Extraction of chitin-glucan complex from *Komagataella pastoris* cell-wall using a biocompatible ionic liquid

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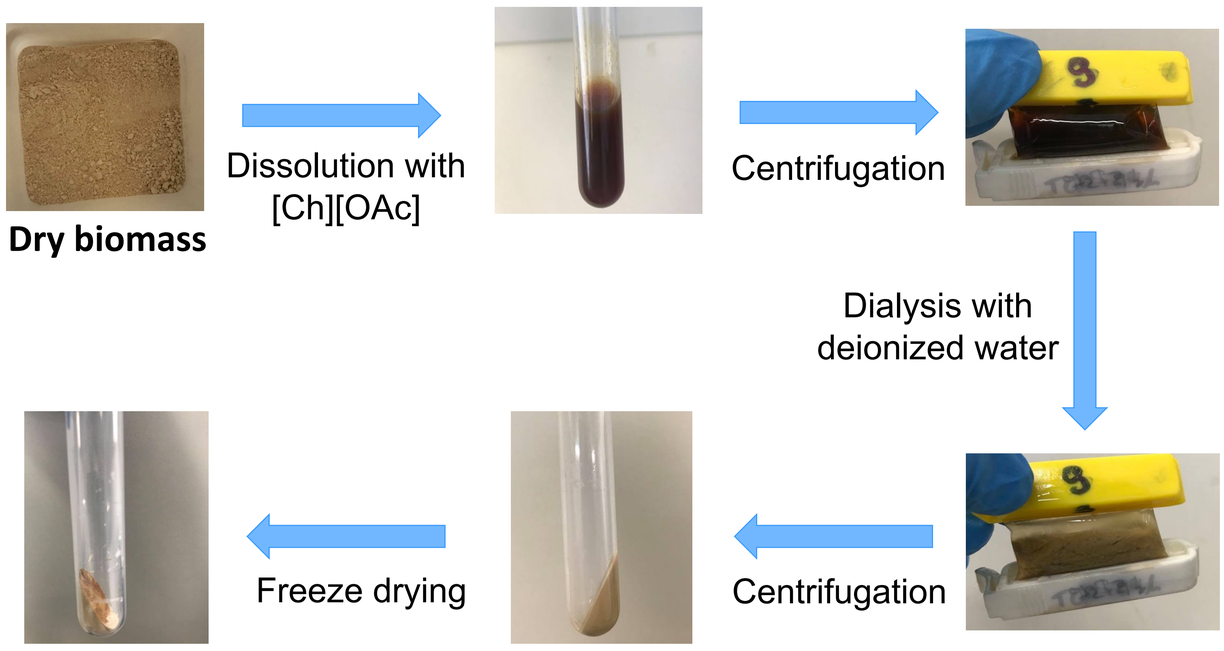
Introduction

- **Chitin–Glucan Complex (CGC)** is a co-polymer composed of covalently linked chitin and β-glucans moieties. It is a biocompatible and biodegradable biopolymer, combining antioxidant, antimicrobial and anti-inflammatory properties. It is commonly extracted from fungal cell-wall by hot alkaline treatment followed by a neutralization step with HCl and a purification step with water [1]. **Thus, the search for safer and more environmentally friendly solvents for CGC extraction is of utmost importance**.

- **Ionic liquids (ILs)** were already used to extract biopolymers similar to CGC (e.g., chitin) from several crustacean sources. ILs are low melting organic salts, possessing non-flammability, negligible volatility and tunable chemical and physical properties.

This work aims at the extraction of CGC from the cell-wall of the yeast *Komagataella pastoris* using a biocompatible IL, choline acetate ([Ch][OAc]), which has been reported to be capable to dissolve CGC [2]. A Design of Experiments (DoE) was used to evaluate the influence of the extraction conditions: temperature (ºC), reaction time (h) and biomass concentration (% w/w) to define the optimal CGC extraction conditions.

CGC extraction from *K. pastoris* biomass using [Ch][OAc]



Design of experiments (DoE)

Response surface methodology (RSM)

Variables (X_i)
- Reaction time: 8 – 24 h
- Temperature: 80 – 120 ºC
- Biomass concentration: 5 – 15 % (w/w)

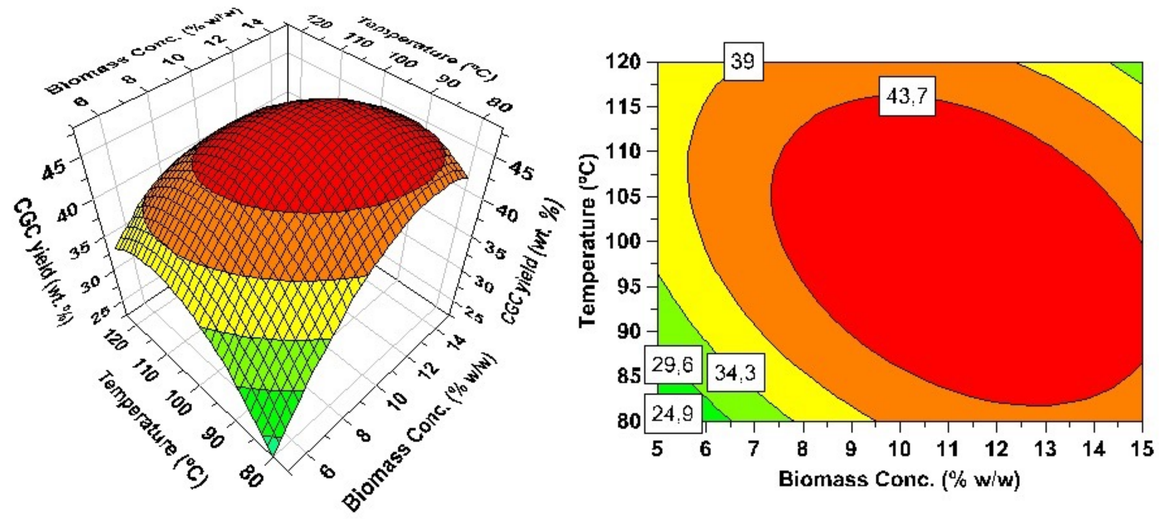
Responses (Y_i)
- CGC extraction yield (wt. %)

Results

- The model fitting was evaluated by analysis of variance (ANOVA) and multiple linear regression (MLR). The second order model showed a good fit (R^2 = 0.89) with statistical meaning (*p*-value < 0.05, for a 95% confidence level) and with no lack of fit (*p*-value > 0.05), i.e. the model error was in the same range as the pure error.

- The factors and their interaction were also evaluated by *p*-value at 95% confidence level, showing that the CGC extraction yield was mostly affected by the temperature and biomass concentration.

Response plots (reaction time – 24h)



Optimal interval for a fixed time of 24 h:
- Temperature: 82 – 116 ºC
- Biomass concentration: 7.3 – 15 % (w/w)

→ Selected optimal conditions: 90 ºC, 11 % (w/w), 24 h.
  - CGC extraction yield (wt. %): 46.2 ± 1.1
  - Protein content (wt. %): 26.4 ± 2.9

Conclusions

✓ CGC was successfully extracted from the yeast biomass using choline acetate as extraction solvent.

✓ Through DoE experiments the optimal conditions for CGC extraction were determined.

✓ The model presented a **good fitting** with the experimental data.

✓ This method showed high extraction yields (46.2 wt. %), comparing to the hot alkaline treatment (13 - 22 wt. %) although presenting also higher protein content (26.4 compared to 3 wt.%)

✓ On going work is being carried out on the development of a CGC polymeric structure obtained from the yeast biomass using the defined optimal conditions.

References
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