Design of experiments for the Monod model - robust and efficient designs

Holger Dette
Ruhr-Universität Bochum
Fakultät für Mathematik
44780 Bochum
Germany
email: holger.dette@ruhr-uni-bochum.de
FAX: +49 2 34 70 94 559

Viatcheslav B. Melas
St. Petersburg State University
Department of Mathematics
St. Petersburg
Russia
email: v.melas@pobox.spbu.ru

Andrey Pepelyshev
St. Petersburg State University
Department of Mathematics
St. Petersburg
Russia
email: andrey@ap7236.spb.edu

Nikolay Strigul
Princeton University,
Department of Ecology and Evolutionary Biology
Princeton NJ, 08544
USA
email: nstrigul@princeton.edu

Abstract

In this paper the problem of designing experiments for a model which is called Monod model and is frequently used in microbiology is studied. The model is defined implicitly by a differential equation and has numerous applications in microbial growth kinetics, environmental research, pharmacokinetics, and plant physiology. The designs presented so far in the literature are locally optimal designs, which depend sensitively on a preliminary guess of the unknown parameters, and are for this reason in many cases not robust with respect to their misspecification. Uniform designs and maximin optimal designs are considered as a strategy to obtain robust and efficient designs for parameter estimation. In particular standardized maximin D- and E- optimal designs are determined and compared with uniform designs, which are usually applied in these microbiological models. It is shown that standardized maximin optimal designs are always supported on a finite number of points and it is demonstrated that maximin optimal designs are substantially more efficient than uniform designs. Parameter variances can be decreased by a factor two by simply sampling at optimal times during the experiment. Moreover, the maximin optimal designs usually provide the possibility for the experimenter to check the model assumptions, because they have more support points than parameters in the Monod model.

Keywords and phrases: robust designs, maximin optimal designs, microbial growth, biodegradation kinetics, Monod model.

1 Introduction

The Monod model can describe several important characteristics of microbial growth in a simple periodic culture of microorganisms. This model was proposed by Nobel Laureate J. Monod more than 60 years ago and it is one of the basic models for quantitative microbiology [see Monod (1949), Koch (1997), Ferenci (1999) among many others]. Although some limitations of this model as well as restrictions of its applications are known [see Pirt (1975), Baranyi and Roberts (1995), Koch (1997) or Ferenci (1999)] and modifications have been proposed in some specific cases [Ellis, Barbeau, Smets, and Grady (1996), Fu and Mathews (1999), Schirmer, Butler, Roy, Frind and Barker (1999), Vanrolleghem, Spanjers, Petersen, Ginestet, and Takacs (1999)], the Monod model is still used very often in its original form, especially in such fields as environmental and industrial microbiology. For example, it is the most common model for describing the dynamics of organic pollutant biodegradation [see Blok and Struys (1996), Knightes and Peters (2000), Goudar and Ellis (2001)]. Much of the versatility of the Monod model is due to the fact that it can describe biodegradation rates following zero-one first order kinetics with respect to the target substrate concentration [see Holmberg (1982)]. At the same time the similarity of the Monod model and the Michaelis-Menten equation provides a very wide application of this model type throughout biological and biomedical sciences. This type of equation is very often used in biochemistry, plant physiology, biophysics and pharmacology. The model is determined by a first-order differential equation which determines the regression function implicitly and the parameters enter in the model nonlinearly.

In a recent paper Dette, Melas, Pepelysheff and Strigul (2003) discussed the problem of designing experiments for the Monod model. These authors considered locally optimal designs in the sense of Chernoff (1953), which depend on an initial guess of the "true" but unknown parameters in the regression model, and demonstrated that these designs are rather efficient with respect to minor misspecifications of the "true" parameters. On the other hand, it is also shown in this paper that the loss of efficiency by using a locally optimal design can be substantial, if the initial guess of the "true" parameters is completely wrong. The purpose of the present paper is to construct non-sequential robust designs for the Monod model, which are on the one hand less sensitive with respect to a misspecification of the unknown parameters and on the other hand still efficient for parameter estimation.

