

**Water and Environmental Research Center  
Annual Technical Report  
FY 2013**

# Introduction

## Research Program Introduction

The UAF Water and Environmental Research Center is a vibrant, relevant, and expanding research center for the State of Alaska. As WERC is housed in the UAF Institute of Northern Engineering, WERC researchers have historically focused on water resource and environmental engineering/science. However, WERC has diversified its interests and expertise in recent years, in recognition of the interdisciplinary nature of contemporary water resource research questions. The FY2013 104(b) grants reflect the diverse nature of our current research. The 104(b) grants we received for the funding period were instrumental in allowing a group of WERC-affiliated students to pursue research projects important to the State of Alaska. The topics covered were broad, and included studies related to bioremediation of oil spills, the toxicity of sulfolane in groundwater, developing climate records using stable isotopes obtained from fossil willow wood, evaluating sediments in arctic rivers, and methane cycling in thermokarst lakes.

# Characterization of sediment grain-size distributions from several Alaskan rivers

## Basic Information

<b>Title:</b>	Characterization of sediment grain-size distributions from several Alaskan rivers
<b>Project Number:</b>	2013AK115B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	AK-1
<b>Research Category:</b>	Climate and Hydrologic Processes
<b>Focus Category:</b>	Sediments, Surface Water, None
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Horacio Toniolo

## Publications

There are no publications.

## NIWR Project Report

**Project Title:** Characterization of sediment grain-size distributions from several Alaskan rivers

**Project Type:** Research

**Focus Categories:** SED, SW

**Keywords:** Suspended sediment, grain-size distributions, river discharge

**Start Date:** 03/01/13

**End Date:** 02/28/14

**PI:** Horacio Toniolo  
E-mail: hatoniolo@alaska.edu

**State:** AK

### Abstract:

In recent years, researchers at the Water and Environmental Research Center (WERC), University of Alaska Fairbanks, have conducted several studies focused on hydraulic and hydrologic conditions in streams located in the Interior and North Slope of Alaska. While preliminary work on sediment transport allowed the initial development of sediment rating curves (i.e., suspended sediment concentration vs. river discharge) in those streams, no information is available on sediment grain-size distributions. Thus, the need for gathering data on sediment size is clear. **The objectives of the proposed research are (1) to characterize suspended sediment sizes and (2) to compare temporal (in a given stream) and spatial (between streams) variations of suspended sediment distributions.** Extensive field data collection efforts on the Tanana River near Nenana; Anaktuvuk and Chandler Rivers near Umiat; and Ambler, Koyoukuk, Reed and Alatna

Rivers near Bettles will be conducted, as well as laboratory analysis, to accomplish these objectives.

**Results:**

Due to substantial budget cuts (only 43 % of the original budget was awarded) the scope, and consequently the results were reduced.

The research efforts were focused on streams located on the North Slope of Alaska, such as: Anaktuvuk, Chandler, Itkillik (Lower and Upper sites), Prince, and Seabee. Most of the streams were sampled during breakup. One site (Lower Itkillik) was also sampled in early summer. Available  $D_{50}$  are shown in Table 1.

**Table 1: Representative sediment sizes in different streams**

<b>River</b>	<b>Date</b>	<b>D50 (<math>\mu\text{m}</math>)</b>
Anaktuvuk	6/2/2013	52.80
Anaktuvuk	6/5/2013	27.63
Chandler	6/1/2013	23.5
Chandler	6/2/2013	17.94
Chandler	6/6/2013	14.46
Lower Itkillik	5/31/2013	50.03
Lower Itkillik	6/2/2013	38.2
Lower Itkillik	6/8/2013	32.49
Lower Itkillik	7/12/2013	24.8
Prince	6/11/2013	58.82
Seabee	6/12/2013	22.93
Upper Itkillik	6/3/2013	27.63

Suspended sediment sizes reported in the table indicate a temporal reduction in streams sampled multiple times. This, in fact, shows a diminution of sediment transport capacity

in those streams during the break up period. Additionally, the diameters correspond to silt.

### **Products of Project**

Sediment data gathered by the project will be used in an article describing sediment transport characteristics in the Anaktuvuk, Chandler, and Itkillik Rivers. Erica Lamb is the lead author of this manuscript. The expected submission date is July 2014.

# Creating long-term records of changes in past precipitation in northern Alaska using stable isotopes from willow wood.

## Basic Information

<b>Title:</b>	Creating long-term records of changes in past precipitation in northern Alaska using stable isotopes from willow wood.
<b>Project Number:</b>	2013AK116B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	AK-1
<b>Research Category:</b>	Climate and Hydrologic Processes
<b>Focus Category:</b>	Climatological Processes, Geomorphological Processes, Geochemical Processes
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Matthew John Wooller

## Publications

There are no publications.

**Title:** Creating long-term records of changes in past precipitation in northern Alaska using stable isotopes from willow wood.

**Principal Investigators:** Dr. Matthew J. Wooller & Benjamin V. Gaglioti

**5/31/14**

**Background:**

The current results of this project surround several datasets of modern and ancient willow isotopes across the North Slope of Alaska to infer modern precipitation sources for the region, as well as past climate change over the last 15,000 years.

The oxygen isotope value of cellulose from wood can serve as a proxy for recent and past episodes of climate change. The oxygen isotope ratio of precipitation reflects the climatic conditions in which it fell. Cellulose isotopes in wood take on the isotopic signature of the climate, and in the proper conditions can be preserved for millennia. Willow (*Salix* spp.) is a ubiquitous plant on the North Slope and commonly preserved in many depositional environments (i.e. lake and river sediments) on the North Slope today. Therefore, the isotopic signature will serve as an excellent proxy to understand climate change in this remote region.

**Preliminary Results:**

-Willow cellulose isotopes from five individuals at a single location suggest that intra-site isotopic variability is around 1.5-2 per mill. This serves as the error that a single individual willow isotope value represents for a given site on the North Slope.

-Oxygen isotope values of willow cellulose isotopes at 14 locations (n=70) spanning an approximate study area of 180,000 square kilometers indicate that summer precipitation sources from the Arctic Ocean are an important source for willow growth on much of the North Slope. This suggests that the extent of summer sea ice may regulate the isotopic nature and amount of precipitation supplying the tundra vegetation there.

-Dendrochronology analysis (ring-width measurements and counting) on modern willow shrubs is testing this hypothesis further. The goal of this work is to test how years with variable sea ice extent and climate affects the isotopic signature of the cellulose from a single ring representing that calendar year.

-Based on the ring-width measurements and dating conducted on 78 individuals at 15 locations, the amount of growth put on in a given year for willows on the North Slope is dependent on mean June Temperature over the last 30 years. This is counter to the often-held belief that arctic tundra vegetation is limited by mean July temperature.

-The isotopic signature of individual rings will be sampled, and analyzed next month.

-Preliminary results from 200 willow cellulose samples from a 15,000-year time series of radiocarbon dated willow wood from a lake sediment record in the Arctic Foothills region suggest that rapid and far-ranging climate changes occurred at the end of the last ice age.

-Two time periods between 14,500 and 12,500, and between 11,500 and 8,000 had cellulose isotope values that were greater than those across the North Slope today

suggesting either warmer-than-present conditions or more proximal precipitation sources from the Arctic Ocean.

-Cellulose isotope values of willow wood dating to the Younger Dryas cold interval (12,500-11,500 years ago) that occurred between the warm intervals had lower-than-present values that occur on the North Slope today. These data suggest a cooler-than-present, and/or more distal source of precipitation moderated by the capping of the Arctic Ocean by sea ice.

-The end of the Younger Dryas was marked by a rapid (a few centuries) shift in isotope values and may offer the best analogue for the rate and magnitude of current and future warming.

-The quantified climatic meaning of this time series will be completed later this summer when the individual ring values can give us a better data for modeling what climate the isotopic signatures of the past represent.

# Simulating the effect of wave action on fate of crude oil spilled at Alaskan shores

## Basic Information

<b>Title:</b>	Simulating the effect of wave action on fate of crude oil spilled at Alaskan shores
<b>Project Number:</b>	2013AK117B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	AK-1
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Treatment, Toxic Substances, None
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Silke Schiewer

## Publications

1. Iverson, A. Public Poster Presentation (March 2013). Simulation of the effect of wave action on fate of crude oil spilled at Alaskan shores. BP Energy Center, Anchorage, AK.
2. Iverson, A. Public Poster Presentation (2014). Assessing the fate and transport of crude oil through an arctic coastline sediments, based on sediment structure and wave action. Birch Hill Ski Area Recreation Center, Fairbanks, AK.

**Title: Simulating the effect of wave action on fate of crude oil spilled at Alaskan shores**

**Start date: March 1, 2012**

**End date: Feb 28, 2013**

**Focus Category:** Treatment (TRT), Toxic Substances (TS)

**Descriptors:** bioremediation, contaminated soil, crude oil, contaminants in cold regions, contaminant transport

**PI:** Silke Schiewer, Associate Professor, WERC, UAF,  
[sschiewer@alaska.edu](mailto:sschiewer@alaska.edu), (907) 474 2620

**Products of Project:**

Poster Presentation

Iverson, A. *Public Presentation* (March 2013). Simulation of the effect of wave action on fate of crude oil spilled at Alaskan shores. BP Energy Center, Anchorage, AK.

