

Assessment of methanogen survival in anaerobic enrichment cultures degrading long-chain fatty acids

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Long-chain fatty acids (LCFA) are generally considered highly toxic towards methanogens. However, high methane yields have been obtained from high-load anaerobic digestion of LCFA, suggesting that methanogens can tolerate these compounds. In this work, hydrogenotrophic and acetoclastic methanogens were added to LCFA-degrading enrichments in order to evaluate their survival in the presence of unsaturated-LCFA (C18:1, oleate) and saturated-LCFA (C16:0, palmitate). Two hydrogenotrophs (*Methanobacterium formicicum* and *Methanospirillum hungatei*), and two acetoclasts (*Methanosarcina mazei* and *Methanosaeta concilii*) were tested. Oleate- and palmitate-enrichment cultures (OM and PM, respectively) were amended with each of the methanogens and incubated with 1 mM of the corresponding LCFA. Survival of methanogens after several transfers was evaluated by PCR-DGGE. For the hydrogenotrophs, results showed that *M. formicicum* survived in both OM and PM cultures, while *M. hungatei* only grew in the PM culture. Moreover, viability tests using live/dead staining coupled to fluorescent microscopy observation and cell counting indicated that *M. hungatei* is indeed more sensitive to oleate than *M. formicicum*. The percentage of damaged cells, caused by the exposure to low concentration of oleate (i.e. 0.5 mM), was very high in the case of *M. hungatei* (79%) contrasting with *M. formicicum* that was only slightly affected by this LCFA (8%). Regarding acetoclastic methanogens, both tested species prevailed in OM and PM cultures, although more abundant in PM enrichment. These results suggest that oleate is a more toxic compound for methanogens than palmitate. Nevertheless, all the methanogens studied, except *M. hungatei*, were found in the OM cultures.

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