An industrial perspective on in vivo kinetics of central metabolite pools and dynamic flux responses in Penicillium chrysogenum during periodic dissolved oxygen feast-famine cycles in a scale-down system

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August 4, 2023

Abstract

Limitations in mixing and mass transfer coupled with high hydrostatic pressures lead to significant spatial variations in dissolved oxygen (DO) concentrations in large-scale bioreactors. While traveling through different zones in the bioreactor, microbes are subjected to fluctuating DO conditions at the timescales of global circulation time. In this study, to mimic industrial-scale spatial DOT gradients, we present a scale-down model based on dynamic feast/famine regime (150 s) that leads to repetitive cycles with rapid changes in DO availability in glucose-limited chemostat cultures of *Penicillium chrysogenum*. The results revealed that the exposure time to the low DO level (less than 10%) imposed a significant impact on the biomass growth and penicillin productivity was considerably reduced by a factor of two, while the averaged substrate consumption rates were comparable under the DO oscillation condition compared to that of 60% DO steady-state condition. Quantitative metabolomics data showed that the DO feast/famine induced a stable and repetitive pattern with a reproducible metabolic response in time. The dynamic response of intracellular metabolites under such DO oscillating conditions showed specific differences in comparison to repetitive substrate pulse experiments. Due to invariable the specific glucose uptake rate (q s) during a cycle, the variation in the intracellular pools size of amino acids, sugar phosphates and organic acids was less pronounced in terms of both coverage and magnitude under DO fluctuations than under repetitive substrate pulses featured with a marked variation in the q s . Remarkably, intracellular sugar polyols were considerably increased as the hallmark metabolites to reserve carbon source and reducing equivalent, which likely provide short-term benefits in such changing environments. Furthermore, the calculated cytosolic NADH/NAD $^+$ ratio under the DO oscillating condition indicated a dynamic and higher redox state of the cytosol, which has been reported to negatively affect the maintenance of penicillin productivity. Despite the increased availability of NADPH for penicillin production under the oscillatory DO conditions, this positive effect may be counteracted by the decreased ATP supply. From an economical point of view, it is interesting to note that not only the penicillin productivity was reduced under such oscillating DO conditions, but also that of the unrecyclable byproduct ortho-hydroxyphenyl acetic acid (o-OH-PAA) and degeneration of penicillin productivity induced by low extracellular glucose sensing. Furthermore, dynamic metabolic flux analysis based on constraining time-resolved metabolite data into genome-scale metabolic model showed that *Penicillium chrysogenum* metabolism shifted from penicillin production to maintaining biomass growth upon a reduction of oxygen supply. The relative decreasing fluxes of amino acid metabolic pathways and fatty acid biosynthetic pathways were assumed to relieve the energy demand for balanced cellular metabolism. Taken together, the metabolic responses of *Penicillium chrysogenum* to DOT gradients reported here are important for elucidating metabolic regulation mechanisms, improving bioreactor design and scale-up procedures as well as for constructing robust cell strains to cope with heterogenous industrial culture conditions.

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