Iverson, A. *Public Presentation* (March 2014). Assessing the fate and transport of crude oil through an arctic coastline sediments, based on sediment structure and wave action. Birch Hill Ski Area Recreation Center, Fairbanks, AK.

Future Publications:

Iverson, A. *Master's Thesis* (2015). Assessing the fate of crude oil through an arctic coastline sediments, based on sediment structure and wave action.

**Introduction: problem, objectives, and relevance**

Problem

The U.S Department of Energy states that the United States currently relies on foreign countries for 45% of the oil we use today. The recent prospect of drilling in the Beaufort Sea indicates that the chances of an oil spill may increase. Historic oil spills such as that of the Exxon Valdez show that long term effects on ecosystems can occur as a result of oil spills, with consecutive impacts on the fisheries industry. A number of studies have been performed in the past evaluating different response strategies to help clean up oil spills in the ocean. Very little research has been done to investigate the fate of crude oil once it reaches the shoreline. Experience with the Exxon Valdez oil spill shows that though a large percentage of the oil can be removed from the shore in a relatively short time frame by a combination of physical and biological processes, some quantities of crude oil persist over extended time periods e.g. trapped under a layer of gravel. However, no systematic study is available investigating the fate of crude oil after it reaches the shore. This study will look at the movement of both fresh and degraded crude oil through the soil profile on the Arctic Coast, with special consideration of wave action. The oil will move through the soil profile at different rates depending on the tide zone it is located in. This study will

investigate the rate at which the oil moves through the soil, oil pooling, the amount of oil remaining in the soil (not washed away), and the extent to which biodegradation can contribute to oil removal.

### Goal

From this experiment we hope to gain a better understanding of how both fresh and degraded crude oil interacts with the shoreline. The interaction that occurs between the oil and sediment is not fully understood. The 1989 Valdez spill in Prince William Sound is still having negative environmental effects. The oil at different sites pooled in the sediment and is still present there today, impacting marine mammals found in the area. Identifying the location where the oil pools will help future research better isolate the microorganisms in the soil that are capable of degrading hydrocarbons.

### Objectives

The objectives of this study were:

- 1) To investigate the fate of crude oil as it moves through an Arctic shoreline based on wave action.
- 2) Determine how the addition of fertilizer will impact the biodegradation of crude oil on a shore line

### Relevance

This study is part of a two part project looking at the fate of crude oil on an Arctic shoreline. By the completion of this project the effects of crude oil and fertilizer on microbial biodegradation and the transport of crude oil will be determined. This knowledge, along with the ability to predict how an environment will react to a spill under specific environmental conditions, will help to create a better environmental response plan if a spill were to occur.

### **Methods**

Soil samples from the inter-tidal zone of typical beaches in Barrow (at the intersection of the Beaufort and Chukchi seas) will be used to obtain soil profiles representative for an Arctic Alaskan coast. The samples will be characterized in terms of grain size distribution, hydraulic conductivity, porosity and bulk density. The collected beach sediment will be used in laboratory experiments to study a) contaminant transport and b) biodegradation.

Alaska North Slope Crude obtained from Flint Hills Resources will be used as the contaminant to be studied.

Experiments will be conducted in the well-equipped laboratories of the WERC at UAF.

### *Setup:*

Using soil samples obtained from Barrow, researchers created a scale model of the soil composition typical of a tidal zone in a Plexiglas wave tank as shown in Fig. 1. The tank will include a simulated beach slope with an adjacent water body of simulated sea water. An electric motor will move a paddle in the tank to generate waves that will simulate those crashing against the shore. Since only one such setup will be constructed, only a limited number of experiments can be performed in the wave tank. Each experiment will run for one week.

Additional experiments will be performed in mini-columns (length = 30 cm, diameter = 3.8 cm) as shown in Fig. 3 to compare crude oil and nutrient transport under a wider variety of conditions. Mini-column experiments will run for 3 or 24 days, during which the column will be flushed every 12hrs as shown in Fig. 2.

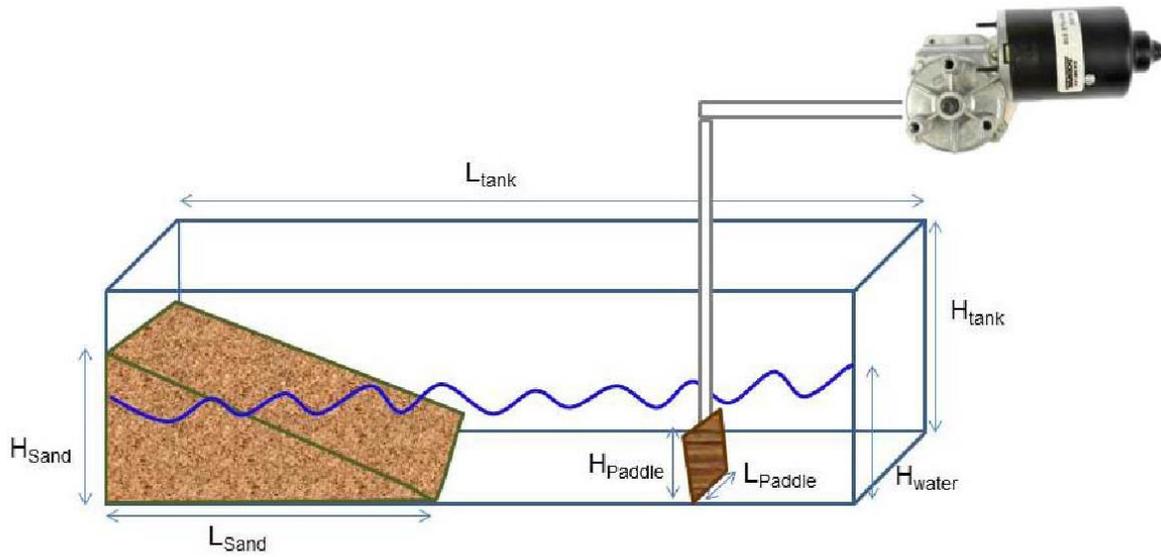


Figure 1: Scheme of Wave Tank

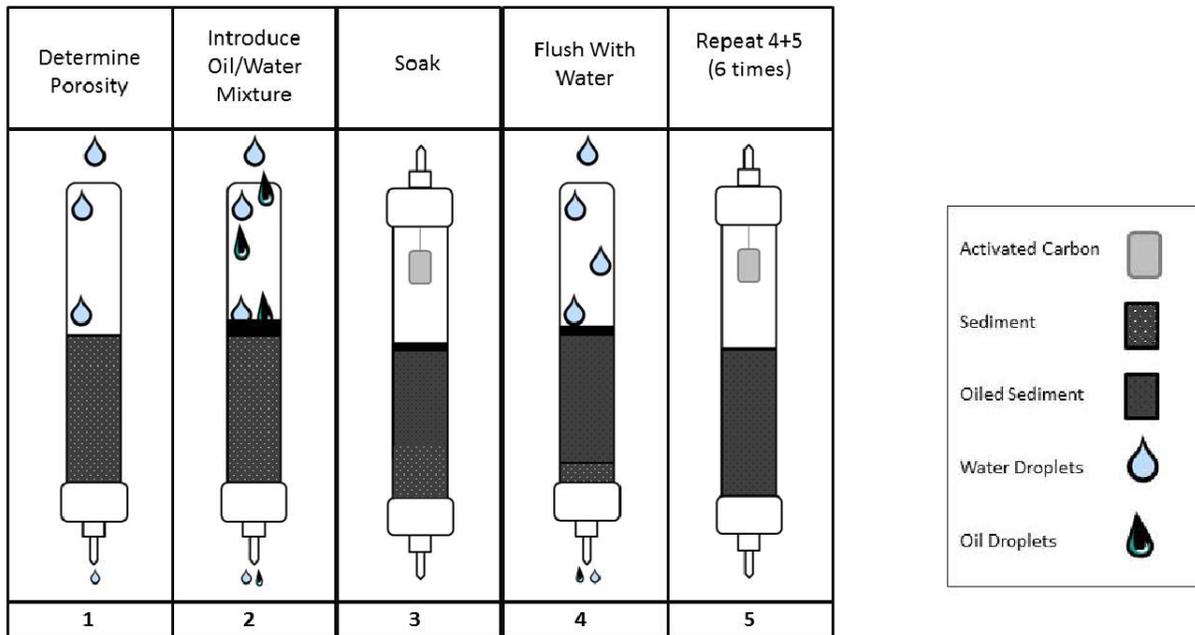


Figure 2: Scheme of column experiment

*Parameters varied:*

Generating waves at different frequencies and soil submersion levels will help to simulate the fate of oil due to wave action.

The soil type will be varied. Experiments will be performed using different beach sediment types (mostly sand, mostly gravel, sand/gravel mixture) obtained from sampling in the Barrow area.

However, sand/gravel matrices are prevalent along much of Alaska’s Artic shore, so that the conditions of this study will also be representative of other areas.

Experiments at different temperatures (4 or 20 °C) will be performed. 4 °C is considered as a typical Barrow summer temperature (near average daily temperature in July & August), and 20 °C was included as a reference value for comparison to typical temperatures in warmer areas (though record highs in Barrow may exceed this temperature) as in other biodegradation studies.