There are essentially two strategies to construct non-sequential robust designs for nonlinear regression models, the Bayesian- [see Pronzato and Walter (1985), Chaloner and Larntz (1989) or Haines (1995)] and the (standardized) maximin concept [see Müller (1995), Dette (1997), or Dette and Biedermann (2003)]. An application of a Bayesian design assumes that the experimenter is able to specify a prior for the unknown parameters in the regression model, while standardized maximin optimal designs require only the specification of a certain range for the unknown parameters. In this paper we will apply the latter method for the construction of robust and efficient designs for the Monod model, because we observed in many studies of microbial growth that the experimenter was able to specify certain intervals for the unknown parameters, but had not enough information, which could be used for the specification of a prior [see Grady, Smets and Barbeau (1996), Sommer, Spliid, Holst and Arvin (1998), Liu and Zachara (2001)]. The maximin approach determines the design such that the minimum efficiency taken over a certain region of the unknown parameters becomes maximal. In other words this approach minimizes the maximal loss of accuracy with respect to the best design, which requires knowledge of the "true" parameters. Throughout this paper these designs will be called standardized maximin optimal designs.

In Section 2 we give some background on the Monod model and briefly review some basic facts from the theory of optimal design of experiments for this model in order to make this paper self-contained. In Section 3 we study some analytical properties of locally optimal uniform and standardized maximin optimal designs, while we explain an algorithm for the numerical construction of maximin optimal designs in Section 4. Section 5 contains some numerical results and a comparison of standardized maximin optimal designs with uniform designs, which are commonly applied in microbial growth models. Dette et al. (2003) showed that these designs usually require 2-3 times more observations than locally optimal designs in order to achieve the same accuracy. However the locally optimal designs depend sensitively on the unknown parameters of the model. In the present paper we demonstrate that the maximin efficient designs determined in Section 4 and 5 are on the one hand substantially more robust with respect to misspecification of the "true" parameters and on the other hand considerably more efficient than the commonly used equidistant designs. Moreover, it is also demonstrated that standardized maximin optimal designs usually have at least 4 support points, while locally optimal designs are supported at only 3 points. There appear 3 unknown parameters in the Monod equation, and consequently the new designs can also be used for checking the model assumptions of the Monod model by means of a goodness-of-fit test. Finally some technical details regarding the proofs of the results in Section 3 are given in the Appendix.

2 Design of experiment for the Monod model

The Monod model for periodic culture (batch) experiments may be presented as a first order differential equation

$$\eta'(t) = \mu(t)\eta(t), \tag{2.1}$$

where

$$\mu(t) = \theta_1 \frac{s(t)}{s(t) + \theta_2}, \, s(t) - s_0 = (\eta_0 - \eta(t))/\theta_3 \tag{2.2}$$

[see Pirt (1975) or Koch (1997)]. Here $s_0 = s(0)$ and $\eta_0 = \eta(0)$ are given initial conditions, i.e., initial concentrations of the consuming substrate and microbial biomass, respectively. Three parameters θ_1, θ_2, θ_3 characterize microbial growth. Each parameter has its own traditional notation and name: θ_1 is called the maximal specific growth rate usually denoted by $\mu_{\max}$, θ_2 the saturation (affinity) constant denoted by K_s, θ_3 the yield coefficient usually denoted by Y. The variable t represents time, which varies in the closed interval $[0, T]$. Typical minimum values of T are several hours for optimal microbiological media, whereas the maximum is one year or more for specialized groups of microorganisms. All three parameters, initial conditions and variables are positive because of natural biological conditions. Parameter estimation and experimental design for this model have recently been discussed by extensive empirical studies [see Vanrolleghem, Van Daele and Dochaine (1995), Merkel, Schwarz, Fritz, Reuss and Krauth (1996) or Ossenbruggen, Spanjers and Klapwik (1996) among many others]. Note that the functions η, μ and s in the differential equation (2.1) depend on the parameter $\theta = (\theta_1, \theta_2, \theta_3)$ and we will make this dependence explicit in our notation, whenever it is necessary. We assume that in principle at each experimental condition $t \in [0, T]$ an observation could be obtained, which is described by the stochastic model

