	04/23/07	172.30	5.616	0.7349
0.8 mg/L - 2	04/13/07	223p11.ASC	2357	04/23/07	164.80	5.616	0.7029
1.0 mg/L - 1	04/13/07	223p12.ASC	2358	04/23/07	220.15	5.600	0.9389
1.0 mg/L - 2	04/13/07	223p13.ASC	2359	04/23/07	222.50	5.583	0.9490
223-11	04/18/07	223p24.ASC	2370	04/23/07	34.80	5.600	0.1484
223-12	04/18/07	223p25.ASC	2371	04/23/07	31.90	5.633	0.1361
223-13	04/18/07	223p26.ASC	2372	04/23/07	74.65	5.600	0.3184
223-14	04/18/07	223p27.ASC	2373	04/23/07	69.50	5.633	0.2964
223-15	04/18/07	223p28.ASC	2374	04/23/07	95.80	5.600	0.4086
223-16	04/18/07	223p29.ASC	2375	04/23/07	106.10	5.600	0.4525
223-17	04/18/07	223p55.ASC	2401	04/24/07	128.00	5.616	0.5459
223-18	04/18/07	223p31.ASC	2377	04/23/07	129.50	5.650	0.5523
223-19	04/18/07	223p32.ASC	2378	04/23/07	172.80	5.633	0.7370
223-20	04/18/07	223p33.ASC	2379	04/23/07	175.80	5.616	0.7498

Table B.74. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 2 biomass extraction 168 raw data

HPLC Data: Biomass Extraction									
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Mass Nap. Extracted (mg)	Dry Mass Biomass (mg)	Nap. Conc. (mg/g Dry Mass)
223-11	04/18/07	223p44	2390	04/24/07	105.90	5.483	0.00027	7.5	0.0362
223-12	04/18/07	223p45	2391	04/24/07	68.80	5.483	0.00018	7.6	0.0232
223-13	04/18/07	223p46	2392	04/24/07	198.50	5.550	0.00051	8.2	0.0620
223-14	04/18/07	223p47	2393	04/24/07	156.40	5.516	0.00040	7.3	0.0545
223-15	04/18/07	223p48	2394	04/24/07	182.40	5.516	0.00047	7.3	0.0636
223-16	04/18/07	223p49	2395	04/24/07	229.80	5.483	0.00059	7.4	0.0797
223-17	04/18/07	223p50	2396	04/24/07	309.10	5.483	0.00079	7.3	0.1083
223-18	04/18/07	223p51	2397	04/24/07	390.15	5.466	0.00100	7.9	0.1262
223-19	04/18/07	223p52	2398	04/24/07	434.70	5.500	0.00111	8.8	0.1268
223-20	04/18/07	223p53	2399	04/24/07	466.30	5.466	0.00119	7.9	0.1515

Table B.75. Equilibrium partitioning of naphthalene between seawater and heat-killed *A. coalita* tissue: Trial 2 mass balance

Initial Naphthalene Conc. (mg/L)	Vial	Total Mass	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
0.1695	11	0.00339	0.00297	87.5	0.00027	7.99	0.00015	4.46
0.1695	12	0.00339	0.00272	80.3	0.00018	5.19	0.00049	14.56
0.3632	13	0.00726	0.00637	87.7	0.00051	6.99	0.00039	5.34
0.3632	14	0.00726	0.00593	81.6	0.00040	5.51	0.00093	12.87
0.5173	15	0.01035	0.00817	79.0	0.00047	4.51	0.00171	16.51
0.5173	16	0.01035	0.00905	87.5	0.00059	5.68	0.00071	6.85
0.7189	17	0.01438	0.01092	75.9	0.00079	5.50	0.00267	18.56
0.7189	18	0.01438	0.01105	76.8	0.00100	6.94	0.00233	16.22
0.9440	19	0.01888	0.01474	78.1	0.00111	5.89	0.00303	16.03
0.9440	20	0.01888	0.01500	79.4	0.00119	6.32	0.00269	14.25

Table B.76. Phenanthrene desorption: experiment conditions

Run Description	
Run ID #:	AC225
Time Stage 1 Started:	11:45
Date Stage 1 Started:	05/09/07
Time Transfer:	12:30
Date Transfer:	05/11/07
Date Stage 2 Ended:	13:30
Time Stage 2Ended:	05/16/07
Experiment Duration:	170 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	ACI-38
Age of Inoculum:	27 days
Initial Biomass Density Stage 1:	1.5157 g FW/L
Phenanthrene Solution Prep.:	
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
PAH:	Phenanthrene
PAH Stock Prep.:	35.3 mg phen dissolved in 3.5 mL EtOH
PAH Stock Conc.:	10.086 mg/mL
Vol. PAH Stock:	49.6 µL
Calc. Initial Conc. Phen	0.5
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extraction 1 start:	04/25/07
Extraction 1 end:	05/04/07
Sonicated from 15:00-16:30 05/03/07	
Extraction Volume (mL):	
Samples 1-4	3.0
Samples 5-6	4.0
Samples 7-8	5.0
Samples 9-10	6.0
Samples 11-12	7.0
Samples 13-14	8.0

Table B.76. Continued

HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 μL
Standard Method:	External Calibration Curve
Response Factor:	0.000618 area/0.1 μg PAH injected
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 μm particle size
Dilution Factor (Liquid Samples):	1:4, 500 μL diluted w/ 2 mL acetonitrile
FW to DM Conversion	0.358
Test Parameters: Start-up Summary	
Incident Light Intensity Stage 1	80 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	4 in
Incident Light Intensity Stage 2	65 ($\mu E/m^2$-s):
Light Distance From Vessel Surface:	3.25 in
Photoperiod :	16:8
Dark Period:	22:00-6:00
Reactor Type 1:	1 L Bottle Reactor
Mixing:	stir bar/stir plate
Reactor Type 2:	20 mL Vials w/ Teflon Lined Caps
Mixing:	Orbital Shaker
Mixer Speed (rpm):	162
Stage 1	Uptake
Stage 2	Desorption
Stage 1 Initial Conditions	
System location	VWR incubator
Lab room number	302
temp set point	12.0 ºC
temp reading	12.6 ºC
pH	NA
Stage 1 Final Conditions	
System location	VWR incubator
Lab room number	302
temp set point	12.0 ºC
temp reading	12.0 ºC
pH	9.894
Total Biomass weight	1.6234 g FW

Table B.76. Continued

Stage 2 Inital Conditions	
System location	Precision Science 1
Lab room number	302
temp set point	12.0 ºC
temp reading	12.0 ºC
pH	8.622
Stage 2 Final Conditions	
System location	Precision Science 1
Lab room number	302
temp set point	12.0 ºC
temp reading	21.8/18.7 ºC
pH	See Experiment summary

Table B.77. Phenanthrene desorption: vial summary

Vial #	Initial Biomass Weight Measured (mg FW)	Final Biomass Weight Measured (mg FW)	Final pH	X_f/X_0
Stage 1 Final	NA	31.3	NA	NA
Stage 1 Final	NA	29.9	NA	NA
1	20.3	20.5	9.887	1.01
2	20.4	20.9	9.943	1.02
3	29.9	30.5	9.919	1.02
4	30.6	27.0	9.940	0.88
5	41.1	36.8	9.976	0.90
6	40.5	40.7	10.008	1.00
7	50.7	45.1	10.005	0.89
8	50.1	49.4	10.012	0.99
9	99.8	91.0	9.988	0.91
10	100.6	109.3	9.936	1.09
11	150.5	148.2	9.914	0.98
12	150.5	161.4	9.866	1.07
13	204.1	201.8	9.788	0.99
14	199.6	205.6	9.783	1.03

Table B.78. Phenanthrene desorption: liquid phase raw data

HPLC Data: Liquid Samples							
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Phenanthrene Concentration in Liquid (mg/L)
Stage 2 initial	05/16/07	225p5.ASC	2434	05/18/07	0.00	NA	0.0000
Stage 1 Final	05/16/07	225p6.ASC	2435	05/18/07	41.00	8.433	0.1267
225-1	05/16/07	225p7.ASC	2436	05/18/07	26.10	8.400	0.0806
225-2	05/16/07	225p8.ASC	2437	05/18/07	26.15	8.466	0.0808
225-3	05/16/07	225p9.ASC	2438	05/18/07	28.45	8.416	0.0879
225-4	05/16/07	225p10.ASC	2439	05/18/07	34.40	8.433	0.1063
225-5	05/16/07	225p11.ASC	2440	05/18/07	34.90	8.466	0.1078
225-6	05/16/07	225p12.ASC	2441	05/18/07	42.20	8.466	0.1304
225-7	05/16/07	225p13.ASC	2442	05/18/07	37.70	8.500	0.1165
225-8	05/16/07	225p14.ASC	2443	05/18/07	41.70	8.466	0.1289
225-9	05/16/07	225p15.ASC	2444	05/18/07	47.40	8.400	0.1465
225-10	05/16/07	225p16.ASC	2445	05/18/07	49.30	8.416	0.1523
225-11	05/16/07	225p17.ASC	2446	05/18/07	58.90	8.383	0.1820
225-12	05/16/07	225p18.ASC	2447	05/18/07	64.90	7.266	0.2005
225-13	05/16/07	225p19.ASC	2448	05/18/07	62.40	8.450	0.1928
225-14	05/16/07	225p20.ASC	2449	05/18/07	65.15	8.450	0.2013