Another factor varied is nutrient addition. Experiments will be performed adding soluble fertilizer as typically used for gardening applications, or slow-release fertilizer.

The crude oil concentration will be 1 g/kg.

*Parameters analyzed:*

Crude oil remaining at different soil depths and accumulated in the activated carbon will be determined by gas chromatography mass spectrometry (GC-MS) after extraction at the end of the experiment.

Microbial respiration, i.e. CO<sub>2</sub> production will be measured as an indicator of hydrocarbon mineralization as described below.

Nutrient concentrations will be determined by ion chromatography.

**Results**

An experiment was run at 20° C to show how the movement of crude oil changed with the variation of amount of time between each flush and how fertilizer will altered the biodegradation of the crude oil. Below you will see 3 different graphs. Showing the volatilization rate of hydrocarbons, the amount of CO<sub>2</sub> released throughout the experiment, and the concentration of crude oil throughout the column.

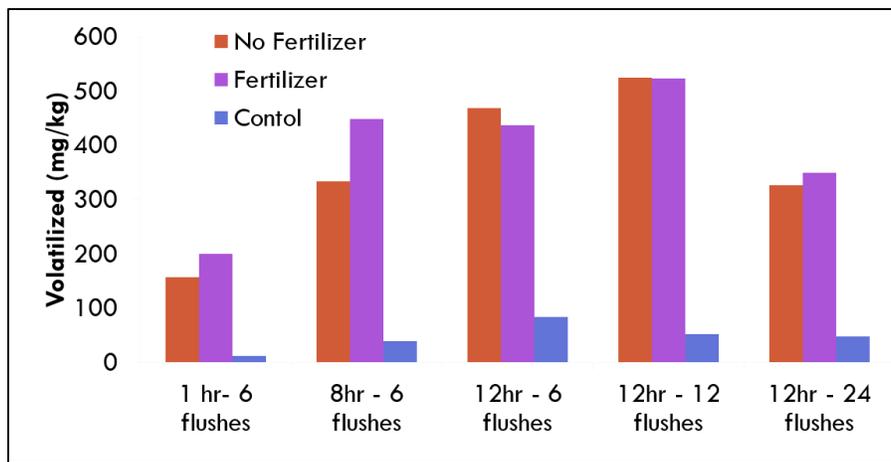


Figure 3: Amount of crude oil volatilized during each trail.

Figure 3 shows a steady volatilization rate as the amount of time that the cycles were flushed increased. 3 trails were ran were each column was flushed 6 times, but were differed in the amount of time between each flush (first 3 bar groups). From this as the amount of time between flushes increase an increase also occurs in the amount of volatilization. For these 3 columns it appears that the fertilizer has some impact in the amount of volatilization, but not enough information has been gathered to conclude anything yet. The 3 last bar columns, each had a 12hr wait between flushes, but were varied in the amount of flushes they underwent. It is interesting to see that at 24 flushes there is a decrease in volatilization rate. This is believed to be due to the lag time it takes for the microbes to begin to degrade oil. As the sediment used is actual beach sediment, it is possible that the microbes for the first 6+ days were degrading material already found in the sediment and it took them a longer time to get around to degrading the crude oil.

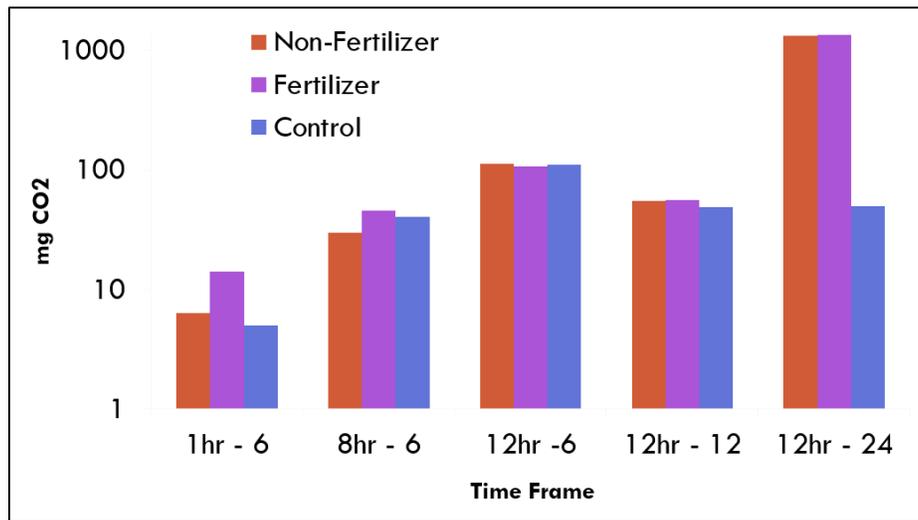


Figure 4: Amount of CO<sub>2</sub> released during each trail

Figure 4, show the amount of CO<sub>2</sub> that was released during each trail. By looking at the first 3 columns it is shown that as the amount of time between flushes grows a larger release of CO<sub>2</sub> from the columns occurs. However, when looking at the last 3, were the amount of flushes are varied, there is a decrease in volatilization at 12 flushes, and then a giant increase at 24 flushes. This is believed to be attributed to the lag time previously discussed. This lag time will also explain why the control columns were always very similar to the columns with crude oil added. Once the lag time was over 6+ days, a giant increase in the amount of CO<sub>2</sub> released is seen, indicating that crude oil is now biodegrading.

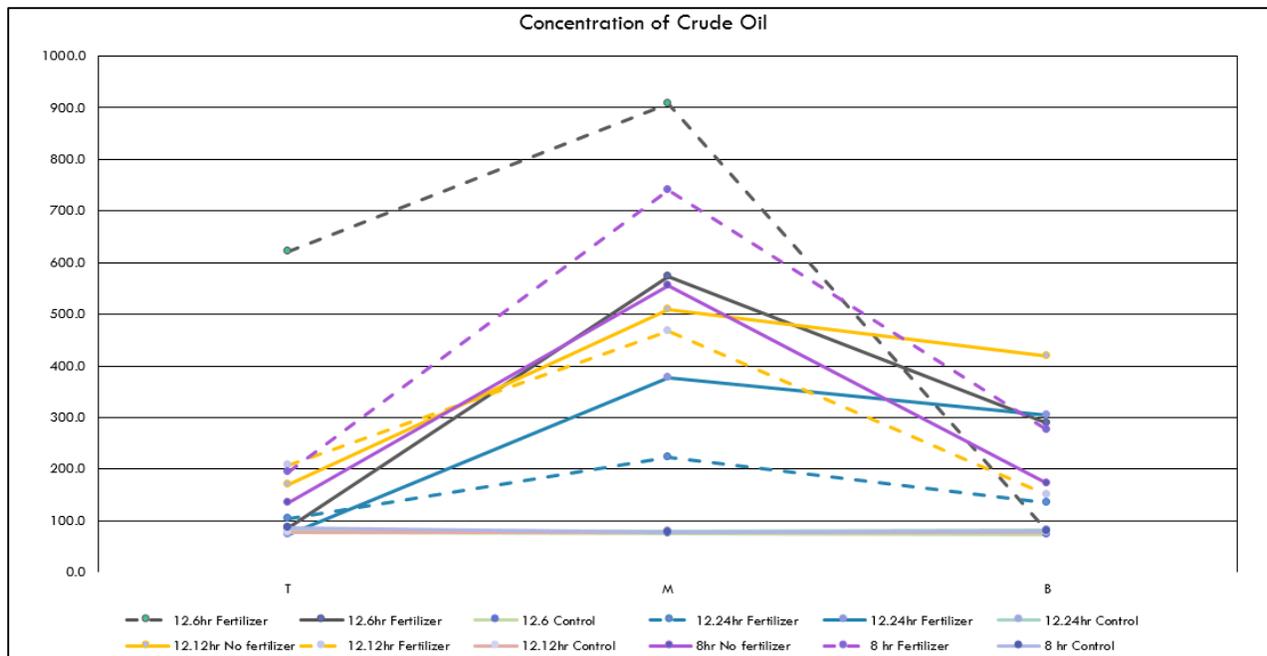


Figure 5: Graph showing concentration of crude oil in mg/kg of soil at the Top (T), Middle (M) and Bottom (B) of columns

Figure 5, shows the actual movement of crude oil through the columns. In all trials, the majority of the oil pools in the middle of the column, with lower levels in the top versus the bottom of the column. This shows that oil is being moved through the top of the column and starts to pool in the middle. The dotted lines indicate the columns where fertilizer was added, and for both the 12 and 24 flush cycles the fertilizer appears to assist in the biodegradation process. This however, is not the case when the flush cycles occur at a faster rate. This is most likely due to the fertilizer being washed out at too fast of a rate and not having enough time to properly assist in the biodegradation process.

These results are all preliminary with additional studies occurring in the future. The studies currently in progress have an additional trial added that will have flushes every 12hrs for 48 flushes (24days), this should help to definitively prove if there is a lag time. Additionally, more columns will be added so that both liquid and solid fertilizer can be tested,

# Toxicity of Sulfolane Breakdown Products in Contaminated Groundwater

## Basic Information

<b>Title:</b>	Toxicity of Sulfolane Breakdown Products in Contaminated Groundwater
<b>Project Number:</b>	2013AK118B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	AK-1
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Groundwater, Toxic Substances, Water Supply
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Mary Beth Leigh, David L. Barnes

## Publications

There are no publications.

