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Development of extraction techniques for the detection of signature lipids from oil *

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Pure cultures, including *Desulfovibrio vulgaris* and *Methanococcus maripaludus*, were combined with model oil samples and oil/diesel mixtures to optimize extraction techniques of signature lipids from oil in support of investigation of microbial communities in oil deposit samples targets for microbial enhanced hydrocarbon recovery. Several technique were evaluated, including standard phospholipid extraction, ether linked lipid for Archaeal bacterial detection, and high pressure extraction techniques. Recovery of lipids was ranged from 50-80% as compared to extraction of the pure culture. Extraction efficiency was evaluated by the use of internal standards. Field samples will also be tested for recovery of signature lipids with optimized extraction techniques.

Why Phospholipid analysis?

PLFA analysis for community profiling may come in question with recent developments in high throughput molecular techniques. However, PLFA analysis can be useful for certain situations and is part of a complete eco-genomics-proteomics approach.

Advantages

•PLFA are only viable in intact organisms, therefore it provides insight into the active and most dominant species present in the sample

•Since PLFA analysis involves an organic extraction, analysis can be done directly on oil samples, making one of the few techniques for estimates community structure in crude oil

•When used in conjunction with isotope analysis information on C-utilization can be obtained

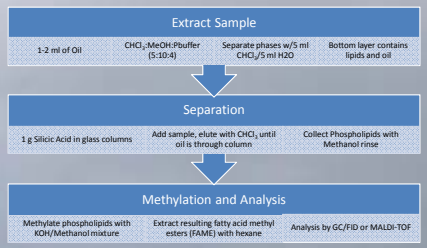
•It is a useful way to estimate biomass in complex environmental samples

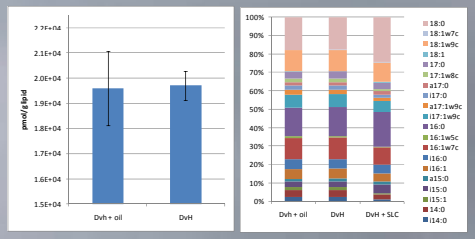
Disadvantages

•low specificity compared to DNA techniques and labor-intensive extraction and analysis

Extraction of pure cultures in oil

To test recovery of bacterial lipids in oil, a model oil mixture was prepared by mixing diesel#2 (Chevron) with SAE 30 non-detergent motor oil (Valvoline) and spiked with pure cultures of *Desulfovibrio vulgaris*, Hildenborough (DvH). Lipid signatures from DvH spiked in oil and also DvH spiked into model crude oil SLC (Southern Louisiana Crude Oil, RT Corp, Laramie, WY) were similar. Plots represents averages of triplicate samples.



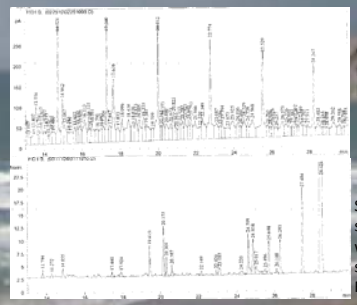


Successful extraction of lipids from oil required modifications of the standard PLFA protocol. More than 2 ml of oil overwhelmed the Silicic acid for separation, resulting in oil carryover in the phospholipid extract which gave high background during analysis. To address this problem, silicic acid in the column was increased to 1 g from 0.5 g. Also, after the sample was placed on the silicic acid, the column was rinsed with CHCl3 until the eluting solvent ran clear to assure removal of the majority of the oil.

Extraction of Crude Oil samples – method development

First try: 1-5 ml oil samples were extracted. No attempt was made to remove oil layer –it was included in the total extract (very messy) and resulting PLFA on GC was unusable due to high background

2nd try: ~ 1 ml of oil sample was extracted. Oil layer was removed (by centrifugation and cooling) during primary extraction. Resultant extract results are shown in next few slides. Still fairly low levels of biomass in most samples, but peaks can be identified



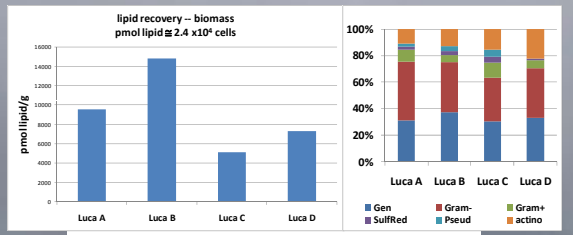
Oil residue in sample results in high background

