Pervaporative fermentation for continuous anaerobic production of *n*-butanol with high titer, yield and productivity

Marwen Moussa[1], Lizeth Galindo Alvarez[2], Mailys Pinault[2], Isabelle Meynial-Salles[2], Violaine Athès-Dutour[1]

1- UMR GMPA, AgroParisTech-INRA, Université Paris-Saclay, Thiverval-Grignon (78), France
2- UMR LISBP, INRA-INSA, CNRS-INSA, Toulouse (31), France

*marwen.moussa@agroparistech.fr

Keywords: *in vivo* evolution, membrane bioreactor, pervaporation, integrated process, *n*-butanol

Within the framework of the global climate change and decline of natural resources, the substitution of petrochemical products by biobased products is drawing an increasing attention. Butanol has a major interest due to its multifunctional characters and considerable possibilities on various applications sectors: energy, cosmetic and pharmaceutical formulations, chemistry, etc. In this context, it is widely believed that biotechnology will provide a more sustainable route to produce butanol from renewable biomass-derived feedstocks. Nevertheless, to develop robust biotechnological processes for full scale industrial implementation, it is necessary to resolve technical issues, such as product inhibition of butanol producing microorganisms. One way to tackle product inhibition and thus increase productivity and the overall sustainability of the process is the implementation of an "*In Situ* Product Recovery" (ISPR) using pervaporation [1].

Pervaporation represents a promising process for the extraction of bio-butanol. This membrane-based process has several advantages over the existing processes, mainly with regard to energy consumption. Further considerable advantages of this process are related to its compactness and ability to operate at variable scales while being easily integrated with fermentation. In hydrophobic pervaporation, organic compounds like *n*-butanol undergo a selective permeation through the membrane [2]. The liquid bulk (feed) is in direct contact with the organo-selective upstream side of the membrane while a butanol-enriched vapor phase (permeate) is removed from the opposite side.

Even though the Acetone-Butanol-Ethanol (ABE) production by *C. acetobulylicum* has been developed at the industrial scale (Weizmann Process), some limitations are remaining, such as i) the co-production of acetone, ii) the low growth rate of the producing cells and their possible degeneration during a continuous process, iii) the complexity of the downstream process and its high environmental footprint. Recently, an engineered *Escherichia coli* strain was successfully developed by our group to produce *n*-butanol as the main metabolite obtained from glucose, with a high yield (0.28 g/g, *i.e.* 73% of the theoretical yield) [3].

The present work was aimed to develop an integrated process for the continuous production of *n*-butanol using the *E. coli* strain in a membrane bioreactor, coupled to a pervaporation unit, according the following strategy:

- *In vivo* evolution of the *E. coli* strain: this step allowed selecting highly solvent-resistant mutants.
- The implementation of the obtained mutants using a membrane bioreactor: this step allowed to increase the *n*-butanol production rate (+50%) and the glucose consumption rate (+85%)
- A comparative study of various organo-selective pervaporation membranes for the extraction of bio-butanol from model media (pure compounds and in binary or ternary mixtures) and real media (resulting from fermentation): this step allowed to highlight the role of competitive effects and facilitated transfer between the components, as well as the effect of the medium complexity on transport mechanisms.
- The use of the best performing membranes for the *in situ* recovery of *n*-butanol.

This work brings new insights towards the implementation of a robust and intensified biotechnological process for the continuous production of *n*-butanol with high titer, yield and productivity. The work strategy is valuable for similar integrated bioprocesses.

References

[1] Van Hecke W, Kaur G,, De Wever H. (2014) Advances in in-situ product recovery (ISPR) in whole cell biotechnology during the last decade. Biotechnol Adv. 32:1245-55.

[2] Vane L. (2008) Separation technologies for the recovery and dehydration of alcohols from fermentation broths. Biofuels, Bioprod. Bioref. 2:553–588

[3] Soucaille P, Meynial-Salles I, Foulquier C, Rivière A. (2015) New polypeptide having ferredoxin NADP+ reductase activity polynucleotide encoding the sale and uses thereof, PCT INSA/INRA/CNRS EP15306225 27 juillet.