## NIWR Progress Report

**Title:** Toxicity of Sulfolane Breakdown Products in Contaminated Groundwater

**Start date:** March 1, 2013

**End date:** February 28, 2014

**Focus Category:** GW, TS, WS

**Descriptors:** sulfolane, toxicity, groundwater, drinking water, biodegradation, North Pole, Alaska

**PI:** Dr. Mary Beth Leigh

**CoI:** Dr. David Barnes

### Products of Project:

#### Oral Presentations:

Leigh, MB and DL Barnes. Sulfolane contamination in North Pole's Groundwater: Movement and biodegradation potential. UAF Research Showcase Lecture. Feb. 19, 2014. UAF Campus, Fairbanks, AK.

Leigh, MB and DL Barnes. Sulfolane contamination in North Pole's Groundwater: Movement and biodegradation potential. Fairbanks Economic Development Corporation Meeting. March 4, 2014. Fairbanks, AK.

### Introduction: problem, objectives, and relevance

#### Problem

Sulfolane is a toxic groundwater contaminant released from some petroleum refineries and natural gas sweetening facilities. A major plume of sulfolane-contaminated groundwater has been detected in North Pole, Alaska, which has affected over 250 private drinking water wells within a 2-mile by 4-mile region from a refinery operated by Flint Hills Resources. Sulfolane can undergo degradation in the environment through natural microbial or chemical processes. Sulfolane degradation may also be promoted using an experimental system to accelerate sulfolane degradation through injection of air into the subsurface (air sparging), which shows signs of reducing sulfolane concentrations in groundwater, and may be proposed for widespread implementation. However, little is known about how sulfolane degradation occurs, what degradation products may be produced, and the toxicity of these compounds. Knowing the identity and toxicity of sulfolane breakdown products is essential to ensuring that household water purification systems are effective at removing any compounds of concern, and that methods like air sparging reduce rather than increase groundwater toxicity. Given the enormous number of drinking water wells affected by

sulfolane contamination, it is of critical importance to conduct these investigations in order to ensure that drinking water supplies are safe for the affected residents of North Pole.

### Objectives

Our proposed research aims to identify possible breakdown products of sulfolane generated through natural and accelerated microbial and chemical processes, and then to assess the toxicity of these compounds.

### Relevance

Our results are relevant to human health and will be informative to cleanup decisions by regulators and stakeholders. The project aims to reveal the identity of sulfolane breakdown products that show potential for toxicity, which will enable regulators and others to monitor them in the environment and in drinking water supplies.

### **Methods**

#### ***Putative biodegradation products***

A list of putative (predicted) products of sulfolane degradation that might be generated during both biological and abiotic degradation processes was generated using information from the literature and from site reports. To date, no pathway for sulfolane degradation has been elucidated fully. Possible strategies for biodegradation were considered, based on a proposed pathway for sulfolane biodegradation analogous to a known pathway for dibenzothiophene (Greene et al., 2000). Aerobic and anaerobic metabolic strategies for biodegradation of other related compounds were also considered. For abiotic (chemical oxidation) products, those proposed by Barr Engineering in a technical memorandum summarizing sulfolane remediation knowledge (Barr Engineering, 2012) were considered. Analogs of sulfolane were also included on the list. ADEC assisted greatly in the compilation of this information. Efforts to identify intermediates are continuing through our collaborations with OU to identify intermediates generated during sulfolane degradation.

The specific compounds that have been tested to date were selected by commercial availability, and feasibility for use in the Microtox Acute Toxicity Test. Following the compilation of a list of possible degradation products, we searched for CAS numbers and attempted to identify commercial sources of the compounds for use in toxicity studies. Not all compounds were found to be commercially available. Others were not suited for use in the aqueous toxicity assays, such as gaseous chemicals.

We obtained and tested the toxicity of 6 different putative degradation products, plus sulfolane itself, using the Microtox assay. The compounds tested thus far were 1-butanol, 1-heptanol, butyraldehyde, butyric acid, thiolane (tetrahydrothiophene), 3-hydroxysulfolane, and sulfolane.

### ***Culture supernatant test***

Because the actual products of sulfolane biodegradation are not yet known, we also performed a preliminary screening test of the toxicity of culture medium after biodegradation of sulfolane was performed by a culture obtained from the site. Details regarding culture isolation methods were provided in Section 4.2. Culture 13 was selected, which was isolated from POE-GAC and identified using 16S rRNA gene sequencing as a member of the genus, *Brevundimonas*. The culture was grown for 5 weeks in 100 ml of SOCS liquid medium containing 500 ppm sulfolane on a shaker at 21°C. The culture was then centrifuged to pellet the cells and the supernatant was decanted and used in the Microtox Toxicity Assay.

### ***Microtox Acute Toxicity Test***

Toxicity assays were performed according to the Microtox Acute Toxicity Test, using the sdx, Microtox Omni software version 4.1 (Strategic Diagnostics, Inc., Newark DE) for data capture and processing. The Basic SPT assay utilises light emissions produced by a live nonpathogenic, bioluminescent marine bacterium, *Vibrio fischeri*, following exposure to a dilution series of the suspected toxic substance. The test works by exposing the bioluminescent bacterium, *Vibrio fischeri*, to different concentrations of a test compound for 5, 15, and 30 minutes, and then measuring the resulting changes in light output by *V. fischeri*. This allows for an immediate estimate of the concentration of sample required to effectively reduce *V. fischeri* light output by 50% (EC50 concentration) (Johnson, 2005).

Total light emitted was measured using a Model 500 Analyser (AZUR Environmental, Carlsbad, CA). The Model 500 Analyser, a temperature-controlled photometer was used to maintain samples and the bacterial cell suspension at a constant temperature during the test and for measuring light output; temperature is maintained at 15°C +/- 0.5°C for 20 sample cuvettes plus a “reagent” cuvette containing the bacterial cell suspension.

It is important to note that the Microtox assay is an acute bacterial screening test, and cannot detect some modes of action or chronic effects that may occur in mammals, such as endocrine disruption, mutagenesis or carcinogenicity. The acute toxicity of a substance toward *V. fischeri* may also be greater or lesser than observed in animals (King et al., 2013). Therefore, results of the Microtox screening tests should not be considered a substitute for further toxicity testing to determine human health risks, rather, it should be considered a preliminary screening tool.

In order to determine the toxicity of potential sulfolane degradation products in relation to sulfolane, standard Microtox Toxicity assays were run on sulfolane, 1-butanol, 1-heptanol, butyric acid, butyraldehyde, tetrahydrothiophene, and 3-hydroxysulfolane. All Microtox toxicity assays were conducted according to the standard Microtox Acute Toxicity Assay protocols.

To obtain an initial estimate of the EC50 concentrations of these compounds for the purpose of selecting appropriate dilutions for focused testing, a 15 minute Microtox 45% Screen Test was conducted on sulfolane, butyraldehyde, thiolane, and 1-heptanol. Additionally, for butyric acid and 1-butanol, a 15 minute Microtox 2% Screen test was performed in order to obtain an initial EC50 estimate. After obtaining these initial EC50

estimates, a 30 minute 2% Microtox toxicity assay was performed on sulfolane, butyraldehyde, thiolane, 1-heptanol, and butyric acid. To obtain a more accurate assessment of the EC50 concentration for 1-butanol, a 30 minute 45% Microtox toxicity assay was also performed. For 3-hydroxysulfolane, a 30 minute 1% Microtox toxicity assay was also performed to obtain an accurate EC50 value.

### ***Effects of sulfolane concentration on putative sulfolane-degrading bacteria from the plume***

In order to determine if high concentrations of sulfolane might be inhibitory to growth of sulfolane-degrading bacteria, we performed growth tests on 3 taxonomically different bacterial isolates (isolate #2, #13, and #15) isolated from the North Pole plume with different concentrations of sulfolane. Isolate #2 (*Pseudomonas*) was isolated from groundwater in the air sparge system, #13 was a *Brevundimonas* strain isolated from a point-of-entry granular activated charcoal water treatment system (POE-GAC), and #15 was a *Variovorax* strain isolated from the experimental air sparge system at the Flint Hills Refinery. Each of these isolates was inoculated into 7 different 250 ml Erlenmeyer flasks per isolate, each containing 100 ml SOCS liquid medium which has sulfolane as the sole carbon and sulfur source. Different concentrations of sulfolane (3ppm, 30ppm, and 300ppm) at, well above, and well below the EC50 of sulfolane, as previously determined by the 30 minute Microtox assay. Cultures were incubated on a shaker at room temperature (approximately 21°C). Bacterial growth was determined over time by collecting a subsample of culture once per week and then performing plate counts by plating liquid culture media onto pH agar with tetrahydrothiophene-1,1-dioxide (PAT) plates according to the protocol devised by Greene and Fedorak (1998). These plates were then allowed to grow over a 4-day period, before being counted in order to determine CFU/ml in the culture at the time of sampling.