$$y(t) = \eta(t, \theta) + \varepsilon \ , \tag{2.3}$$

where $\eta(t, \theta)$ is the solution of the differential equation (2.1) and ε is a normally distributed random variable with mean 0 and variance $\sigma^2 > 0$. Moreover, throughout this article we assume that different observations are independent and following Kiefer (1974) we call a discrete probability measure with masses $w_1, \ldots, w_n$ at points $t_1, \ldots, t_n \in [0, T]$ an approximate experimental design. These points define the distinct experimental conditions at which observations have to be taken and

$w_1, \ldots, w_n > 0$, $\sum_{j=1}^n w_j = 1$ are positive weights representing the proportions of total observations taken at the corresponding points [see Silvey (1980) or Pukelsheim (1993) for more details]. If N observations can be taken by the experimenter a rounding procedure is applied to obtain integers r_j from the not necessarily integer valued quantities $w_j N$. The values r_j represent the number of observations taken at experimental condition t_j, $j = 1, \ldots n$, and satisfy $\sum_{j=1}^n r_j = N$ [see Pukelsheim and Rieder (1992)]. The analogue of the (appropriately normalized) Fisher information matrix for an approximate design is the matrix

$$M(\xi, \theta) = \left(\sum_{k=1}^n w_k \frac{\partial}{\partial \theta_i} \eta(t_k, \theta) \frac{\partial}{\partial \theta_j} \eta(t_k, \theta) \right)_{i,j=1}^3 , \tag{2.4}$$

which is called the information matrix of the design ξ in the literature. If N observations are taken according to an approximate design (possibly by applying a rounding procedure) it was shown in Dette et al. (2003) that the least squares estimate in the model (2.3) is consistent and asymptotically normal distributed with mean θ^* and variance-covariance matrix $\frac{\sigma^2}{N} M^{-1}(\xi, \theta^*)$, where θ^* denotes the "true" but unknown parameter in the regression model (2.3). An optimal design maximizes an appropriate real valued function, say Φ, of the information matrix and there are numerous criteria proposed in the microbiological literature to compare competing designs for statistical inference in the Monod model [see Vanrolleghem et al. (1995), Versyck, Bernaerts Geeraerd and Van Impe (1999)]. Note that the "true" parameter is not known and also estimates are not available before any experiments have been carried out.
Following Chernoff (1953) we assume that some prior knowledge about the unknown parameter is available, say θ, and call a design ξ_θ^* locally Φ-optimal (for the parameter θ) if ξ_θ^* maximizes the function $\Phi((M(\xi, \theta))$. Throughout this paper Φ denotes an information function in the sense of Pukelsheim (1993), where we are particularly interested in the D-criterion

$$\Phi_D(M(\xi, \theta)) = (\det M(\xi, \theta))^{1/3} ,$$

the E-criterion

$$\Phi_E(M(\xi, \theta)) = \lambda_{\min}(M(\xi, \theta))$$

and in the criterion for estimating the individual coefficients θ_1, θ_2, θ_3, i.e.

$$\Phi_i(M(\xi, \theta)) = (e_i^T M^-(\xi, \theta) e_i)^{-1} \quad (i = 1, 2, 3).$$

Here A^- denotes a generalized inverse of the matrix A, $e_i \in \mathbb{R}^3$ is the ith unit vector and we assume that the parameter θ_i is estimable by the design ξ, that is $\text{range}(e_i) \subset \text{range}(M(\xi, \theta))$ $(i = 1, 2, 3)$. For the definition of a more robust optimality criterion let $\Omega \subset \mathbb{R}_+^3$ denote a given subset of the parameter space, then we call a design ξ^* standardized maximin Φ-optimal if ξ^* maximizes

$$\Psi_\Omega(\xi) \;=\; \min_{\theta \in \Omega} \frac{\Phi(M(\xi, \theta))}{\Phi(M(\xi_\theta^*, \theta))} . \tag{2.5}$$