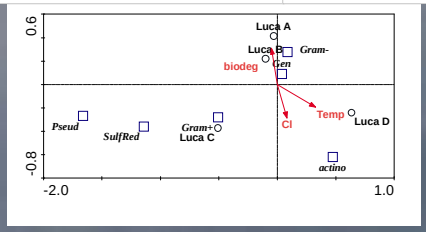
Sample showing PLFA signature, analyzed with sufficient separation of oil From lipid

Community Structure

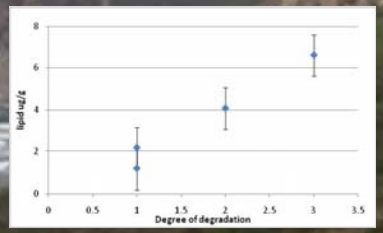
Four samples from different wells were obtained from Luca Technologies (Golden, CO). The wells, labeled A, B, C, D, contained crude oil and formation water mixtures. The same extraction technique used on the model oils was used on the crude oil samples.

Sample	Cl mg/L	In situ temp (°C)	Oil biodegradation
A	100	20	High
B	17000	27	Medium
C	24000	31	Low
D	27000	65	Low





Results showed approximately 10^7 cells per ml in the samples. Since only 1-2 ml was used for each extraction, this resulted in a low lipid recovery and only major peaks were identified. Differences were seen between the samples. The CCA analysis differentiates samples. Sample C was more closely associated with sulfate reducers and gram positive bacteria while A and B were predominantly gram negative, although sulfate reducer biomarkers were found in all samples.



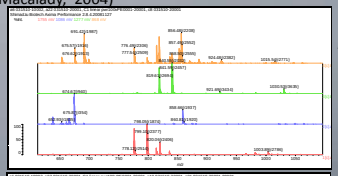
If Luca C,D were assigned to have low degradation (1) and Luca B medium degradation (2) and Luca A high degradation. There is an increase in biomass with increase in degradation. This relationship and range of biomass recovery has been shown previously in oil samples (Hallman, 2008)

Detection of Archaeal Lipids

Use of traditional GC/MS analysis for diether and tetraether lipids found in archaea requires additional extraction and derivatization and in most cases provides just a presence/absence results.

The same information was obtained using a MALDI-TOF analysis on total Lipid extract

In the top series of spectra below is shown *M. maripaludus (Mm)*, a methanogen, compared to a phospholipid standard (19:0 PC) and two diether standards. The PC was used as an internal standard in the Mm so it is present in the sample. There are also matches for the diether matches as well as a 3rd peak at 856, demonstrating presence of diether lipids in this archaea. It has been shown that Mass recoveries in 700-1000 range indicate archaeal lipids (Macalady, 2004)

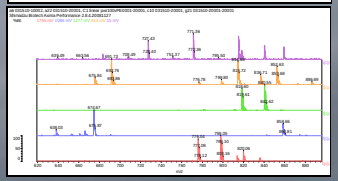


M.maripaludus

19:0 PC

Diether lipid 1

Diether lipid 2



Luca C oil

Luca B oil

Diether lipid 1

Diether lipid 2

In the lower plot, Luca sample B and C are compared with two diether lipid standards. Both show matches with the known standards indicating presence of archaeal lipids

Further development of the technique will include optimization of the MALDI matrix and/or a NIMS approach. In addition, more standards will be developed to focus on tetraether lipids which are in the range of 1000-1700 m/z

Conclusions

•Combining pure culture cell pellets with crude and model oil did Not lower recovery of phospholipids

•It is critical that residual oil be removed in the silicic acid separation step to avoid high background when quantifying PLFA on the GC

•PLFA recovered from Luca samples differentiate between wells and show a biomass in the oil of 10^7 to 10^ cells/ml

•Archaeal lipids can be detected by MALDI-TOF. Further work is needed to develop standards and increase detection of low biomass samples.

References

Hallmann, C., Schwark, L., Grice, K., 2008. Community dynamics of anaerobic bacteria in deep petroleum reservoirs. Nature Geosci 1, 588-591.

Macalady, J.L., Vestling, M.M., Baumler, D., Boekelheide, N., Kaspar, C.W., Banfield, J.F., 2004. Tetraether-linked membrane monolayers in Ferroplasma spp: a key to survival in acid. Extremophiles 8, 411-419.

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