

## Cheese-whey permeate as a sustainable inducer for the production of a cold-active $\beta$ -galactosidase in *Escherichia coli* cells

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Cheese whey is a by-product of the cheese making process, consisting of the liquid phase obtained after separation of the curd. Whey proteins - used as nutritional supplements - can be separated by ultrafiltration to produce cheese whey permeate (CWP), a secondary by-product rich in lactose (165 g/L) and micro-nutrients. In a circular economy perspective, we designed a new recombinant enzyme production process aimed at valorizing CWP collected from an Italian plant. Specifically, we studied the effects of CWP on the production of a recombinant  $\beta$ -galactosidase from *Marinomonas* sp. ef1 (M- $\beta$ Gal) in a lactose-inducible pET-*Escherichia coli* BL21 system. M- $\beta$ Gal is robust, active over a wide temperature range (4°C-55°C), and capable of hydrolyzing lactose from complex matrices such as CWP itself and skim milk. Due to its high lactose content, CWP can be used as an effective inducer, maintaining a high level of M- $\beta$ Gal production, comparable to a traditional inducer such as pure lactose, in both flask and bioreactor scale. To conclude, CWP has proven to be a valuable and cost-effective alternative inducer for the production of recombinant enzymes for biotechnological applications.

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