JoVE: Science Education

Extraction of Lipid Biomarkers from Geological Archive Sediments - 3. ASE

--Manuscript Draft--

Manuscript Number:	10097
Full Title:	Extraction of Lipid Biomarkers from Geological Archive Sediments - 3. ASE
Article Type:	Manuscript
Section/Category:	Manuscript Submission
Corresponding Author:	Jeff Salacup UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Jeff Salacup
First Author Secondary Information:	
Order of Authors:	Jeff Salacup
Order of Authors Secondary Information:	

Author: Jeff Salacup, Ph.D, UMass – Amherst

Title: Extraction of Lipid Biomarkers from Geological Archive Sediments – 3. ASE

Overview:

Comment [DM1]: Same overview as the previous 2 extraction technique (Soxhlet; Sonication) manuscripts.

The material comprising the living "organic" share of any ecosystem (leaves, fungi, bark, tissue; **Figure 1**) differs fundamentally from the material of the non-living "inorganic" share (rocks and their constituent minerals, oxygen, water, metals). Organic material contains carbon linked to a series of other carbon and hydrogen molecules (**Figure 2**), which distinguishes it from inorganic material. Carbon's wide valency range (-4 to +4) allows it to form up to four separate covalent bonds with neighboring atoms, usually C, H, O, N, S, and P. It can also share up to three covalent bonds with a single other atom, such as the triple bond in the often poisonous cyanide, or nitrile, group. Over the past 4.6 billion years, this flexibility has led to an amazing array of chemical structures which vary in size, complexity, polarity, shape, and function. The scientific field of organic geochemistry is concerned with the identification and characterization of the whole range of detectable organic compounds, called biomarkers, produced by life on this planet, as well as others, through geologic time.

Principles:

Accelerated solvent extraction is a trademarked (Thermo Scientific Dionex) method of extraction that utilizes high temperatures (~100 °C) and pressures (~1200 psi) to increase the kinetics of the extraction process. The extractor, called an Accelerated Solvent Extractor, or ASE (**Figure 3**), can hold up to 24 individual samples. Once the ASE is loaded and set to run, it is completely automated. The ASE allows electronic control of the entire extraction process: extraction temperature, pressure, solvent volume, solvent mixture, duration, rinse, and repetition are all adjustable from sample to sample. Most organic geochemistry laboratories now use the ASE as the standard method of solvent extraction.

Biomarkers contained in the sample dissolve into the organic phase based on the rules of solubility, which with organic compounds, are controlled primarily by the polarity of both the biomarker and the solvent. This is summarized by the so-called "like dissolves like" rule, whereby relatively apolar biomarkers (those containing exclusively C and H; isoprene) dissolve in apolar solvents (such as hexane, polarity = 0.1) and more polar biomarkers (those containing O, N, S, P; GDGTs) dissolve in more polar solvents (such as methanol or dichloromethane, polarity = 5.1 and 3.1). A mixture of dichloromethane and methanol (9:1) is commonly used.

Procedure:

1. Collect the necessary materials:

    1.1. Extract 22 samples. Samples (leaves, dirt, fungi, bark, tissue), usually frozen, freeze-dried, crushed, and homogenized prior to extraction, are extracted in groups to maximize efficiency.

    Comment [JS2]: We can do fewer. I just wanted to highlight one of the major differences between this method and the others.

    1.2. Depending on the size of the sample, use vials with volumes ranging from 4-60 mL. For this experiment, use borosilicate glass vials (40 mL) and solvent safe caps. Combust vials, borosilicate glass pipettes, and weighing tins at 550 °C for 6 hr prior to ensure removal of possible organic contaminants.

    1.3. Dichloromethane and methanol are common in most organic geochemistry laboratories. Use them individually to rinse lab tools and glassware before use. A mixture of dichloromethane (DCM) to methanol (MeOH; 9:1) is used in many labs to efficiently extract biomarkers with a wide range of polarities. Solvents should be free of organic contaminants.

    1.4. Use an Accelerated Solvent Extractor (Thermo Scientific Dionex) for this experiment.

1.5. Gather the remaining materials: ASE sample cells and collection vials (cell and collection vial volume varies according to sample size), combusted (14 mm diameter) glass fiber filters (such as Whatman GF/F), an ASE filter plunger, lab spatula(s), a lab scale, and combusted diatomaceous earth or Ottowa sand (Fisher Scientific).

2. Preparation of sample cells.

2.1. Assemble a sample cell for each sample to be extracted, plus one blank.

2.1.1. For each cell, screw an end cap onto one end of the cell body.

2.1.2. Place a combusted glass fiber filter on top of each cell using solvent-rinsed tweezers. Then, gently and slowly press the filter down into the cell using the filter plunger.

2.1.3. Label the cell bodies by number (1 – 22) for each sample and write 'blank' on the blank.

2.1.4. Fill the blank with diatomaceous earth (or sand) and cap with a second end cap. Tighten by hand.

3. Preparation of Sample.

3.1. Place a combusted weighing tin on the lab scale and then tare.

3.2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.

3.2.1. The mass of the sample varies depending on its organic matter content. Relatively organic matter lean material (marine mud) may require several grams, while organic matter rich material (leaf tissue) may require much less.

