Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0502.79**

OPTIMAL MODEL TO REPRESENT GROWTH AND PROTEIN EXPRESSION DYNAMICS IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT: A growth kinetics model has been developed to simulate the growth of *Saccharomyces cerevisiae* under different substrate combinations. The optimal model is developed to describe microbial growth in varied microbial growth phenomenon, which involves co-metabolism, sequential and simultaneous utilization of substrates in aerobic and anaerobic fermentation. We have further, extended the growth kinetics model to capture the expression dynamics of galactose-regulated GAL genes of *Saccharomyces cerevisiae*. The model predictions capture the growth profile of *Saccharomyces cerevisiae* on single and mixtures of substrates involving glucose, galactose, glycerol and lactate under different pre-culturing conditions. The constraints applied in the model present the multiple levels of control inside the cell to describe diauxic and triauxic growth. The model predictions also, show close agreement with galactose-induced β-galactosidase synthesis dynamics in *Saccharomyces cerevisiae.*

KEYWORDS: *Saccharomyces cerevisiae*, multiple-substrate, growth kinetics, sequential utilization, optimal model, fermentation, anaerobic, protein expression.

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1.INTRODUCTION

The modelling of fermentation processes has been a challenge for over the years because of its complex nature of cellular reactions. Though the Monod's model can't describe the kinetics of the lag phase of microbes on the single and multi-substrate environment, which is generally the situation in real fermentation processes [1, 2]. The different growth phenomenon of micro-organisms are

observed in the presence of multiple substrates and multiple modes (aerobic and anaerobic). These involve growth due to: (1) sequential utilization of substrates, (2) simultaneous consumption of substrates; and, (3) co-metabolism of substrates. Scientists have tried to explain the diauxic growth of microbes, but have not been very successful in order to explain the simultaneous and sequential modes growth. Yoon et al., [3] have modified Monod's growth model equation to present an unstructured model for multiple substrates, but could not explain the complex cellular regulatory process. Narang and co-workers [4] gave a detailed study on the growth of *E.coli* of multiple substrates. Ramakrishna [5] has developed the concept of cybernetic modelling to explain the behaviour of the cells in the multi-substrate environment. Kompala et al, [6] developed a cybernetic model for the triauxic growth of microbes using matching law. The matching law model was found to be able to explain the sequential utilization of substrates [7]. This model was further modified by Baloo and Ramakrishna [8, 9] to include cell maintenance and the response of the cell to the transient growth. But, the rigid nature of the matching law model did not allow it to describe the simultaneous utilization of substrates. In order to overcome this problem, Straight and Ramakrishna [10] proposed the cybernetic variables for all four basic structures of metabolic pathways assuming them to be independent. Using these parameters, Ramakrishna et al. [11] developed a cybernetic model, which was able to explain the sequential as well as simultaneous utilization of substrates assuming that the 12 essential precursors are responsible for microbial growth. But, it had few drawbacks, such as it is not necessary that only precursors are responsible for growth. Also, the intracellular parameters involved in the model are very difficult to be determined experimentally. The Cornell model developed by Domach et al. [12] was a single cell model for *E. coli*. In this model, the cell was divided into various compartments and enormous biological information was provided about the cell. But, the drawback of this model was its complexity and so it was not easy to apply it for real life fermentation processes. The Cornell model was simplified by reducing the number of compartments to three [13, 14], but many parameters were still required to explain the model which were not possible to be determined experimentally. Nielson et al. [15] also developed a compartmental model for microbial growth in mixed substrates environment. Nikollajsen et al. [16] presented a simple compartmental model, which gave various metabolic details for sequential utilization of substrates. Doshi et al. [17] developed an optimal model to present the diauxic and triauxic growth of microbes. They followed the principle of instantaneous maximization of specific growth rate and described the microbial growth in multiple substrates environment as a problem of multivariable constrained optimisation. This model involves a simple representation of complex cell structure as an optimisation function, which regulates the interplay of cellular machinery. The model was found to match well with the published experimental data of bacterial growth of *Klebsiella oxytoca* on the mixture of sugars. The most interesting feature of this model was the ability to prove that the microbial growth in presence of two sugars will be diauxic if one of the substrates has a

