

# Towards sustainable processes: use of agro-food waste biomasses to produce recombinant proteins

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*Escherichia coli* cells are the most widely used microbial cell factories for the production of recombinant proteins and enzymes. Typically, recombinant production involves the use of well-defined media and expensive inducers of gene expression, such as isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG). Agro-food waste biomasses can be valuable growth substrates for microbial cultures due to their organic matter content, offering potential solutions to reduce costs and improve process sustainability.

This study focuses on the valorization of cheese whey permeate (CWP) and crude glycerol, collected from two Italian plants. CWP is derived from the extraction of whey protein, while crude glycerol is obtained from the hydrolysis of waste oils to produce fatty acids. We exploited both biomasses for the production of various recombinant proteins in *E. coli* BL21 (DE3) cells. Our results indicated that CWP is a cost-effective alternative inducer for recombinant protein production. In addition, the micronutrients present in CWP, such as vitamins and coenzymes, reduce the cellular stress caused by heterologous expression<sup>1</sup>. To improve recombinant protein production and maximize the use of crude glycerol and CWP, a 1.5 L fed-batch cultivation process was developed. This process used 250 mL crude glycerol (125 g) and 300 mL CWP (52.5 g lactose) to produce  $52.9 \pm 2.2$  g dry cell weight and  $1980 \pm 135.9$  kU of a recombinant  $\beta$ -galactosidase with biotechnological potential. In conclusion, crude glycerol and CWP can serve as alternative carbon sources and inducers in high-density cell cultivations, resulting in water conservation and a sustainable and economical recombinant protein production process.

## References

<sup>1</sup> de Divitiis et al. (2023). Cheese-whey permeate improves the fitness of *Escherichia coli* cells during recombinant protein production. *Biotechnology for Biofuels and Bioproducts*, 16(1), 1-19.

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