3.3. Transfer all of the material in the weighing tin into a prepared ASE cell.

3.4. Place another glass fiber filter onto the top of the cell, then slowly and gently press down until it reaches the top of the sample using the filter plunger.

3.5. Add diatomaceous earth (or sand) to the cell until it is almost full. Be careful to remove any debris from the cell body threads.

3.6. Cap the top of the cell with another end cap.

3.7. Repeat steps 3.1 – 3.6 for each sample.

4. Preparation of collection vials.

4.1. Label each vial with the number of a corresponding cell (1 – 22 or blank) and cap with ASE collection vial cap.

5. Extraction.

5.1. Place each sample cell into a numbered slot on the upper ASE tray.

5.2. Place the corresponding collection vial in the same number slot on the lower ASE tray.

5.3. Create the extraction method using the keypad on the ASE. Extract at 100 °C and 1200 psi. Extract each sample three times with a static hold of 10 min and flush the cell body with 50% of its total volume between static holds.

5.4. Make sure the solvent bottle contains enough solvent to extract all of the samples.

5.5. Rinse the ASE three times before starting the run by pushing the "rinse" button on the ASE control pad.

5.6. Press start.

Representative Results:

At the end of the extraction, there will be a total lipid extract (TLE) for each sample. Each vial now contains the extractable organic matter from a sediment, soil, or plant tissue. These TLEs can be analyzed, and their chemical constituents identified and quantified.

Applications:

Comment [DM3]: Same as the Soxhlet and Sonication manuscripts.

Different classes of biomarkers impart information on specific aspects of the Earth system. For example, in its infancy, organic geochemistry was primarily concerned with the formation, migration, and alteration of petroleum, and many of the chemical tools organic geochemists use today are based on those initial investigations. It was through the investigation of a class of compounds called isoprenoids, having a repeating five carbon pattern (**Figure 2**), that scientists discovered petroleum comprised the chemically-altered remains of ancient primary producers, such as plankton in the ocean (converting to oil)(**Figure 4**) or peat bogs on land (coal)(**Figure 5**). Chemists at large oil companies used the ratios of a variety of compounds, each with its own known rate of alteration, to estimate how old petroleum was, where it came from, and if it was worth exploiting. Today, new biomarkers are being discovered, identified, and characterized in modern and ancient samples analyzed in organic geochemistry labs around the world. Many of today's applications seek to extract environmental information from biomarkers obtained in modern samples (leaves, soil, microbes, water samples, etc.) in order to extend the biomarker's utility to ancient sediments in an effort to reconstruct the climates,

environments, and ecosystems of the past. For example, the distribution of a group of biomarkers called glycerol-dialkyl glycerol-tetraethers (GDGTs for short), produced by a suite of archaea and bacteria, were found in modern sediments to change in a predictable manner in response to air or water temperature. Therefore the distribution of these biomarkers in ancient sediments, or through a series of sediments of known age, can be used to reconstruct air and water temperature back several million years.

Legend:

Figure 1: Organic material, such as trees, leaves, and moss, are chemically and visually distinct from inorganic material, such as pavement.

Figure 2: Isoprene comprises five carbon atoms and two double bonds. When added together in biosynthesis reaction, they can form complex molecules diagnostic for the presence of life. For example, 2, 6, 10, 15, 19-pentamethyleicosane, commonly found in cyanobacterial mats.

Figure 3. An Accelerated Solvent Extractor (ASE).

Figure 4: Illumination of plankton at Maldives.

Figure 5: Peat bog at 4500 m elevation in the Ecuadorian Andes.

Author: Jeff Salacup, Ph.D, UMass – Amherst

Title: Extraction of Lipid Biomarkers from Geological Archive Sediments – 3. ASE

Overview:

~~The material comprising the living "organic" share of any ecosystem (leaves, fungi, bark, tissue; **Figure 1**) differs fundamentally from the material of the non-living "inorganic" share (rocks and their constituent minerals, oxygen, water, metals). Organic material contains carbon linked to a series of other carbon and hydrogen molecules (**Figure 2**), which distinguishes it from inorganic material. Carbon's wide valency range (-4 to +4) allows it to form up to four separate covalent bonds with neighboring atoms, usually C, H, O, N, S, and P. It can also share up to three covalent bonds with a single other atom, such as the triple bond in the often poisonous cyanide, or nitrile, group. Over the past 4.6 billion years, this flexibility has led to an amazing array of chemical structures which vary in size, complexity, polarity, shape, and function. The scientific field of organic geochemistry is concerned with the identification and characterization of the whole range of detectable organic compounds, called biomarkers, produced by life on this planet, as well as others, through geologic time.~~

T~~t~~he distribution of a group of organic biomarkers called glycerol-dialkyl glycerol-tetraethers (GDGTs ~~for short~~), produced by a suite of archaea and bacteria, were found in modern sediments to change in a predictable manner in response to air or water temperature [*Schouten et al.*, 2002; *Weijers et al.*, 2007]. Therefore, the distribution of these biomarkers in ~~ancient sediments, or through~~ a ~~series~~ sequence of sediments of known age~~,~~ can be used to reconstruct the evolution of air and/or water temperature ~~back several million years~~ on decadal to millennial ~~,~~timescales ~~(~~**Figure 1**~~, 1)~~. The production of long high-resolution records of past climates, called paleoclimatology, depends on the rapid analysis of hundreds, possibly ~~if not~~ thousands of samples. Older extraction techniques, such as sonication or Soxhlet, are too slow. However, the newer Accelerated Solvent Extraction~~-~~ technique (Thermo Scientific Dionex) was designed with efficiency in mind.