Table B.79. Phenanthrene desorption: biomass extraction raw data

HPLC Data: Biomass Extraction											
Sample Name	Date Sampled	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time (min)	Mass Phen. Extracted (mg)	FW Biomass (mg)	Phen. Conc. (mg/ g FW)	Bio-mass DW (mg)	Phen. Conc. (mg/g dry weight)
Stage 1 Final	05/16/07	225p21.ASC	2450	05/18/07	1875.90	8.350	0.00348	31.3	0.11112	11.2	0.3104
Stage 1 Final	05/16/07	225p22.ASC	2451	05/18/07	2209.30	8.350	0.00410	29.9	0.13699	10.7	0.3827
225-1	05/16/07	225p23.ASC	2452	05/18/07	392.50	8.283	0.00073	20.5	0.03550	7.3	0.0992
225-2	05/16/07	225p24.ASC	2453	05/18/07	512.90	8.283	0.00095	20.9	0.04550	7.5	0.1271
225-3	05/16/07	225p25.ASC	2454	05/18/07	827.80	8.300	0.00153	30.5	0.05032	10.9	0.1406
225-4	05/16/07	225p26.ASC	2455	05/18/07	835.90	8.283	0.00155	27.0	0.05740	9.7	0.1603
225-5	05/16/07	225p27.ASC	2456	05/18/07	1014.10	8.300	0.00251	36.8	0.06812	13.2	0.1903
225-6	05/16/07	225p28.ASC	2457	05/18/07	1002.15	8.216	0.00248	40.7	0.06087	14.6	0.1700
225-7	05/16/07	225p29.ASC	2458	05/19/07	1207.10	8.283	0.00373	45.1	0.08270	16.1	0.2310
225-8	05/16/07	225p30.ASC	2459	05/19/07	1119.40	8.200	0.00346	49.4	0.07002	17.7	0.1956
225-9	05/16/07	225p31.ASC	2460	05/19/07	2143.05	8.200	0.00795	91.0	0.08732	32.6	0.2439
225-10	05/16/07	225p32.ASC	2461	05/19/07	2426.30	8.333	0.00900	109.3	0.08231	39.1	0.2299
225-11	05/16/07	225p33.ASC	2462	05/19/07	3524.40	8.300	0.01525	148.2	0.10288	53.1	0.2874
225-12	05/16/07	225p34.ASC	2463	05/19/07	4025.80	8.266	0.01742	161.4	0.10790	57.8	0.3014
225-13	05/16/07	225p35.ASC	2464	05/19/07	4576.70	8.250	0.02263	201.8	0.11213	72.2	0.3132
225-14	05/16/07	225p36.ASC	2465	05/19/07	4817.40	8.383	0.02382	205.6	0.11584	73.6	0.3236

Table B.80. Phenanthrene desorption: mass balance

Initial Biomass FW (mg)	Vial	Total Mass (mg)	Mass Recovered in Liquid (mg)	Recovered in Liquid (% initial)	Mass Recovered in Biomass (mg)	Recovered in Biomass (% initial)	Mass Unaccounted For (mg)	Unaccounted For (% initial)
20.3	1	0.00703	0.00161	22.9	0.00073	10.3	0.00469	66.7
20.4	2	0.00707	0.00162	22.9	0.00095	13.5	0.00450	63.7
29.9	3	0.01036	0.00176	17.0	0.00153	14.8	0.00707	68.2
30.6	4	0.01060	0.00213	20.0	0.00155	14.6	0.00693	65.3
41.1	5	0.01424	0.00216	15.1	0.00251	17.6	0.00958	67.3
40.5	6	0.01403	0.00261	18.6	0.00248	17.7	0.00895	63.8
50.7	7	0.01757	0.00233	13.3	0.00373	21.2	0.01151	65.5
50.1	8	0.01736	0.00258	14.8	0.00346	19.9	0.01132	65.2
99.8	9	0.03458	0.00293	8.5	0.00795	23.0	0.02371	68.6
100.6	10	0.03486	0.00305	8.7	0.00900	25.8	0.02282	65.5
150.5	11	0.05215	0.00364	7.0	0.01525	29.2	0.03326	63.8
150.5	12	0.05215	0.00401	7.7	0.01742	33.4	0.03072	58.9
204.1	13	0.07072	0.00386	5.5	0.02263	32.0	0.04424	62.6
199.6	14	0.06917	0.00403	5.8	0.02382	34.4	0.04132	59.7

Table B.81. Metabolite Detection: experiment conditions

Run Description	
Run ID #:	AC219
Date Started:	02/20/07
Time Started:	14:12
Date Ended:	02/26/07
Time Ended:	12:40
Date Biomass Added:	02/20/07
Time Biomass Added:	14:12
Initial Biomass Weight:	1.562 g FW
Experiment Duration:	142.5 hr
Culture Loading	
Culture:	*Acrosiphonia coalita*
Cell Line I.D.:	AC1-33
Age of Inoculum:	41 days
Test Parameters: Start-up Summary	
System Location:	Precision Science #2
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	12.6/12.4 ºC
Incident Light Intensity (Reactor 1):	80 μE/m^2-s
Incident Light Intensity (Reactor 2):	80 μE/m^2-s
Photoperiod :	8:16
Dark Period:	20:00-6:00
Reactor Type:	1.0 L bottle reactor
Mixing:	stir bar/stir plate
PAH:	Phenanthrene
PAH Stock Prep.:	31.7 mg dissolve in 3 mL EtOH
PAH Stock Conc.:	10.57 mg/L
Vol. PAH Stock:	139.1 μL
Calculated Initial PAH Conc.:	0.7 mg/L
Measured Initial PAH Conc.:	0.6688 mg/L
Date PAH Added:	02/20/07
Time PAH Added:	14:12

Table B.81. Continued

Solution Preparation	
Nutrient Stock:	50 X PES
Nutrient Stock Conc.:	2 mL per 100 mL Soln.
Base Medium:	Natural Seawater
Sterilization Method:	Autoclave
Medium Volume:	1000 mL per reactor
Initial pH Medium:	8.341
Shut-down Summary	
Final Weight Biomass Reactor 1:	1.614 g FW
Final Medium pH Reactor 1:	9.395
Final Medium pH Reactor 2:	8.320
Temperature Readout:	22.7/18.1 ºC
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Response Factor:	0.00124 Area/0.1 µg PAH injected
Mobile Phase:	80% acetonitrile 20% H20 (v/v)
Flow Rate:	1 mL/min
UV Absorbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm particle size
Dilution Factor (Liquid Samples):	1:4, 500 µL diluted w/ 2 mL acetonitrile
SPE Extraction	
Column	C18
Volume Concentrated	200 mL per reactor
Column Rinse	5 mL DI water
Ellution	3.5 mL Acetonitrile
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extract Volume Initial	3.0 mL
Extract Volume Sample 1-2	6.0 mL
Extract Volume Sample 3	5.0 mL
GC-MS Analysis	
Method #:	KMC01
Injection Volume:	1 µL
File Director	May07
Mobile Phase:	Helium
Flow Rate:	0.9 mL/min
Detection:	Total ion count
Column:	DB-5 .25mm x 30m

Table B.82. Metabolite detection: liquid phase raw data

HPLC Data: Liquid Samples										
Sample Name	Date Sampled	Sample Time	Elapsed Time (hr)	HPLC file	Injn #	Date of Analysis	Peak Area	Reactor #	Reten-tion Time	Phen. Conc. (mg/L)
219-1-1	02/20/07	14:12	0.0	219p2.ASC	2093	02/28/07	108.60	1	8.416	0.6733
219-2-2	02/20/07	14:12	0.0	219p3.ASC	2094	02/28/07	107.15	2	8.433	0.6643
219-1-3	02/26/07	12:40	142.5	219p4.ASC	2095	02/28/07	42.60	1	8.416	0.2641
219-2-4	02/26/07	12:40	142.5	219p5.ASC	2096	02/28/07	93.60	2	8.433	0.5803

