Microbial Diversity and Methanogen Survival in Anaerobic Communities Degrading Long-Chain Fatty Acids

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Anaerobic digestion of wastes/wastewaters is a way of treating polluted streams with the concomitant production of biogas. Lipids are main components of municipal and industrial wastes/wastewaters, and the efficient conversion of these high energy substrates is a condition to optimize biogas production. Despite this, there has been some concern regarding anaerobic conversion of lipids mainly due to the reported inhibitory/toxic effect of long-chain fatty acids (LCFA), main intermediates of lipids degradation, on anaerobic microorganisms. The main objective of the work described here was to study the effect of LCFA on the microbial composition of anaerobic sludge. Bacterial and archaeal clone libraries were constructed from LCFA-degrading sludges. LCFA-degrading bacteria clustering within the *Syntrophomonadaceae* were detected in all the samples. *Clostridiaceae*-related bacteria were also present, although their function in these systems is not yet clear. Two major groups of methanogens were identified in LCFA-degrading sludges: hydrogen-utilizing organisms, related to *Methanobacterium*, and acetoclastic organisms affiliated with *Methanosaeta* and *Methanosarcina* spp. Quantification of archaea by real-time PCR showed that the relative abundance of this group increased during continuous-batch LCFA degradation (i.e. relative archaea percentage increased from 42±15% to 85±29% and to 75±14% in reactors fed with oleate (C18:1, unsaturated) and palmitate (C16:0, saturated), respectively). To get further insight on the effect of LCFA towards methanogens, hydrogenotrophic (*Methanobacterium formicicum* and *Methanospirillum hungatei*) and acetoclastic (*Methanosarcina mazei* and *Methanosaeta concilii*) methanogens were added to LCFA-degrading enrichments in order to evaluate their survival in the presence of LCFA. 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. These results suggest that oleate is a more toxic compound for methanogens than palmitate. Nevertheless, methanogens could endure the presence of LCFA proving that toxicity/inhibitory effects of these compounds do not impair anaerobic digestion of lipid-rich wastewaters.

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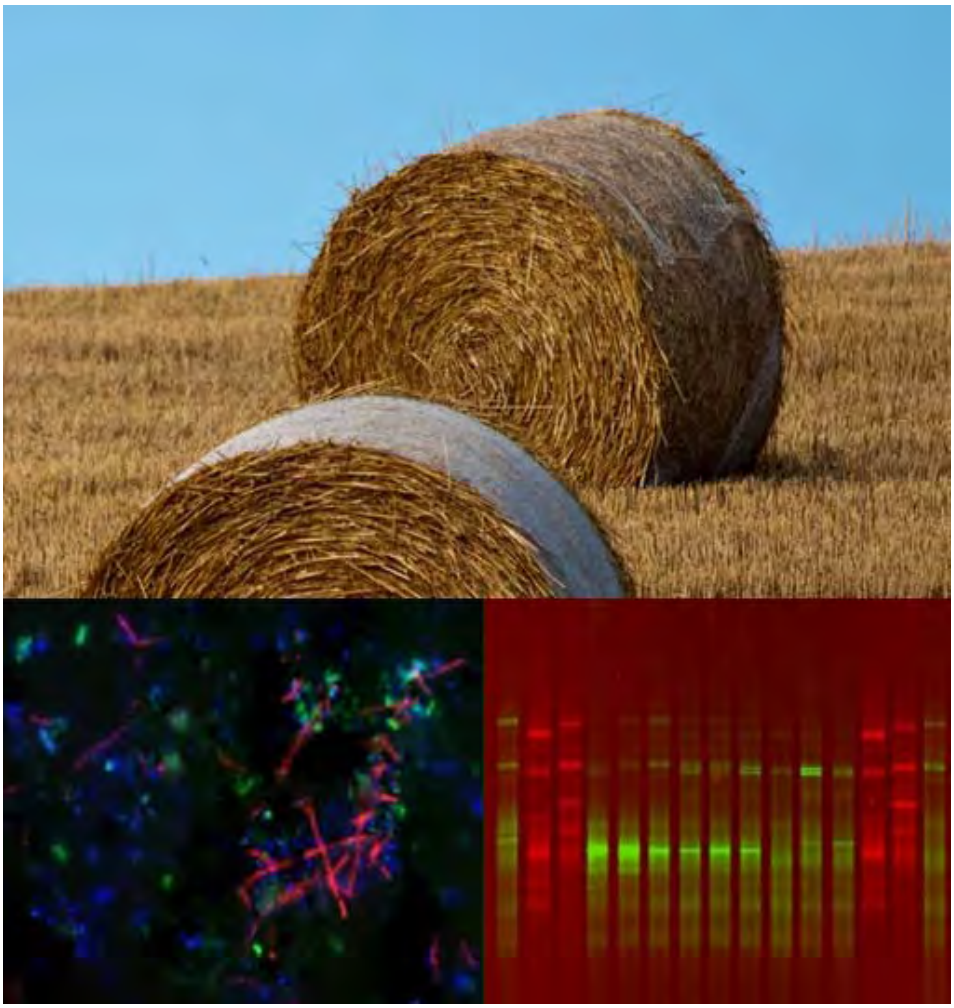

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