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A practical guide for optimal designs of experiments in the Monod model

Nikolay Strigul [a,*], Holger Dette [b,1], Viatcheslav B. Melas [c]

[a] Stevens Institute of Technology, Department of Mathematical Sciences, Hoboken, NJ 07030, USA
[b] Ruhr-Universität Bochum, Fakultät für Mathematik, 44780 Bochum, Germany
[c] St. Petersburg State University, Department of Mathematics, St. Petersburg, Russia

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ABSTRACT

The Monod model is a classical microbiological model often used in environmental sciences, for example to evaluate biodegradation processes. The model describes microbial growth kinetics in batch culture experiments using three parameters: the maximal specific growth rate, the saturation constant and the yield coefficient. However, identification of these parameter values from experimental data is a challenging problem. Recently, it was demonstrated theoretically that the application of optimal design theory in this model is an efficient method for both parameter value identification and economic use of experimental resources [Dette, H., Melas, V.B., Pepelyshev, A., Strigul, N., 2003. Efficient design of experiments in the Monod model. J. R. Stat. Soc. B 65 (Part 3), 725–742]. The purpose of this paper is to provide this method as a computational "tool" such that it can be used by practitioners without a strong mathematical and statistical background for the efficient design of experiments in the Monod model. The paper presents careful explanations of the principal theoretical concepts, and a simple algorithm for practical optimal design calculations.

1. Introduction

The Monod model was suggested by Nobel Laureate J. Monod in 1942 and for more than 60 years has been one of the most frequently used models in microbiology (Monod, 1949; Pirt, 1975; Koch, 1997; Kovarova-Kovar and Egli, 1998). Most models of chemostat growth are based on the Monod equations (Pirt, 1975; Smith and Waltman, 1995), and numerous models of microbial ecology incorporate Monod's growth kinetics (Koch, 1997; Strigul and Kravchenko, 2006). One of the very important practical applications of this model is the evaluation of the biodegradation kinetics of organic pollutants in environmental systems (Blok, 1994; Blok and Struys, 1996). The Monod model describes microbial growth with three parameters:

1) maximal specific growth rate;
2) a saturation constant;
3) a yield coefficient.

In the case of biodegradation kinetics, these parameters can be used as criteria for the biodegradability of organic pollutants (Blok, 1994).

One of the most important problems in practical applications of the Monod model is evaluating the parameters' values from experimental data. It was shown that in many cases, reasonable estimates of the parameters cannot be obtained by a simple application of non-linear least squares estimators (Holmberg, 1982; Saez and Rittmann, 1992). A possible way to circumvent this problem is the application of optimal experimental design techniques (Dette et al., 2005a,b). Optimal design theory is a branch of statistics actively developed since 1960, when basic results by Kiefer and Wolfowitz were published (Kiefer and Wolfowitz, 1960; Kiefer, 1974). In general, the application of optimal experimental designs can be useful in

1) reducing the number of necessary experimental measurements;
2) improving the precision of model parameter value determinations

(Fedorov, 1972). Optimal design theory is well developed for linear regression models, and for several simple non-linear regression models (Dette et al., 2005a,b). Recently, a complete theoretical examination of optimal designs for the Monod model was presented (Dette et al., 2003, 2005a), where two different approaches to the problem of designing experiments for the Monod model were investigated, namely local optimal experimental designs (Dette et al., 2003); and maximin optimal experimental designs (Dette et al., 2005a).

* Corresponding author. Tel.: +1 2019524260.
E-mail addresses: nstrigul@stevens.edu (N. Strigul), holger.dette@ruhr-uni--bochum.de (H. Dette), v.melas@pobox.spbu.ru (V.B. Melas).
[1] Fax: +49 2 34 70 94 559.

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While optimal designs have numerous advantages, they are not widely used in practice. Indeed, applications of local optimal experimental designs for the different modifications of the Monod model have been recommended in the literature, and supported by demonstrative case studies (Munack, 1991; Vanrolleghem et al., 1995; Versyck et al., 1999; Berkholz et al., 2000; Smets et al., 2002). In particular, this technique was recommended for the design of biodegradation experiments (Merkel et al., 1996). However, there are only a few cases where this technique has been applied in real experimental research as a tool for parameter identification (Dette et al., 2005b). We suspect that this gap between statistical theory and experimental practice is caused by the fact that practitioners often do not have the mathematical background or experience in numerical methods to comfortably use these methods. A theoretical method must not only be well described and mathematically investigated, but must also be delivered "as a tool" for practitioners, in order to be widely accepted in practice. It should include full explanations of the practical meaning of all explicit and implicit mathematical assumptions, and a readily available computer program for its applications in real experiments. While least squares estimators for models of non-linear differential equations are incorporated in numerous software products, for instance in the ModelMaker (SB Technology Ltd), sophisticated algorithms for optimal experimental design calculation are not included in widely distributed software products.

