Integration of yield factor expression into Haldane's model for substrate inhibition.

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Abstract. Haldane equation is a mathematical expression that has been widely used in growth kinetics to give a proper fit to experimental data in case of substrate inhibition during enzymatic processes. It determines the specific growth rate of a microorganism based on the substrate concentration, the half saturation constant, the inhibitory constant and the maximum growth rate achievable. However, for practical and experimental design purposes it is important to describe Haldane equation in terms of the initial concentration of substrate, since this information is required to know the proper amount of initial substrate to be used. For this reason, in the present paper we proposed to integrate the expression of yield factor and the definition of specific growth rate in a batch system into Haldane's equation and to solve analytically the mathematical equations in order to obtain a final expression that correlates the maximum growth rate, the limiting nutrient concentration at which the specific growth is half its maximum value, the inhibitory constant, the initial concentration of substrate and the initial amount of biomass required in time. Accordingly, simulation and numerical studies are presented to analyze and discuss the importance of the obtained model.

1 Introduction

Biochemical reactions are generally performed using cells or living organisms which in the presence of specific nutrients are able to grow and perform biochemical operations. Research using living cells for the production of new compounds, secondary metabolites and high value added products (i.e. biopharmaceuticals, antibiotics, proteins, and chemicals used in industry) is a recent trend in microbiology and biotechnology. For this purpose, different forms of microorganism (algae, bacteria or fungi) and cells (human, vegetable or animal) are widely used [1].

Microorganisms are able to grow either by increasing the population number or the cell density after consuming the nutrients of the medium under optimum conditions of temperature, pH, turbulent regime, and concentration of particular elements and compounds found in the culture medium [2]. The modeling of this process requires assuming the absence of intracellular reactions, and considering the biomass as homogenous and in a steady environment. This leads to a balance growth state. Under these considerations, it has been observed that the growth velocity of microorganisms is proportional to the existing population, that is $dx/dt = \mu x$, where μ is the specific growth rate, x is the cell concentration and t represents the time. The yield ($Y_{X/S}$) is the ratio of product to reactant consumed [3]. It is important to note that the specific growth rate does not remains constant during the process as it generally depends on the concentration of nutrients [4]. The concentration of substrate is important because it allows the growth of the microorganism or to increase the synthesis of products. However, excessive concentration can be detrimental due to inhibition or poisoning effects. Several mathematical models have been proposed aiming to describe the process of growth kinetics. Some common models generally used were proposed by Monod, Haldane, Tessier, Andrews, Moser, Aiba, and Contois to fit the values of experimental data reported [5-8]. In the present paper we aim to study the Haldane model. This model is used to describe the inhibitory behavior of microorganisms or cells, which can occur at specific values of substrate concentration. Haldane model has been used to fit experimental data of several kinetic models, i.e. process in anaerobic reactors that describes sulfate reduction process considering different concentrations of sulfate, biomass and sulfide [9], phenol degradation in batch operations by means of description of the influence of concentration of an inhibition substrate on specific growth rate and the proposal of analytical expressions that correlates the biomass and substrate using homotopy perturbation methods [10].

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2. Description of the model

Haldane kinetic model is represented by equation (1)

$$\mu = \frac{\mu_{max}S}{Ks+S+\frac{S^2}{Ki}} \tag{1}$$

where μ_{max} is the maximum growth rate, Ki is the inhibitory constant, S is the substrate concentration, Ks is the half saturation constant. As we can observe, from this equation we can obtain information about the rate of growth and the concentration of substrate that cause inhibition. However, from this equation there is no correlation to the initial substrate concentration (S_0). This can be approached by including the biomass yield factor equation (2), which represents the proportion of biomass that has been produced to the amount of substrate consumed

$$Y_{\frac{X}{S}} = \frac{X-Xo}{So-S} \tag{2}$$

where $Y_{X/S}$ represents the yield, and X_0 and X are the initial and final concentration of biomass respectively.
From equation (2) we can obtain (3). Then, substitution of (3) into (1) leads to (4), which can be also represented as in expression (5) after solving the square of the trinomial.