very high maximum specific growth rate and very low value for Monod's substrate saturation constant. The model was found to match with the cybernetic model. The advantage here is that in the cybernetic model, two control parameters are required for each substrate while in the optimal model, only one parameter is required. The concept of optimal modelling was further developed by Venkatesh et al. [18] to present the simultaneous consumption of substrates. A simple multi-variable constrained optimisation was aimed to maximize the specific cell growth. It was assumed that the different growth phenomena occur due to different levels of controls present inside the cell. These controls have been taken care of in the optimization formulation in the form of constraints. The model predictions were found to match well with the experimental growth data of *E.coli* K12 on glucose and organic acids. This model was further extended by Doshi and Venkatesh [19] for the sequential and simultaneous utilization of substrates. The model was found to present the two levels of control inside the cell, which represented the catabolite repression of lactose in the presence of glucose and the simultaneous consumption of glucose-acetate and lactose-acetate. In this article, an attempt has been made to use this model to describe the sequential utilization of glucose, galactose and alcohol by *Saccharomyces cerevisiae* in aerobic and anaerobic fermentation. The effect of pre-culturing has also been discussed. We have extended the model of Venkatesh et al. [18] to capture the transcription dynamics of glucose-galactose (glc-gal) regulated GAL genes for the production of recombinant proteins. The model predicted pattern was verified by experimentally measuring β-galactosidase expression in *Saccharomyces cerevisiae*.

2. MATERIALS AND METHODS

Model Development

The key equations of the optimal model for microbial growth on single and multiple substrates are represented as follows:

$$\frac{dx_i}{dt} = \mu_i^{\max}\left(\frac{e_i}{e_i^{\max}}\right)\left(\frac{s_i}{k_i + s_i}\right)x_i \tag{1}$$

$$\frac{ds}{dt} = -\left(\frac{\mu_i}{Y_i}\right)x \tag{2}$$

$$\frac{d\left(e_i / e_{\max}\right)}{dt} = \left(\mu_i^{\max} + \beta\right)\left(\frac{s_i}{k_i + s_i}\right) - \left(\mu + \beta\right)\left(\frac{e_i}{e_i^{\max}}\right) \tag{3}$$

The maximization has done under the following constraints:

$$\max(\mu) = \max\left(\sum_i \alpha_i \mu_i\right) \quad (4)$$

We have further extended this model for capturing the protein expression dynamics including the binding mechanism of the repressor protein (Gal80p) and inducer (Gal3p) proteins for GAL genes of *S. cerevisiae* [20-28].

The equilibrium equations for the interactions of transcriptional activator Gal4 protein, β-galactosidase gene integrated in the gal-operon are as follows [20-24];

$$\text{Gal4p} + \text{Gal4p} = \text{Gal4p} \ldots \text{Gal4p} \quad (5)$$

$$K_d = \frac{[Gal4p]^2}{[Gal4p \ldots Gal4p]} \quad (6)$$

$$\text{Gene} + \text{Gal4p} \ldots \text{Gal4p} = \text{Gene} \ldots \text{Gal4p} \ldots \text{Gal4p} \quad (7)$$

$$K_{d1} = \frac{[Gal4p \ldots Gal4p].[Gene]}{[Gal4p \ldots Gal4p]} \quad (8)$$

$$\text{Gene} \ldots \text{Gal4p} \ldots \text{Gal4p} + \text{Gal4p} \ldots \text{Gal4p} = \text{Gal4p} \ldots \text{Gal4p} \ldots \text{Gene} \ldots \text{Gal4p} \ldots \text{Gal4p} \quad (9)$$

$$K_{d2} = \frac{[Gal4p \ldots Gal4p].[Gene \ldots Gal4p \ldots Gal4p]}{[Gal4p \ldots Gal4p..Gene \ldots Gal4p \ldots Gal4p]} \quad (10)$$