The present paper is an attempt to close this gap between applied mathematicians and practitioners, and is addressed to microbiologists, environmental scientists and other experimentalists. Our objective is to present optimal experimental designs for the Monod model "as a tool" which can be easily applied by practitioners. Firstly, we describe the Monod model with its theoretical background, and give general explanations of the application of optimal experimental designs in the Monod model. Secondly, we present a computer program for the numerical calculation of efficient experimental designs, which can be used directly in experimental studies. We try to avoid a technical description here: all mathematical details of local optimal design theory for the Monod model are presented in Dette et al. (2003); additionally, we direct practitioners to the recent review of optimal design applications in microbiology by Dette et al. (2005b).

2. The Monod model for a simple batch culture and its properties

In a simple homogeneous batch culture it is assumed that the growth conditions are similar for all cells (Pirt, 1975). Traditionally, a typical growth curve is divided into six phases: 1) lag, 2) accelerating, 3) exponential, 4) decelerating, 5) stationary, and 6) declining growth (Monod, 1949). In many cases cultures do not demonstrate this typical growth; also, the experimenter might be interested in only particular aspects of growth: for instance, in predictive microbiology the duration of the lag phase is the main parameter characterizing growth inhibition. This has led to models simulating only a few focal phases of growth, for example in predictive microbiology (Dette et al., 2005b), and to more sophisticated structured models able to describe typical growth curves in general (Koch, 1997). The role of the experimenter is to select the model that best reflects the characteristics of microbial growth that are his current focus, and to balance a model's complexity and its flexibility.

The Monod model uses a very convenient approximation of the batch growth process, especially when describing biodegradation kinetics (Blok, 1994; Dette et al., 2005b); it recognizes only 3 growth phases, i.e. exponential and decelerating growth, and a stationary phase. It is assumed that lag and accelerating and declining growth phases do not exist. This assumption is satisfied in many practical cases. The cells used to inoculate batch cultures are often taken from another actively growing culture, and, therefore, growth starts immediately in the exponential phase. However, one should not try to obtain Monod's model parameters directly from experimental data if a lag phase is observed. In this case, it is important to estimate the duration of the lag phase in preliminary experiments and take the time when this phase ends as a starting moment of the Monod experiment. The declining growth phase is usually not a consideration in biodegradation studies, but it can be analyzed separately, using, for example, the negative exponential model, for which optimal experimental designs have been also constructed (Dette et al., 2005b). Another important assumption of the Monod model is that microbial growth is limited solely by the substrate concentration and, therefore, it is very important that experimental conditions satisfy this requirement.

The Monod model suggests that the microbial growth rate $\mu(t)$ and the substrate concentration $s(t)$ at time t are related by the Michaelis–Menten function:

$$\mu(t) = \theta_1 \frac{s(t)}{s(t) + \theta_2}. \tag{1}$$

The microbial growth rate for the population is then given by the equation:

$$\eta'(\mathrm{t}) = \mu(t)\eta(t) \tag{2}$$

Finally, the model assumes that some constant fraction of the consumed substrate is transformed into microbial biomass:

$$s(t) - s_0 = \frac{\eta_0 - \eta(t)}{\theta_3} \tag{3}$$

where η_0 and s_0 are the initial values of microbial biomass and substrate concentration respectively, and θ_1, θ_2, and θ_3 denote the three model parameters, maximal specific growth rate (μ_m), saturation constant (K_s), and yield coefficient (Y), respectively.

The Monod differential equations (1)–(3) can be easily integrated by separation of variables and the solution gives a function $\eta(t)$ implicitly given by the equation:

$$t = \frac{1}{\theta_1}\left[\left(1 + b\right)\ln(\eta/\eta_0) - b\ln\frac{c-\eta}{c-\eta_0}\right], \tag{4}$$

where constants b and c are defined by

$$b = \frac{\theta_2\theta_3}{s_0\theta_3 + \eta_0}, \quad c = \eta_0 + \theta_3 s_0. \tag{5}$$

This function is depicted for a typical example in Fig. 1 and has remarkable properties (Pirt, 1975):

1. It is a sigmoidal growth curve with one inflection point.
2. The microbial biomass tends to a horizontal asymptote determined by $\theta_3 s_0 - \eta_0$. This value is the microbial biomass at the stationary state. Only the yield coefficient determines the horizontal asymptote. The other two parameters do not affect it.

It is necessary to emphasize two important assumptions that should be taken into consideration by a practitioner attempting to identify Monod's parameter values from experimental data. First, the sigmoidal function, which is a solution of the Monod model, starts in the exponential growth phase. Therefore, an experimenter must exclude measurements reflecting a lag phase. It is equivalent to shifting the experiment starting time to the right, to a time equal to the observed lag phase. Second,