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Improving the prediction of *Pseudomonas putida* mt-2 growth kinetics with the use of a gene expression regulation model of the TOL plasmid

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ABSTRACT

The molecular and genetic events responsible for the growth kinetics of a microorganism can be extensively influenced by the presence of mixtures of substrates leading to unusual growth patterns, which cannot be accurately predicted by mathematical models developed using analogies to enzyme kinetics. Towards this end, we have combined a dynamic mathematical model of the *Ps*/*Pr* promoters of the TOL (pWW0) plasmid of *Pseudomonas putida* mt-2, involved in the metabolism of *m*-xylene, with the growth kinetics of the microorganism to predict the biodegradation of *m*-xylene and succinate in batch cultures. The substrate interactions observed in mixed-substrate experiments could not be accurately described by models without directly specifying the type of interaction even when accounting for enzymatic interactions. The structure of the genetic circuit–growth kinetic model was validated with batch cultures of mt-2 fed with *m*-xylene and succinate and its predictive capability was confirmed by successfully predicting independent sets of experimental data. Our combined genetic circuit–growth kinetic modelling approach exemplifies the critical importance of the molecular interactions of key genetic circuits in predicting unusual growth patterns. Such strategy is more suitable in describing bioprocess performance, which current models fail to predict.

1. Introduction

Microbial growth kinetics is an essential tool for the design of optimal bioprocesses. Despite more than half a century of research, many fundamental questions about the validity and application of growth kinetics are still unanswered [1]. One of the various cases in biotechnology where understanding of the kinetics of microbial growth is limited is when a culture is grown on mixed-substrates [2]. A multitude of utilisation patterns may occur depending on the metabolic effects of each compound [3] and various substrate interactions have been identified including sequential [4] and simultaneous [5] utilisation. Although substrate interactions can be either positively or negatively influenced by the presence of other compounds [6], in some cases unusual substrate interactions have been reported but not modelled [7].

In general there is no simple rule for the prediction of substrate interactions [8]. When no substrate interactions are identified, simple Monod terms [9] can be added in sum kinetics. However, if substrate interactions occur, growth rate equations accounting for these interactions are used [10]. Usually an analogy to enzyme kinetics is made, because if a reaction is enzyme catalysed then the inhibition of enzyme activity results in the inhibition of microbial growth by the same pattern. Nevertheless, although the determination of the model giving the most accurate description of the experimental data might suggest the mechanism of the interactions, this might not always hold true when unusual substrate interactions occur. Thus, for a certain combination of substrates none of the developed models may accurately fit the experimental data or the interaction indicated may not be valid for a wide range of conditions. Although the metabolic events taking place in mixed-substrate cultivation have been previously studied [6], the failure of models to predict the growth kinetics in some cases underlines the need for inclusion of the exact mechanism for the production of enzymes [11]. The genetic information required for the production of enzymes used for the metabolism of substrates in a certain

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Nomenclature

$I_{1,2}$	interaction parameter of *m*-xylene on succinate [–]
$I_{2,1}$	interaction parameter of succinate on *m*-xylene [–]
i	inhibition constant [$mM^{-1}\ h^{-1}$]
$K_{I,1}$	*m*-xylene inhibition constant [mM]
$K_{I,1,2}$	*m*-xylene inhibition on succinate constant [mM]
$K_{I,1\text{-}P,2}$	*m*-xylene by-product inhibition on succinate constant [mM]
$K_{Iq,1,2}$	*m*-xylene inhibition on succinate consumption constant [mM]
$K_{Iq,1\text{-}P,2}$	*m*-xylene by-product inhibition on succinate consumption constant [mM]
$K_{Pr,XylR_i}$	$XylR_i$ translation coefficient [–]
$K_{S,1}$	*m*-xylene saturation constant [mM]
$K_{s,1}$	saturation constant for *m*-xylene consumption [mM]
$K_{S,2}$	succinate saturation constant [mM]
$K_{s,2}$	saturation constant for succinate consumption [mM]
$K_{SUC,Pr}$	inhibition constant of succinate on *Pr* promoter activity [mM^{-2}]
$K_{SUC,Ps}$	inhibition constant of succinate on *Ps* promoter activity [mM]
K_{XylR_a}	repression coefficient of *Pr* promoter (due to $XylR_a$ binding) [mM]
$K_{XylR_a,Ps}$	activation coefficient of *Ps* promoter [mM]
K_{XylR_i}	repression coefficient of *Pr* promoter (due to $XylR_i$ binding) [mM]
MWt_1	*m*-xylene molecular weight [mg $mmol^{-1}$]
n	exponent indicating the type of relation between μ_2 and S_2 [–]
$n_{Pr,a}$	hill coefficient of *Pr* promoter (due to $XylR_a$ binding) [–]
$n_{Pr,i}$	hill coefficient of *Pr* promoter (due to $XylR_i$ binding) [–]
$n_{Ps,a}$	hill coefficient of *Ps* promoter (due to $XylR_a$ binding) [–]
$q_{s,1}$	*m*-xylene metabolic quotient [$mM_{m\text{-xylene}}\ mg_{biomass}^{-1}\ h^{-1}$]
$q_{s,2}$	succinate metabolic quotient [$mM_{succinate}\ mg_{biomass}^{-1}\ h^{-1}$]
$r_{R,XylR}$	$XylR_a$ dissociation constant [$mM^{-1}\ h^{-1}$]
r_{XylR}	$XylR_i$ oligomerization constant [$mM^{-1}\ h^{-1}$]
$S_{0,1}$	initial *m*-xylene concentration [mM]
S_1	*m*-xylene concentration [mM]
S_2	succinate concentration [mM]
S_m	maximum *m*-xylene concentration above which growth is completely inhibited [mM]
S_θ	threshold *m*-xylene concentration below which there is no inhibition [mM]
t	time [h]
Y_1	yield coefficient for biomass on *m*-xylene [$mg_{biomass}\ mg_{m\text{-xylene}}^{-1}$]
Pr_{TC}	*Pr* promoter relative activity [–]
Ps_{TC}	*Ps* promoter relative activity [–]
$R_{max,1}$	maximum *m*-xylene metabolic quotient [$mmol_{m\text{-xylene}}\ mg_{biomass}^{-1}\ h^{-1}$]
$R_{max,2}$	maximum succinate metabolic quotient [$mmol_{succinate}\ mg_{biomass}^{-1}\ h^{-1}$]
X	biomass concentration [$mg\ L^{-1}$]
$XylR_a$	$XylR_a$ protein concentration [mM]
$XylR_i$	$XylR_i$ protein concentration [mM]