$$K_{d1} = m.K_{d2} \quad (11)$$

The mass balances of various moieties in the transcriptional regulations are;

$$[Gal4p]_t =$$
$$2.[\text{Gal4p} \ldots \text{Gal4p}]+2.[\text{Gene}\ldots\text{Gal4p..Gal4p}]+4.[\text{Gal4p}\ldots\text{Gal4p}\ldots\text{Gene}\ldots\text{Gal4p}\ldots\text{Gal4p}] \quad (12)$$

$$[Gene]_t = [\text{Gene}]+[\text{Gene}\ldots\text{Gal4p..Gal4p}]+[\text{Gal4p}\ldots\text{Gal4p}\ldots\text{Gene}\ldots\text{Gal4p}\ldots\text{Gal4p}] \quad (13)$$

Percentage transcriptional and translational expressions of gene are mathematically defined as follows [20];

$$f_{transcription} * 100 = \frac{[Gene \ldots Gal4p \ldots Gal4p]+[Gal4p \ldots Gal4p \ldots Gene \ldots Gal4p \ldots Gal4p]}{[Gene]_t}.100 \quad (14)$$

$$f_{translation}.100 = f_{trancription}^{n}.100 \quad (15)$$

where n is co-response coefficient for translation [20-27], its average value is about 0.3 for *S. cerevisiae* [20-24, 28].

The dynamic expression of β-galactosidase under gal-operon in the GAL80 knockout strain was modelled as below;

$$\frac{d[P]}{dt} = \alpha . f_{transcription}^{0.5} - (\mu_{Gly} + \beta).[P] \quad (16)$$

where α and β are protein synthesis rate constant and protein degradation constants respectively

The glucose repression of Gal4p was modelled using the Hill equation as given below [28-30];

$$f_{Gal4p} = \frac{[Ga4p_t^h]}{[K_{0.5Glc}^h] + [Gal4p_t^h]} \quad (17)$$

where 'h' is hill coefficient and it's value is 3.2 adopted from Verma et al. [28]. $K_{0.5Glc}$ is half glucose inhibition constant for Gal4p and has estimated value is 3.2 mM [20-24, 28-29].

Glucose is the inhibitor of Gal4p, the maximum β–galactosidase protein expression would be in the absence of glucose when $f_{transcription}$ = 1.0 at this point P/P^{max}= 1.0 indicating that dP/dt = 0

$$\propto = (\mu_{Gly} + \beta).P^{max} \quad (18)$$

Growth kinetics and protein expression dynamics model were solved using fsolve algorithm in MATLAB using model parameters given in table 1.

Experimental Methods

Strains, media, growth conditions: The YM 3543 yeast strain with genotype MTAa ura3-52 his3-200 ade2-101 trp1-901 CAN^r metGAL80 LEU2::GAL1-lacZ lys2-801::GAL4 gal4-CAT-URA3 [26] was used in this study, which was obtained from Yeast Genomics lab, Department of Biosciences and Bioengineering, IIT Bombay (India). The YEP media contained peptone (10.0 g/l), Yeast extract powder (5.0 g/l), adenine (25.0 mg/l) carbon sources glucose, galactose, glycerol and sodium lactate P^H 5.5. Fermentations were carried out in 500 ml Erlenmeyer flasks with working volume 100-110 ml in a rotary shaker at shaking speed 240 rpm.

Analyses

The samples were taken at regular intervals. The supernatant was frozen for further analysis. Biomass was determined by measuring the absorbance using a standard curve of absorbance against dry cell weight. Absorbance was measured at 600nm in a Shimadzu Spectrophotometer. The β-galactosidase assay for strain YM 3543 was carried out as described by Rose and Botstein [30]. The frozen samples were analyzed for glucose, galactose, glycerol and lactate by high-performance liquid chromatography using UV and RI detector in series on LaChrom L-7490, Merck, Germany and were separated on an Aminex HPX-87H column (Biorad) at 65^0 C using 5 mM H_2SO_4 as mobile phase at a flow rate 0.6 mL/min [20-24, 29]. All the experiments were at-least reproduced on the different days and the deviation in data was within acceptable limits (maximum variation: 9.0 %).

