Investigating relationship between oxygen level in *E.coli* culture chamber and carbon isotopic composition of headspace CO_2: Relevance to early organic evolution

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Bacterial population during 3.2 billion years of early earth was responsible for modifying the green house gas concentration in the early earth. Here we studied the effect of phosphate limiting conditions in two primitive facultative anaerobes *E.coli* K12 and DH5 alpha, known to contain pathways of mixed acid fermentation in stressed oxygen deficient environment. We carried out chamber experiments where bacteria were grown in M9 minimal media containing 0.3 % glucose in crimp sealed chambers for a period of 7 days-12 days. Growth rate for the bacteria were monitored using optical density measurements and CFU values on LB agar. O_2 utilization together with CO_2, and CH_4 production inside the culture chambers were measured by passing an aliquot of headspace gaseous mixture through FID of a gas chromatograph. $\delta^{13}C$ of CO_2 was analysed using Gas Bench peripheral connected with IRMS MAT 253.

Enrichment of $\delta^{13}C$ by 2.7‰ coincides with a drop in oxygen concentration of ~ 11.5% for K 12 and 1.7 ‰ coincides with a drop in oxygen concentration of ~ 12% for DH5 alpha. The relationship between $\delta^{13}C$ of CO_2 and the oxygen level is grossly correlated ((R^2 coefficient 0.35 for K 12 and 0.8 for DH5 alpha). We suggest that enrichment in $\delta^{13}C$ captured the CH_4 production due to bio-degradation of organophosphonates under phosphate limiting conditions [1]. The process documented in this experiment can explain the $\delta^{13}C$ excursion recorded in the Precambrian succession [2].

[1] S S Kamat et al (2011) *Nature* **480**, 570-573 [2] Grotzinger. J.et al (2011) *Nature Geoscience* **4**, 285–292