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Addition of co-substrates stimulates hexadecene conversion to methane by an enriched microbial consortium

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Linear olefins with 16 to 18 carbon atoms are frequently used as hydrophobic groups in oil-soluble surfactants and as lubricating fluids. The production of olefins in petrochemical plants generates olefin-contaminated wastewater that can be treated anaerobically in methanogenic bioreactors, coupling degradation to energy recovery. However, this conversion is generally slow, due to olefins´ insolubility in water and poor bioavailability for microorganisms. Addition of an easy degradable carbon source may enhance the growth of hydrocarbon-degrading methanogenic communities. In this study, hexadecene degradation by a methanogenic enrichment was stimulated by addition of yeast extract (0.5 $g \cdot L^{-1}$), lactate (4.5 $mmol \cdot L^{-1}$) or crotonate (4.5 $mmol \cdot L^{-1}$) as co-substrates. After stimulation with yeast extract or lactate, the microbial communities were able to convert hexadecene to methane 5 and 2.5 times faster, respectively, than non-stimulated cultures. Hexadecene conversion to methane was not enhanced by crotonate addition. Further incubations with fermented yeast extract did not improve methane production from hexadecene, which suggests that the positive stimulatory effect of yeast extract was due to the extra carbon source and not to the supply of essential co-factors. The microbial community composition of the hexadecene-degrading enrichments was studied by 16S rRNA sequencing. Bacteria from the *Chloroflexi*, *Firmicutes*, *Proteobacteria* (*Deltaproteobacteria*), *Spirochaetes*, *Synergistetes* and *Thermotogae* phyla were identified, with *Syntrophobacterales*, *Spirochaetales* and *Synergistales* as the most abundant orders. Hydrogenotrophic methanogens predominated over acetoclastic methanogens. Currently the isolation and identification of key microbial players involved in hexadecene degradation are ongoing. This study can be useful for improving the treatment of olefin-contaminated wastewater using methanogenic conditions.