Implementation of a repeated fed-batch process for the production of CGC by *Komagataella pastoris*

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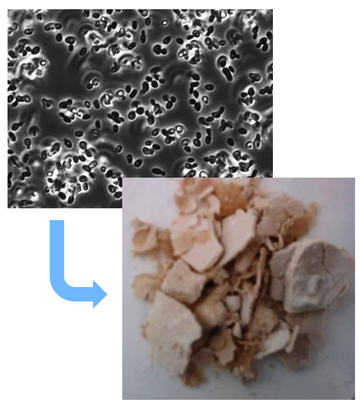
Introduction

Chitin-glucan complex (CGC) is a natural cell wall component of most fungi and yeasts, conferring stability and rigidity to the microbial cells. This biopolymer is a copolymer of chitin and β-glucan moieties and combines the physico-chemical properties of the two polysaccharides, being an interesting material for pharmaceutical, cosmetic or food applications [Farinha et al., Carbohydr Pol 2015;130:455-64].

Recently, a process for the production of CGC by cultivation of the yeast *Komagataella pastoris* (formerly known as *Pichia pastoris*) using glycerol as carbon source was developed and patented [International Patent WO2010/013174 and WO2015177622].



With the objective of developing a CGC continuous production process, a repeated fed-batch strategy was implemented. This strategy consisted of 7 daily fed-batch cycles (8 operation days), in order to achieve high cell densities and high CGC production, keeping the stability of the culture during the hole process.

Methodology

CGC Production

- Yeast strain *K. pastoris* DSM 70877

- Culture medium: BSM + PTMs + glycerol (60 g/L)
- Feeding solution: glycerol supplemented with PTMs

- Operational conditions:
  5 L bioreactor (initial working volume: 3 L)
  T = 30 ºC
  pH = 5.0 ± 0.2
  pO_2 = 50%, controlled by automatic stirrer control and oxygen supplementation

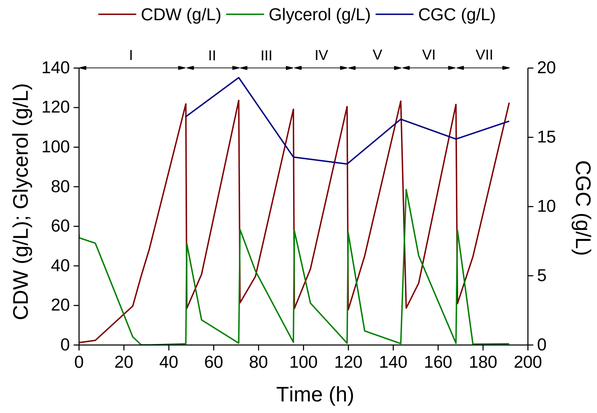
Repeated Fed-batch Procedure

- 1st cycle: 47 h (batch phase – 24 h)

- Repeated fed-batch cycles:
  - 23 h cycles (batch phase – 6 h)
  - Fed-batch phase: glycerol feeding profile started with a flow rate of 13.7 g/L.h that was increased to 15.7 g/L.h
  - End of the cycle: 3.5 L was purged from the bioreactor. The remaining 380 mL was kept in the bioreactor, serving as inoculum for the next cycle
  - Beginning of the next cycle: refill the bioreactor with 2.6 L of fresh BSM medium

Results

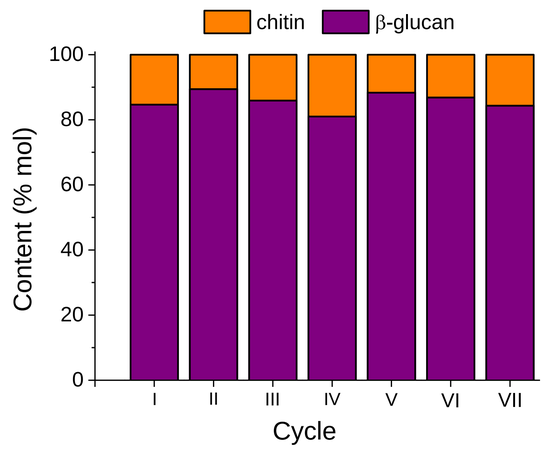
Repeated fed-batch production



Cycle	$Y_{X/S}$ (g_X/g_S)	$Y_{CGC/S}$ (g_{CGC}/g_S)	r_{CGC} (g/L.day)	r_X (g/L.day)
I	0.492	0.067	8.316	60.868
II	0.434	0.077	20.007	108.901
III	0.388	0.052	13.714	98.829
IV	0.405	0.050	13.353	104.238
V	0.419	0.063	16.712	108.115
VI	0.393	0.055	16.104	111.475
VII	0.409	0.063	16.809	105.648

- 958.5±23 g of feed solution was consumed in each repeated cycles.
- a daily production of 121.8±1.5 g/L of biomass with a CGC content of 12.9±1.6 wt% was achieved, which results in a biomass yield of 0.41±0.02 $g_{CDW}/g_{glycerol}$ and a product productivity of 16.1±2.2 g_{CGC}/L.day.

CGC sugars composition



Conclusions

- In this work, a repeated fed-batch strategy was implemented for CGC continuous production.
- This fermentation process revealed to be a promising strategy for high cell density cultivation of *K. pastoris* and, consequently, for the enhancement of CGC production.
- The process was reproducible and yielded high cell density production associated to a daily high biopolymer productivity.

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