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Constraints based analysis of extended cybernetic models

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ABSTRACT

The cybernetic modeling framework provides an interesting approach to model the regulatory phenomena occurring in microorganisms. In the present work, we adopt a constraints based approach to analyze the nonlinear behavior of the extended equations of the cybernetic model. We first show that the cybernetic model exhibits linear growth behavior under the constraint of no resource allocation for the induction of the key enzyme. We then quantify the maximum achievable specific growth rate of microorganisms on mixtures of substitutable substrates under various kinds of regulation and show its use in gaining an understanding of the regulatory strategies of microorganisms. Finally, we show that *Saccharomyces cerevisiae* exhibits suboptimal dynamic growth with a long diauxic lag phase when growing on a mixture of glucose and galactose and discuss on its potential to achieve optimal growth with a significantly reduced diauxic lag period. The analysis carried out in the present study illustrates the utility of adopting a constraints based approach to understand the dynamic growth strategies of microorganisms.

1. Introduction

The complexity of the dynamic growth of microorganisms manifests itself in the diverse substrate uptake patterns they exhibit when growing on two substitutable substrates. The most commonly observed pattern is the diauxie, wherein microorganisms grow in two exponential phases, each characterized by the consumption of a single substrate (Monod, 1949). Another pattern that is observed relatively frequently is the simultaneous consumption, wherein microorganisms grow by utilizing both the substrates simultaneously (Kovarova-Kovar and Egli, 1998). The maximum specific growth rate of the microorganisms during simultaneous consumption either lies in between the maximum specific growth rates on the individual substrates or is substantially higher than either of the maximum specific growth rates on individual substrates (Narang et al., 1997b). In a few instances, the substrate consumption pattern of microorganisms also depends on the preculture (Narang et al., 1997b). This diversity of microorganism growth behavior has prevented the derivation of any correlations between single substrate and mixed substrate maximum specific growth rates (Narang et al., 1997b; Ramkrishna, 1987).

The growth behavior exhibited by microorganisms is dependent on the internal regulatory mechanisms they have evolved to grow optimally in their natural environment. Various kinds of regulatory mechanisms found in microorganisms include direct regulation (Jacob and Monod, 1961), asymmetric anticipatory regulation (Mitchell et al., 2009), symmetric anticipatory regulation (Tagkopoulos et al., 2008) etc. While the explicit details of specific regulatory processes in microorganisms are known, an understanding of the overall regulatory process that guides the microorganisms to exhibit various growth behaviors is still absent and various regulatory strategies are still being discovered (Siegal, 2015). Consequently, modeling the growth of microorganisms has relied on mathematical models that do not account for internal regulation. Examples of such mathematical models include Monod model, Contois model, sum kinetics with interaction parameters model etc. (Reardon et al., 2000; Shuler and Kargi, 2002). The utilization of these models does not provide much insight into the internal regulatory structure of the microorganisms, an aspect that is essential for understanding their growth.

The absence of the knowledge of regulatory processes and the development of omic technologies such as genomics has led to the development of constraint-based modeling approaches, specifically stoichiometric models and flux balance analysis (FBA) (Covert et al., 2003; Orth et al., 2010). Constraint-based models attempt to model cellular behavior by successively imposing various

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physico-chemical constraints such as stoichiometric, thermodynamic, regulatory etc. (Covert et al., 2003). The stoichiometric models infer the numerous reactions that occur in the cell by analyzing the enzymes encoded in the genome and formulate a stoichiometric matrix of these reactions. FBA then assumes that the substrate uptake rate of the microorganisms is measured/specified a priori and that the microorganisms have evolved to become optimal with respect to cellular objectives such as cellular yield (Schuster et al., 2008) to infer the internal fluxes of the cells. In cases where the microorganisms behave sub-optimally, adaptive evolution experiments result in the generation mutant microorganism strains that are consistent with FBA predictions (Ibarra et al., 2002). The stoichiometric models provide a base onto which the regulatory information can be integrated (Covert et al., 2004; Covert et al., 2001) and analysis of these models can provide insights into the regulation of various reactions (Stelling et al., 2002). However, these models require the measurement/identification of substrate uptake rates and further constraints for modeling the dynamic growth behavior of microorganisms on multiple substitutable substrates.

The need for incorporating regulation in the mathematical models of microorganisms has led to the development of the cybernetic modeling framework by Ramkrishna and co-workers (Ramkrishna and Song, 2012). This framework is inspired from the principles of the discipline cybernetics, which considers the control and communication processes in both animals and machines (Ashby, 1957). Cybernetic models view microorganisms as optimal control systems that control the induction/repression and activation/inhibition of key enzymes to achieve cellular goals (Dhurjati et al., 1985; Kompala et al., 1984). These models assume that microorganisms possess limited internal resources for enzyme induction and have utilized the matching and proportional laws for the control of enzyme induction and activity respectively (Kompala et al., 1986).