## **Results**

### ***List of potential degradation intermediates***

A draft list of 28 putative sulfolane breakdown products and analogs was generated and is presented in Table 1. The compounds are organized based on whether they are predicted to be generated during aerobic biodegradation, aerobic biodegradation, abiotic degradation, or are sulfolane analogs. One compound, 3-hydroxysulfolane, was reported in the literature to have accumulated in wetland plants exposed to sulfolane (Headley et al., 1999). Development of the list and compilation of literature, CAS numbers, analytical detection methods, and other information regarding each compound is still in progress.

### ***Microtox assays***

The EC50 concentration for sulfolane, as determined by the Microtox Toxicity Assay, was 23 ppm with 5 min exposure, 25 ppm with 15 min exposure, and 30 ppm with 30 min of

exposure. Many of the other compounds tested, 1-butanol, 1-heptanol, 3-hydroxysulfolane, butyraldehyde, thiolane, and the cell growth supernatant had higher EC50 values at 5, 15 and 30 min, indicating lower toxicity than sulfolane. It is notable that the EC50 of thiolane was similar to sulfolane for 5-min exposures, at 28 ppm for thiolane and 23 ppm for sulfolane. Butyric acid was the only intermediate that had lower EC50 concentrations (greater toxicity) than sulfolane. 1-Butanol had the highest EC50 concentrations of all the pure degradation products tested. The EC50 concentration of the cell culture supernatants was higher than any of the pure compounds tested (Table 2).

Table 1. Draft list of potential sulfolane degradation products and sulfolane analogs. Compilation of potential intermediates and their CAS#, detection methods and other information is still in progress.

Compound Name	Synonyms	CAS #	Detection methods
<b>Aerobic Biodegradation Intermediates</b>			
4-Hydroxybutane-sulfinic acid		785010-16-4	
1-Butanol	Propylcarbinol	71-36-3	GC/MS or LC/MS
1-Heptanol	Heptyl alcohol	111-70-6	
Sulfite			Oxidize to sulfate
Sulfate			Ion chromatography
Carbon Dioxide			GC/TCD or carbonic acid
Methane			GC/FID
7-Hydroxy-butane-sulfinic acid			
Sulfur dioxide			Monitor pH or GC/TCD
1-Octanol	Octanol	111-87-5	GC/MS or LC/MS
Octane 1-8 disulfinic acid			
8-Hydroxy-octane sulfinic acid			
Butyraldehyde	Butanal	123-72-8	
Butyric acid	butanoic acid	107-92-6	
<b>Anaerobic Biodegradation Intermediates</b>			
Thiolane	Tetrahydrothiophene	110-01-0	GC/MS or LC/MS
Butanethiol	Butyl mercaptan	109-79-5	
Butane		106-97-8	
Hydrogen sulfide		7783-06-4	
<b>Plant Transformation Product</b>			
3-Hydroxysulfolane		13031-76-0	GC/MS or LC/MS
<b>Aerobic Abiotic Intermediates</b>			
Butanesulfinic acid			
4-Hydroxy-butanesulfinic acid			
Octane-1,8-disulfinic acid			
Octane-1-sulfinic acid			
8-Hydroxy-octanesulfinic acid			
<b>Sulfolane Analogs</b>			
Sulfolane		77-79-2	GC/MS or LC/MS
Butane sultone		1633-83-6	GC/MS or LC/MS
3-Methylsulfolane		872-93-5	GC/MS or lc/ms
2,4-Dimethylsulfolane		1003-78-7	GC/MS or LC/MS

Table 2. Microtox EC50 results, expressed in ppm, for sulfolane, its potential intermediates, and culture supernatant following growth of a sulfolane-degrading bacterium. EC50 and 95% CI values are reported for Microtox assays performed using the 5 min, 15 min, and 30 min exposure tests. The Microtox assay measures toxicity in terms of reduction in bioluminescence by a test bacterium (*Vibrio fischeri*), and toxicity measured in this assay does not translate directly to mammalian toxicity.

Compound	Microtox Test (max % v/v)	5 min test		15 min test		30 min test	
		EC50 (ppm)	95% CI	EC50 (ppm)	95% CI	EC50 (ppm)	95% CI
Sulfolane	0	23	18-29	25	20-31	30	24-37
Butyric acid	2%	14	11-19	15	9-25	18	16-19
Thiolane	2%	28	22-36	49	37-65	63	48-84
3-Hydroxysulfolane	1%	150	75-298	133	57-306	312	300-325
1-Heptanol	2%	167	144-194	180	149-217	202	157-259
Butyraldehyde	2%	596	370-963	492	304-798	457	258-809
1-Butanol	45%	6377	4506-9026	6872	5545-8517	6781	4942-9304
Culture supernatant	45%	60285	58020-68070	60971	60220-70820	70216	63180-82410

### ***Effects of sulfolane concentration on sulfolane-degrading bacterial cultures***

The EC50 of sulfolane measured for *V. fisheri* in the Microtox assay (ranged from 23-30 ppm, depending on exposure time) was used as the basis for selecting sulfolane concentrations for studies of bacteria isolated from the site. Three concentrations of sulfolane were selected (3 ppm, 30 ppm, and 300 ppm) for tests designed to determine if the toxicity of sulfolane inhibits growth and activity of sulfolane-degrading bacteria isolated from the contaminated site. These concentrations were selected to represent the EC50 measured for *V. fisheri* (30 ppm), as well as 10-fold lower and 10-fold higher levels.

The growth of the three cultures in medium containing sulfolane as the sole carbon source at concentrations of 3, 20 and 300 ppm is shown in Figs 1A, B and C. All of the cultures tested demonstrated cell growth of at least 3 orders of magnitude during the first week of incubation, followed by a plateau and/or slow decline in cell numbers in subsequent weeks. For all three bacteria tested, there was no detectable difference in cell growth patterns between the different sulfolane concentrations.

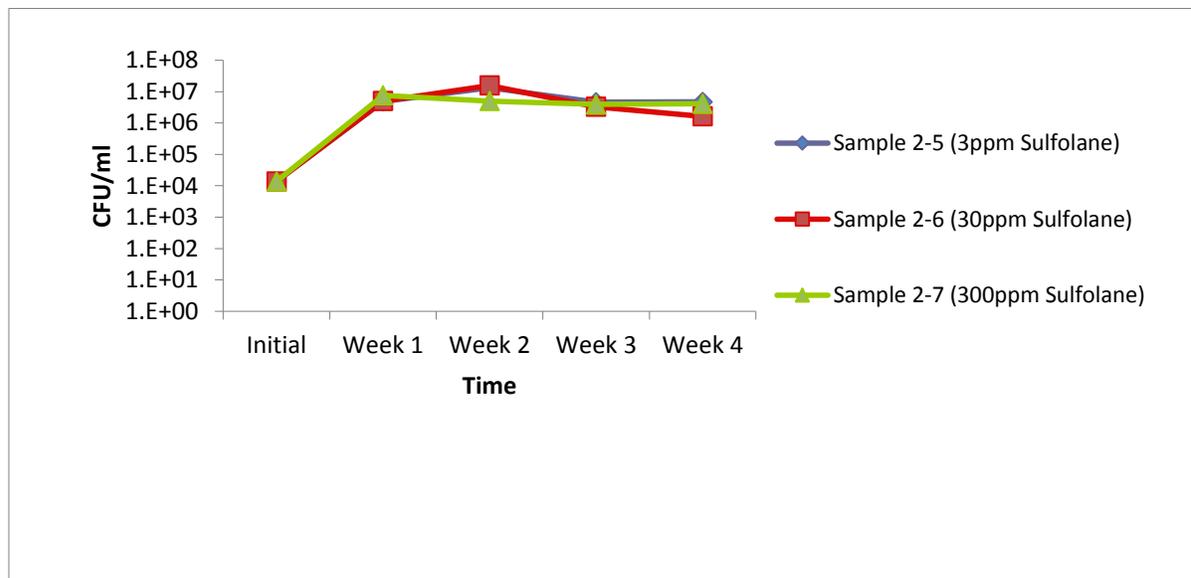


Fig. 1A. Growth of a *Pseudomonas* strain isolated from the air sparge system in liquid medium containing different concentrations of sulfolane (3 ppm, 30 ppm, and 300 ppm). Growth is expressed as colony forming units (CFU) per ml.