Principles:

Accelerated solvent extraction is a trademarked (Thermo Scientific Dionex) method of extraction that utilizes high temperatures (~100 °C) and pressures (~1,200 psi) to increase the kinetics of the extraction process. The extractor, called an Accelerated Solvent Extractor, or ASE (**Figure 2**), can hold up to 24 individual samples. Once the ASE is loaded and set to run, it is completely automated. The ASE allows electronic control of the entire extraction process: extraction temperature, pressure, solvent volume, solvent mixture, duration, rinse, and repetition are all adjustable from sample to sample. Most organic geochemistry laboratories now use the ASE as the standard method of solvent extraction.

Comment [DM1]: Same overview as the previous 2 extraction technique (Soxhlet; Sonication) manuscripts.

Comment [A2]: We can't have the same Overview section in 3 (maybe 4) videos. If you want to demonstrate multiple ways to perform extractions, there needs to be 1) different components being extracted, 2) comparison between the techniques, and when you'd choose one over another.

Comment [A3]: Then why are we demonstrating sonication and Soxhlet? That question should be answered in those write-ups.

Comment [JS4]: I think that is now better addressed in all manuscripts

~~Biomarkers contained in the sample dissolve into the organic phase based on the rules of solubility, which with organic compounds, are controlled primarily by the polarity of both the biomarker and the solvent. This is summarized by the so-called "like dissolves like" rule, whereby relatively apolar biomarkers (those containing exclusively C and H; isoprene) dissolve in apolar solvents (such as hexane, polarity = 0.1) and more polar biomarkers (those containing O, N, S, P; GDGTs) dissolve in more polar solvents (such as methanol or dichloromethane, polarity = 5.1 and 3.1). A mixture of dichloromethane and methanol (9:1) is commonly used.~~

Comment [A5]: This is that same as in the other 2. These videos need to be differentiated.

Procedure:

1. Collect the necessary materials:

    1.1. Extract 22 samples. Samples (leaves, dirt, fungi, bark, tissue), usually frozen, freeze-dried, crushed, and homogenized prior to extraction, are extracted in groups to maximize efficiency.

    Comment [JS6]: We can do fewer. I just wanted to highlight one of the major differences between this method and the others.

    1.2. Depending on the size of the sample, use vials with volumes ranging from 4-60 mL. For this experiment, use borosilicate glass vials (40 mL) and solvent safe caps. Combust vials, borosilicate glass pipettes, and weighing tins at 550 °C for 6 hr prior to ensure removal of possible organic contaminants.

    1.3. Dichloromethane and methanol are common in most organic geochemistry laboratories. Use them individually to rinse lab tools and glassware before use. A mixture of dichloromethane (DCM) to methanol (MeOH; 9:1) is used in many labs to efficiently extract biomarkers with a wide range of polarities. Solvents should be free of organic contaminants.

    1.4. Obtain~~Use~~ an Accelerated Solvent Extractor (Thermo Scientific Dionex) to use for this experiment.

    ~~1.5. Gather the remaining materials: ASE sample cells and collection vials (cell and collection vial volume varies according to sample size), combusted (14 mm diameter) glass fiber filters (such as Whatman GF/F), an ASE filter plunger, lab spatula(s), a lab scale, and combusted diatomaceous earth or Ottowa sand (Fisher Scientific).~~

2. Preparation of sample cells.

    2.1. Assemble a sample cell for each sample to be extracted, plus one blank.

2.1.1. For each cell, screw an end cap onto one end of the cell body.

2.1.2. Place a combusted glass fiber filter on top of each cell using solvent-rinsed tweezers. Then, gently and slowly press the filter down into the cell using the filter plunger.

2.1.3. Label the cell bodies by number (1 – 22) for each sample and write "blank" on the blank.

2.1.4. Fill the blank with diatomaceous earth (or sand) and cap with a second end cap. Tighten by hand.

3. Preparation of Sample.

3.1. Place a combusted weighing tin on the lab scale and then tare.

3.2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.

3.2.1. The mass of the sample varies depending on its organic matter content. Relatively organic matter lean material (marine mud) may require several grams, while organic matter rich material (leaf tissue) may require much less.

3.3. Transfer all of the material in the weighing tin into a prepared ASE cell.

3.4. Place another glass fiber filter onto the top of the cell, then slowly and gently press down until it reaches the top of the sample using the filter plunger.

3.5. Add diatomaceous earth (or sand) to the cell until it is almost full. Be careful to remove any debris from the cell body threads.