3. RESULTS AND DISCUSSION

Determination of Model Parameters

Model parameters were determined using single substrate batch growth data of *S. cerevisiae* on glucose, galactose, alcohol, glycerol and lactate under different pre-culturing conditions. The log phase data was used to calculate the maximum specific growth rate ($\mu_{i,m}$) and substrates' half-saturation constants ($K_{s,i}$). The initial enzyme concentration was fixed to yield minimum least square errors. The growth parameters determined for the different substrate are listed in Table 1. The initial relative enzyme concentrations (e_i/e_{max}) for different pre-culturing conditions were taken a best-fit.

Sequential Utilization of Substrates for Growth

The model equations were solved to simulate the growth of *S. cerevisiae* on multiple substrates. The predictive capabilities of the model were tested by comparing model predictions with experimental data. Figure 1 shows the experimental data and the model simulation for the growth of *S. cerevisiae* on glucose in aerobic and anaerobic fermentation. Figure 2 shows a diauxic growth on galactose and alcohol. Table 1 shows that the sum of specific growth rates of two substrates (glucose + alcohol) is 0.24 h^{-1}, which is equal to μ^{max} (0.24 h^{-1}), maximum specific growth rate obtained experimentally for the growth on glucose (pre-cultured on glucose + alcohol). Therefore the cell was not able to take both the substrate simultaneously. Under such circumstances, the cell prefers to take glucose first because it has a higher maximum specific growth rate (0.24 h^{-1}). Once the glucose is run out in the medium, the flux for growth comes from alcohol and diauxic growth on alcohol was observed. The model was able to show that the triauxic growth of *S. cerevisiae* is governed by genetic control, which repressed the enzyme for galactose uptake by glucose [29, 31-34]. Figure 3 shows the model prediction and the experimental data for the growth on the (glucose + galactose) in aerobic and anaerobic fermentation.

Figure 4 shows that the model captures the experimental fractional expression profile of β-galactosidase. The expression of GAL genes start after 7.0 h of fermentation when all the glucose is run-out in the vessel growing *S. cerevisiae* mutant of GAL80 gene, later cells are growing glycerol, the non-inducing non-repressing (NINR) medium [28-30].

Table 1. Model parameters for growth and protein expression dynamics in *S. cerevisiae*

Parameters	Glucose	Galactose	Alcohol
μ_{max}	0.25	0.20	0.09
K_s	0.02	0.40	0.50
$Y_{x/s}$	0.32	0.26	0.49
b	0.08	0.06	0.03
K	-----	10	

Parameters	Values	Source of Information
$[Gene]_t$	1.66×10−4 μM	Model calculation
m	30	[20-25, 29]
*K*d	2×10^{-4} μM	[21-23, 29]
*K*1	1×10^{-1} μM	[20-23, 29]
β	0.08	Assumed

