1st International Congress on Bioenergy
Portalegre (Portugal), 23-25 May 2013

Flow cytometric method for cell viability evaluation of *Gordonia alkanivorans* strain 1B in fossil fuels biodesulfurization processes

A.V. Teixeira, T.P. Silva, T. Lopes da Silva, S.M. Paixão, L. Alves*

LNEG – U. Bioenergia, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal

**Corresponding author:* luis.alves@lneg.pt;

Introduction - The most commonly method used for sulfur removal from fossil fuels is hydrodesulfurization, a physico-chemical process at very high temperatures and pressures. An alternative to this process is biodedesulfurization (BDS), a microbiological process that works at atmospheric pressure and temperature making it easier to work with and less expensive. It also as the advantage of easily desulfurizing recalcitrant sulfur compounds which are hard to remove by hydrodesulfurization [1]. Several bacteria species, such as *Gordonia alkanivorans* strain1B [2], are able to desulfurize dibenzothiophene, a model compound used commonly in BDS studies, to 2-hydroxybiphenyl (2-HBP) via the 4S pathway without destroying the carbon structure [3], therefore maintaining the fuel potential energy. BDS limitations are related with process parameters and with the cost of maintaining bacterial cultures so to enhance the BDS process, it is necessary to monitor how changes in the experimental system affect the microbial cells viability and consequently the process efficiency. An alternative method to conventional microbial techniques to determine cell viability is flow cytometry. This method provides a fast and accurate quantitative method for measurement of thousands of individual cells, based on scattered light and fluorescence emitted by specific dyes. The goal of this study was to develop a rapid method for viability assessment of *G. alkanivorans* cells using flow cytometry for further application to monitor and optimize BDS processes.

Experimental - A mixture of dyes was used to obtain information about the cells physiological state. This mixture contained propidium iodide (PI), a nucleic acid dye that only enters into cells whit a compromised cytoplasmatic membrane, and 5(6)-carboxyfluorescein diacetate (CFDA), an indicator of cell viability as a function of enzymatic activity. *G. alkanivorans* cells at different physiological states were used to establish controls with positive staining for PI (PI^+) and CFDA ($CFDA^+$).

Results and Discussion - The results allowed the identification of three distinct subpopulations: viable cells ($CFDA^+$, PI^-), stressed or injured cells ($CFDA^+$, PI^+) and dead cells (PI^+, $CFDA^-$). These controls were then used to perform physiological studies of 2-HBP toxicity, a very toxic end product of the desulfurization process, on *G. alkanivorans* resting cells. Five different concentrations of 2-HBP (0.25, 0.45, 1, 5 and 10 mM) were tested and the percentages of viable, dead and stressed or injured cells for each concentration of toxicant over time were obtained by analysis of the flow cytometry density plots. The lower concentrations of 2-HBP did not seemed to affect significantly the viability of the resting cells, but with the increase of the concentration the decrease of viable cells was faster as well as the increase of stressed or injured cells. The higher concentrations of 2-HBP showed to be very toxic to the resting cells, presenting high percentages of dead cells.

Conclusion - Considering the obtained results, the use of this technique seems to be a promising tool to monitor the viability of microbial desulfurizing cells during BDS processes, since the possibility of performing frequent evaluations of how cells viability vary in response to parameters changes could enable a faster optimization and a better control of the process.

Acknowledgments: The authors gratefully acknowledge the financial support of the project Carbon4Desulf (FCOMP-01-0124-FEDER-013932) by FCT (Fundação para a Ciência e a Tecnologia).

References

[1] N. Gupta, P.K. Roychoudhury, J.K. Deb, *Appl Microbiol Biotechnol*, **66** (2005) p. 356–366.
[2] L. Alves, R. Salgueiro, C. Rodrigues, E. Mesquita, J. Matos, F.M. Gírio, *Appl Biochem Biotechnol*, **120**, (2005) p. 199–208.
[3] V.C. Srivastava, *RSC Advances* **2** (2012) p. 759-783.