Activity-based profiling of β-mannanases in Aspergillus niger

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Syngas fermentation to ethanol or other useful compounds is an upcoming type of industrial process that allows valorization of gasified waste biomass or other CO/CO2/H2 mixtures. Therefore, it can contribute to defossilization of chemical industry.

Understanding of microbial metabolism and fermentation operation is required to advance the technology. We studied the individual impact of dilution rate, gas transfer rate, and acetic acid addition on metabolic shifts, product titers, and rates in continuous CO fermentation by Clostridium autoethanogenum, the microbe used in industry. These variables jointly determine ethanol production rates, presumably because ethanol production requires a certain minimum undissociated acetic acid concentration.

Consequently, some acetate is co-produced, and becomes a waste. Biomass production is also at the expense of the yield of ethanol on syngas. On the other hand, a high biomass concentration is desired to obtain a high ethanol production rate.

To maximize both the yield of ethanol on syngas and the ethanol production rate, we designed a process with a recycle of aqueous acetate and biomass to the continuous fermentation, after ethanol recovery in a separate unit. The recovery method is vacuum distillation at fermentation temperature, to maintain microbial viability. The distillation removes all ethanol and a small part of the water, while keeping the rest of the broth in the system. Distillate was upgraded to 99.9% fuel-grade ethanol. Vapor recompression minimized energy use substantially, leading to modest costs of downstream processing at industrial scale.

This process integration is an attractive option for further development.

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Activity-based profiling of β-mannanases in *Aspergillus niger*

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β-Mannanases are endo-acting glycosyl-hydrolases (GHs) capable of hydrolyzing β-1,4 linkages of mannan-containing polysaccharides. Such a process has applications in many industrial sectors including bioethanol production. The Aspergillus species is amongst the main producers of these enzymes. β-Mannanases of Aspergillus niger were studied by activity-based protein profiling (ABPP). ABPP is an effective tool to characterize GHs in complex mixtures. ABPP relies on a simple yet powerful concept in which activity-based probes (ABPs) irreversibly inhibit an enzyme by covalently binding to its active site. ABPs are endowed with a reporter entity (either a fluorophore like Cy5 or a capture agent such as biotin) which allows detection and isolation of the enzyme bound to the ABP. In this study ABPs for β-mannanases were developed. Mannobiose and mannotriose were chosen as recognition elements, and an epoxide was employed as electrophilic warhead. The synthesized ABPs were evaluated on A. niger secretomes obtained from cultivations on mannan-containing substrates. AnManA was pulled-down from the secretome with a biotinylated ABP and identified by LC-MS. The ABPs were also employed to test the temperature and pH stability of the labelled enzymes directly in the secretome. Furthermore, AnManA was overexpressed in a genetically modified strain of A. niger. The recombinant mannanase was purified from the secretome of the overexpressing strain. The purified protein will be further characterized by ABPP and by X-ray crystallography. This research highlights the utility of ABPP in the characterization of fungal mannan-degrading GHs and its applicability in the field of industrial biotechnology.

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Exploring yeast's energy dynamics: the impact of general stress response on maintenance energy

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Understanding and quantifying cellular energetics is crucial in bioprocesses. Besides growth and product formation, cells also need energy for cellular homeostasis. This amount of energy, the maintenance energy requirement (MER), must be included in the cultivation design to supply cells with sufficient substrate to remain alive and productive. We examined the impact of growth rate and the general stress response (GSR) on the MER of the yeast Saccharomyces cerevisiae through various cultivation methods. In chemostats with growth rates from 0.025 to 0.2h-1, the MER was estimated at 0.07 mmol/g/h, but glucose-limited fed-batch cultures in which growth rates approached zero, showed a lower MER of 0.04 mmol/g/h. This suggests that part of the MER is growth-rate dependent.

To explore the effect of the GSR on cellular energetics, including the MER, we characterized a mutant strain lacking the transcription factors Msn2 and Msn4. The absence of Msn2 and 4 resulted in increased stress-sensitivity. Our findings indicate a complex relationship between the GSR and energy management. The absence of Msn2 and Msn4 did not affect the maximal biomass yield but did result in a significant increase in MER, both in chemostat and especially slow-growing fed-batch cultures. These observations demonstrate a critical role of GSR in maintaining cellular efficiency under varying conditions. Additionally, we propose a model for how maintenance energy correlates with growth rate as a function of GSR activation. Together these insights pave the way to design and construct more efficient yeast cell factories.

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MixMatters project: saccharification of bio-waste streams to obtain simple sugars for the fermentation of a 2,3-butanediol recombinant *E. coli* producer

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MixMatters aims to set up and demonstrate an Integrated System that is able to separate and valorise three types of mixed bio-waste streams containing impurities from three agri-food sectors into six high value-added outputs. The Integrated System consists of a Separation Unit for sorting, separation and pre-conditioning of the