$$S = \frac{\frac{SoY_{\frac{X}{S}}+Xo-X}{Y_{\frac{X}{S}}}} \tag{3}$$

$$\mu = \frac{\mu_{max}\frac{\frac{SoY_{\frac{X}{S}}+Xo-X}{Y_{\frac{X}{S}}}}}{Ks+\frac{\frac{SoY_{\frac{X}{S}}+Xo-X}{Y_{\frac{X}{S}}}}+\frac{\left(\frac{SoY_{\frac{X}{S}}+Xo-X}{Y_{\frac{X}{S}}}\right)^2}{Ki}} \tag{4}$$

$$\mu = \frac{\mu_{max}\left(\frac{SoY_{\frac{X}{S}}+Xo-X}{Y_{\frac{X}{S}}}\right)}{Ks+\left(\frac{SoY_{\frac{X}{S}}+Xo-X}{Y_{\frac{X}{S}}}\right)+\left[\left(\frac{So^2Y_{\frac{X}{S}}^2+2SoY_{\frac{X}{S}}Xo-2SoY_{\frac{X}{S}}X-2XXo+Xo^2+X^2}{KiY_{\frac{X}{S}}^2}\right)\right]} \tag{5}$$

After simplification of the denominator, it follows that:

$$\mu = \frac{\mu_{max}\left(\frac{SoY_{\frac{X}{S}}+Xo-X}{Y_{\frac{X}{S}}}\right)}{\frac{b}{KiY_{\frac{X}{S}}^2}} \tag{6}$$

where $b = (KsY_{\frac{X}{S}}^2Ki + KiSoY_{\frac{X}{S}}^2 + KiXoY_{\frac{x}{S}} + So^2Y_{\frac{X}{S}}^2 + Xo^2 + 2SoY_{\frac{X}{S}}Xo - KiXY_{\frac{X}{S}} - 2SoY_{\frac{X}{S}}X-2XXo + X^2).$

$$\mu = \frac{\mu_{max}(SoY_{\frac{X}{S}}+Xo-X)Y_{\frac{X}{S}}^2Ki}{b*Y_{\frac{X}{S}}} \tag{7}$$

$$\mu = \frac{\mu_{max}(SoY_{\frac{X}{S}}+Xo-X)Y_{\frac{X}{S}}Ki}{c} \tag{8}$$

where $c = KsY_{\frac{X}{S}}^2Ki + KiSoY_{\frac{X}{S}}^2 + KiXoY_{\frac{x}{S}} + So^2Y_{\frac{X}{S}}^2 + Xo^2 + 2SoY_{\frac{X}{S}}Xo + X(X − KiY_{\frac{x}{S}} − 2SoY_{\frac{X}{S}}-2Xo)$

We can substitute the value of μ in the definition of growth velocity to have equation (9)

$$\frac{dx}{dt} = \frac{X\mu_{max}\left[SoY_{\frac{X}{S}}+Xo-X\right]Y_{\frac{X}{S}}Ki}{KsY_{\frac{X}{S}}^2Ki+KiSoY_{\frac{X}{S}}^2+KiXoY_{\frac{x}{S}}+So^2Y_{\frac{X}{S}}^2+Xo^2+2SoY_{\frac{X}{S}}Xo+X(X−KiY_{\frac{x}{S}}−2SoY_{\frac{X}{S}}-2Xo)} \tag{9}$$

