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PRODUCTION OF CHITIN-GLUCAN COMPLEX (CGC) FROM BIODIESEL INDUSTRY BY-PRODUCT

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Chitin-glucan complex (CGC) is a natural material composed by two types of biopolymers, namely, chitin and β-glucans, linked by glycosidic linkages. It is one of the cell-wall components of several yeasts and fungus that confer the microorganisms' cell stability and rigidity. Chitin, β-glucans and their derivatives are widely used in several applications, including the food, cosmetics and biomedical industries. Currently, chitin is mainly obtained from crustacean, while β-glucans are obtained from plants. The seasonal character of those raw materials and the variability of the composition of the organisms, make the processes rather expensive and with a lower reproducibility. Yeast production of CGC allows for the use of inexpensive raw materials, such as crude glycerol from the biodiesel industry, with no availability restrictions, and for the continuous optimization of the process with high cell density. Moreover, both the composition and the properties of the polymers are more stable than the ones obtained by the traditional extraction method from crustacean.
In this work, we present a new bioprocess[1] for the production of CGC by *Pichia pastoris* using low-cost crude glycerol as the carbon source. *P. pastoris* DSM 70877 was cultivated using either pure glycerol or crude glycerol from the biodiesel industry. CGC was extracted using the procedure proposed by Synowiecki and Al-Khateeb[2].

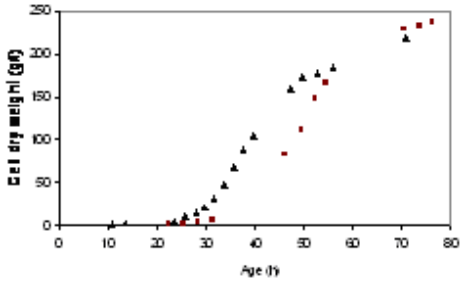

Fig. 1. Cell dry weight during *Pichia pastoris* fermentation with ▪ pure glycerol and ▲ glycerol from biodiesel industry.

The results have shown that similar CDW was obtained for both substrates (237 and 224 g/L, respectively) (Fig. 1).The biomass produced during growth on crude glycerol had a CGC content of 16%, out of which 6% corresponded to chitin. CGC extracted from *P. pastoris* cultivation on pure and crude glycerol were compared with commercial CGC (Kitozyme).
The molecular characteristics of the CGC were studied by capilar viscosimetry using the Mark-Houwink-Sakurada constants. An aprotic dipolar solvent, N,N-dimethylacetamide containing 5% lithium chloride at 30ºC was used to dissolved the CGC. The average molecular weight (*Mw*) of the CGC extracted from *P. Pastoris* ranged between 4.3-4.9x10^4 (Table 1). These values are higher than those determinated for commercial CGC and are in agreement with the results found in the literature[3].

Table 1.Mass characterization of the fractions by Capilar Viscosimetry.

Sample	weight fraction (g)	[η] (dl/g)	Mw
Commercial CGC (Kitosyme)	0.002504	1.5936	3.54x10^4
P. pastoris CGC (crude glycerol)	0.002528	1.9081	4.28x10^4
P. pastoris CGC (pure glycerol)	0.002572	2.1784	4.92x10^4

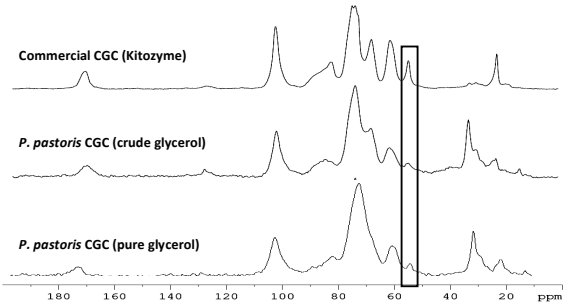

Fig. 2. C-NMR spectra of CGC.

The solid-state NMR spectra are presented in Fig. 2. The signal at 33 ppm is due to impurities, residual proteins and/or lipids[4]. Being a glucose derivative polymer, CGC spectra displays a characteristic range of resonances between 55–104 ppm. The signal near 50 ppm may be assigned to the presence of the acetyl groups of chitin.

In conclusion, crude glycerol was shown to be a suitable carbon source for *P pastoris* growth. CGC

extracted from *P. pastoris* cell wall had *Mw* similar to the commercial biopolymer (Kitozyme).

ACKNOWLEDGEMENTS
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