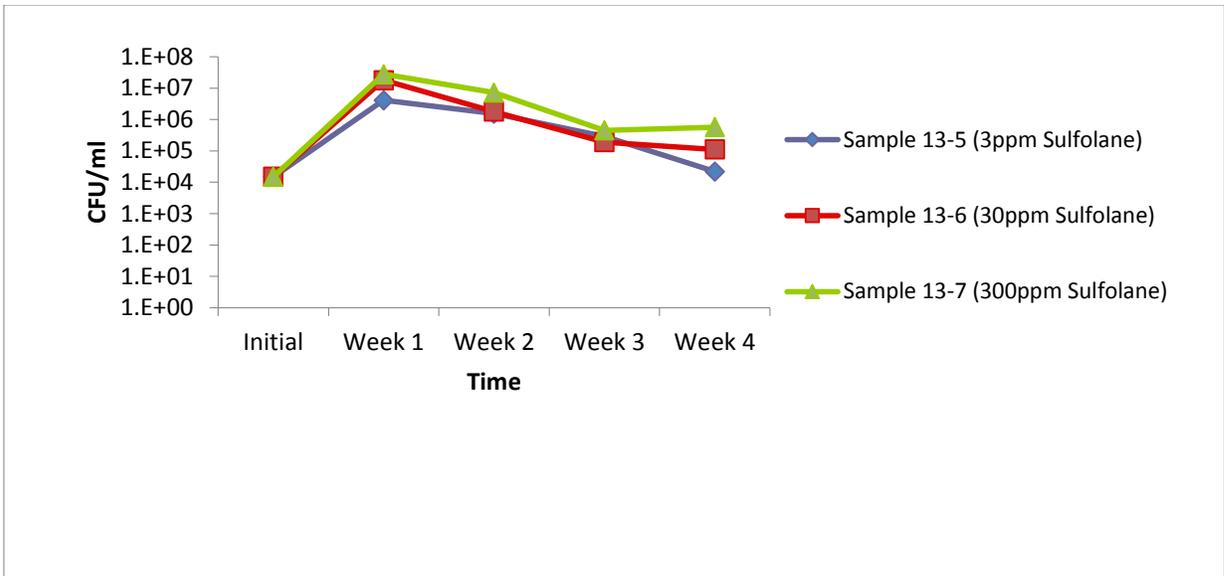


Fig. 1B – Growth of a *Brevundimonas* strain isolated from spent POE-GAC material in liquid medium containing different concentrations of sulfolane (3 ppm, 30 ppm, and 300 ppm). Growth is expressed as colony forming units (CFU) per ml.

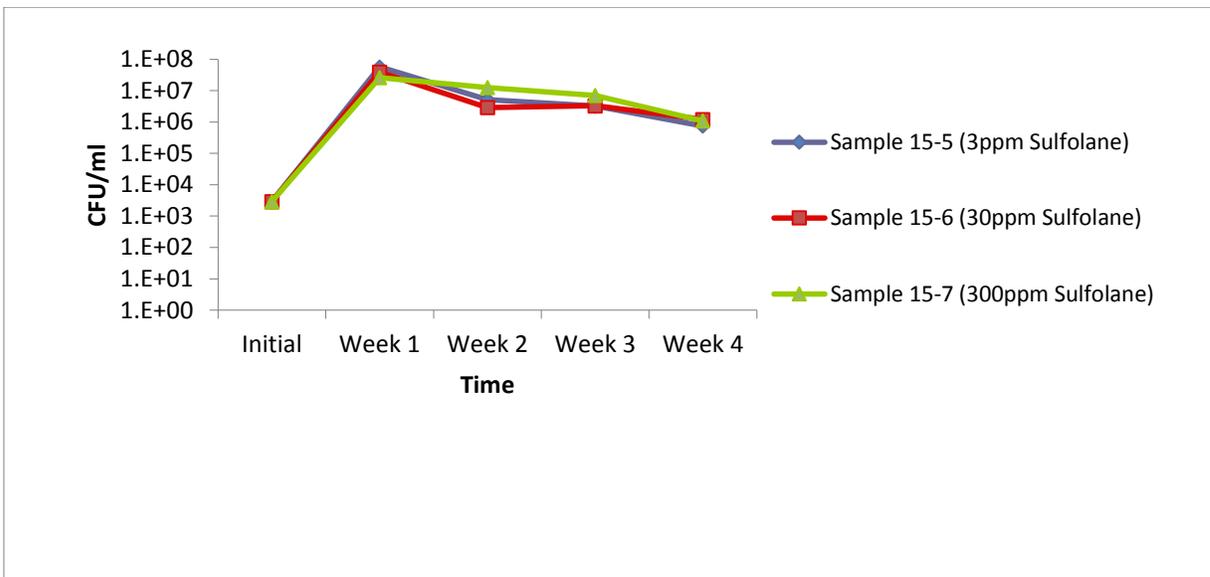


Fig. 1C – Growth of a *Variovorax* strain isolated from the air sparge system in liquid medium containing different concentrations of sulfolane (3 ppm, 30 ppm, and 300 ppm). Growth is expressed as colony forming units (CFU) per ml.

## Discussion

The EC50 of sulfolane to *V. fischeri* ranged from 23-30 ppm depending on exposure time. This is the first determination of the toxicity of pure sulfolane using the Microtox assay that has been performed, to our knowledge. A prior study using Microtox assays was performed using contaminated soils, and it was reported that soils contaminated with sulfolane ranging from 450 mg/kg to 3400 mg/kg were toxic to the test organism, a bioluminescent bacterium (*Vibrio fischeri*) (Agatonovic and E. Vaisman, 2005). The exact threshold for toxic effects to microbes was not assessed in this study.

Based on the EC50 concentrations determined in our Microtox toxicity assays, sulfolane and all of the putative intermediates tested showed toxicity toward *V. fischeri*, with toxicity varying greatly among the compounds tested.

Only one compound tested was more toxic than sulfolane, butyric acid, with EC50s of 14-18 ppm. The toxicity of butyric acid may have been due to its acidity, which could result in interruption of bioluminescence of *V. fischeri* and result in a positive signal. Butyric acid is a common fermentation product generated in the environment and in the GI tract, and is readily metabolized further by microorganisms under aerobic and anaerobic conditions. For these reasons, the production of butyric acid during sulfolane degradation is unlikely to result in substantial environmental accumulation of the compound. Nonetheless, future literature research should be conducted to investigate potential health hazards of butyric acid, and the potential value of screening groundwater and treatment systems for butyric acid should be considered.

The toxicity of thiolane was only slightly lower than sulfolane based on the Microtox assay. Thiolane generated EC50 values of 28-63 ppm (depending on exposure time), which were similar to sulfolane (23-30 ppm) especially in the 5-min test in which the EC50 of thiolane was 28 ppm compared to sulfolane's EC50 of 23 ppm. The potential production, accumulation, and health risks to humans of thiolane should be the subject of focused investigation.

Supernatant derived from a bacterial culture following sulfolane biodegradation was significantly less toxic than sulfolane or other pure compounds tested in this study. While the supernatant was found to have low toxicity relative to other compounds tested, it is worth noting that the supernatant was still toxic to *Vibrio fischeri*. We did not quantify the amount of sulfolane remaining in the culture supernatant, but it is likely that some remained and may have contributed to the toxicity. Considerably more research is needed to identify metabolites present following sulfolane biodegradation by cultures and soil/groundwater, and to assay them in a focused way for toxicity, before solid conclusions can be drawn. This study was just an initial screening test, but indicated that for this particular bacterial culture, biodegradation of sulfolane resulted in decreased toxicity in the growth medium based on the Microtox assay.

While sulfolane was found to be highly toxic to *V. fischeri* in the Microtox assay, bacterial isolates from the genera *Pseudomonas*, *Brevundimonas* and *Variovorax*, all of which were isolated from the sulfolane plume, were able to grow equally efficiently when sulfolane was present at concentrations ranging from 3-300 ppm. Growth is also commonly observed for

these strains at 500 ppm sulfolane, which is the concentration commonly used in PAT and SOCS media used to culture sulfolane-degraders.

The results suggest that EC50 concentrations determined by the Microtox Toxicity Assay do not hold true for all bacterial species, which is not unexpected due to the huge metabolic and physiologic diversity found in bacteria. As a result of this, a compound that may be highly toxic to *V. fischeri* may not be very toxic at all to a different bacterial genus or species. It is also important to note that the two tests (Microtox and our growth test on different concentrations of sulfolane) differ substantially and are not directly comparable. EC50 concentrations are determined in Microtox assays by measuring the percentage of light loss from the luminescent *V. fischeri*. Although a compound may be causing measurable light loss in *V. fischeri*, there is no measurement of whether or not the compound is actually killing the cells or inhibiting their growth. The cultures isolated from the sulfolane plume may be experiencing some toxicity, but are nonetheless able to grow and biodegrade sulfolane regardless of any possible inhibitory effects.

This study was very limited in terms of the number of potential intermediates tested. For example, one of the major aerobic degradation intermediates, 4-hydroxybutane-1-sulfinic acid, proposed by Greene et al. (2000), was not able to be obtained for testing in this study, and therefore the toxicity of this compound remains widely unknown. Also, the intermediate compounds tested in this study are only proposed intermediates, since the actual degradation pathway(s) of sulfolane remain unknown. Work is underway to identify more potential or actual metabolites, and as they are identified, additional toxicity tests should be performed.

### **Preliminary conclusions**

The results of this study provided some new insight into the toxicity of sulfolane and several of its potential degradation products. The results of this study provide further confirmation that sulfolane is a toxic compound, thereby underscoring concerns regarding human and environmental health. Of the intermediates that were tested, butyric acid was more toxic than sulfolane, which is likely due to its low pH having inhibitory effects on light production by *V. fischeri*. Butyric acid is a common metabolite of microbial fermentation in the GI tract and in the environment, and is a common food additive, so is not an intermediate of concern. The most concerning potential metabolite identified in these tests was thiolane, which was found to be only slightly less toxic than sulfolane based on the Microtox assay. Although the other potential intermediates tested showed less toxicity than sulfolane, they were all found to be toxic at varying levels. Further investigation is needed to better understand the degradation pathways of sulfolane and its true degradation intermediates, followed by toxicity testing of those compounds. Collaborative work with the University of Oklahoma is underway with separate funding from the Alaska Department of Environmental Conservation to apply advanced analytical chemical analysis methods to determine if any intermediates accumulate in lab incubations following sulfolane biodegradation, as well as in groundwater and in household point-of-entry (POE) water treatment systems, and to identify any potentially toxic intermediates that may be present.