3.6. Cap the top of the cell with another end cap.

3.7. Repeat steps 3.1 – 3.6 for each sample.

4. Preparation of collection vials.

4.1. Label each vial with the number of a corresponding cell (1 – 22 or blank) and cap with ASE collection vial cap.

5. Extraction.

5.1. Place each sample cell into a numbered slot on the upper ASE tray.

5.2. Place the corresponding collection vial in the same number slot on the lower ASE tray.

5.3. Create the extraction method using the keypad on the ASE. Extract at 100 °C and 1200 psi. Extract each sample three times with a static hold of 10 min and flush the cell body with 50% of its total volume between static holds.

5.4. Make sure the solvent bottle contains enough solvent to extract all of the samples.

5.5. Rinse the ASE three times before starting the run by pushing the “rinse” button on the ASE control pad.

5.6. Press start.

Representative Results:

At the end of the extraction, there is a total lipid extract (TLE) for each sample. Each vial now contains the extractable organic matter from a sediment, soil, or plant tissue. These TLEs can be analyzed, and their chemical constituents identified and quantified.

Applications:

~~Different classes of biomarkers impart information on specific aspects of the Earth system. For example, in its infancy, organic geochemistry was primarily concerned with the formation, migration, and alteration of petroleum, and many of the chemical tools organic geochemists use today are based on those initial investigations. It was through the investigation of a class of compounds called isoprenoids, having a repeating five carbon pattern (**Figure 2**), that scientists discovered petroleum comprised the chemically-altered remains of ancient primary producers, such as plankton in the ocean (converting to oil)(**Figure 4**) or peat bogs on land (coal)(**Figure 5**). Chemists at large oil companies used the ratios of a variety of compounds, each with its own known rate of alteration, to estimate how old petroleum was, where it came from, and if it was worth exploiting. Today, new biomarkers are being discovered, identified, and characterized in modern and ancient samples analyzed in organic geochemistry labs around the world. Many of today’s applications seek to extract environmental information from biomarkers obtained in modern samples (leaves, soil, microbes, water samples, etc.) in order to~~

Comment [DM7]: Same as the Soxhlet and Sonication manuscripts.

Comment [A8]: What makes these videos different are the extraction methods, not the extractant. This should be an Applications section unique to ASE.

~~extend the biomarker's utility to ancient sediments in an effort to reconstruct the climates, environments, and ecosystems of the past. For example, the distribution of a group of biomarkers called glycerol dialkyl glycerol tetraethers (GDGTs for short), produced by a suite of archaea and bacteria, were found in modern sediments to change in a predictable manner in response to air or water temperature. Therefore the distribution of these biomarkers in ancient sediments, or through a series of sediments of known age, can be used to reconstruct air and water temperature back several million years.~~

The TLEs of the extracted samples contain a wide spectrum of different organic compounds, including the GDGTs ~~we want~~ to be used to reconstruct ancient temperatures. Glycerol-dialkyl glycerol-tetraethers ~~(**Fig. 3**)~~ are a large suite of biomarkers that show sensitivity to growth temperatures. There are two groups of GDGTs, branched and isoprenoid, which differ in the character of the branching patterns on the core alkyl groups (**Figure 3**) ~~(Fig 3)~~. In the ocean, a cosmopolitan group of archaea, called Thaumarchaeota, produce isoprenoidal GDGTs [*Damste et al.*, 2002]. Branched GDGTs are produced on land in soils [*Hopmans et al.*, 2004], lakes, and in lake sediments [*Tierney and Russell*, 2009] by as yet unidentified bacteria, likely Acidobacteria [*Damste et al.*, 2011]. Both archaea and bacteria adjust the number of methyl branches in the core alkyl group according to growth temperature, and because GDGTs are stable in sediments for millions of years, ~~we generate~~ long high resolution records of climate change are generated using them.

Legend:

~~Figure 1: Organic material, such as trees, leaves, and moss, are chemically and visually distinct from inorganic material, such as pavement.~~

~~Figure 2: Isoprene comprises five carbon atoms and two double bonds. When added together in biosynthesis reaction, they can form complex molecules diagnostic for the presence of life. For example, 2, 6, 10, 15, 19-pentamethyleicosane, commonly found in cyanobacterial mats.~~

~~Figure 1. An Accelerated Solvent Extractor (ASE).~~

Figure 1~~2~~. An example of a paleoclimate record showing changes in sea surface temperature (SST) in the eastern Mediterranean Sea during the past ~27,000 years [*Castaneda et al.*, 2010]. This record comprises ~115 samples and is based on the isoprenoidal GDGT-based -TEX86 SST proxy.

Figure 2. An Accelerated Solvent Extractor (ASE).

~~Figure 3. An Accelerated Solvent Extractor (ASE).~~

Figure 3. Chemical structures of isoprenoidal and branched GDGTs.

~~Figure 4: Illumination of plankton at Maldives.~~
~~Copyright PawelG Photo.~~
~~Figure 5: Peat bog at 4500 m elevation in the Ecuadorian Andes.~~
~~Copyright Dr. Morley Read.~~

~~REFERENCES~~References:

Castaneda, I. S., E. Schefuss, J. Patzold, J. S. S. Damste, S. Weldeab, and S. Schouten (2010), Millennial-scale sea surface temperature changes in the eastern Mediterranean (Nile River Delta region) over the last 27,000 years, *Paleoceanography*, *25*, 13.

