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PSEUDOMONAS EMPOWER SYNTROPHIC FATTY ACIDS DEGRADATION IN THE PRESENCE OF OXYGEN

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Anaerobic digestion (AD) processes specifically directed towards biogas production are currently of great interest worldwide, due to the urgent need of more sustainable energy sources.

Although not consensual, oxygen has been shown as an ally of AD processes, resulting in more efficient biogas production when added to the systems in vestigial doses. It has been suggested that it stimulates facultative anaerobic bacteria (FAB), which are generally present in the anaerobic communities. These bacteria are involved in several steps of AD (fermentation and acidogenesis) but have also been referred to protect the strict anaerobes from oxidative stress [1].

In this work, the influence of FAB in the degradation of short-, medium- and long-chain fatty acids (C4, butyrate; C8, octanoate; C16, hexadecanoate) by two syntrophic co-cultures was investigated. *Syntrophomonas wolfei* (Sw)/ *Methanospirillum hungatei* (Mh) and *S. zehnderi* (Sz)/ *Methanobacterium formicicum* (Mf) were pre-grown and *Pseudomonas* sp. (FAB) were further added, along with each substrate over a range of O_2 concentrations (0-2 % v/v). In a second transfer, each culture was exposed to the same O_2 concentration range.

Both syntrophic co-cultures (Sw+Mh and Sz+Mf) had their activity highly reduced, or even completely inhibited, in the presence of O_2. Interestingly, the theoretical CH_4 production expected from C8 was reached by consortium Sw+Mh+Ps at days 3, 8 and 28 under 0%, 1% and 2% O_2, respectively. The same trend was observed for consortium Sz+Mf+Ps.

C4 and C16 degradation occurred similarly to C8 degradation, presenting similar results and the same tendency for both tested consortia.

This data suggests a positive interaction and network establishment between these organisms. Apparently, *Pseudomonas* consumed the oxygen allowing the creation of a reduced environment, a requirement for an effective development of the strict anaerobic syntrophic co-cultures.

In the 2nd transfer, the protective support of *Pseudomonas* was maintained. Moreover, it was verified that the cultures (Sw+Mh+Ps and Sz+Mf+Ps) previously exposed to O_2 preserved their activity either under anoxic or microaerophilic conditions.

These results show the essential role of *Pseudomonas* in the protection of syntrophic co-cultures activity, empowering fatty acids degradation under microaerophilic conditions. Furthermore, it highlights the FAB/*Pseudomonas* importance in real AD systems (where vestigial amounts of O_2 can be detected) for the stability and resilience of the system maintaining syntrophic communities' functionality and biogas production.

[1] D. Nguyen and S. K. Khanal, *Biotechnol. Adv.*, vol. 36, no. 7, pp. 1971–1983, 2018.