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OLEATE DEGRADATION IN A CONTINUOUS MICROAEROPHILIC BIOREACTOR BY A SYNTROPHIC CO-CULTURE TOGETHER WITH FACULTATIVE ANAEROBIC BACTERIA

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Introduction: In high-rate bioreactors, to degrade long-chain fatty acids (LCFA), syntrophic bacteria convert LCFA to acetate and hydrogen/formate, and hydrogenotrophic methanogens keep the H₂/formate at low levels, which is essential to allow the continuous LCFA degradation. However, syntrophic bacteria are usually detected in low numbers and facultative anaerobic bacteria (FAB) were shown to be relatively more abundant, although their role is not yet clear. This work aims to study the microbial relationships between syntrophic LCFA-degrading bacteria, methanogens and FAB in oleate degradation, under anaerobic and microaerophilic conditions.

Methodology: A synthetic microbial consortium composed by *Syntrophomonas zehnderi* (a syntrophic LCFA-degrading bacterium, Sz), *Methanobacterium formicicum* (a hydrogenotrophic methanogen, Mf) and two *Pseudomonas* spp. (FAB isolated from an oleate degrading bioreactor, I1 and I2) was studied in a continuous bioreactor fed with oleate, under strict anaerobic and microaerophilic conditions. During the first 112 days, the bioreactor was operated with the syntrophic co-culture (Sz+Mf) under strict anaerobic conditions. After, the two *Pseudomonas* isolates (I1+I2) were added to the bioreactor and microaerophilic conditions were provided by allowing the entrance of low oxygen amounts through continuous feeding from a tank containing oxygen in the headspace. The bioreactor operation was divided in 10 different periods that differ in oleate concentration in the feed (0.25 to 4.67 mmol L⁻¹), hydraulic retention time (19 to 10 days), and oxygen concentration in the feeding tank headspace (21 %, 10 % and 5 %). In parallel, I1 and I2 were characterized in batch assays for their capability to degrade oleate or acetate under aerobic conditions, as well as formate and hydrogen under microaerophilic conditions (5% O₂).

Results: In the continuous microaerophilic bioreactor, oleate was degraded by the synthetic community composed of Sz+Mf+I1+I2. Acetate was the main product and methane was detected only in low amounts. The highest oleate conversion to acetate was achieved at 5% O₂. Our data indicate that the production of acetate from oleate, under microaerophilic conditions, was performed largely by *S. zehnderi*, and that *Pseudomonas* isolates were potentially alternative syntrophic partners. Indeed, additional batch tests support this suggestion, as they confirmed that *Pseudomonas* isolates (I1+I2) were able to consume formate and hydrogen under microaerophilic conditions.

Conclusions: Our results suggest that FAB, particularly *Pseudomonas* spp., besides protecting the strict anaerobic community from oxygen toxicity, may also act as alternative hydrogen/formate scavengers for syntrophic LCFA-degrading bacteria.