POLITECNICO MILANO 1863

LEAP
Laboratorio Energia e Ambiente Piacenza

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materials & energy from refuse

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INNOVATIONS & TECHNOLOGIES IN WASTE RECOVERY

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BACKGROUND

Waste is normally perceived with a host of negative attributes. Its very generation signifies the willingness to get rid of it, while its treatment and disposal raise apprehension from several standpoints: sanitary, ethical, social, energy, environmental, economic. Yet waste is simply the output of a number of processes taking place in our society. Its treatment should be approached as simply one of the many practices that contribute to the operation of our economic system, hopefully in a way that meets our expectations on sustainability, energy efficiency, environmental consciousness, economic viability. In this framework, the recovery of useful material and the recovery of energy are the two means that can turn waste from a fearsome concern into a valuable asset. But the real world can be very far from best practices. Inadequate waste management policies and/or treatment plants can result in emergency situations that create widespread concern and hostility in the public opinion. Also, the debate on strategies and technologies to be adopted is often misled by ideology, with little or no account of scientific evidence and technological capabilities.

MISSION

The MatER Research Center aims at establishing sound scientific bases for the many issues related to recovery from waste, without being influenced by any ideological or political consideration, independently of the expectations of any interest group. The ultimate goal is to give a rigorous scientific representation of the technologies and the policies adopted for material and energy recovery, contributing to identify the most effective options for sustainable, economically viable waste management practice. These goals are pursued by the following actions.

- Identify and analyze best available technologies for the recovery of material and energy from waste.
- Establish connections and collaborations among academic institutions, public and private organisations, business operators and technology providers.
- Promote and undertake studies and researches concerning material and energy recovery from waste.
- Organize course and events (conferences, seminars, workshops) to promote and spread proper scientific information.
- Monitor the evolution of processes, technologies and practices for the recovery of material and energy from waste.
- Review and promote the improvement of legislation on waste management, recovery from waste and waste treatment.

BOARD

The MatER Study Center is a project undertaken by LEAP s.c.a r.l. with the scientific support of DICA (Department of Civil and Environmental Engineering) and the Energy Department of the Politecnico di Milano, and with the collaboration of Utilitalia.

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"POWER TO GAS": PROCESS MONITORING OF HYDROGENOTROPHIC METHANOGENESIS TO BIOMETHANE PRODUCTION

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Power to Gas (PtG) is a promising solution for storing the surplus electricity as CH_4 and for upgrading biogas to biomethane. The Power to Gas technologies exploit chemical conversion and/or biological activity of anaerobic microorganisms. The H_2 and CO_2 produced (4:1) via electrolysis of water are used to produce methane (Luo et al., 2012). Especially for small-scale biogas plants, where conventional upgrading technologies are too expensive, the PtG technology in combination with biological methanation can be a valuable approach (Reuter and Klueckers, 2017). Methanogenesis from H_2/CO_2 is mainly carried out by hydrogenotrophic methanogens that reduce CO_2 with H_2 to produce CH_4, which can happen in enriched or pure culture in a separate reactor (Bassani et al., 2015). Biogas upgrading strategies that use pure robust cultures could be more promising in industrial application due to better withstand to oxygen and hydrogen sulfide exposure, contamination of bacteria or phages present in the continuously fed biogas (Martin et al., 2013). The first results with pure culture demonstrated that the bacteria contamination did not compromise the microorganism's proliferation, while the performance were reduced. The CO_2 could be provided as untreated biogas or pure gas. The use of untreated raw biogas need a proper monitoring plan to control the bacteria contamination in the pure archaea culture.
This work was aimed at define a specific useful monitoring tool for identifying and quantifying bacteria and archaea methanogens respectively. Our tests were performed on samples of *Methanothermobacter marburgensis* and *Desulfovibrio desulfuricans* by applying microbiological and molecular methods. The final aim was to define the accuracy and sensitivity of each method to quantify the possible occurrence of *Desulfovibrio desulfuricans* contamination in the archaea culture. This work is a part of a preliminary study to verify the feasibility of the PtG technology introduction in mountain areas to combine the use of surplus electricity and syngas/biogas available to produce biomethane.

Methods
Two methods were applied for the identification and quantification of each microorganism considered: 1) citofluorimetry and 2) DNA extraction and quantification by RT-qPCR.
The pure cultures of each type of microorganism were performed in Hungate tubes with specific growing medium, i.e. "Medium 63" for methanogens and "Postgate medium" for sulfate-reducing bacteria. The quantification with citofluorimetry was performed by using a specific kit (Live/Death™ BacLight™ Bacterial Viability- Thermofisher) to quantify the cell concentration of single population and also to distinguish between live and dead cells. After DNA extraction with the kit (Quiagen QIAamp PowerFecal DNA Kit) combining with proteinase K use, the amount of DNA was measured by Nanodrop ND 8000 8 sample spectrometer" at 230 nm, 260 nm and 280 nm absorbance. The protocol for quantify by RT-qPCR (Roche LightCycler 480) was assayed. At first it was identified the specific primer from both and then the specific reaction condition for PCR reactions.
The detection of bacterial contamination at very small cell concentration was done for both DNA and cell quantification approach. In these tests three different mix with different percentage of bacterial contamination in the pure culture of methanogens were performed: 5%, 2,5% and 1%. The mix were calculated based on the number of cell counted in the pure culture.

First results

The cell counting of pure culture and for the mix results defined by citofluorimetry were reported in Table 1.

Table 1: Cell counting.