Throughout this paper the standardized maximin D- and E-optimal designs will be denoted ξ_D^* and ξ_E^*, respectively. The most important case for the choice of the set Ω in the maximin criterion arises, if the experimenter is able to specify intervals for the location of each parameter θ_i, that is

$$(\theta_1, \theta_2, \theta_3) \in \Omega = [z_{1,L}, z_{1,U}] \times [z_{2,L}, z_{2,U}] \times [z_{3,L}, z_{3,U}], \tag{2.6}$$

where $0 < z_{i,L} \leq z_{i,U} < \infty$ $(i = 1, 2, 3)$. In this paper we will compare standardized maximin Φ-optimal designs with uniform designs of the form

$$\xi_{\mathcal{U}(N),\bar{T}} = \begin{pmatrix} \frac{1}{N}\bar{T} & \ldots & \frac{N-1}{N}\bar{T} & \bar{T} \\ \frac{1}{N} & \ldots & \frac{1}{N} & \frac{1}{N} \end{pmatrix} , \tag{2.7}$$

which are commonly applied in microbiological models [see Dette, Melas and Strigul(2004)]. In (2.7) the parameter $\bar{T} \leq T$ is a given bound for the largest support point of the design while N characterizes the number of different experimental conditions. It was demonstrated by Dette et al. (2003) that for moderate sample size $N \in \mathbb{N}$ the impact of the number of support points N on the performance of the uniform design is negligible, and the important characteristic of this type of design is its largest support point $\bar{T}$. For this reason we will restrict our theoretical investigations to the continuous uniform design denoted by $\xi_{\bar{T}}$, that is

$$d\xi_{\bar{T}} = I_{[0,\bar{T}]}\frac{dt}{\bar{T}} \tag{2.8}$$

and determine the largest support point $\bar{T}$ in an optimal way. A continous uniform design $\xi_{T^*,\theta}$ maximizing the function $\Phi(M(\xi_{\bar{T}}, \theta))$ in the class of all continuous uniform designs is called locally Φ-optimal uniform design.

Note that the definition of the standardized maximin optimality criteria requires the knowledge of the locally optimal design for any $\theta \in \Omega$ and that the regression function η in the model (2.3) is only implicitly given by the differential equation (2.1). Nevertheless, it is possible to obtain an explicit representation of the information matrix (2.4) of an approximate design. In order to make this paper self-contained we briefly recall some results established in Dette et al. (2003), where a general machinery is developed to determine locally optimal designs for the Monod model. These authors show that for fixed θ the function $\eta(t, \theta)$ is strictly increasing with existing limit $c = \lim_{t\to\infty} \eta(t, \theta) < \infty$ (note that this limit depends on the parameter θ). Define $\bar{c} = \eta(T, \theta) < c$ and consider the induced design space

$$\mathcal{X} = \{\eta(t, \theta) \mid t \in [0, T]\ \} \;=\; [\eta_0, \bar{c}] \ . \tag{2.9}$$

Any design of the form

$$\zeta = \begin{pmatrix} x_1 & \ldots & x_n \\ w_1 & \ldots & w_n \end{pmatrix}, \ \eta_0 \leq x_1 < x_2 < \ldots < x_n \leq \bar{c} \tag{2.10}$$

on the induced design space $\mathcal{X}$ corresponds in a one-to-one manner to a design ξ_ζ on the interval $[0, T]$ with weights w_i at points $t_i = t(x_i, \theta)$ $(i = 1, \ldots, n)$ by the transformation

$$t(x) \;=\; t(x, \theta) \;=\; \frac{1}{\vartheta_1}\left((1 + b)\ln\frac{x}{\eta_0} - b\ln\frac{c - x}{c - \eta_0}\right) , \tag{2.11}$$

where $b = b(\theta) = \theta_2\theta_3/c$ and $c = c(\theta) = s_0\theta_3 + \eta_0$ [see Dette et al. (2003)]. The information matrix in the nonlinear regression model (2.3) can now be represented as