Table B.83. Metabolite detection: biomass extraction raw data

HPLC Data: Biomass Extraction Samples											
Sample Name	Date Sampled	Sample Time	Elapsed Time (hr)	HPLC file	Injn #	Date of Analysis	Peak Area	Retention Time	Phen. Extracted (mg)	Biomass FW (mg)	Phen. Conc. (mg/g FW)
219-Biomass - Initial	02/20/07	14:12	0.0	219p8	2099	02/28/07	0.00	NA	0.00000	26.4	0.00000
219-Biomass - Final-1	02/26/07	12:40	142.5	219p9	2100	02/28/07	563.90	8.333	0.00420	51.0	0.08226
219-Biomass - Final-2	02/26/07	12:40	142.5	219p10	2101	02/28/07	731.90	8.333	0.00545	62.4	0.08727
219-Biomass - Final-3	02/26/07	12:40	142.5	219p21	2112	02/28/07	14678.65	8.283	0.09101	478.3	0.19027

Table B.84. Metabolite detection: mass balance

Mass Balance Reactor 1: With Biomass			
Initial Mass in Reactor (mg)	Final Mass in Liquid (mg)	Final Mass in Biomass (mg)	Mass Unaccounted For (mg)
0.6688	0.5803	0.1936	-0.1051
Initial (%)	Final in Liquid (%)	Final in Biomass (%)	Unaccounted For (%)
100.0	86.8	28.9	-15.7
Mass Balance Reactor 2: Control			
Initial Mass in Liquid (mg)	Final Mass in Liquid (mg)	Final Mass in Biomass (mg)	Mass Unaccounted For (mg)
0.6688	0.5803	0.0000	0.0885
Initial (%)	Final in Liquid (%)	Final in Biomass(%)	Unaccounted For (%)
100.0	86.8	0.0	13.2

Table B.85. Metabolite detection: sample identification

Sample ID	Sample Information	GC-MS File Number
AC-219-1-AQ	Concentrated aqueous sample from reactor with phenanthrene and biomass	CO1266
AC-219-2-AQ	Concentrated aqueous sample from control reactor with phenanthrene only (no biomass)	C01267
AC-219-3-AQ	Concentrated aqueous sample from spent medium in culture flasks	C01268
AC-219-1-BM	Biomass extraction sample from reactor with biomass and phenanthrene	C01269
AC-219-3-BM	Biomass extraction sample from biomass in culture flasks not exposed to PAH	C01270
AC-222-AQ	Concentrated aqueous sample from reactor with phenanthrene and biomass	C01271
AC-222-BM	Biomass extraction sample from reactor with biomass and phenanthrene	C01272

Table B.86. Lipid analysis: experiment conditions

Preparation of Stock Solution of Palmitic Acid:	
Palmitic Acid Initial Stock Preparation:	5.0 mg dissolved in 5.0 mL chloroform
Palmitic Acid Initial Stock Concentration:	1.0 mg/mL
Preparation of Dichromate Solution:	
Mass of $K_2Cr_2O_7$ Measured (g)	0.1
Volume of Concentrated Sulfuric Acid	40 mL
Solution Concentration (mg/L)	2.5
Culture Identification:	
Culture	Acrosiphonia coalita
Culture ID	ACI-39
Age	35 days
Weight Sample 1 (mg FW):	49.7
Weight Sample 2(mg FW):	50.8
Weight Sample 3 (mg FW):	50.3
Biomass Extration:	
Extration 1:	10 mL 2:1 v/v chloroform:methanol
Start Time:	16:20
End Time:	16:35
Extration 2:	5 mL 2:1 v/v chloroform:methanol
Start Time:	17:35
End Time:	17:35

Table B.87. Lipid analysis: determination of lipid content

Biomass Sample	Absorbance at 350 nm	Lipid Conc. (mg/L)	Dilution Factor	Mass Lipid in Sample (mg)	Biomass Weight (g FW)	Mass Lipid/Mass Biomass (mg lipid/ mg FW biomass)
Sample 1	0.33061	13.13	20	0.2627	49.7	0.00528
Sample 2	0.37310	12.72	20	0.2545	50.8	0.00501
Sample 3	0.40762	12.39	20	0.2478	50.3	0.00493
				Average Lipid Fraction		**0.00507**

Table B.88. Biomass extraction study: experiment conditions

Run ID #:	**Biomass Extraction Study**
Date Started:	08/28/06
Time Started:	16:05
Date Ended:	08/29/06
Time Ended:	10:20
Experiment Duration:	18.25 hr
Performed by:	Kristi Christensen
Purpose: To determine the efficiency of multiple extractions of PAH from algal Biomass	
Culture Loading	
Culture:	Acrosiphonia coalita
Cell Line I.D.:	ACI-25
Initial Biomass Weight Vial 1:	12.2 mg FW
Initial Biomass Weight Vial 1:	12.2 mg FW
Nutrient Stock:	50 x PES
Nutrient Stock Conc.:	2 mL/100 mL soln.
Base Medium:	Natural seawater
Sterilization Method:	Autoclave
Medium Volume:	10 mL/vial
Initial Biomass Density:	1.22 g FW/L
Test Parameters: Start-up Summary	
System Location:	Precision Science #1 incubator
Lab Room #:	302
Temperature Setpoint:	12.0 ºC
Temperature Readout:	17.4/15.8 ºC
Incident Light Intensity:	0, wrapped in tin foil
Photoperiod :	NA
Dark Period:	NA
Reactor Type:	20 mL vials
Mixing:	Orbital Shaker
Mixer RPM:	120
Substrate:	Phenanthrene
Substrate Stock Prep.:	22.7 mg phen. 2 mL EtOH
Substrate Stock Conc.:	11.35 µg/mL
Vol. Substrate Stock:	13.2 mL added to 250 mL medium
Calculated Initial Substrate Conc.:	0.6 mg/L
Measured Initial Substrate Conc.:	0.5084 mg/L

Table B.88. Continued

Shut-down Summary	
Final Weight Biomass Vial 1:	29.1 mg FW
Final Weight Biomass Vial 2:	35.5 mg FW
Temperature Readout:	16.8/14.3 ºC
HPLC Analysis	
Procedure #:	Phen2
Injection Volume:	100 µL
Standard Method:	External Calibration Curve
Calibration Conversion Factor:	0.00124
Mobile Phase:	80% acetonitrile 20% H_20 (v/v)
Flow Rate:	1 mL/min
UV Abosrbance Setting:	249 nm
Column:	Waters spherisorb ODS2, 5 µm particle size, Serial # 01483500511651
Dilution Factor (Liquid Samples):	1:4, 500 mL diluted w/ 2 mL acetonitrile
Biomass Extraction	
Extraction Solvent:	Acetonitrile
Extract Volume (mL):	0.5

Table B.89. Biomass extraction study: HPLC Results

Sample Name	Date Sampled	HPLC file	Injn #	Date of HPLC Analysis	Peak Area	Reten-tion Time (min)	Mass Phen in Sample (mg)
Initial	8/28/07	208p9.ASC	1372	8/30/06	82.00	8.500	5.084E-03
1-AQ	8/29/07	208p10.ASC	1373	8/30/06	27.15	8.500	1.683E-03
1-EX1	8/29/07	208p18.ASC	1381	8/30/06	3161.80	8.400	1.960E-03
1-EX2	8/29/07	208p24.ASC	1387	9/22/06	230.00	8.300	1.426E-04
1-EX3	8/29/07	208p25.ASC	1388	9/22/06	19.70	8.250	1.221E-05
2-AQ	8/29/07	208p11.ASC	1374	8/30/06	27.30	8.466	1.693E-03
2-EX1	8/29/07	208p19.ASC	1382	8/30/06	4595.05	8.400	2.849E-03
2-EX2	8/29/07	208p27.ASC	1390	9/22/06	376.10	8.350	2.332E-04
2-EX3	8/29/07	208p28.ASC	1391	9/22/06	27.05	8.266	1.677E-05

HPLC Calibration: Phenanthrene Quantification at 249 nm

Table C.1. Calibration data for phenanthrene detection at 249 nm

Solution Concentration (mg/L)	Peak Area
0	0.00
0.05	41.85
0.05	39.15
0.5	401.95
0.5	401.90
1	803.90
1	791.40
2	1603.10
2	1616.30

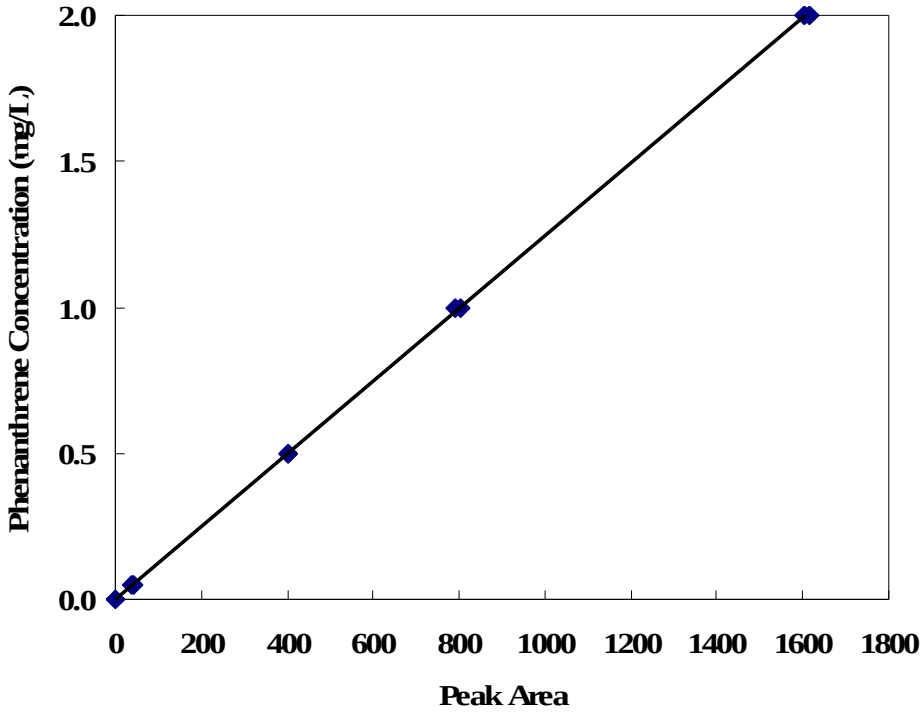

Figure C.1. Calibration curve for phenanthrene detection at 249 nm.