Damste, J. S. S., S. Schouten, E. C. Hopmans, A. C. T. van Duin, and J. A. J. Geenevasen (2002), Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota, *J Lipid Res*, *43*(10), 1641-1651.

Damste, J. S. S., W. I. C. Rijpstra, E. C. Hopmans, J. W. H. Weijers, B. U. Foesel, J. Overmann, and S. N. Dedysh (2011), 13,16-Dimethyl Octacosanedioic Acid (iso-Diabolic Acid), a Common Membrane-Spanning Lipid of Acidobacteria Subdivisions 1 and 3, *Appl Environ Microb*, *77*(12), 4147-4154.

Hopmans, E. C., J. W. H. Weijers, E. Schefuss, L. Herfort, J. S. S. Damste, and S. Schouten (2004), A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids, *Earth and Planetary Science Letters*, *224*(1-2), 107-116.

Schouten, S., E. C. Hopmans, E. Schefuss, and J. S. S. Damste (2002), Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures?, *Earth and Planetary Science Letters*, *204*(1-2), 265-274.

Tierney, J. E., and J. M. Russell (2009), Distributions of branched GDGTs in a tropical lake system: Implications for lacustrine application of the MBT/CBT paleoproxy, *Organic Geochemistry*, *40*(9), 1032-1036.

Weijers, J. W. H., S. Schouten, J. C. van den Donker, E. C. Hopmans, and J. S. S. Damste (2007), Environmental controls on bacterial tetraether membrane lipid distribution in soils, *Geochimica et Cosmochimica Acta*, *71*(3), 703-713.

Photo or Graphic File
Click here to download Photo or Graphic File: Jeff Salacup_ASE_Figure 1.jpg