Microorganisms and MIX	cell ml^{-1}
Desulfovibrio desulfuricans	9.47*10E6
Methanotermobacter marburgensis	9.46*10E7
MIX_5%	1.28*10E7
MIX_2.5%	1.25*10E7
MIX_1%	1.18*10E7

M. marburgensis growth was very slow; on the contrary the *D. desulfuricans* grown very fast in the anaerobic Hungate tubes. The hydrogenotrophic methanogens grown very hardly in the pure culture, but they are readily available in the mixed culture of effluents from the anaerobic degradation of organic matter (Szuhay et al., 2016).
For each microorganism, the DNA extractions was carried out in accordance with the commercial kit protocol. This protocol was applied for both. The data of total DNA extracted are reported in Table 2.

Table 2: DNA extraction.

Microorganisms and MIX	ng μl^{-1}
Desulfovibrio desulfuricans	14.83 ± 4.74
Methanotermobacter marburgensis	4.75 ± 0.55
MIX_5%	8.92 ± 1.16
MIX_2.5%	8.63 ± 0.80
MIX_1%	7.46 ± 1.13

For performing the qPCR reaction, the specific primers were identified by melting peaks and were Meth_F and Meth_667_R for *Methanothermobacter marburgensis* (Reitschler et al., 2014) and DSVsp G11f and DVSsp G11r for *Desulfovibrio desulfuricans* (Junicke te al., 2014).
The standard for quantify the bacteria and archaea respectively were performed at the specific conditions, varying the annealing temperature. Table 3 shows the quantification of *Methanothermobacter marburgensis* by qPCR reactions, using the standard curve and primers for archaea. In the Table 4 the quantification of

Desulfovibrio desulfuricans by qPCR reactions are reported, using the standard curve and primers for sulfate-reducing bacteria.

Table 3: Microorganisms identification and quantification by RT-qPCR.

***M. marburgensis* in the MIX**	**cell ml^{-1}**
MIX_5%	2.67*10E6 ± 2.07*10E4
MIX_2.5%	3.17*10E6 ± 4.85*10E5
MIX_1%	3.13*10E6 ± 4.99*10E5

Table 4: Microorganisms identification and quantification by RT-qPCR.

***D. desulfuricans* in the MIX**	**cell ml^{-1}**
MIX_5%	3.24*10E5 ± 3.36*10E4
MIX_2.5%	2.46*10E5 ± 4.85*10E4
MIX_1%	1.31*10E5 ± 4.99*10E4

The amplification of the DNA of *D. desulfuricans* detected by the primers DSVsp G11 201f e DSVsp G11 171r in the different MIX tested are reported in Figure 1. The MIX 5% is in orange, MIX 2.5% in violet and MIX 1 % in green.

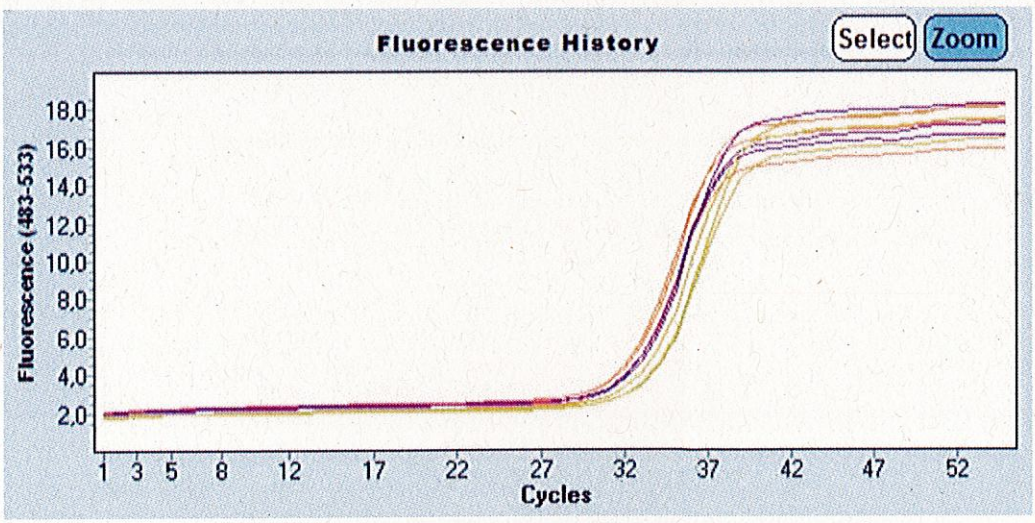

Figure 1: DNA Amplification from D. desulfuricans in the MIX tested.

Conclusions

The results obtained are the first approach to perform a monitoring protocol for biomethanation with hydrogenotrophic methanogens, exploitable in the PtG technology. The most difficult issue concerned the very slow growth of the archaea, the choose of the extraction protocol for extracting with the same efficiency DNA from archaea e from bacteria and finally the exploitation of the process conditions suitable for identifying and quantifying both the microorganisms. The methods chosen have several advantage for applying in the biotechnological industrial process monitoring and therefore their use could improve the monitoring effectiveness in terms of time reduction and information accuracy. After the first phase of this work, it was possible to develop a specific protocol for process monitoring from the microbiological and molecular point of view for the scaling up of the biological process in the PtG technology. The microorganisms monitoring may be integrated with data of operational parameter and process performance control for better managing in the industrial plants.

Future works will aim to apply this protocol at the microorganisms sampled in the pilot or industrial scale continuous reactor. The individuation of effective monitoring will allow the integration of the PtG technologies with the use of biogas/syngas from industrial plant for feeding the reactors and at the same time to use directly the fermentation effluent of biogas plants to enriched culture of hydrogenotrophic methanogens.

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