Table C.2. Calibration data for naphthalene detection at 254 nm

Solution Concentration (mg/L)	Peak Area
0	0.00
1	52.75
1	52.20
5	259.30
5	257.70
10	497.70
10	499.45
20	1016.55
20	1036.45

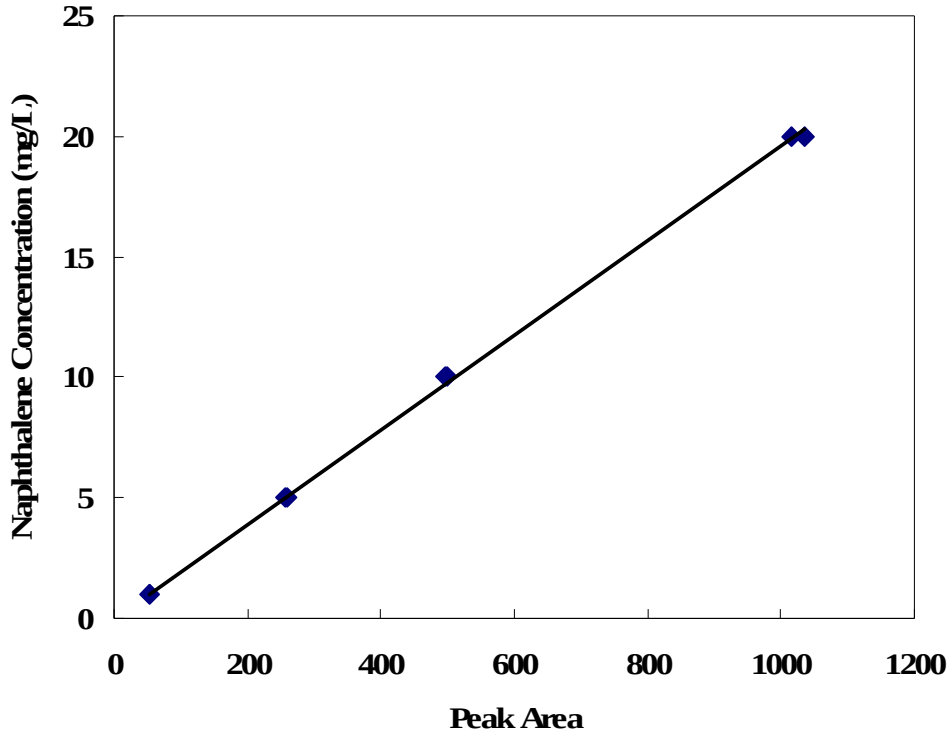

Figure C.2. Calibration curve for naphthalene detection at 254 nm.

Table C.3. Calibration data for naphthalene detection at 214 nm

Solution Concentration (mg/L)	Peak Area
0	0.00
0.05	72.50
0.05	59.65
0.5	611.30
0.5	611.80
1	1262.10
1	1272.60
5	5845.05
5	5831.60

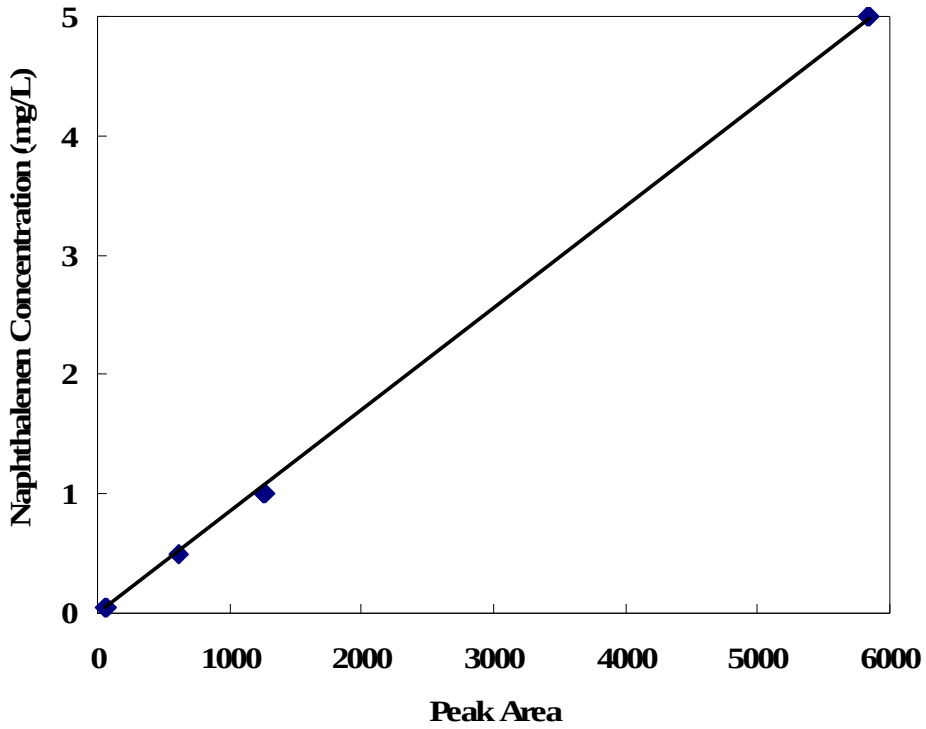

Figure C.3. Calibration curve for naphthalene detection at 214 nm. .

Table C.4. Summary of response factors for calibration curves

PAH	UV Wavelength (nm)	Response Factor (area/0.1 µg PAH)	R^2, n
Phenanthrene	249	0.00124	0.99996, n = 9
Naphthalene	254	0.0196	0.9998, n = 9
Naphthalene	214	0.000853	0.9997, n = 9

Table C.5. Calibration curve data for calibration of the UV spectrophotometer for lipid analysis

Solution Concentration	Total Mass Palmitic Acid (mg)	Absorbance at 350 nm
0 mg/L Palmitic Acid	0.00	1.70080
2.5 mg/L Palmitic Acid	0.05	1.46216
5.0 mg/L Palmitic Acid	0.10	1.15586
7.5 mg/L Palmitic Acid	0.15	0.90468
10.0 mg/L Palmitic Acid	0.20	0.64680
12.5 mg/L Palmitic Acid	0.25	0.37136
15.0 mg/L Palmitic Acid	0.30	0.17457

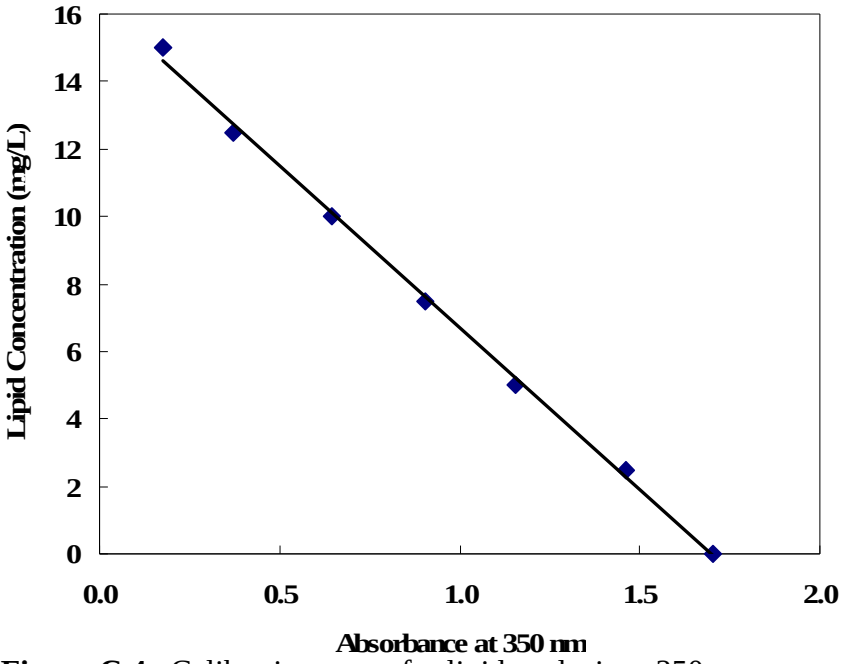

Figure C.4. Calibration curve for lipid analysis at 350 nm.