$$M(\xi_\zeta, \theta) \;=\; K\bar{M}(\zeta, \theta)K^T , \tag{2.12}$$

where the matrix $\bar{M}(\zeta, \theta)$ is given by

$$\bar{M}(\zeta, \theta) = \sum_{j=1}^{n} w_j\varphi(x_j, \theta)\varphi(x_j, \theta)^T,$$

and the matrix K is defined by

$$K = \begin{pmatrix} \frac{1+b}{\theta_1} & -\frac{b}{\theta_1} & 0 \\ -\frac{b}{\theta_2} & +\frac{b}{\theta_2} & 0 \\ -\frac{b\eta_0}{c\theta_3} & +\frac{b\eta_0}{c\theta_3} & +\frac{b}{\theta_3} \end{pmatrix} . \tag{2.13}$$

Here

$$\varphi(x,\theta) = (\varphi_1(x,\theta), \varphi_2(x,\theta), \varphi_3(x,\theta))^T \tag{2.14}$$

denotes a vector of regression functions with components

$$\varphi_1(x,\theta) = v(x)\ln\frac{x}{\eta_0},\ \varphi_2(x,\theta) = v(x)\ln\frac{c-x}{c-\eta_0},\ \varphi_3(x,\theta) = v(x)\frac{x-\eta_0}{c-x}, \tag{2.15}$$

and

$$v(x) = v(x,\theta) = \frac{x(c-x)}{(1+b)c-x} . \tag{2.16}$$

[see Dette et al. (2003) for more details]. Consequently, it is sufficient to construct locally D-optimal designs for the regression model

$$\beta^T\varphi(x,\theta) , \tag{2.17}$$

and E- and e_k-optimal designs for the regression model $\beta^T K\varphi(x,\theta)$. For a fixed θ the locally optimal designs for the Monod model (2.1) are simply obtained by transforming the design ζ in (2.10) to the design ξ_ζ using the mapping $t(x,\theta)$.

3 Some theoretical results

In the present section we derive some important characteristics of standardized maximin optimal designs and also discuss properties of locally Φ-optimal uniform designs defined in (2.8). Dette et al. (2003) proved that the for a sufficiently small initial condition η_0 the locally D-, E- and e_i- optimal designs have three support points including the right boundary of the design space. They also observed this property numerically for arbitrary η_0. As a consequence the locally D-optimal design has equal masses at three points [see Silvey (1980)] and formulas representing the weights of the E- and e_i- optimal designs are also available [see Pukelsheim and Torsney (1991) or Dette et al. (2003)]. In all cases the locally optimal designs have to be found numerically, but with the transformation onto the induced design space introduced in the second part of Section 2 the calculation is straightforward and easy implementable in standard software as Mathematica or Matlab. Our first results of the present paper provide some properties of the locally optimal continuous uniform design defined by (2.8), which simplify their calculation substantially.

Lemma 3.1. *Let $f(t,\theta) = \frac{\partial\eta}{\partial\theta}(t,\theta)$ denote the gradient of the regression function in the Monod model (2.3)*

(i) If $\xi_{T^*,\theta}$ is a locally D-optimal uniform design, then

$$f^T(T^*,\theta)M^{-1}(\xi_{T^*,\theta},\theta)f(T^*,\theta) \;=\; 3 \tag{3.1}$$

(ii) *If $\xi_{T^*,\theta}$ is a locally E-optimal uniform design and the minimum eigenvalue of the corresponding information matrix $M(\xi_{T^*,\theta},\theta)$ has multiplicity 1 , then*

$$(p^T f(T^*,\theta))^2 \;=\; \lambda_{\min}(M(\xi_{T^*,\theta},\theta)) \tag{3.2}$$

where p is a normalized eigenvector corresponding to the minimum eigenvalue of $M(\xi_{T^*,\theta},\theta)$.

