## Effect of carbon nanotubes on methane production in pure cultures of methanogens and in a syntrophic co-culture

<u>G. Martins</u><sup>1</sup>, A.F. Salvador<sup>1</sup>, M. Melle-Franco<sup>2</sup>, R. Serpa<sup>1</sup>, A.J.M. Stams<sup>3</sup>, A.J. Cavaleiro<sup>1</sup>, M.A. Pereira<sup>1</sup>, M.M. Alves<sup>1</sup>

<sup>1</sup>Centre of Biological Engineering, University of Minho, Centre of Biological Engineering, Braga, Portugal, <sup>2</sup>Ciceco - Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal, <sup>3</sup>Laboratory of Microbiology, Wageningen University, Laboratory of Microbiology, Wageningen, Netherlands

Conductive materials have been reported to enhance methane production by anaerobic microbial communities from a wide diversity of substrates <sup>1</sup>. The mechanisms involved are far from being fully understood. Many studies suggest that these materials facilitate direct interspecies electron transfer (DIET) between electrogenic bacteria and methanogens and that this mechanism is even dominant over interspecies hydrogen and formate transfer <sup>2,3</sup>. The effect of conductive materials in pure cultures of methanogens or in co-cultures of typical fatty acid-degrading syntrophs with methanogenic partners was never studied. In this work, the effect of carbon nanotubes (CNT) on the activity of pure cultures of *Methanobacterium formicicum, Methanospirillum hungatei, Methanosarcina mazei* and *Methanosaeta concilii*, and in the co-culture of *Syntrophomonas wolfei* and *Methanospirillum hungatei* was evaluated. The results showed that CNT affect methane production by methanogens. Initial methane production rate (MPR) increased 17 and 6 times when *M. formicicum* and *M. hungatei* were incubated with 5g·L<sup>-1</sup> CNT, respectively. *M. mazei* and *M. concilii* activities were higher when exposed to CNT concentrations

of 0.1 to  $1g \cdot L^{-1}$ , but lower with  $5g \cdot L^{-1}$ . Increasing CNT concentrations resulted in more negative redox potentials, which correlated with the increased methanogenic activity. Remarkably, in the absence of a reducing agent, but in the presence of CNT, the MPR was higher than in incubations with reducing agent, while no growth was observed without reducing agent and without CNT. MPR from butyrate increased 1.5 fold in the presence of CNT ( $5g \cdot L^{-1}$ ), showing a positive effect of CNT on the syntrophic co-culture. Indications of DIET by the presence CNT were not obtained. Rather, CNT directly affects the activity of methanogens, which creates new opportunities to improve methane production from waste and wastewater in anaerobic digesters.

## References

1 Zhao et al., 2016. Bioresource Technol 209:148-156.

2. Rotaru et al., 2014. AEM 80(15):4599-4605.

3. Lovley, 2016. ISME J, doi:10.1038/ismej.2016.136.