Table D.1. Linear regression analysis of phenanthrene partitioning between living *A. coalita* tissue and seawater: Trial 1, based on biomass FW. Experiment ID: AC215

Regression Statistics	
R Square	0.6985
Standard Error	0.0099
Observations	21

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0186	0.0186	192.1	2.20E-11
Residual	20	0.0019	9.71E-05		
Total	21	0.0206			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1855	0.0134	13.8589	1.03E-11	0.158	0.2134

Table D.2. Linear regression analysis of phenanthrene partitioning between living *A. coalita* tissue and seawater: Trial 1, based on biomass DW. Experiment ID: AC215

Regression Statistics	
R Square	0.6984
Standard Error	0.0275
Observations	21

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.1455	0.1455	192.1	2.20E-11
Residual	20	0.0152	0.0008		
Total	21	0.1607			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.5182	0.0374	13.8589	1.03E-11	0.440	0.5962

Table D.3. Linear regression analysis of phenanthrene partitioning between living *A. coalita* tissue and seawater: Trial 2, based on biomass FW. Experiment ID: AC220

Regression Statistics	
R Square	0.8121
Standard Error	0.0074
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0173	1.73E-02	317.3	5.37E-10
Residual	13	0.0007	5.47E-05		
Total	14	0.0181			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1591	0.0089	17.81	1.63E-10	0.140	0.1784

Table D.4. Linear regression analysis of phenanthrene partitioning between living *A. coalita* tissue and seawater: Trial 2, based on biomass DW. Experiment ID: AC220

Regression Statistics	
R Square	0.8123
Standard Error	0.0207
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.1353	0.1353	317.3	5.37E-10
Residual	13	0.0055	4.26E-04		
Total	14	0.1409			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.4445	0.0250	17.8128	1.63E-10	0.391	0.4984

Table D.5. Linear regression analysis of phenanthrene partitioning between heat-killed *A. coalita* tissue and seawater: Trial 1, based on biomass DW. Experiment ID: AC220

Regression Statistics	
R Square	0.6143
Standard Error	0.0451
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.2895	0.2895	142.2	5.20E-08
Residual	13	0.0265	0.0020		
Total	14	0.3159			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	1.8165	0.1524	11.9229	2.26E-08	1.487	2.1457

Table D.6. Linear regression analysis of phenanthrene partitioning between heat-killed *A. coalita* tissue and seawater: Trial 2, based on biomass DW. Experiment ID: AC221

Regression Statistics	
R Square	0.8410
Standard Error	0.0461
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.6300	0.6300	296.2	7.99E-10
Residual	13	0.0276	0.0021		
Total	14	0.6576			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	1.9780	0.1149	17.2107	2.50E-10	1.730	2.2263

Table D.7. Linear regression analysis of naphthalene partitioning between living *A. coalita* tissue and seawater: high concentration, based on biomass FW. Experiment ID: AC214

Regression Statistics	
R Square	0.9700
Standard Error	0.0353
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.8981	0.8981	721.5	3.97E-09
Residual	9	0.0112	0.0012		
Total	10	0.9093			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.0470	0.00175	26.8605	6.65E-10	0.0430	0.0509

Table D.8. Linear regression analysis of naphthalene partitioning between living *A. coalita* tissue and seawater: high concentration, based on biomass DW. Experiment ID: AC214

Regression Statistics	
R Square	0.9700
Standard Error	0.0986
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7.0075	7.0075	721.5	3.972E-09
Residual	9	0.0874	0.0097		
Total	10	7.0949			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1312	0.00488	26.8605	6.65E-10	0.1201	0.1422

Table D.9. Linear regression analysis of naphthalene partitioning between living *A. coalita* tissue and seawater: low concentration Trial 1, based on biomass FW. Experiment ID: AC218

Regression Statistics	
R Square	0.8958
Standard Error	0.0027
Observations	10

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0036	0.0036	482.2	1.95E-08
Residual	9	6.75E-05	7.5E-06		
Total	10	0.0037			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.0449	0.0020	21.9594	3.98E-09	0.0402	0.0495

Table D.10. Linear regression analysis of naphthalene partitioning between living *A. coalita* tissue and seawater: low concentration Trial 1, based on biomass DW. Experiment ID: AC218

Regression Statistics	
R Square	0.8960
Standard Error	0.0077
Observations	10

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0282	2.82E-2	482.2	1.95E-08
Residual	9	0.0005	5.86E-5		
Total	10	0.0288			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1253	0.0057	21.9594	3.98E-09	0.1124	0.1382

Table D.11. Linear regression analysis of naphthalene partitioning between living *A. coalita* tissue and seawater: low concentration Trial 2, based on biomass FW. Experiment ID: AC223

Regression Statistics	
R Square	0.7572
Standard Error	0.0063
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0069	0.0069	173.4	1.05E-06
Residual	9	0.0004	4.0E-05		
Total	10	0.0073			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.0539	0.0041	13.1672	3.48E-07	0.0446	0.0632

Table D.12. Linear regression analysis of naphthalene partitioning between living *A. coalita* tissue and seawater: low concentration Trial 2, based on biomass DW. Experiment ID: AC223

Regression Statistics	
R Square	0.7572
Standard Error	0.0176
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0539	0.0539	173.4	1.05E-06
Residual	9	0.0028	3.1E-04		
Total	10	0.0567			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1506	0.0114	13.1672	3.48E-07	0.1247	0.1764

Table D.13. Linear regression analysis of naphthalene partitioning between heat-killed 193 *A. coalita* tissue and seawater: low concentration Trial 1, based on biomass DW.
Experiment ID: AC221

Regression Statistics	
R Square	0.8751
Standard Error	0.0117
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0480	0.0480	349.8	6.90E-08
Residual	9	0.0012	1.37E-4		
Total	10	0.0493			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1371	0.0073	18.7024	1.64E-08	0.1205	0.1536

Table D.14. Linear regression analysis of naphthalene partitioning between heat-killed *A. coalita* tissue and seawater: low concentration Trial 2, based on biomass DW.
Experiment ID: AC223

Regression Statistics	
R Square	0.9372
Standard Error	0.0107
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0847	0.0847	736.1	3.67E-09
Residual	9	0.0010	1.2E-04		
Total	10	0.0858			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1915	0.0071	27.1314	6.08E-10	0.1755	0.2075

Table D.15. Linear regression analysis of phenanthrene desorption, based on biomass FW. Experiment ID: AC225

Regression Statistics	
R Square	0.9203
Standard Error	0.0073
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0912	0.0912	1702.9	2.66E-14
Residual	13	0.0007	5.4E-05		
Total	14	0.0919			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.5640	0.0137	41.2661	3.59E-15	0.5345	0.5936

Table D.16. Linear regression analysis of phenanthrene desorption, based on biomass DW. Experiment ID: AC225

Regression Statistics	
R Square	0.9203
Standard Error	0.0204
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.7113	0.7113	1702.9	2.7E-14
Residual	13	0.0054	4.2E-04		
Total	14	0.7168			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	1.5755	0.0382	41.2661	3.59E-15	1.4930	1.6580

Table D.17. Linear regression analysis of phenanthrene calibration curve at 249 nm

Regression Statistics	
R Square	0.99992
Standard Error	0.0070
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	10.5046	10.5046	215149	5.72E-17
Residual	8	3.91E-04	4.9E-05		
Total	9	10.5050			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.00124	2.68E-06	464	0.0000	0.0012	0.0013

Table D.18. Linear regression analysis of naphthalene calibration curve at 254 nm

Regression Statistics	
R Square	0.9995
Standard Error	0.1768
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1052	1052	33643	1.77E-12
Residual	7	0.2188	0.0313		
Total	8	1052			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.0196	1.07E-04	183.4209	0.0000	0.0193	0.0198

Table D.19. Linear regression analysis of naphthalene calibration curve at 214 nm

Regression Statistics						
R Square		0.9995				
Standard Error		0.0465				
Observations		8				
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	52.5	52.5	24308	4.70E-12	
Residual	7	0.0151	0.0022			
Total	8	52.5				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	8.53E-04	5.47E-06	155.9	1.18E-13	8.4E-4	8.66E-04

Table D.20. Linear regression analysis of phenanthrene partitioning between seawater and living *A. coalita* tissue: combined trials, based on FW

Regression Statistics						
R Square		0.7279				
Standard Error		0.0092				
Observations		35				
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	0.0358	0.0358	425	2.03E-20	
Residual	34	0.0029	8.42E-05			
Total	35	0.0386				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1708	8.29E-03	20.6124	8.61E-21	0.1539	0.1876

