

## **Development of a flow method for monitoring cell membrane damage: application to the study of phosphate release from wine lactic acid bacteria**

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Leakage of cellular metabolites like nucleotides, amino acids and inorganic ions, is a primary indication of membrane damage in bacteria that can be caused by membrane-active chemical agents. The measurement of the efflux rates of potassium or phosphate ions (using potentiometric or photometric methods) can be used to monitor cell membrane damage and to compare the effects of different chemical compounds on the cellular membrane.

In this work, a flow injection system was developed with the objective of the determination of phosphate efflux from microbial cells. The developed system allowed the direct injection of the cell suspension, avoiding the filtration step with minimum sample treatment and minimized reagent consumption. The determination of phosphate efflux was based on the formation of phosphomolybdate heteropoly acid and on its spectrophotometric detection. Cell suspensions of lactic acid bacteria were introduced via a 6 port rotary valve allowing continuous sample/cell recirculation. The system is characterized with a linear response concentration range between  $3 \times 10^{-5}$  –  $5 \times 10^{-4}$  M, and with repeatability better than 2.9% even in the case of direct injection of cell cultures. The efflux of phosphate was used to compare the effect of different phenolic acids on cell membrane of two strains of wine lactic acid bacteria (*Oenococcus oeni* VF and *Lactobacillus hilgardii* 5).

### Acknowledgements:

Francisco Campos and Ildikó Tóth thank Fundação para a Ciência e a Tecnologia (FCT) and FSE (III Quadro Comunitário) for the grants PRAXIS XXI/BD/19909/99 and SFRH/BPD/5631/2001, respectively. FCT is also thanked for funding this research via project POCTI/AGG/40724/2001.