(iii) *If $i \in \{1,2,3\}$, $\xi_{T^*,\theta}$ is a locally e_i-optimal uniform design and the corresponding information matrix $M(\xi_{T^*,\theta},\theta)$ is non-singular, then*

$$(f^T(T^*,\theta)M^{-1}(\xi_{T^*,\theta},\theta)e_i)^2 \;=\; e_i^T M^{-1}(\xi_{T^*,\theta},\theta)e_i \tag{3.3}$$

For the application of this result we note that the matrix $M(\xi_{T,\theta},\theta)$ can be calculated by formula (2.12) and the gradient of the response function can be represented as $\frac{\partial\eta}{\partial\theta}(t,\theta) = K\varphi(x)$, where the matrix K and the vector φ are given by (2.13) and (2.14), respectively. Note that the function $\bar{T} \to \Phi(M(\xi_{\bar{T}},\theta))$ is not necessarily concave and as a consequence only a necessary condition for the local optimality of a continuous uniform design can be established. Nevertheless, we could show numerically that the equations (3.1) - (3.3) have always a unique solution. We have checked this property for all optimality criteria under consideration and always found a unique solution. A typical example is shown in Figure 1, where we considered the case of the D-optimality criterion and depicted the function

$$\psi(\bar{T}) \;=\; (\det M(\xi_{\bar{T}},\theta))^{1/3} \tag{3.4}$$

for the parameters $\eta_0 = 0.03$, $s_0 = 1$, $\theta_1 = 0.25$, $\theta_2 = 0.5$ and $\theta_3 = 0.25$ which corresponds to a typical situation observed in studies of microbial growth [see Pirt (1975) or Blok (1994)].
Because the existence of an optimal uniform design is obvious (due to the continuity of the problem) the locally optimal uniform design could be obtained directly for any θ by solving the equation (3.1) (or (3.2), (3.3)) with respect to T^*. As a consequence the determination of locally optimal uniform designs reduces to a very simple numerical solution of a (one-dimensional) nonlinear equation. If the optimal largest support point T^* has been determined an implementable (locally optimal) uniform design for a given sample size N is obtained by (2.7).
The calculation of standardized maximin optimal designs is substantially more difficult. However, the following result restricts the dimension of the optimization problem and is the basis for the numerical determination of standardized maximin optimal designs, which will be described in the next section.

Theorem 3.2. *In the Monod model (2.3) defined by the differential equation (2.1) there always exists a standardized maximin D-, E- or e_i-optimal design with a finite number of support points, if the minimum in the standardized maximin optimality criterion is taken over a set Ω of the form (2.6). Moreover, the right boundary point of the design space $[0,T]$ is always a support point of the standardized maximin optimal design.*

It should pointed out here that Theorem 3.2 cannot be obtained by Caratheodory's Theorem [see e.g. Silvey (1980)], because the set $\{\varphi(x,\theta)|x \in \mathcal{X}, \theta \in \Omega\}$ is in general not of finite dimension. By the same reason no upper bound for the number of support points of the standardized maximin optimal designs in the Monod model is available. Nevertheless, based on an intensive numerical study described below, we obtain the following conjecture regarding the maximal number of support points in the Monod model.

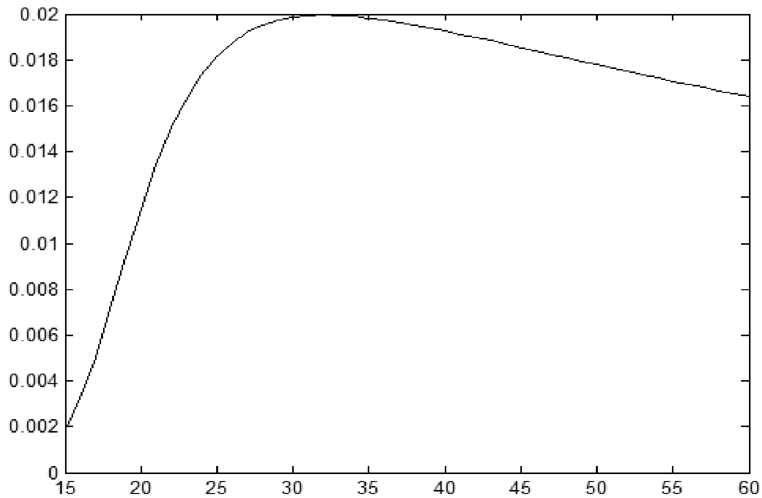

Figure 1: *The function $\psi(\bar{T})$ defined in (3.4) for the parameters $\eta_0 = 0.03$, $s_0 = 1$, $\theta_1 = 0.25$, $\theta_2 = 0.5$ and $\theta_3 = 0.25$.*

