

**Title:** Identifying optimal metabolic nodes and intervention strategies for dynamically controlled microorganisms using two stage minimal cut sets

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**Abstract:** Microorganisms are capable of producing a large variety of valuable chemicals. Due to stoichiometric constraints, metabolic optimization of these cell factories for chemical production can come at the expense of native functionality, such as cell growth. This trade-off is of immense importance due to its significant impact on overall productivity. To overcome such limitations, there has been recent interest in implementing sensor-actuator circuits to dynamically control metabolism, and gain temporal control of the microbial phenotype. These systems can be used to implement a two-stage fermentation, where the first stage is dedicated to cell growth and the second stage to production. Here, we present the first algorithm to determine optimal interventions to dynamically control metabolism for chemical production, by eliminating unwanted (low yield) phenotypes. This algorithm is based on the minimal cut set (MCS) algorithm and searches for a suitable minimal combination of static and dynamic (valve) interventions. The static interventions allow for a high growth rate (first stage), while additionally switching off the valves in the production stage enables high-yield production. We exploit the computational efficiency of this algorithm to determine metabolic valves which can be applied to a broad spectrum of products, leading to targets for the creation of platform strains. Furthermore, we explore the efficacy of controlling the phenotype based on oxygen availability, which has historically been a preferred method to implement two-stage fermentation. This algorithm presents for the first time a direct and efficient route to determine optimal interventions for dynamically controlled microbes.