Untangling the role of facultative bacteria in LCFA conversion to methane

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Palmitate accumulation is frequently reported in continuous methanogenic bioreactors fed with lipid-rich wastewater, and facultative bacteria were suggested to be involved in this conversion. In this work, the possible effects of limited oxygen conditions in triggering palmitate formation from oleate were studied. Two bioreactors were operated in parallel, one under strict anaerobic conditions (AnR) and the other with the feeding tank open to the air (FR). Palmitate was the main LCFA in both reactors, but it reached approximately 7 times higher concentrations in the FR than in the AnR (16 and 2 $g{\cdot}L^{-1}$ in COD, respectively). Moreover, oleate was more abundant in the AnR than in the FR, presenting concentrations of 1.3 and 0.5 $g{\cdot}L^{-1}$, and oxidation-reduction potential values (ORP) of -366 mV and -255 mV were measured. Batch incubations of samples collected from the reactors showed that methanogens were completely inhibited in the AnR, while the FR sludge exhibited methanogenic activity, possibly due to the lower toxicity of palmitate when comparing with oleate. In a second experiment, hydraulic pressure was used to promote a selective washout of the microorganisms that did not perform oleate conversion to palmitate. A continuous stirred tank reactor (CSTR) and a plug flow reactor (PFR) were assembled in series and were fed with oleate from a tank open to the air. Biological activity occurred mainly in the biofilm, where palmitate accounted for up to 82 % of the LCFA and Pseudomonas was the predominant genus (42-58 % relative abundance), which highlights the important role of this genus in oleate to palmitate bioconversion. From the PFR biofilm, two different Pseudomonas sp. were isolated and further tested. These isolates were able to degrade oleate with oxygen as electron acceptor, but not in anaerobic conditions, and palmitate formation was not observed. Therefore, the formation of a biofilm and/or the presence of other microbial partners in a complex microbial community appear to be necessary conditions. All these results show that the presence of vestigial amounts of oxygen, directly related with the activity of Pseudomonas and higher redox potentials, are imperative for oleate conversion to methane.