Table D.21. Linear regression analysis of phenanthrene partitioning between seawater and living *A. coalita* tissue: combined trials, based on DW

Regression Statistics	
R Square	0.7279
Standard Error	0.0256
Observations	35

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.2792	0.2792	425	2.03E-20
Residual	34	0.0223	6.57E-04		
Total	35	0.3015			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.4770	0.0231	20.6124	8.61E-21	0.4300	0.5241

Table D.22. Linear regression analysis of phenanthrene partitioning between seawater and heat-killed *A. coalita* tissue: combined trials, based on DW

Regression Statistics	
R Square	0.7879
Standard Error	0.0454
Observations	28

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1	0.9180	0.9180	446
Residual	34	27	0.0556	0.0021	
Total	35	28	0.9736		

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	1.9211	0.0910	21.1133	2.57E-18	1.7344	2.1077

Table D.23. Linear regression analysis of naphthalene partitioning between seawater and living *A. coalita* tissue: combined trials, based on FW

Regression Statistics	
R Square	0.7826
Standard Error	0.0052
Observations	20

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0104	0.0104	389	1.22E-13
Residual	19	5.10E-04	2.69E-05		
Total	20	0.0110			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.0500	0.0025	19.7246	4.10E-14	0.0447	0.0553

Table D.24. Linear regression analysis of naphthalene partitioning between seawater and living *A. coalita* tissue: combined trials, based on DW

Regression Statistics	
R Square	0.7827
Standard Error	0.0145
Observations	20

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0815	0.0815	389	1.22E-13
Residual	19	0.0040	2.10E-04		
Total	20	0.0855			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1397	0.0071	19.7246	4.10E-14	0.1249	0.1545

Table D.25. Linear regression analysis of naphthalene partitioning between seawater and heat-killed *A. coalita* tissue: combined trials, based on DW

Regression Statistics	
R Square	0.7942
Standard Error	0.0176
Observations	20

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.1291	0.1291	418	6.58E-14
Residual	19	0.0059	3.09E-04		
Total	20	0.1350			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable 1	0.1629	0.0080	20.4451	2.13E-14	0.1462	0.1796

Table D.26. Statistical comparison of phenanthrene uptake by living *A. coalita* tissue vs. phenanthrene uptake by heat-killed *A. coalita* tissue

Analysis of Variance for Biomass Phen Concentration - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
COVARIATES					
Liquid PAH Conc	0.158497	1	0.158497	51.17	0.0000
MAIN EFFECTS					
Alga Condition	0.194582	1	0.194582	62.81	0.0000
RESIDUAL	0.185863	60	0.00309772		
TOTAL (CORRECTED)	0.444474	62			

Algal condition (living vs. heat-killed) has a P-values less than 0.05, this factors is statistically significant at the 95.0% confidence level

Table D.27. Statistical comparison of naphthalene uptake by living *A. coalita* tissue vs. naphthalene uptake by heat-killed *A. coalita* tissue

Analysis of Variance for BIOMASS Naphthalene Concentration DW - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
COVARIATES					
Liquid Naphthalene Concentration	0.0367555	1	0.0367555	134.77	0.0000
MAIN EFFECTS					
A:Alga Condition	0.000965755	1	0.000965755	3.54	0.0678
RESIDUAL	0.0100912	37	0.000272736		
TOTAL (CORRECTED)	0.0491024	39			

All F-ratios are based on the residual mean square error.
Algal condition is not statistically significant at the 95.0% confidence level.

Table D.28. Statistical comparison of naphthalene uptake by living *A. coalita* tissue vs. phenanthrene uptake by living *A. coalita* tissue

Analysis of Variance for Biomass Conc FW - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
COVARIATES					
Liquid Concentration	0.00789418	1	0.00789418	82.48	0.0000
MAIN EFFECTS					
A:PAH	0.00620331	1	0.00620331	64.81	0.0000
RESIDUAL	0.00497685	52	0.0000957086		
TOTAL (CORRECTED)	0.0136061	54			

All F-ratios are based on the residual mean square error.
PAH (phenanthrene vs. naphthalene) has a P-values less than 0.05, this factors is statistically significant at the 95.0% confidence level

Table D.29. Linear regression analysis for UV spectrophotometer calibration for lipid analysis 201

Regression Statistics	
R Square	0.9982
Standard Error	0.2514
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	174.7	174.7	2764	4.71E-08
Residual	5	0.316	0.0632		
Total	6	175			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	16.31	0.1926	84.67	4.36E-09	15.815	16.81
X Variable 1	-9.61	0.1828	-52.6	4.71E-08	-10.08	-9.14

Table E.1. Chromatogram identification summary

Sample Name	HPLC File	UV analysis Wavelength (nm)	PAH	Phase Sampled	Biomass Condition
1.0 mg/L Phen	215p21	249	Phenanthrene	Standard in Acetonitrile	NA
215-0.3-2	215p9	249	Phenanthrene	Aqueous	No Biomass Control
215-26-AQ	215p56	249	Phenanthrene	Aqueous	Live
215-26-BM	215p77	249	Phenanthrene	Biomass	Live
220-26-AQ	220p41	249	Phenanthrene	Aqueous	Heat-Killed
220-26-BM	220p69	249	Phenanthrene	Biomass	Heat-Killed
1.0 mg/L Nap	218p7	214	Naphthalene	Standard in Acetonitrile	NA
218-0.4	218p13	214	Naphthalene	Aqueous	No Biomass Control
218-4-AQ	218p23	214	Naphthalene	Aqueous	Live
218-4-BM	218p33	214	Naphthalene	Biomass	Live
221-34-AQ	222p37	214	Naphthalene	Aqueous	Heat-Killed
221-34-BM	222p51	214	Naphthalene	Biomass	Heat-Killed

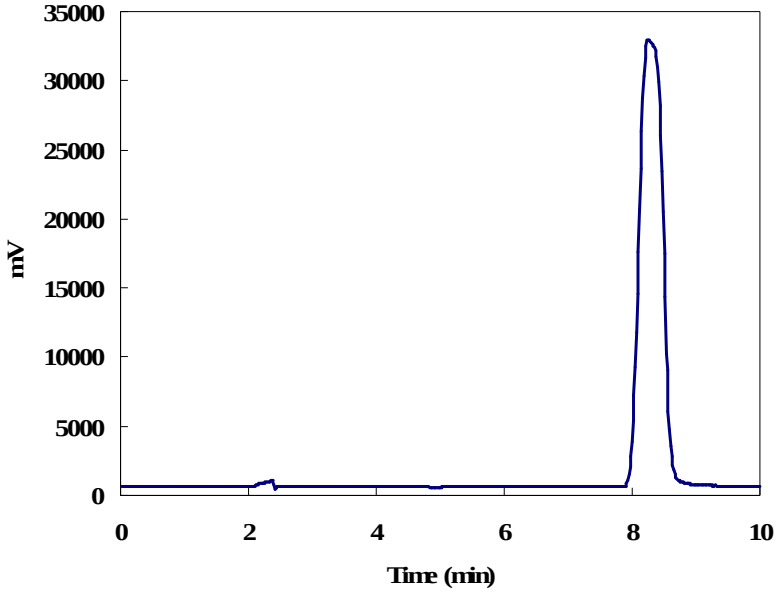

Figure E.1. HPLC Sample Chromatogram: 1.0 mg/L phenanthrene standard in acetonitrile. HPLC File: 215p21.ASC.

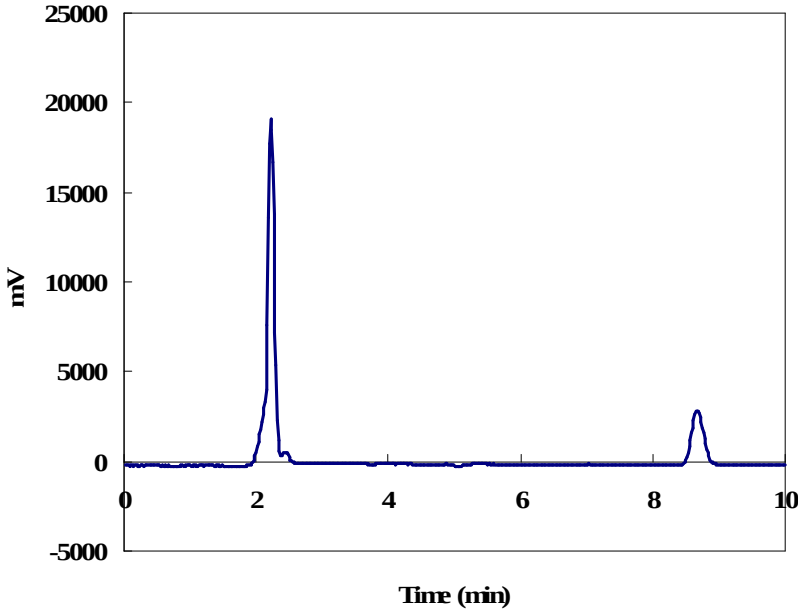

Figure E.2. HPLC Sample Chromatogram: Aqueous sample from a no biomass control experiment with phenanthrene. HPLC File: 215p9.ASC.