The cybernetic modeling efforts of Ramkrishna and co-workers assume a priori that microorganisms have evolved to optimal dynamic growth behavior under the constraint of limited internal resources (Ramkrishna and Song, 2012). While this goal seeking behavior is an important aspect of cybernetics, the discipline is also interested in all the possible behaviors that a system can exhibit and the constraints under which the system exhibits a particular behavior (Ashby, 1957). This aspect of cybernetics is similar to the core philosophy of the constraint-based modeling approaches that typically only consider the pseudo-steady state behavior of the microorganisms. In the present work, we extend the cybernetic modeling framework of Ramkrishna and colleagues by analyzing the dynamic growth behavior of microorganisms under various constraints and show its use in gaining an understanding of regulation in microorganisms. First, we show that the cybernetic model exhibits linear growth behavior, a characteristic of the unstructured Contois model at high cellular concentrations, under the assumption of no resource allocation for the induction of the key enzyme. We then analyze the dynamic behavior of the extended equations of the cybernetic model under various constraints to derive correlations between single substrate and mixed substrate maximum specific growth rates and show its utility in gaining an understanding of the growth strategies of microorganisms. Finally, we show that *S. cerevisiae* exhibits suboptimal dynamic behavior with a long diauxic lag phase when growing on a mixture of glucose and galactose and discuss on its potential to achieve optimal growth with a significantly reduced diauxic lag period.

2. Analysis of cybernetic model

The extended governing dynamic equations of the cybernetic model for the growth of microorganisms C on N growth limiting substitutable substrates S_i ($i = 1, 2, ...N$) derived in a previous study are written as (Mandli and Modak, 2014)

$$\frac{dc}{dt} = \left(\sum_{j=1}^{N} r_j^c v_j\right) c = \mu^c c, \quad \frac{ds_i}{dt} = -\frac{1}{Y_i} r_i^c v_i c,$$

$$r_i^c = \frac{\mu_i^m s_i}{K_i + s_i} \frac{e_i}{e_i^{\max}}, \quad i = 1, 2, ..N \tag{1}$$

$$\frac{de_i}{dt} = u_i r_{E_i} + \sum_{\substack{j=1 \\ j \neq i}}^{N} u_j \frac{e_j}{e_i} \frac{\alpha_i^2}{\alpha_j^2} \frac{(\mu_j^m)^2}{(\mu_i^m)^2} r_{E_j} - \mu^c e_i, \quad i = 1, 2, ..N \tag{2}$$

where c is the concentration of cells, s_i is the concentration of substrate S_i, Y_i is the yield of cells on substrate S_i, e_i are the specific levels of key enzyme required for the consumption of S_i, r_i^c is the specific growth rate of microorganisms on S_i and μ^c is the overall specific growth rate. $r_i{}^c$ is specified using the modified Monod kinetics that depends not only on μ_i^m, the maximum specific growth rate and K_i, the half saturation constant but also on the level of key enzyme e_i and the maximum level of the key enzyme $e_i^{\max}$. The rate of synthesis of enzyme e_i in the presence of substrate S_i, r_{E_i}, is specified as $r_{E_i} = \alpha_i s_i/(K_i + s_i)$ where α_i is the maximum enzyme synthesis rate. The maximum level of key enzyme e_i when growing on a single substrate S_i, $e_i^{\max}$, can be derived as $e_i^{\max} = \alpha_i/\mu_i^{\max}$. The control of enzyme induction/repression and activation/inhibition is specified using the cybernetic variables u_i and v_i respectively. The first term on the right hand side of Eq. (2) represents the production of e_i due to substrate S_i, the second term represents the contributions of substrates $S_j (j \neq i)$ to the production of e_i and the third term represents the dilution of e_i. The presence of second term in Eq. (2) for all e_i, $i = 1, 2, ..N$ represents symmetric regulation as all the substrates contribute to the induction of not only the enzymes required for their consumption but also for the consumption of other substrates. On the other hand, the absence/removal of the second term in Eq. (2) results in each of the substrates contributing only to the induction of the enzymes required for their consumption and represents direct regulation. The presence of these terms only for e_i, $i = m + 1, m + 2, ..N$ where $m \geq 1$ results in asymmetric regulation. Symmetric anticipatory regulation was observed by Tagkopoulos et al. (2008) wherein they have shown that *E. coli* induces the expression of genes needed for coping with both increased temperature and reduced oxygen availability when only one of the above two conditions is encountered. Asymmetric anticipatory regulation was observed by Mitchell et al. (2009) wherein they have shown that *E. coli* induces the genes required for maltose consumption upon encounter with lactose alone whereas no induction of genes coding for lactose consumption is observed when *E. coli* encounters maltose alone. In the following sections, we analyze the nonlinear behavior of Eqs. (1) and (2) under various constraints.

2.1. Linear growth of microorganisms

The typical growth curve of microorganisms in a batch culture displays (a) a lag phase during which the microorganisms adapt to the environment (b) an exponential phase wherein the microorganisms grow exponentially and (c) a stationary phase where growth stops due to the exhaustion of nutrients (Shuler and Kargi, 2002). However, during the interpretation of exponential growth curves, Monod (1949) noted that microorganisms grow linearly when the synthesis of a rate determining enzyme ceases. More recently, Avraham et al. (2013) have observed that when the budding yeast *S. cerevisiae* are shifted to a low-metal environment,