THE CHALLENGE

Methane is an energy-carrier that results from the anaerobic digestion of organic matter present in wastewater. Microbiology of anaerobic processes is complex and the last steps of anaerobic digestion are rather sensitive to the presence of wastewater contaminants, such as heavy metals or sulphate; both positive or negative effects have been observed, depending mainly on contaminant concentration. At present, the use of metals and sulphate-derived compounds to enhance methane production is not fully understood. Furthermore, new developments indicate that cofactor-engineering strategies can advance the production of added-value compounds (methane, acetate) and application to wastewater valorisation should be researched.

THE PROJECT

One of the challenges proposed in the project was to gain more knowledge on the effects of pollutants, such as heavy metals and sulphate, on biogas production and on the microbial communities involved. In particular, the possible beneficial effect of trace metals on biogas production was evaluated.

On the other hand, the toxic concentrations of heavy metals at which methane production was affected were also investigated, as well as the possible use of biogenic sulphide to mitigate metals toxicity.

Furthermore, the interference of sulphate on biogas production using new technologies, such as Microbial Fuel Cells (MFCs), for energy production was also studied. Finally, to address the last challenge, the increase of cofactor levels and its effect on the production of acetate and methane from wastewaters was evaluated.
METHODOLOGY

To address most of the questions proposed for this project, different batch experiments were designed using anaerobic sludge as inoculum. Cobalt and nickel were the heavy metals chosen due to their presence as cofactors of the main enzymes of methanogenesis and presence in high concentrations in wastewaters. Trace concentrations and toxic concentrations of metals were tested, and the effect on biogas production and on the microbial communities evaluated. The effect of sulphate and sulphide were also tested, individually and in the presence of metals, to test to possible use of biogenic sulphide as a metal detoxification strategy.

Nicotinic acid (NA) is the precursor of NAD/NADH, one of the main cofactors used by microorganisms. NA was added in different concentrations to anaerobic cultures to evaluate its possible stimulatory effect on the production of methane and acetate. Finally, the interference of sulphate on biogas production in a MFC was also tested.

APPLICABILITY

The results obtained in this project indicate that metal supplementation can be used to increase biogas production. On the other hand, if this compounds are present in high concentrations, sulphate reduction, a natural process occurring in wastewater treatment, can be used as a possible metal detoxification process. Furthermore, the addition of nicotinic acid can be supplemented to anaerobic processes resulting in a substantial increase of methane and acetate production.

New technologies, such as MFCs, can be successfully applied to the treatment of wastewaters, especially the ones with low COD content. Results obtained from our work showed that the process is quite flexible and even in the presence of high concentrations of sulphate and methane production is not severely affected.

RESULTS

I. Effect of sulphate and heavy metals on methanogenesis:

- Nickel (2 - 8µM) and cobalt (10µM) stimulated methanogenesis from H₂/CO₂ but not from acetate.
- Addition of sulphate or sulphide did not restore methane production from acetate in the presence of nickel or cobalt, while low amounts of sulphide enhanced methane production from H₂/CO₂.

II. Cofactor engineering:

- Methane or acetate production could be differentially stimulated by amending different NA concentrations to enriched methanogenic cultures. Higher NA concentrations promoted acetogenesis over methanogenesis.
- NA can play an important role in redistributing the metabolic flux of anaerobic enrichments producing methane and acetate.

III. Effect of sulphate on a biogas-producing MFC:

- Complete sulphate (10 mM) reduction in the MFC cathode. Methane production was decreased after sulphate addition, but the system was able to quickly recover methanogenic activity within 3 days.

BENEFITS

Metal supplementation or cofactor engineering can be used to increase the production of biogas during anaerobic processes. Biogas can be properly recovered and used as energy source, making the process more sustainable by not only diminishing the emissions of greenhouse gases, but also by reducing the consumption of fossil fuel-derived energy. Moreover, the addition of different concentrations of cofactor precursors seems to play a role in shifting the metabolic fluxes towards the production of different valuable products. This opens the door to a selective reduction of CO₂ to desired end products.

MFCs are an alternative technology to the traditional anaerobic reactors and have been proven to be efficient for biogas production, linking wastewater treatment to CO₂ fixation. The results indicate that the system can be applied to treat wastewaters containing sulphate without the issues associated with the production of hydrogen sulphide, which is highly toxic, corrosive and odorous.

Figure 1 - A - Methane production over time at different added nicotinic acid concentrations. B – Detail of graphic A for the first 140h. C - Acetate production over time at different NA concentrations.

Figure 2 - Schematic of the MFC operated in this project; B – The MFC.