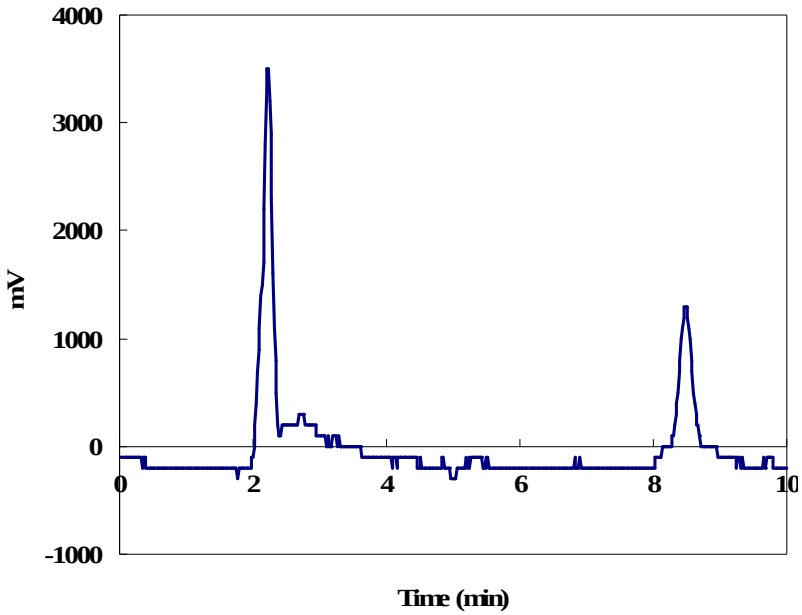

Figure E.3. HPLC Sample Chromatogram: Aqueous sample from a phenanthrene experiment with living *A. coalita* tissue. HPLC File: 215p56.ASC.

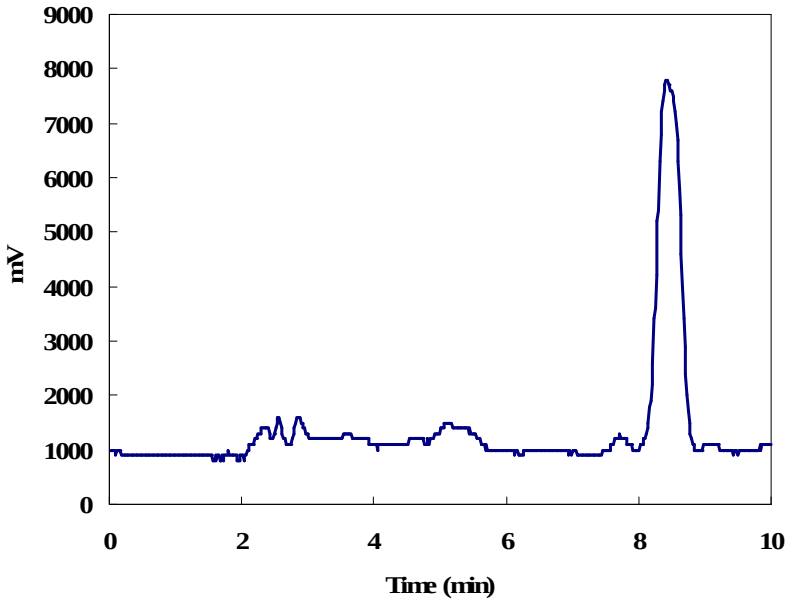

Figure E.4. HPLC Sample Chromatogram: Biomass extract of biomass from living *A. coalita* tissue exposed to phenanthrene. HPLC File: 215p77.ASC.

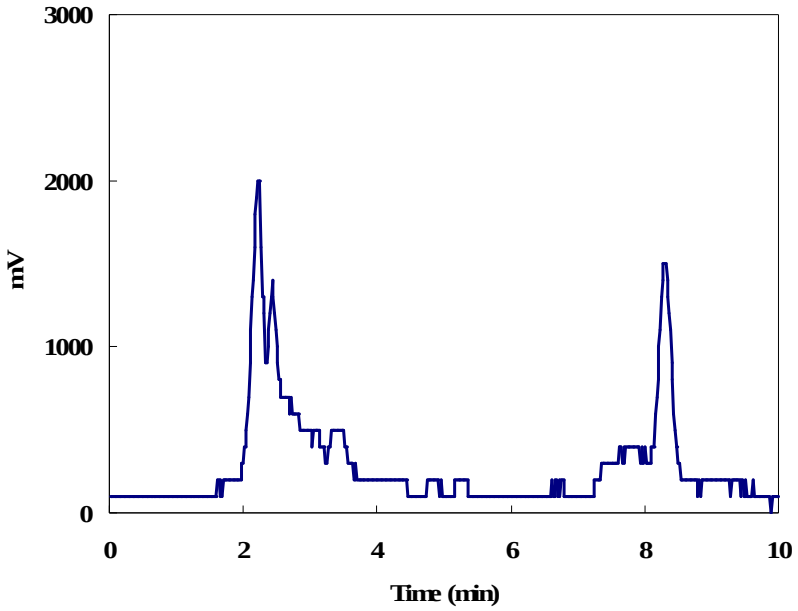

Figure E.5. HPLC Sample Chromatogram: Aqueous sample from a phenanthrene experiment with heat-killed *A. coalita* tissue. HPLC File: 220p41.ASC.

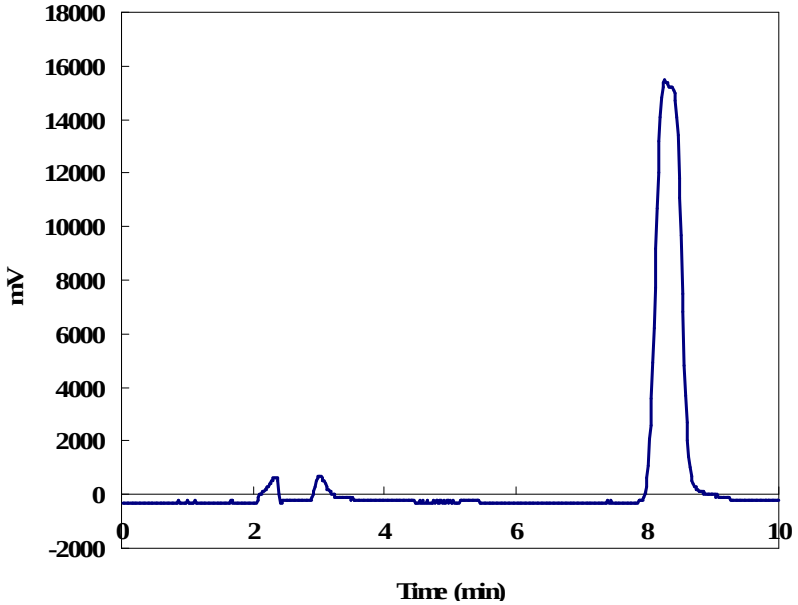

Figure E.6. HPLC Sample Chromatogram: Biomass extract of biomass from heat-killed *A. coalita* tissue exposed to phenanthrene. HPLC File: 220p69.ASC.

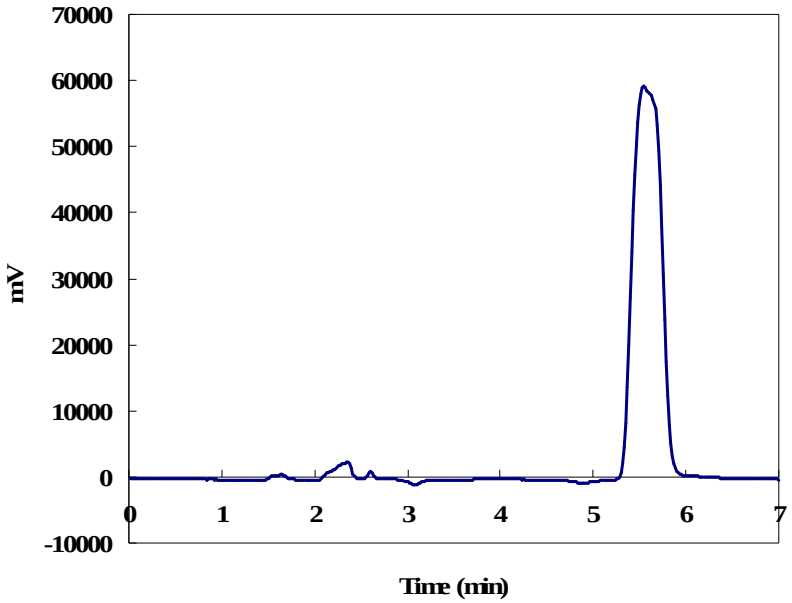

Figure E.7. HPLC Sample Chromatogram: 1.0 mg/L naphthalene standard in acetonitrile. HPLC File: 218p7.ASC.