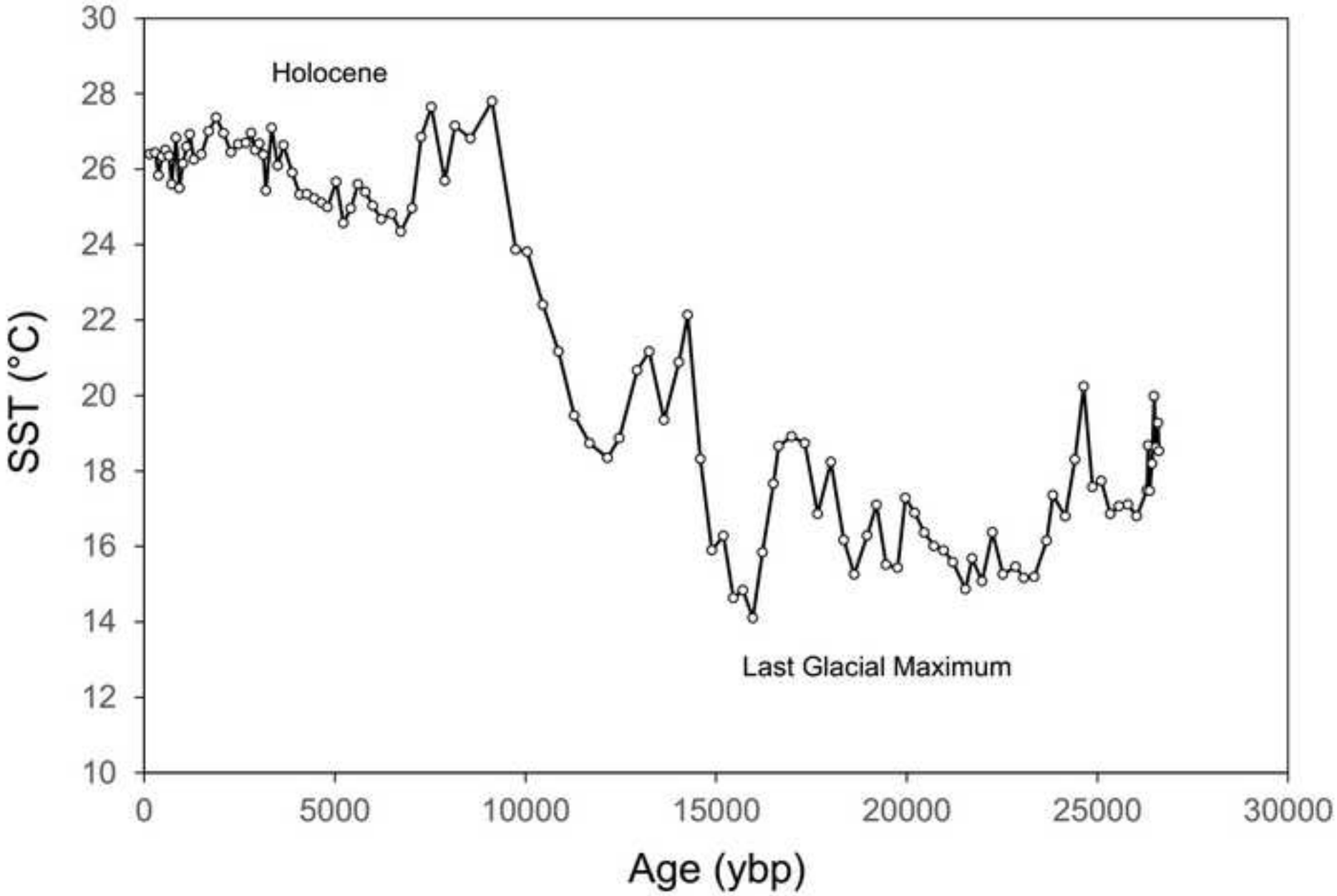
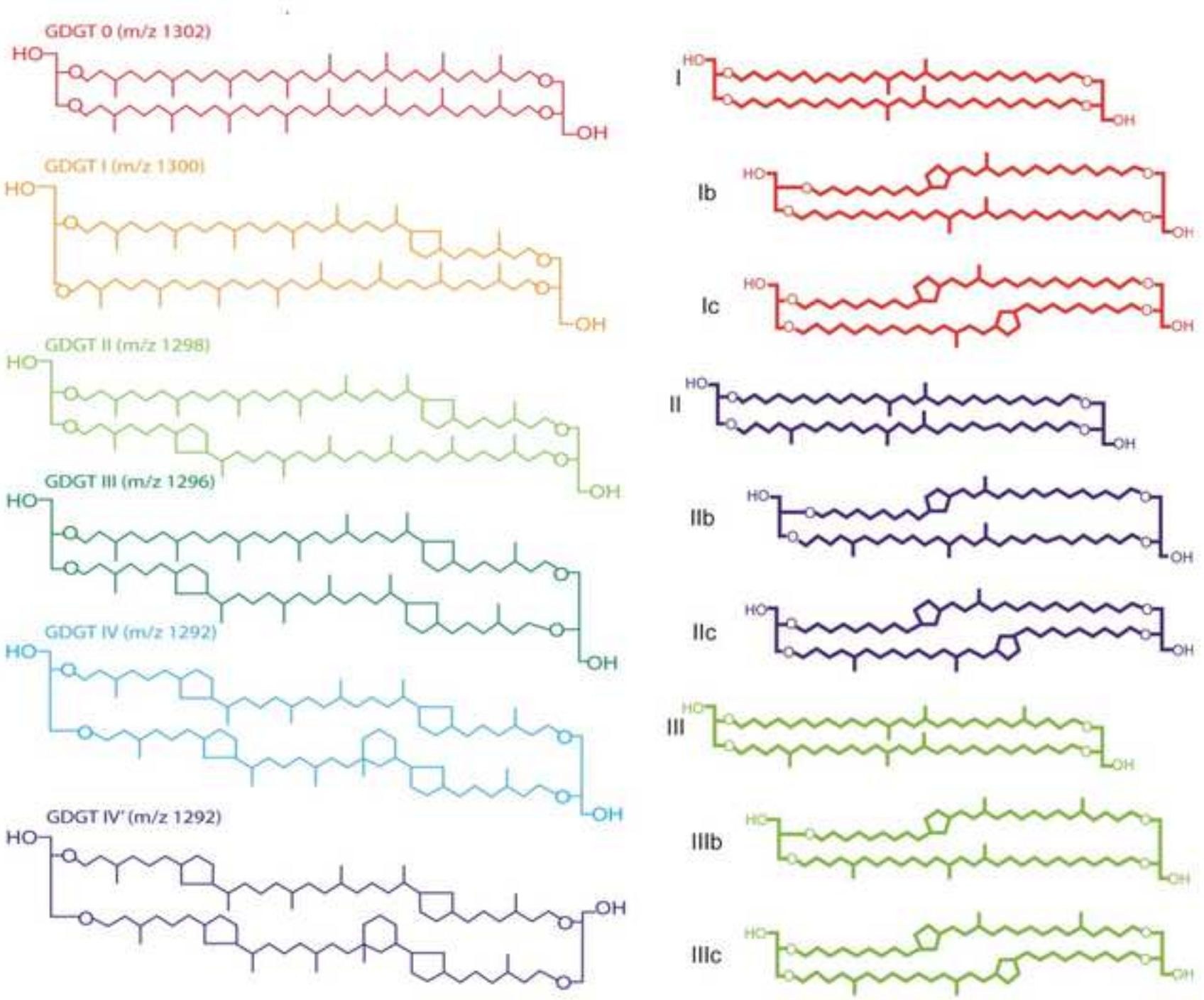


Photo or Graphic File
Click here to download Photo or Graphic File: Jeff Salacup_ASE_Figure 2.jpg

Photo or Graphic File

Click here to download Photo or Graphic File: Jeff Salacup_ASE_Figure 3.jpg



Author: Jeff Salacup, Ph.D, UMass – Amherst

Title: Extraction of Lipid Biomarkers from Geological Archive Sediments – 3. ~~ASE~~Accelerated Solvent Extraction

Overview:

The distribution of a group of organic biomarkers called glycerol-dialkyl glycerol-tetraethers (GDGTs), produced by a suite of archaea and bacteria, were found in modern sediments to change in a predictable manner in response to air or water temperature [*Schouten et al.*, 2002; *Weijers et al.*, 2007]. Therefore, the distribution of these biomarkers in a sequence of sediments of known age can be used to reconstruct the evolution of air and/or water temperature on decadal to millennial timescales (**Figure 1**). The production of long high-resolution records of past climates, called paleoclimatology, depends on the rapid analysis of hundreds, possibly thousands of samples. Older extraction techniques, such as sonication or Soxhlet, are too slow. However, the newer Accelerated Solvent Extraction technique ~~(Thermo Scientific Dionex)~~ was designed with efficiency in mind.