## References

Agatonovic, V. and E. Vaisman. 2005. Sulfolane impacted soil and groundwater treatability study. EBA Engineering Consultants, Ltd. And University of Calgary Tomographic Imaging and Porous Media Laboratory.

Barr Engineering (DeJournett, T). North Pole Refinery – Summary of Sulfolane Remediation Knowledge. Technical Memorandum. Barr Engineering. March 20, 2012.

Greene, E.A., Beatty, P.H., & Fedorak, P.M. 2000. Sulfolane degradation by mixed cultures and bacterial isolate identified as a *Variovorax* sp. *Arch Microbiol.* 174, 111-119.

Greene, E. A., & Fedorak, P. M. 1998. A differential medium for the isolation and enumeration of sulfolane-degrading bacteria. *Journal of microbiological methods*, 33(3), 255-262.

Headley, J. V., Peru, K. M., & Dickson, L. C. 1999. Gas chromatographic–mass spectrometric determination of sulfolane in wetland vegetation exposed to sour gas-contaminated groundwater. *Journal of Chromatography A*, 859(1), 69-75.

Johnson, B. T. 2005. Microtox® acute toxicity test. In *Small-scale freshwater toxicity investigations* (pp. 69-105). Springer Netherlands.

King, M., Catranis, C., Soria, J. A., & Leigh, M. B. 2013. Phytochemical and toxicological analysis of *Albizia falcataria* sawdust.

# Surface water and sub-permafrost groundwater exchange effects on Alaska thermokarst-lake methane cycling

## Basic Information

<b>Title:</b>	Surface water and sub-permafrost groundwater exchange effects on Alaska thermokarst-lake methane cycling
<b>Project Number:</b>	2013AK119B
<b>Start Date:</b>	3/1/2013
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	AK-1
<b>Research Category:</b>	Climate and Hydrologic Processes
<b>Focus Category:</b>	Climatological Processes, Groundwater, Wetlands
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Katey WalterAnthony, Katey WalterAnthony

## Publications

1. Sepulveda-Jauregui, A., Martinez-Cruz, K., Walter Anthony, K., Anthony, P., et al. Seasonal and Latitudinal Variations in Dissolved Methane from 42 Lakes along a North-South Transect in Alaska. (December 2013) 2013 AGU Fall Meeting, San Francisco, California (poster).
2. Martinez-Cruz, K., Sepulveda-Jauregui, A., Walter Anthony, K., Thalasso, F. Aerobic Methane Oxidation in Alaskan Lakes along a Latitudinal Transect. (December 2013) 2013 AGU Fall Meeting, San Francisco, California.
3. Walter Anthony, K. 2012. Methane seepage from lakes along boundaries of thawing permafrost and melting glaciers. SMAP/ICESat-2 Joint Mission Applications Tutorial, ASF & NASA.
4. Gonzalez-Valencia, R., Magana-Rodriguez, F., Gerardo-Nieto, O., Sepulveda-Jauregui, A., et al. In situ measurement of dissolved methane and carbon dioxide in freshwater ecosystems by Off-Axis Integrated Cavity Output Spectroscopy. (Conditionally accepted, 2014). Environmental Science and Technology.
5. Martinez-Cruz, K., Sepulveda-Jauregui, A., Walter-Anthony, K. and Thalasso, F. Latitudinal and seasonal variation of aerobic methane oxidation in Alaskan lakes (Conditionally accepted, 2014). Global Change Biology.
6. Sepulveda-Jauregui, A., Anthony, K.M.W., Martinez-Cruz, K., Thalasso, F. Methane and carbon dioxide emissions from 42 lakes along a north-south latitudinal transect in Alaska. Biogeosciences, in review.

**Title: Surface water and sub-permafrost groundwater exchange effects on Alaska thermokarst-lake methane cycling**

**Start date: March 1, 2012**

**End date: Feb. 28, 2013**

**Focus Category:** Hydro Geochemistry - HYDGEO, Ecology - ECL, Biogeochemistry – BIOGEO.

**Descriptors:** Alaskan thermokarst lakes, permafrost type, methane, carbon, carbon isotopes, microbial activity.

**PI: Dr. Katey Walter Anthony**

**Products of Project:**

Oral presentation:

- Heslop, J. Incubation study of methane production in a thermokarst-lake system, CH<sub>4</sub> Workshop, International Arctic Research Center, Fairbanks, Alaska, Sep. 6, 2013.
- Martinez-Cruz, K., Walter Anthony, K., Sepulveda-Jauregui, A. *Public Presentation*. (September 2013). The role of methane oxidation in Alaskan lakes. CH<sub>4</sub> Workshop International Arctic Research Center (IARC), Akasofu Building, University of Alaska, Fairbanks.
- Martinez-Cruz, K., Sepulveda-Jauregui, A., Walter Anthony, K., Thalasso, F. Aerobic Methane Oxidation in Alaskan Lakes along a Latitudinal Transect. (December 2013) 2013 AGU Fall Meeting, San Francisco, California.
- Sepulveda-Jauregui, A., Walter Anthony, K., Martinez-Cruz, K. *Public Presentation*. (September 2013). Seasonal and annual methane emissions from lakes along a N-S transect in Alaska. CH<sub>4</sub> Workshop International Arctic Research Center (IARC), Akasofu Building, University of Alaska, Fairbanks.
- Walter Anthony, K. 2012. Methane seepage from lakes along boundaries of thawing permafrost and melting glaciers. *SMAP/ICESat-2 Joint Mission Applications Tutorial, ASF & NASA*.
- Walter Anthony, K. 2012. Methane emissions from arctic lakes- the role of permafrost thaw and glacier melt. *University of Alaska Climate Change Seminar Series, UAF SFOS-Juneau*.
- Walter Anthony, K. 2012. Bubble trouble - methane in Alaska's lakes. *Undergraduate Research and Scholarly Activity (URSA), UAF Research Showcase*.
- Walter Anthony, K. 2013. Overview of permafrost thaw and methane emissions and isotopes in Alaskan lakes. CH<sub>4</sub> Workshop, International Arctic Research Center, Fairbanks, Alaska, Sep. 6, 2013.

### Poster Presentation:

Sepulveda-Jauregui, A., Martinez-Cruz, K., Walter Anthony, K., Anthony, P., *et al.* Seasonal and Latitudinal Variations in Dissolved Methane from 42 Lakes along a North-South Transect in Alaska. (December 2013) 2013 AGU Fall Meeting, San Francisco, California.

### Journal Publications (published or near publication):

Gonzalez-Valencia, R., Magana-Rodriguez, F., Gerardo-Nieto, O., Sepulveda-Jauregui, A., *et al.* In situ measurement of dissolved methane and carbon dioxide in freshwater ecosystems by Off-Axis Integrated Cavity Output Spectroscopy. (Conditionally accepted, 2014). *Environmental Science and Technology*.

Martinez-Cruz, K., Sepulveda-Jauregui, A., Walter-Anthony, K. and Thalasso, F. Latitudinal and seasonal variation of aerobic methane oxidation in Alaskan lakes (Conditionally accepted, 2014). *Global Change Biology*.

Sepulveda-Jauregui, A., Anthony, K.M.W., Martinez-Cruz, K., Thalasso, F. Methane and carbon dioxide emissions from 42 lakes along a north-south latitudinal transect in Alaska. *Biogeosciences*, in review.

### Journal Publications (In preparation):

Martinez-Cruz, K., Sepulveda-Jauregui, A., Anthony, K. M. W., Thalasso, F., *et al.* Anaerobic oxidation of methane in two contrasting northern high latitude Alaskan lakes. (To submit, 2014) *Nature Biogeosciences*.

Sepulveda-Jauregui, A., Hoyos-Santillan, J., Martinez-Cruz, K., Anthony K. M. W., Temperature dependence of methanotrophy and methanogenesis in subarctic, subtropical, and tropical lakes with distinct trophic states. (To submit, 2014). *Global Change Biology*.

Sepulveda-Jauregui, A., Martinez-Cruz, K., Anthony, K. M. W., Thalasso, F. The Potential Anaerobic Oxidation of Methane in Alaskan Sediment Lakes and its Response of Different Electron Acceptors. (To submit, 2014). *Biogeochemistry*.

Sepulveda-Jauregui, A., Martinez-Cruz, K., Anthony, K. M. W., Dendooven, L., Thalasso, F. Anaerobic oxidation of methane: a widespread process in global lake ecosystems. (To submit, 2014). *PLoS ONE*.

Thalasso, F. Sepulveda-Jauregui, A., Hoyos-Santillan, J., Martinez-Cruz, K., Belmonte-Izquierdo, Y., Anthony, K. M. W. Eutrophication exacerbates potential methane production response in lake sediments to warming. (To submit, 2014). *Nature Climate Change*.

