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## Implementation of a repeated fed-batch process for the production of chitin-glucan complex by *Komagataella pastoris*

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Nhitin-glucan complex (CGC), a co-polymer composed of chitin and  $\beta$ -glucan units, was produced by yeast *Komagataella pastoris*, Jusing glycerol as sole carbon source. A repeated fed-batch process was developed with daily cycles to achieve high cell density and high CGC production. K. pastoris fermentation was performed in basal salts medium (BSM) supplemented with a trace mineral solution (PTMs) in a 5 L bioreactor, with 3 L initial working volume. Cultivation was performed with a controlled temperature and pH of 30°C and 5.0±0.02, respectively. The dissolved oxygen concentration was kept at 50% air saturation with a constant aeration rate of 1 vvm and by automatic stirrer control and oxygen supplementation. Process stability was shown on a 7 daily cycles repeated fed-batch production. Each cycle had an initial glycerol concentration of approximately 60 g/L. After 6 h of batch cultivation, the reactor was fed with a constant glycerol feeding rate of 44 g/h until the end of the cycle (23 h). At the end of each cycle, around 3.5 L of culture broth were purged from the reactor and the remaining volume (~380 mL) were kept in the reactor, serving as inoculum for the next cycle. The new cycle was initiated by filling the bioreactor with 2.6 L of fresh culture medium. This strategy resulted in a daily production of 120 g/L of biomass with a CGC content of 12.9±1.6 wt%. These results represent an improvement when compared with previous fed-batch experiments, in which 104 gCDW/L were obtained after 41 h of cultivation (Roca et al., 2012). An average biomass yield of 0.42±0.03 gCDW/gglycerol and a volumetric productivity of 16±2.2 gCGC/L.day were achieved. This fermentation process is a promising strategy for high cell density cultivation of K. pastoris for enhanced CGC production. In this work, a continuous CGC production was implemented. The process was reproducible and yielded high cell density production associated to a daily high biopolymer productivity.

## Biography

Maria A M Reis has a PhD in Biochemical Engineering, and is Group Leader of the Biochemical Engineering Group at the Universidade Nova de Lisboa, Portugal. Her research area is Environmental/Industrial Bioengineering, with special focus on the development of sustainable bioprocesses for the removal of pollutants from water and wastewater streams, and for the exploitation of industrial wastes for the production of biopolymers. She is co-author of 4 National patents and 5 International patents. She has published over 200 papers in scientific journals, and she is presently Editor of Water Research.

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