Principles:

Accelerated solvent extraction is a trademarked (Thermo Scientific Dionex) method of extraction that utilizes high temperatures (~100 °C) and pressures (~1,200 psi) to increase the kinetics of the extraction process. The extractor, called an Accelerated Solvent Extractor, or ASE (**Figure 2**), can hold up to 24 individual samples. Once the ASE is loaded and set to run, it is completely automated. The ASE allows electronic control of the entire extraction process: extraction temperature, pressure, solvent volume, solvent mixture, duration, rinse, and repetition are all adjustable from sample to sample. Most organic geochemistry laboratories now use the ASE as the standard method of solvent extraction.

Procedure:

1. Collection of the N~~n~~ecessary M~~m~~aterials~~:~~

    1.1. Extract 22 samples. Samples (~~leaves, dirt, fungi, bark, tissue~~in this case, sediment)~~,~~ ~~usually~~ are frozen, freeze-dried, crushed, and homogenized prior to extraction, and~~are~~ extracted in groups to maximize efficiency.

    1.2. Depending on the size of the sample, use collection vials ~~vials~~ with volumes ~~ranging from~~of 40~~o~~ or ~~-~~60 m~~l~~L ~~are used~~. For this experiment, ~~use~~ borosilicate glass vials (40 m~~l~~L) and solvent safe caps are used. Combust vials, borosilicate glass pipettes, and weighing tins at 550 °C for 6 hr prior to ensure removal of

Comment [DM1]: Jeff, production mentioned they wanted this spelled out, and that they also don't want the video to come across as promoting Dionex too much. I realize that could be a challenge as they own the trademark to ASE. Do you think just mentioning that fact and their name once (as below) will suffice?

Comment [JS2]: Yes I do.

Comment [JS3]: We can do fewer. I just wanted to highlight one of the major differences between this method and the others.

Comment [AW4]: Is this process identical to that found in the Soxhlet manuscript?

Comment [DM5]: Jeff, they're wondering about this as if it is, we can get away with filming it only once and reuse it in each video. Would save you and us some time.

Comment [JS6]: Yes, sample prep will be the same for each video.

possible organic contaminants.

1.3. Dichloromethane (DCM) and methanol are common in most organic geochemistry laboratories. Use them individually~~,~~ (methanol first, followed by DCM)~~,~~ to rinse lab tools and glassware before use. A mixture of dichloromethane ~~(DCM)~~ to methanol (MeOH; 9:1) is used in many labs to efficiently extract biomarkers with a wide range of polarities. Solvents should be free of organic contaminants.

Comment [AW7]: Does the order in which you rinse matter?

1.4. Obtain an Accelerated Solvent Extractor ~~(Thermo Scientific Dionex)~~ to use for this experiment.

2. Preparation of S~~s~~ample C~~c~~ells~~.~~

2.1. Assemble a sample cell for each sample to be extracted, plus one blank.

2.1.1. For each cell, screw an end cap onto one end of the cell body.

2.1.2. Place a combusted glass fiber filter on top of each cell using solvent-rinsed tweezers. Then, gently and slowly press the filter down into the cell using the filter plunger.

2.1.3. Label the cell bodies by number (1 – 22) for each sample and write “blank” on the blank cell.

2.1.4. Fill the blank with diatomaceous earth (or sand) and cap with a second end cap. Tighten by hand.

3. Preparation of Sample~~s~~~~.~~

3.1. Place a combusted weighing tin on the lab scale and then tare.

3.2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.

3.2.1. The mass of the sample varies depending on its organic matter content. Relatively organic matter lean material (marine mud) may require several grams, while organic matter rich material (leaf tissue) may require much less.

3.3. Transfer all of the material in the weighing tin into a prepared ASE cell.

3.4. Place another glass fiber filter onto the top of the cell, then slowly and gently press down until it reaches the top of the sample using the filter plunger.

3.5. Add diatomaceous earth (or sand) to the cell until it is almost full. Be careful to remove any debris from the cell body threads.

3.6. Cap the top of the cell with another end cap.

3.7. Repeat steps 3.1 – 3.6 for each sample.

4. Preparation of Ccollection Vvials.

4.1. Label each vial with the number of a corresponding cell (1 – 22 or blank) and cap with ASE collection vial cap.

5. Extraction.

5.1. Place each sample cell into a numbered slot on the upper ASE tray.

5.2. Place the corresponding collection vial in the same number slot on the lower ASE tray.

5.3. Create the extraction method using the keypad on the ASE. Extract at 100 °C and 1,200 psi. Extract each sample three times3x with a static hold of 10 min and flush the cell body with 50% of its total volume between static holds.

5.4. Make sure the solvent bottle contains enough solvent to extract all of the samples.

5.5. Rinse the ASE three times before starting the run by pushing the “rinse” button on the ASE control pad.

5.6. Press start.

Comment [AW8]: Are these the parameters you use for all samples? Provide some background in the Principles of when you would raise (or lower) the temperature and pressure.