Greek letters

α_{Pr}	*Pr* promoter deactivation rate [h^{-1}]
α_{Ps}	*Ps* promoter deactivation rate [h^{-1}]
α_{XylR_i}	$XylR_i$ degradation/dilution rate [h^{-1}]
α_{XylR_a}	$XylR_a$ degradation/dilution rate [h^{-1}]
β_0	basal expression level of *Ps* promoter [h^{-1}]
β_{Pr}	maximal expression level of *Pr* promoter [h^{-1}]
β_{Ps}	maximal expression level of *Ps* promoter [h^{-1}]
β_{XylR_i}	maximal $XylR_i$ translation rate based on *Pr* activity [$mM\ h^{-1}$]
μ	specific growth rate of biomass [h^{-1}]
μ_1	specific growth rate of biomass on *m*-xylene [h^{-1}]
μ_2	specific growth rate of biomass on succinate [h^{-1}]
$\mu_{max,1}$	maximum specific growth rate of biomass on *m*-xylene [h^{-1}]
$\mu_{max,2}$	maximum specific growth rate of biomass on succinate [h^{-1}]

process, is encoded by genes existing in specific genetic circuits of the cells. Thus, the construction of mathematical models describing the molecular interactions regulating the transcription of these genes might provide the exact mechanism for substrate interactions.

Genetic circuits are groups of elements, which interact producing certain behaviour [12]. These elements include DNA binding regions for RNA polymerase starting transcription of DNA, DNA regions that terminate transcription, mRNA binding sequences for rRNA starting the translation of mRNA, proteins that regulate the synthesis and activity of other proteins, and motifs that determine mRNA and protein stability. Advanced genetic techniques may successfully identify the components of a circuit and the way these interact. With the application of these techniques, several naturally occurring genetic circuits have been studied recently, such as cell cycle regulatory systems [13], bistable switches [14], oscillating networks [15], and circadian clocks [16]. Therefore, given the fact that key genetic circuits are essential for survival and reproduction of microorganisms, the mechanisms of interactions between circuit components may well-define the distinct responses of various cellular functions to changes in the cells environment [17,18]. The current state of the art is rather limited to the work of Bettenbrock et al. [19] utilizing a dynamic gene regulation model of catabolite repression to describe the dynamic behaviour of various metabolites in *Escherichia coli*.

This study attempts to combine a mathematical model of a key genetic circuit with the growth kinetics of the host microorganism. To this end we have previously paved the way with the development of a mathematical model of the *Ps*/*Pr* node of the TOL plasmid encoded by *Pseudomonas putida* mt-2 [20]. Herein, we present a growth kinetic model of the strain and its coupling with the genetic circuit model, demonstrating a new approach for the improvement of growth kinetic models in cases where the use of quantitative genetic information is imperative. The parameter values of the combined model were estimated through independent experiments and its predictive capability was evaluated in a distinct experimental set-up. Analysis of the results showed that there is increased complexity in modelling substrates degradation and growth kinetics due to the substrate interactions. Although, the combined model offered an improved description of the process, different models, either accounting for interactions in analogy to enzyme kinetics or without directly specifying the type of interaction, were unsuccessful in describing the experiments. This modelling framework provides a solid basis for the development