## **Introduction: problem, objectives, and relevance**

### Problem and Goal

Northern latitude lakes (> 55 °N) are important sources of methane (CH<sub>4</sub>), contributing about 20% of the total emissions from lakes (71.6 Tg). However, CH<sub>4</sub> emissions have been increased due to warming. The main response to warming in northern regions is the imbalance on inputs of organic carbon (e.g. permafrost thaws), which creates perturbations in the CH<sub>4</sub> cycling. This effect is

reflected in the CH<sub>4</sub> production (via methanogenesis) and CH<sub>4</sub> consumption (via methanotrophy and/or anaerobic oxidation of methane; AOM). At the moment, the interaction among temperature and organic carbon in the CH<sub>4</sub> production and CH<sub>4</sub> consumption is still unknown. To respond this questions, Alaska provides a valuable opportunity to study CH<sub>4</sub> cycling from lakes spanning wide variability in lake origin, climate, ecology, geology, and permafrost coverage.

Whit the proposed study, we had the opportunity to suggest gross and net balance on CH<sub>4</sub> emissions in Alaskan lakes, different pathways of CH<sub>4</sub> emissions, effect of type of permafrost on CH<sub>4</sub> cycling and microorganisms involved in AOM. This research represented one step forward in advancing our understanding in complex dynamics between northern lakes and CH<sub>4</sub>.

### Objective

To assess CH<sub>4</sub> cycling in northern lakes through the estimation different CH<sub>4</sub> emission pathways; ex situ incubations of CH<sub>4</sub> production and consumption tests and microorganisms involved in AOM.

### Relevance

The proposed work improved the understanding of CH<sub>4</sub> cycling in Alaskan lakes. In particular we established an understanding of the CH<sub>4</sub> production and consumption (aerobic and anaerobic) in lakes with contrasting type of permafrost (yedoma and non-yedoma). We characterized different CH<sub>4</sub> emission pathways of, aerobic oxidation and AOM in 42, 30 and 15 lakes along a North-South latitudinal transect in Alaska, respectively. Besides, to unmask the net and gross CH<sub>4</sub> production in lakes that are strongly influenced to warming; we did an intensive study of the CH<sub>4</sub> cycling in sediment core from thermokarst lakes (Vault lake and Miller Lake).

### **Methods**

This research was conducted in selected lakes in Alaska spanning wide variability in lake origin, climate, ecology, geology, and permafrost coverage along a North-South latitudinal transect in Alaska.

The study was divided in three main activities; field and laboratory lake characterization and ex situ incubation tests. Field and laboratory characterization consists in determining limnological and CH<sub>4</sub> emissions pathways. Incubation tests consists in determining potential CH<sub>4</sub> production via methanogenesis and potential CH<sub>4</sub> consumption via aerobic and anaerobic oxidation.

#### *CH<sub>4</sub> emissions pathways*

We sampled 42 Alaskan lakes during open-water conditions and the end of the winter ice-cover period. Our study lakes were located near the road system along a North-South transect in Alaska that spans a variety of geographic and limnological settings. From these lakes we determined four pathways of emission: (1) ebullition, (2) diffusion, (3) storage flux, and a newly identified (4) ice-sheet bubble storage flux. Additionally, limnological parameters were determined to correlate between lakes.

#### *Aerobic and anaerobic CH<sub>4</sub> oxidation*

We measured aerobic CH<sub>4</sub> oxidation rates in 30 Alaskan lakes (lakes are from the 42 Alaskan lakes studied CH<sub>4</sub> emissions pathways) located near the road system along a North-South transect in Alaska that spans a variety of geographic and limnological settings. The study was made during

winter and summer, with a new field laser spectroscopy method (Headspace Equilibration-Tunable Diode Laser Spectroscopy method). Additionally, dissolved CH<sub>4</sub> and O<sub>2</sub> concentrations were measured.

On the other hand, we measured potential AOM rates in superficial sediments from 15 Alaskan lakes (lakes are from the 42 Alaskan lakes studied CH<sub>4</sub> emissions pathways), with particular focus on Vault and Miller Lakes. To follow the oxidation of CH<sub>4</sub>, isotope <sup>13</sup>CH<sub>4</sub> was used and determined with a new laser spectroscopy system (Cavity Ring-Down Spectroscopy).

*Methane cycling in a thermokarst yedoma lake (Vault lake)*

We made an intensive study in Vault and Miller Lakes, located *ca.* 40 km and 10 km north of Fairbanks, Alaska, in a region characterized by discontinuous yedoma permafrost. From this study, we did analysis of CH<sub>4</sub> emissions pathways, CH<sub>4</sub> production and consumption, and limnological parameters.



**Figure 1.** Armando Sepulveda-Jauregui and Karla Martinez-Cruz sample limnology and surface lake sediments for methane production and anaerobic methane oxidation studies at Vault Lake, concurrent with contracted drilling (drill rig shown in background) of the deep lake sediment core obtained through the entire sediment profile and thaw bulb, into the permafrost underlying the lake. Photo by Katey Walter Anthony

## Results

In this study we observed that ebullition  $\text{CH}_4$  emissions are among the main sources of  $\text{CH}_4$  to the atmosphere and lakes located in yedoma permafrost are the major emitters of this gas. Contracted drilling and hydrology work with piezometers showed that Vault Lake has a closed thaw bulb, while Miller Lake has an open thaw bulb. Vault lake  $\text{CH}_4$  ebullition is dominated by  $\text{CH}_4$  produced by microbial decomposition in permafrost thawing in the closed thaw bulb, while in Miller Lake, groundwater discharge to the lake enhances the release of geologic methane.

With the warming climate scenarios, our results suggest that  $\text{CH}_4$  emissions will increase and yedoma lakes may exacerbate their emissions by the large inputs of organic carbon from the permafrost thaw. Stronger connections between supra-permafrost and sub-permafrost groundwater could accelerate thaw rates.

Further studies have to be done to determine the impact to warming and yedoma permafrost lake emissions. We determine that important amounts of  $\text{CH}_4$  are consumed in the column water and sediments and oxidation processes are widespread in Alaskan lakes and these processes should be incorporated in further estimations and models relative to the  $\text{CH}_4$  cycling.

Our new techniques used in this research allowed combined, real-time and in situ determination of dissolved  $\text{CH}_4$  in lakes, which is beneficial, especially for multiple field measurements in remote areas. The main contribution of our methods were avoid changes in the concentration due to biological activity and not necessary transport and storage samples. Besides, we could determine potential  $\text{CH}_4$  production and consumption in incubation tests (using  $^{13}\text{CH}_4$ ) with a novel low cost method of the Cavity Ring-Down Spectroscopy.

# Information Transfer Program Introduction

None.

# USGS Summer Intern Program

None.

<b>Student Support</b>					
<b>Category</b>	<b>Section 104 Base Grant</b>	<b>Section 104 NCGP Award</b>	<b>NIWR-USGS Internship</b>	<b>Supplemental Awards</b>	<b>Total</b>
<b>Undergraduate</b>	0	0	0	0	0
<b>Masters</b>	2	0	0	0	2
<b>Ph.D.</b>	2	0	0	0	2
<b>Post-Doc.</b>	1	0	0	0	1
<b>Total</b>	5	0	0	0	5

# **Notable Awards and Achievements**

## Publications from Prior Years

1. 2011AK100B ("Integrating Remote Sensing and Local Knowledge to Monitor Seasonal River Ice Dynamics") - Articles in Refereed Scientific Journals - Jones, C.E., K. Kielland, L.D. Hinzman, & W. Schneider. 2014. Integrating local knowledge and science: Economic consequences of driftwood harvest in a changing climate. In review. Submitted to Ecology and Society.
2. 2011AK100B ("Integrating Remote Sensing and Local Knowledge to Monitor Seasonal River Ice Dynamics") - Articles in Refereed Scientific Journals - Jones, C.E., K. Kielland, & L.D. Hinzman. 2014. Modeling groundwater upwelling as a control over river ice thickness. In review. Submitted to Hydrology Research.
3. 2011AK100B ("Integrating Remote Sensing and Local Knowledge to Monitor Seasonal River Ice Dynamics") - Book Chapters - Schneider, WS, Brewster, K, Kielland, K, Jones, C, 2013. Dangerous Ice: Changing ice conditions on the Tanana River, Fairbanks: Oral History Program, Rasmuson Library and the Institute of Arctic Biology, University of Alaska Fairbanks (book).
4. 2010AK87B ("The diminishing role of glacier runoff into Eklutna Lake: potential impacts on hydropower and water supply for the Municipality of Anchorage (year 2 renewal)") - Other Publications - 2014 Ostman JS, Loso MG, Geck JE, Sass LC, Larquier AM, and Liljedahl AK. Modeling fluvial discharge from the glacier-dominated Eklutna watershed, Alaska. Poster presented at the WaSIM users conference, Munich Germany, February 20-21. (presentation).
5. 2010AK94B ("Application of citrus peel biosorbents in repeated adsorption/desorption cycles for removal of heavy metals from waste waters") - Articles in Refereed Scientific Journals - Schiewer, S.; Iqbal, M.: Physicochemical characterization and mechanism analysis of native and protonated grapefruit peels adsorbing cadmium. Desalination and Water Treatment. (2013) in press.