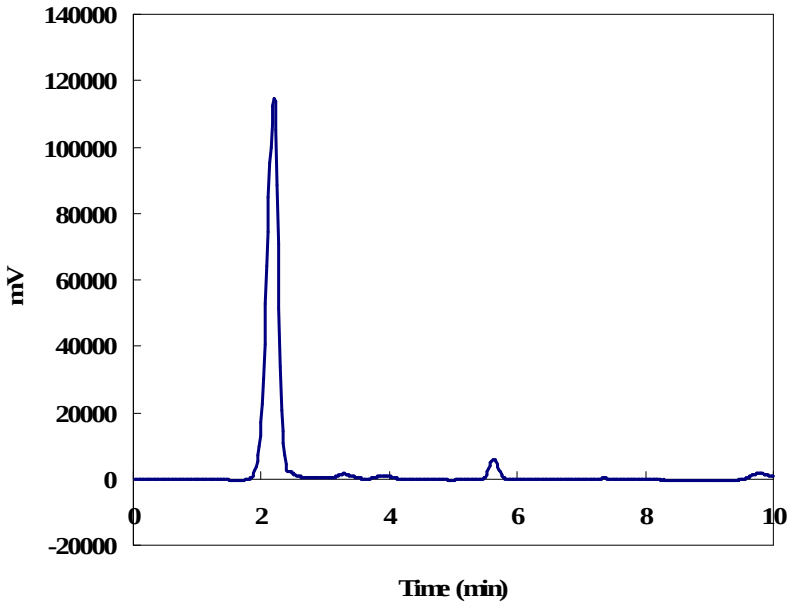

Figure E.8. HPLC Sample Chromatogram: Aqueous sample from a no biomass control experiment with naphthalene. HPLC File: 218p13.ASC.

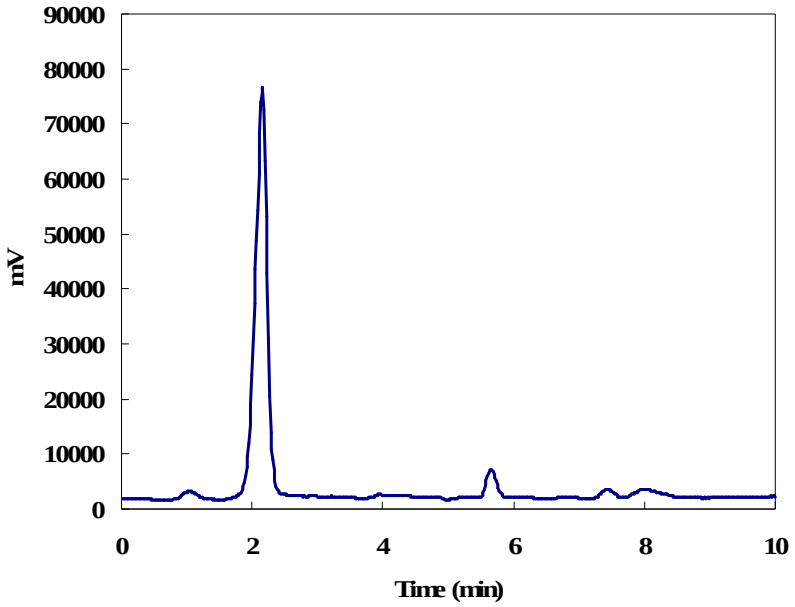

Figure E.9. HPLC Sample Chromatogram: Aqueous sample from a naphthalene experiment with living *A. coalita* tissue. HPLC File: 218p23.ASC.

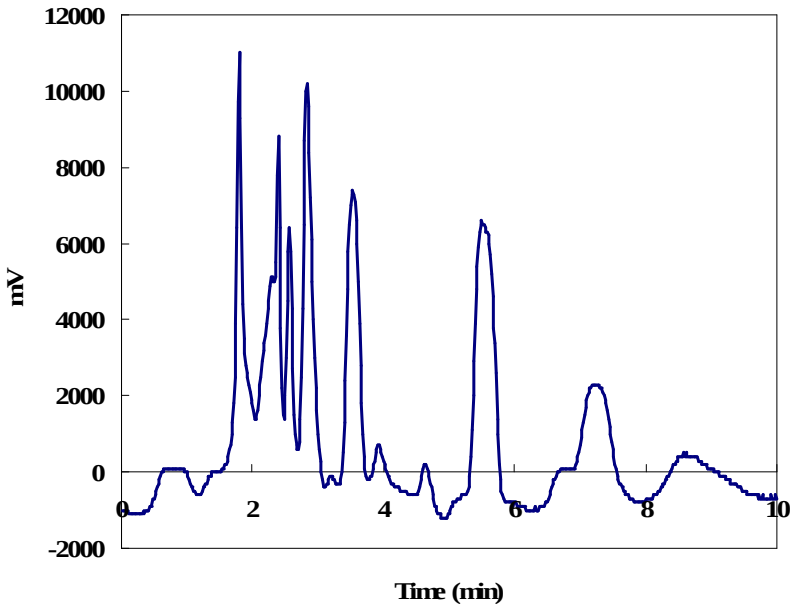

Figure E.10. HPLC Sample Chromatogram: Biomass extract of biomass from living *A. coalita* tissue exposed to naphthalene. HPLC File: 218p33.ASC.

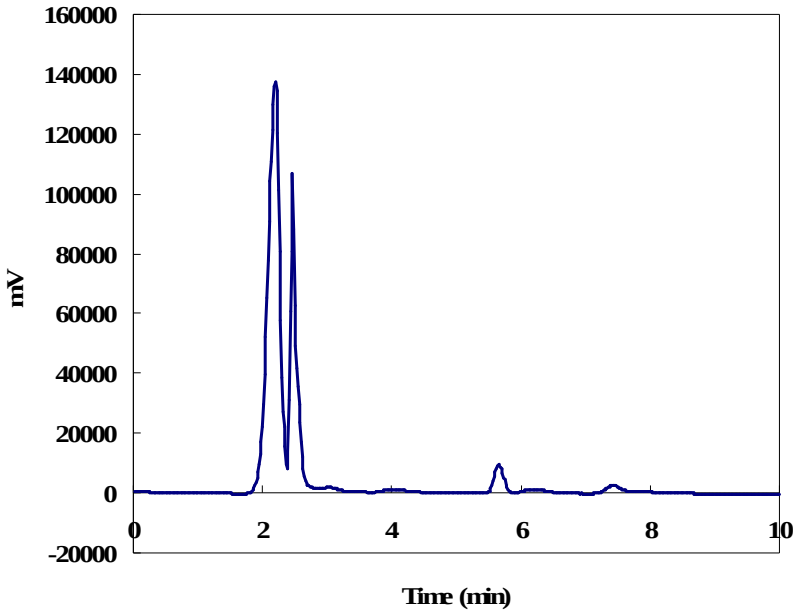

Figure E.11. HPLC Sample Chromatogram: Aqueous sample from a naphthalene experiment with heat-killed *A. coalita* tissue. HPLC File: 222p37.ASC.

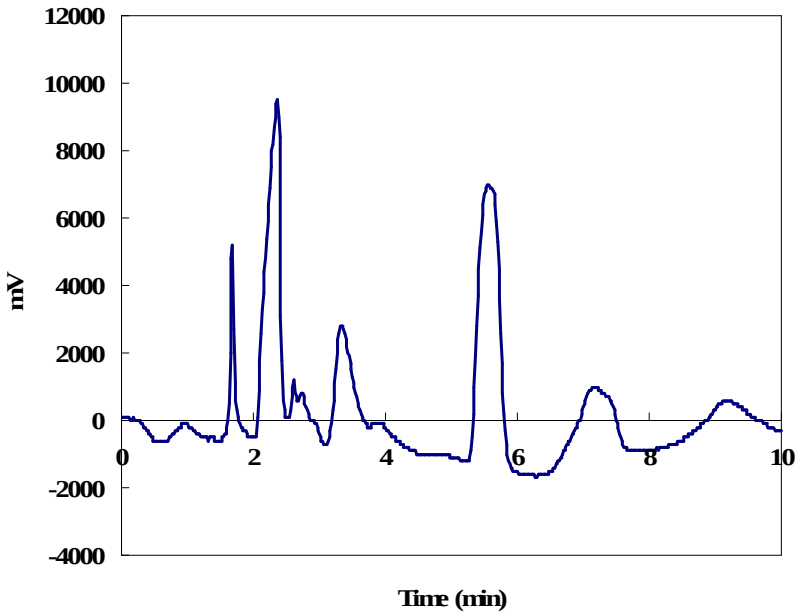

Figure E.12. HPLC Sample Chromatogram: Biomass extract of biomass from heat-killed *A. coalita* tissue exposed to naphthalene. HPLC File: 222p51.ASC.