In-situ assessment of biodegradation potential using bio-traps amended with ^{13}C-labeled benzene or toluene

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Supporting Information (SI)

Calculation of the concentration of ^{13}C carbon derived from the labeled substrate into fatty acids:

The isotope composition of fatty acids methyl esters were determined by gas chromatography combustion isotope-ratio mass spectrometry (GC-C-IRMS). The isotope composition of the fatty acid was corrected for the carbon added by the methylation procedure using equation 1:

$$\left(\frac{^{13}C}{^{12}C}\right)_{fa} = \frac{n+1}{n}\cdot\left(\frac{^{13}C}{^{12}C}\right)_{fame} - \frac{1}{n}\cdot\left(\frac{^{13}C}{^{12}C}\right)_{CH_3} \quad , \qquad (1)$$

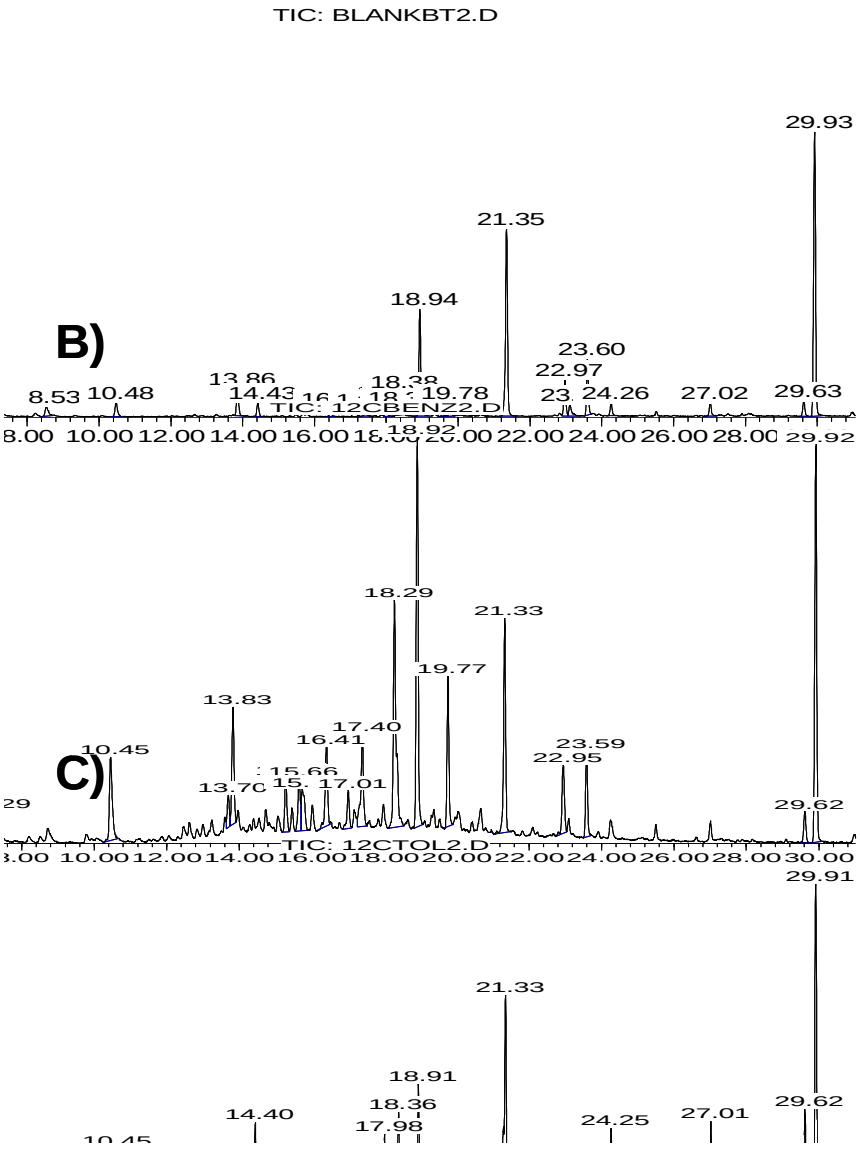
n indicates the number of carbon atoms of the fatty acid. To calculate the isotopic composition of fatty acids (fa) was needed the isotope composition of the fatty acid methyl ester (fame) and the isotope composition of the methyl group (CH_3). In the methylation procedure we used methanol with an isotope composition of -50 ± 0.2‰. The contribution of C from the labeled compound to a given fatty acid was calculated using equation 2.

$$X = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{fa} - \left(\frac{^{13}C}{^{12}C}\right)_{natural}}{\left(\frac{^{13}C}{^{12}C}\right)_{compound} - \left(\frac{^{13}C}{^{12}C}\right)_{natural}} \quad , \qquad (2)$$

where X is the proportion of C derived from the labeled compound, and $^{13}C/^{12}C$ are the isotopic ratios of the fatty acid (fa), of the labeled compound (compound) and of the

natural compound (natural). Here, we used values of 0.98 for the labeled benzene and 0.011 for the natural benzene.

Taking the methyl ester of the 16:1ω7c fatty acid in the benzene microcosm ($\delta^{13}C$ = 13410‰) as an example, is $^{13}C/^{12}C_{benzene}$ = 0.1714 using equation (1), for 98atom% labeled benzene and $\delta^{13}C$ of -50‰ for the methyl group introduced. Equation (2) then yields X = 0.166, indicating that about 17% of the carbon in the 16:1ω7c fatty acid derives from benzene. The other carbons originated from sources not enriched in ^{13}C. However, it should be considered that a certain fatty acid may also derive from microorganisms not involved in the benzene biodegradation.



Supporting Information Figure 1: GC-MS profiles of fatty acids in total lipid extracts obtained from (A) non-amended control bio-traps (B) bio-traps amended with natural benzene ,and (C) bio-traps amended with natural toluene deployed down-well in the aquifer for 32 days. Major fatty acids (RT in min) are 12:0 (8.53), 14:0 (13.83), 15:0 (16.41), 16:1ω9c (18.29), 16:1ω7c (18.36), 16:0 (18.92), 18:1ω9c (22.96), 18:1ω7c (23.10), 18:0 (23.59), internal standard 21:0 (29.91). Peak at RT 21.33-21.35 is a phthalate contamination from extraction process.