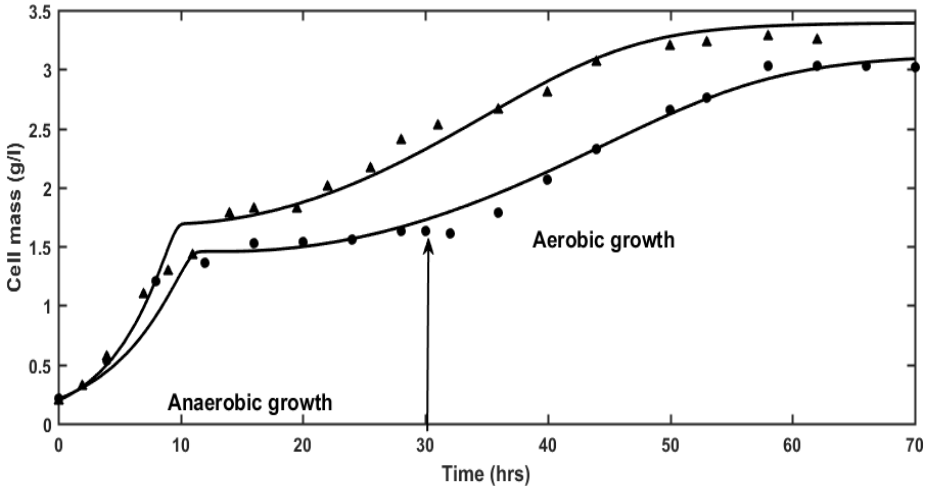

Figure 1. Experimental data and model simulation of cell mass concentration for the growth of Saccharomyces cerevisiae on glucose (6.0 g/l). Symbols (●) denotes aerobic growth and (▲) denotes anaerobic growth. Solid lines show model predictions.

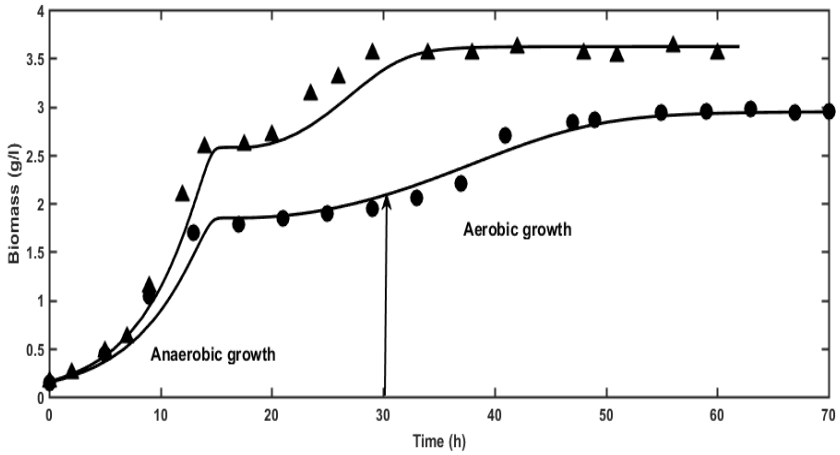

Figure 2. Experimental data and model simulation of cell mass concentration for the growth of Saccharomyces cerevisiae on galactose (6.0 g/l). Symbols (●) denotes aerobic growth and (▲) denotes anaerobic growth. Solid lines show model predictions.

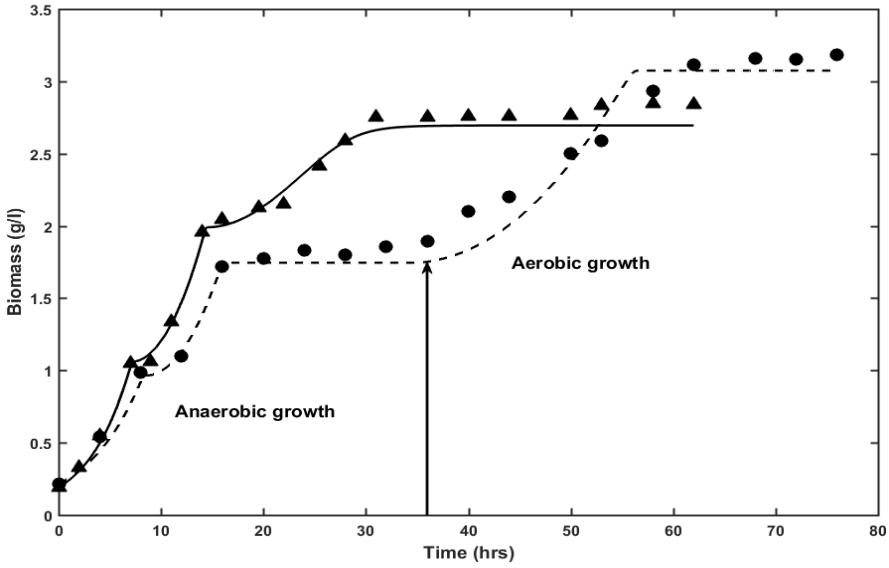

Figure 3. Experimental data and model simulation of cell mass concentration for the growth of Saccharomyces cerevisiae on glucose (3.0 g/l) and galactose (3.0 g/l). Symbols (●) denotes aerobic growth and (▲) denotes anaerobic growth. Solid lines show model predictions.