Then, it follows that

$$\mu_{max}dt = \frac{KsY_{\frac{X}{S}}^2Ki+KiSoY_{\frac{X}{S}}^2+KiXoY_{\frac{x}{S}}+So^2Y_{\frac{X}{S}}^2+Xo^2+2SoY_{\frac{X}{S}}Xo+X\left\{X−KiY_{\frac{x}{S}}−2SoY_{\frac{X}{S}}-2Xo\right\}dx}{Y_{\frac{X}{S}}Ki\left[SoY_{\frac{X}{S}}+Xo-X\right]X} \tag{10}$$

and after performing integration in the limits (X) and (Xo) we observe that we can separate the terms in the second integral

$$\int_0^t \mu_{max}dt = d + e \tag{11}$$

where

$$d = \int_{xo}^{x} \frac{\left(KsY_{\frac{X}{S}}^2Ki + KiSoY_{\frac{X}{S}}^2 + KiXoY_{\frac{x}{S}} + So^2Y_{\frac{X}{S}}^2 + Xo^2 + 2SoY_{\frac{X}{S}}Xo\right)dx}{Y_{\frac{X}{S}}Ki\left(SoY_{\frac{X}{S}} + Xo − X\right)X}$$

$$e = \int_{xo}^{x} \frac{X\left(X−KiY_{\frac{x}{S}}−SoY_{\frac{X}{S}}-2Xo\right)dx}{Y_{\frac{X}{S}}Ki\left(SoY_{\frac{X}{S}}+Xo−X\right)X}$$

Considering that $A/SoY_{\frac{X}{S}} + Xo − X + B/X = 1$, then it follows that $AX + B[SoY_{\frac{X}{S}} + Xo − X] = 1$ and then it can be also expressed as $X(A − B) + B(SoY_{\frac{X}{S}} + Xo) = 1$. Therefore $A − B = 0$. As a result, $B = A = \frac{1}{SoY_{\frac{X}{S}}+Xo−X}$. We can then proceed to perform the integration as described next in (12) to (14)

$$\mu_{max}t = \left(\frac{KsY_{\frac{X}{S}}^2Ki+KiSoY_{\frac{X}{S}}^2+KiXoY_{\frac{x}{S}}+So^2Y_{\frac{X}{S}}^2+Xo^2+2SoY_{\frac{X}{S}}Xo}{Y_{\frac{X}{S}}Ki}\right)$$

$$\int_{xo}^{x}\frac{dx}{\left(SoY_{\frac{X}{S}}+Xo-X\right)X} + \int_{xo}^{x}\frac{\left(X−KiY_{\frac{x}{S}}−2SoY_{\frac{X}{S}}-2Xo\right)dx}{\left(SoY_{\frac{X}{S}}+Xo−X\right)} \tag{12}$$

$$\mu_{max}t = \left(\frac{KsY_{\frac{X}{S}}^2Ki + KiSoY_{\frac{X}{S}}^2 + KiXoY_{\frac{x}{S}} + So^2Y_{\frac{X}{S}}^2 + Xo^2 + 2SoY_{\frac{X}{S}}Xo}{Y_{\frac{X}{S}}Ki}\right)$$

$$\int_{xo}^{x} \frac{dx}{\left(SoY_{\frac{X}{S}}+Xo-X\right)\left(SoY_{\frac{X}{S}}+Xo\right)} + \int_{xo}^{x} \frac{dx}{X\left(SoY_{\frac{X}{S}}+Xo\right)} +$$

$$\int_{xo}^{x} \frac{\left(X-KiY_{\frac{X}{S}}-2SoY_{\frac{X}{S}}-2Xo\right)dx}{\left(SoY_{\frac{X}{S}}+Xo-X\right)} \quad (13)$$

$$\mu_{max}t = \frac{KsY_{\frac{X}{S}}^2Ki+KiSoY_{\frac{X}{S}}^2+KiXoY_{\frac{X}{S}}+So^2Y_{\frac{X}{S}}^2+Xo^2+2SoY_{\frac{X}{S}}Xo}{Y_{\frac{X}{S}}Ki\left(SoY_{\frac{X}{S}}+Xo\right)}$$

