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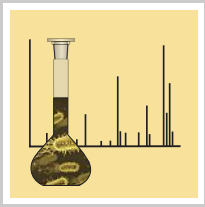

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Reducing Endotoxin Contamination in Biopharmaceuticals for Happier Biotechnologist?

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Bacterial bioreactors are frequently used in the biopharmaceutical industry. About 30% of approved therapeutic proteins are produced in *Escherichia coli* strains and include products such as human insulin or antibodies, *e.g.* Ranibizumab marketed as Lucentis® by Genentech. *E. coli* is a Gram-negative bacterium. Hence, it has endotoxin molecules integrated in its outer membrane. During cell growth, lysis, and environmental adaptation processes, these molecules are released into the culture media. Therefore, endotoxins are widespread contaminations in antibodies, bioplastics, or plasmid DNA produced by bacterial cultivations. They are very potent fever-inducing immunostimulants that can cause organ failure at pg mL^{-1} concentrations when inside the human blood stream. Consequently, products such as injectables or biomaterials for medical applications need to undergo very costly, time-consuming, and tedious downstream processing.

Could the endotoxin contamination of the product be minimized by changing cultivation conditions, thus leading to lower manufacturing costs and increased product safety? To answer this question one needs a method to measure their concentration in a simple and economic way.

The Kdo-DMB-LC assay allows endotoxin quantification in challenging bioreactor matrices in a straightforward way. The sugar acid 3-deoxy-D-manno-2-ulosonic acid (Kdo) is used as an endotoxin marker. Released under mild acid hydrolytic conditions, Kdo is derivatized with the fluorophore 1,2-diamino-4,5-methylenedioxybenzene (DMB), and separated by HPLC from matrix compounds. The endotoxin concentration was analysed in lab- and pilot-scale bioreactors of *E. coli* K12 cultivated under different conditions: one using glucose as the carbon source and the other using a carbon source mix of glucose and arabinose. A comparison of the ratio between the endotoxin concentration in the cell culture media and the dry cell weight of samples at different cultivation times showed that less biomass was produced with the mixed carbon source and more endotoxin was released by the bacteria. Bacteria that grow on their preferred food, *i.e.* in glucose medium, have less adaptation pressure and release less endotoxins.

Our results show that changing bioreactor cultivation conditions can change the endotoxin contamination levels to deal with during downstream processing. Material costs and time expenses can be lowered in the future. **Or: Happy bacteria lead to happy biotechnologists.**

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Fig. 1. Lab scale bioreactor with an *Escherichia coli* K12 culture. This bacterial strain is used to produce injectables such as antibiotics that need to be endotoxin-free.

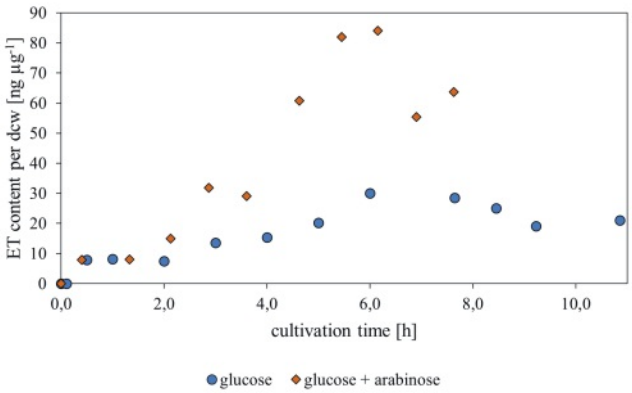

Fig.2. Ratio of released endotoxin amount [ng] per bacterial biomass [µg] for *E. coli* K12 grown on different carbon sources as glucose (blue dots) and glucose / arabinose (orange diamonds).

Reference

A. Hoffmann, K. Pacios, R. Mühlemann, R. Daumke, B. Frank, F. Kalman, *J. Chromatogr. B* **2023** *1228*, 123839, https://doi.org/10.1016/j.jchromb.2023.123839.

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