Comment [JS9]: These are parameters that have been tested to result in the highest TLE yield without submitting the samples to heats and pressures that may degrade them. There is no reason to go cooler or lower pressure in our field. If a certain sample needed delicate extraction techniques, to get a highly labile or volitaile compounds for example, you’d use a different extraction method all together. However, pharma labs also use ASE and may run them at different parameters.

Representative Results:

At the end of the extraction, there is a total lipid extract (TLE) for each sample. Each vial now contains the extractable organic matter from a sediment, soil, or plant tissue. These TLEs can be analyzed, and their chemical constituents identified and quantified.

Applications:

The TLEs of the extracted samples contain a wide spectrum of different organic compounds, including the GDGTs to be used to reconstruct ancient temperatures. Glycerol-dialkyl glycerol-tetraethers are a large suite of biomarkers that show sensitivity to growth temperatures. There are two groups of GDGTs, branched and isoprenoid, which differ in the character of the branching patterns on the core alkyl groups (**Figure 3**). In the ocean, a cosmopolitan group of archaea, called Thaumarchaeota, produce isoprenoidal GDGTs [*Damste et al.*, 2002]. Branched GDGTs are produced on land in soils [*Hopmans et al.*, 2004], lakes, and in lake sediments [*Tierney and Russell*, 2009] by as yet unidentified bacteria, likely Acidobacteria [*Damste et al.*, 2011]. Both archaea and bacteria adjust the number of methyl branches and ring structures in the core alkyl group according to growth temperature, and because GDGTs are stable in sediments for millions of years, long high resolution records of climate change are generated using them.

The TEX_{86} paleo water temperature proxy is based on the ratio of certain isoprenoidal GDGTs, each containing 86 carbon atoms in its core alkyl group (**Figure 3**):

$$TEX_{86} = (GDGT\text{-}2 + GDGT\text{-}3 + GDGT\text{-}4') / (GDGT\text{-}1 + GDGT\text{-}2 + GDGT\text{-}3 + GDGT\text{-}4')$$

Paleo water temperature is then inferred using a calibration, such as the original equation:

$$TEX_{86} = 0.015T + 0.28 \quad (R^2 = 0.92)$$

Proposed by [*Schouten et al.*, 2002], where T is paleotemperature. Several other regional and local calibrations have been developed ~~since~~ to further refine the proxy for use in large lakes or in the tropics, for example.

Formatted: Subscript
Formatted: Font: Bold
Formatted: Subscript
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Subscript
Formatted: Superscript
Formatted: Font: (Default) Arial, 12 pt

Legend:

Figure 1. An example of a paleoclimate record showing changes in sea surface temperature (SST) in the eastern Mediterranean Sea during the past ~27,000 years [*Castaneda et al.*, 2010]. This record comprises ~115 samples and is based on the isoprenoidal GDGT-based TEX_{86} SST proxy.

Figure 2. An Accelerated Solvent Extractor (ASE).

Comment [AW10]: Provide more detail on this. What does the 86 notate? Does this test look at all isoprenoidal GDGTs, or 1 specific one?
Formatted: Subscript
Formatted: Subscript

Figure 3. Chemical structures of isoprenoidal and branched GDGTs.

References:

Castaneda, I. S., E. Schefuss, J. Patzold, J. S. S. Damste, S. Weldeab, and S. Schouten (2010), Millennial-scale sea surface temperature changes in the eastern Mediterranean (Nile River Delta region) over the last 27,000 years, *Paleoceanography*, *25*, 13.

Damste, J. S. S., S. Schouten, E. C. Hopmans, A. C. T. van Duin, and J. A. J. Geenevasen (2002), Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota, *J Lipid Res*, *43*(10), 1641-1651.

Damste, J. S. S., W. I. C. Rijpstra, E. C. Hopmans, J. W. H. Weijers, B. U. Foesel, J. Overmann, and S. N. Dedysh (2011), 13,16-Dimethyl Octacosanedioic Acid (iso-Diabolic Acid), a Common Membrane-Spanning Lipid of Acidobacteria Subdivisions 1 and 3, *Appl Environ Microb*, *77*(12), 4147-4154.

Hopmans, E. C., J. W. H. Weijers, E. Schefuss, L. Herfort, J. S. S. Damste, and S. Schouten (2004), A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids, *Earth and Planetary Science Letters*, *224*(1-2), 107-116.

Schouten, S., E. C. Hopmans, E. Schefuss, and J. S. S. Damste (2002), Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures?, *Earth and Planetary Science Letters*, *204*(1-2), 265-274.

Tierney, J. E., and J. M. Russell (2009), Distributions of branched GDGTs in a tropical lake system: Implications for lacustrine application of the MBT/CBT paleoproxy, *Organic Geochemistry*, *40*(9), 1032-1036.

Weijers, J. W. H., S. Schouten, J. C. van den Donker, E. C. Hopmans, and J. S. S. Damste (2007), Environmental controls on bacterial tetraether membrane lipid distribution in soils, *Geochimica et Cosmochimica Acta*, *71*(3), 703-713.