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## Utilizing cell wall integrity response in *Saccharomyces cerevisiae* for enhanced chitin production : genetic engineering and adaptive laboratory evolution strategies

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economy. In this context, microalgae are prolific sources of added-value biocompounds.

Cyanoflan is a unique extracellular polysaccharidic polymer naturally secreted by a marine unicellular cyanobacterium, requiring minimal isolation steps. Therefore, the biomass surplus can be used to generate value envisaging a biorefinery approach, having the potential to be commercialized as food or feed supplement, for example, due to its high content of proteins, vitamins, and minerals, as well as the blue pigment phycocyanin.

Cyanoflan is a complex and versatile macromolecule that can be applied in cosmetic and pharmaceutical formulations as a rheology modifier, showing high apparent viscosity in aqueous solutions and emulsifying activity. Furthermore, *in vitro* and *in vivo* results demonstrated Cyanoflan biocompatibility with human cells and bioactivity, namely antioxidant and anti-inflammatory properties, which can provide protection to the skin and promoting its regeneration. Other functional and bioactive properties of Cyanoflan are being evaluated mostly envisaging its incorporation into commodities and premium products in the cosmetics and personal care industry.

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IB-062

Investigating the effect of bioreactor parameters on in-line Raman spectra to measure yeast concentration in fermentation

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Many industrial bioprocesses using bioreactors have been well-established around the world. However, there are still some challenges in sampling, monitoring, and controlling them. Process targets like biomass and chemical compound concentrations are measured offline with manual sampling steps. Raman spectroscopy is a valuable and promising process analytical tool (PAT) that can provide real-time information on these measurement targets. However, whereas molecular targets provide clear peaks in Raman spectra, the spectral contribution of yeast biomass is still relatively unknown. This work aims to investigate the spectral contribution of *Saccharomyces cerevisiae* biomass, while screening for the signal attenuation caused by standard bioreactor parameters.

In this work, the Raman spectra attenuation caused by temperature, bubble size, and viscosity was investigated. Using this knowledge, Raman spectra of pure yeast biomass were collected to isolate the spectral effects caused by biomass alone. The complete overview of the spectral effects caused by bioreactor parameters and biomass resulted in the development of a modelling strategy used to quantify biomass concentration. The model predicted the biomass concentration during a batch fermentation with an error of 4.89% (RMSEP/Range). Moreover, a workflow was generated to guide biomass quantification model building for different systems of yeast fermentation.

To improve the quality of biomass quantification with Raman spectroscopy, it is important to understand the spectral markers of yeast and our bioreactor parameters. This will lead to more advanced data processing strategies and aids in the development of robust models.

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IB-063

Utilizing cell wall integrity response in *Saccharomyces cerevisiae* for enhanced chitin production: genetic engineering and adaptive laboratory evolution strategies

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Chitin, a highly valuable biopolymer, is one of the main components in the cell wall of fungi. While microbial chitin production typically involves identifying naturally chitin-rich organisms, some of them can pose risks to humans, crops, and animals. To circumvent the potential hazards, we focus on enhancing chitin production in "generally recognized as safe" (GRAS) yeast *Saccharomyces cerevisiae* that is a common industrial production host.

Previous research indicated that environmental stresses trigger a defense mechanism conserved in fungi known as the cell wall integrity (CWI) response, resulting in increased chitin synthesis as one of the protective measures. In this study, we develop two approaches to utilize CWI response for maximizing chitin content in *S. cerevisiae*'s cell wall: (i) evaluating the use of the key regulatory genes of the CWI response, namely RHO1 and PKC1, along with mutant forms RHO1(Q68H), and PKC1(R398A), to create a genetic switch that provides control over the CWI response for enhancing the chitin content in the cell wall; (ii) developing chitin-rich *S. cerevisiae* strain by harnessing the power of natural selection via adaptive laboratory evolution (ALE) with increasing cell wall stress from the addition of an echinocandin class drug, Caspofungin.

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IB-064

The quest for sustainable N-heterocycle synthesis powered by electricity and light

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Pivoting industrial processes towards more sustainable alternatives is crucial for the mitigation of climate change and reducing greenhouse gas emissions. The bacterium *Rhodospseudomonas palustris* TIE-1 could offer a sustainable alternative towards fine chemical synthesis via photobioelectrosynthesis. *R. palustris* is able to grow photoautotrophically on CO<sub>2</sub> when it harnesses the reducing power of H<sub>2</sub> or electrons from a poised cathode.[1,2] Combining this with the synthesis of N-heterocycles, important pharmaceutical precursors, offers a sustainable approach for fine chemical production.[3] In this study, an imine reductase (IRED), that catalyzes the synthesis of N-heterocycles, was heterologously produced in *R. palustris*. [4] The production levels and activity of IRED in recombinant *R. palustris* were evaluated. Furthermore, *R. palustris* TIE-1 was cultivated, under several conditions, in a bioelectrochemical reactor with CO<sub>2</sub> as sole carbon source and a poised cathode as electron donor. Successful IRED production and activity were shown. Furthermore, *R. palustris* TIE-1 was able to show current consumption under photoelectroautotrophic conditions and several conditions were altered to increase the extracellular uptake of electrons by *R. palustris* TIE-1. Overall, this study provides a crucial step towards