**Cell Immobilization on Affordable Inert Supports for Ethanol Production from Cheese Whey Permeate.**

Rebeca Díez-Antolínez1,2, María Hijosa-Valsero1, Ana Paniagua-García1,2, Jerson Garita-Cambronero1, Xiomar Gómez2

*1 Center of Biofuels and Bioproducts, Instituto Tecnológico Agrario de Castilla y León (ITACyL), Polígono Agoindustrial del Órbigo p. 2-6, E-24358, Villarejo de Órbigo, León, Spain; 2 Chemical and Environmental Bioprocess Engineering Group, Natural Resources Institute (IRENA), University of León, Avda. de Portugal 41, E- 24009, León, Spain*

*\*Corresponding author: dieantre@itacyl.es*

**Highlights**

* The strain *Kluyveromyces marxianus* DSM 5422 was selected among eight yeast strains.
* Cells were immobilised on alumina beads and Raschig rings of plastic, glass and Tygon.
* Cheese whey permeate (130 g/L lactose) was efficiently fermented yielding 60 g/L ethanol.
* Glass Raschig rings and alumina beads allowed a stable performance during 1,000 h.

**1. Introduction**

Cheese whey is a liquid by-product of milk coagulation during cheese production. It is characterized by a high organic load with elevated BOD and COD values, which makes this waste a potential source of pollution if it is incorrectly disposed of in water bodies [1].

Cheese whey can be used in ethanol fermentation processes, thanks to its lactose content. However, not all yeast strains are efficient to deal with lactose concentrations above 100 g/L. In addition, it is essential to improve and reduce the costs of fermentation processes, a fact that can be partially achieved by cell immobilization.

**2. Methods**

The raw material used for fermentations was cheese whey permeate obtained after ultrafiltration of the whey resulting from the production of a mixed cheese of sheep and cow milk, and it was provided by Quesería Entrepinares SAU (Valladolid, Spain). Its lactose content was about 130 g/L with a protein content of 34 g/L.

Eight yeast strains [*Kluyveromyces marxianus* DSM 5418, DSM 5422, DSM 7239 and DSM 70799; *Saccharomyces cerevisiae* CECT 13152, CECT 1383, Ethanol Red and Hércules (Lessafre)] were compared for their ethanol production from cheese whey permeate without nutrient addition. In the case of *S. cerevisae* strains, an enzymatic hydrolysis of lactose before fermentation was necessary. Fermentations were performed in flasks plugged with foam stoppers, at 35°C and 150 rpm. The most efficient strain was selected for further experiments.

Four inorganic materials were employed to immobilize the cells of the selected yeast strain: alumina beads and Raschig rings of plastic, glass and Tygon (5–5.5 mm length). Approximately, 10 g of every support were added to 50 mL of cheese whey permeate and 3.7% (v/v) of the selected yeast cells. Every 48-72 h, fresh whey replaced the exhausted medium.

**3. Results and discussion**

The strain *K. marxianus* DSM 5422 obtained the best performace, with a production of 60.0 g/L ethanol and 100% lactose consumption in 44 h.

Regarding immobilisation experiments, during the first six fermentation batches, the four inorganic supports had a similar behaviour with final ethanol concentrations about 60 g/L. However, the samples containing Tygon and plastic supports suddenly decreased their ethanol production after that moment. On the contrary, the samples with glass Raschig rings and alumina beads kept a good ethanol production until the 14th batch (total time 1,000 h), with average ethanol concentrations of 58-59 g/L, yields of 0.45 g/g and a lactose consumption of 96-98% [1].

**4. Conclusions**

Cheese whey permeate with high lactose concentration is an attractive feedstock for ethanol production. The selection of an appropriate yeast strain is essential to avoid nutrient supplementation, enzymatic hydrolysis steps and osmotolerance problems. Moreover, it is possible to immobilise the yeasts on simple inorganic supports, which clearly reduces yeast cultivation costs.

**References**

1. R. Díez-Antolínez, M. Hijosa-Valsero, A.I. Paniagua-García, J. Garita-Cambronero, X. Gómez, PLoS ONE 13 (2018) e0210002.