**Application of Online Measurement Tools for the Prediction of Residual Substrate Concentration in *Ustilago Maydis* Mixed Cultures on Pectin.**

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**Highlights**

* Prediction of residual substrate concentration without offline sampling
* Determination of the pectinolytic activity of *U. maydis* on polygalacturonic acid
* Mixed cultures increase metabolization rate of complex substrates

**1. Introduction**

The quantification of residual substrate is a major challenge during the microbial conversion of industrial organic waste streams. The pectin fraction of biomasses like sugar beet pulp is robust to acid hydrolysis. Enzymatic hydrolysis during microbial fermentations could thus help to convert complex substrates into fermentable sugars. Galacturonic acid - the main monomeric sugar in pectin - is an unusual carbon source during microbial fermentations. *Ustilago maydis* is well known as expression host for carbon activating enzymes (CAZymes) [1]. It is able to convert galacturonic acid [2]. Therefore, it was chosen as production host for polygalacturonases of intrinsic and heterologous origin.

**2. Methods**

*U. maydis* was cultivated in **R**espiration **A**ctivity **MO**nitoring **S**ystem (RAMOS) shake flasks for the online determination of oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR) and respiratory quotient (RQ). The correlation of the consumed overall oxygen with the consumed substrate was demonstrated previously [2]. Production and secretion of endo- and exo-polygalacturonase in different strains was achieved by promoter exchange in the *U. maydis* genome or introduction of heterologous genes. The generated strains were screened for their potential to metabolize polygalacturonic acid, pectin or sugar beet pulp with or without addition of conventional enzyme cocktails.

**3. Results and discussion**

RAMOS cultivations demonstrated the metabolic activity of an *U. maydis* strain expressing a heterologous exo-polygalacturonase on polygalacturonic acid [2]. However, growth of the culture was limited to the enzymatic activity in the culture supernatant. A screening of three different *U. maydis* strains expressing endo-polygalacturonases of intrinsic, bacterial or eukaryotic origin, respectively, was conducted in mixed cultivation with the exo-polygalacturonase expressing strain. Online monitoring of the metabolic activity revealed that the endo-polygalacturonase of eukaryotic origin showed the highest activity on polygalacturonic acid.

This mixed culture was subsequently cultivated on more complex substrates like pectin or sugar beet pulp. The limited metabolic activity demonstrated that additional pectinases are required for efficient conversion of those substrates. As proof of principle, the metabolic activity of *U. maydis* mixed cultures on pectin and sugar beet pulp was increased by addition of conventional enzyme cocktails. Finally, the potential of *U. maydis* to convert the main pectin sugars was demonstrated by cultivation in a minimal medium containing six different carbon sources.

**4. Conclusions**

A detailed overview of the respiration activity of *U. maydis* during growth on pectic substrates of different complexity was attained. The previously developed methodology for online determination of the residual substrate concentration was applied on a screening of mixed cultures expressing different endo-polygalacturonases. Limitations in the enzyme expression rate were overcome by addition of conventional enzyme cocktails demonstrating the capability of *U. maydis* to convert the main sugars from pectin.

**References**

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