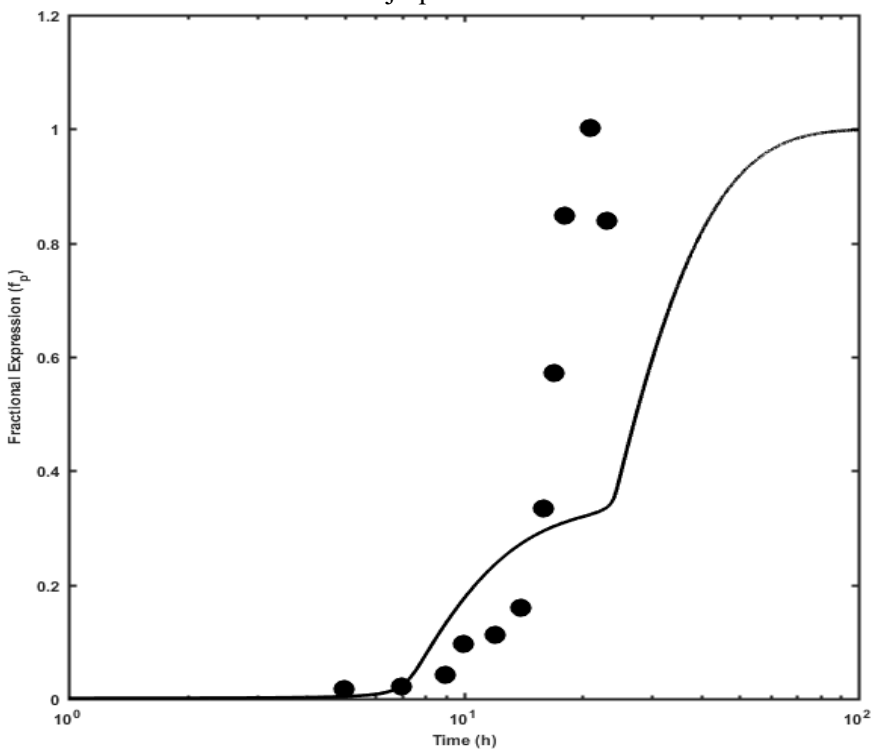

Figure 4. Model prediction and experimental data for the expression dynamics of β-galactosidase in S. cerevisiae. Symbol (●) denotes enzyme expression data and solid lines show model predictions.

4. CONCLUSION

The comprehensive model developed using an optimal strategy is capable of presenting a sequential as well as simultaneous utilization of substrates by micro-organisms. This model is able to present a variety of growth patterns on mixed substrates just considering the macroscopic view of growth. Also, this model is very simple since it requires very few model parameters to describe the growth, which are also easy to be determined experimentally. For the growth of *S. cerevisiae,* it showed that the enzyme alcohol dehydrogenase and galactokinase suffer from catabolic repression in presence of glucose and therefore a diauxic growth is observed with alcohol as less preferred substrate as compared to glucose and triauxic growth on glucose + galactose, the galactose and alcohol both are less preferred substrates. We further extended the optimal model to represent the dynamics of foreign protein synthesis under the control of GAL operon. The dynamics was captured by the model. Further attempt could involve the application of this system for the production of secondary metabolites and commercially important foreign proteins.

ACKNOWLEDGEMENT

Author thanks to the Central University of Punjab, Bathinda, India for seed grant funding in 2016.

CONFLICT OF INTEREST

Author declares that he has no conflicting interests.

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