$$\left(\ln\left|\frac{SoY_{\frac{X}{S}}}{SoY_{\frac{X}{S}}+Xo-X}\right| + \ln\left|\frac{X}{Xo}\right|\right) + \int_{xo}^{x} \frac{\left(X-KiY_{\frac{X}{S}}-2SoY_{\frac{X}{S}}-2Xo\right)dx}{SoY_{\frac{X}{S}}+Xo-X}$$

$$(14)$$

Inserting the result of the integrals, we finally obtain (15)

$$\mu_{max}t = \frac{KsY_{\frac{X}{S}}^2Ki+KiSoY_{\frac{X}{S}}^2+KiXoY_{\frac{X}{S}}+So^2Y_{\frac{X}{S}}^2+Xo^2+2SoY_{\frac{X}{S}}Xo}{Y_{\frac{X}{S}}Ki\left(SoY_{\frac{X}{S}}+Xo\right)}$$

$$\left(\ln\left|\frac{SoY_{\frac{X}{S}}}{SoY_{\frac{X}{S}}+Xo-X}\right| + \ln\left|\frac{X}{Xo}\right|\right) - SoY_{\frac{X}{S}} -$$

$$Xo\left(ln\left|\frac{SoY_{\frac{X}{S}}+Xo-X}{SoY_{\frac{X}{S}}}\right|\right) + (Xo-X) + \left(KiY_{\frac{X}{S}} + 2SoY_{\frac{X}{S}} +\right.$$

$$2Xo\left)\left(\ln\left|\frac{SoY_{\frac{X}{S}}+Xo-X}{SoY_{\frac{X}{S}}}\right|\right) \quad (15)\right.$$

Expression (15) is useful to understand the direct correlation of several parameters, namely the maximum growth rate (μ_{max}), the limiting nutrient concentration at which the specific growth is half its maximum value (Ks), the inhibitory constant (K_i), the yield factor ($Y_{X/S}$) at particular values of biomass (X), the initial concentration of substrate (S_0) and the initial amount of biomass required (X_0) in time (t)

3. Numerical simulation

For the purpose of performing numerical simulation, we take into consideration values of inhibition constant=214.5 mg/L, inhibition by substrate=18.3 mg/L, Yield of biomass per substrate=0.63. We studied an initial concentration of substrate and biomass in the range between 0 mg/L and 2 mg/L as described in Figure 1. Numerical simulation was performed using Matlab software. We can observe that for this particular numerical case, the maximum growth rate is obtained at lower values of initial substrate concentration. Accordingly, the equation can be further used to analyze the effect of time in the maximum growth rate achieved.
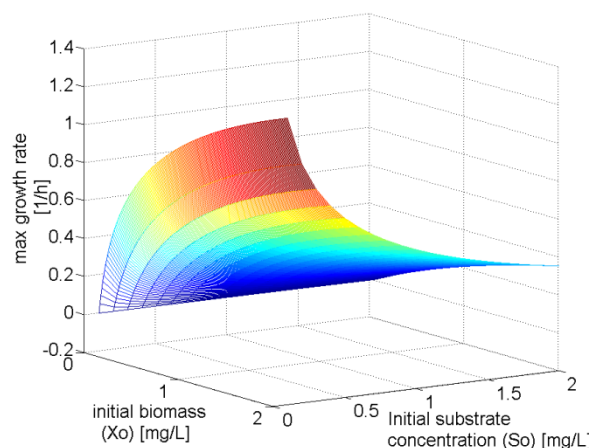
Fig. 1. Numerical simulation. Influence of initial substrate concentration and biomass on maximum growth rate

4. Conclusion

The use of mathematical models in chemical process and biochemical engineering is important because it helps in the understanding of the system. When a reactor is used, we can obtain information about the variation of substrate and product concentration in time and also the requirements of substrate in the feed to change parameters of composition of desired components. Accurate and deep understanding of these parameters can guide to adjust the process, to improve the system by means of prediction and to propose an operation range.

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