Conjecture 3.3. *If the assumptions of Theorem 3.2 are satisfied, any standardized maximin D-, E- or e_i-optimal design in the Monod model (2.3) defined by the differential equation (2.1) has at most 6 support points. Moreover, the right boundary point of the design space $[0, T]$ is always a support point of the standardized maximin optimal design.*

4 A numerical procedure for the calculation of standardized maximin optimal designs

In the following section we will calculate some standardized maximin efficient designs numerically and demonstrate that these designs have excellent efficiencies compared to locally optimal uniform designs. We will now briefly explain the algorithm used for these calculations. The algorithm is based on the following conjecture, which was satisfied in all examples in our numerical study. In this section the function Φ- denotes the D-,E-, or e_i-optimality criterion ($i = 1, 2, 3$) defined in Section 2.

Conjecture 4.1. *For any design ξ the set*

$$(4.1) \qquad \Omega_0 = \Omega_0(\xi) = \Big\{ \theta \;\Big|\; \theta = arg \min_{\theta \in \Omega} \frac{\Phi(M(\xi, \theta))}{\Phi(M(\xi_\theta^*, \theta))} \Big\}.$$

is finite, say $\Omega_0 = \{\theta_{(1)}, \ldots, \theta_{(n_2)}\}$ ($n_2 \in \mathbb{N}$).

In all our considered examples we observed that $n_2 \leq 4$, but a general bound could not be established formally. Now consider the set

$$(4.2) \qquad \mathcal{U}_{n_1} = \Big\{ (u_1, \ldots, u_{2n_1}) = (t_1, \ldots, t_{n_1}, w_1, \ldots, w_{n_1}) \;\Big| \; 0 \leq t_1, \ldots < t_{n_1} \leq T; w_i > 0, \sum_{i=1}^{n_1} w_i = 1 \Big\}$$

and note that each element $u \in \mathcal{U}_{n_1}$ defines a design with n_1 support points, that is

$$\xi_u = \begin{pmatrix} t_1 & \dots & t_{n_1-1} & t_{n_1} \\ w_1 & \dots & w_{n_1-1} & w_{n_1} \end{pmatrix} . \tag{4.3}$$

By Theorem 3.2 there exists an $n_1 \in \mathbb{N}$ and a $u \in \mathcal{U}_{n_1}$ such that the standardized maximin Φ-optimal design is given by $\xi^* = \xi_u$. We will now describe an iterative procedure for the calculation of standardized maximin Φ-optimal design observing that at least 3 support points are required. Thus we set $n_1 = 3$ and chose an arbitrary (possibly locally Φ-optimal) starting design, say $\xi_{u_{(0)}}$ with $u_{(0)} \in \mathcal{U}_{n_1}$. We put $s = 0$, define $u(\alpha) = (1-\alpha)u_{(s)} + \bar{u}$ and determine

$$\begin{aligned} \bar{u}_{(s)} &= \arg\max_{\|\bar{u}\|=1} \frac{\partial}{\partial\alpha} \min_{\theta\in\Omega} \frac{\Phi(M(\xi_{u(\alpha)},\theta))}{\Phi(M(\xi^*_\theta,\theta))}\Big|_{\alpha=0+} \\ &= \arg\max_{\|\bar{u}\|=1} \frac{\partial}{\partial\alpha} \min_{j=1,\dots,n_2} \frac{\Phi(M(\xi_{u(\alpha)},\theta_{(j)}))}{\Phi(M(\xi^*_{\theta_{(j)}},\theta_{(j)}))}\Big|_{\alpha=0+} \\ &= \arg\max_{\|\bar{u}\|=1} \min\Big\{ \sum_{j=1}^{n_2} h_j \frac{\partial}{\partial\alpha} \frac{\Phi(M(\xi_{u(\alpha)},\theta_{(j)}))}{\Phi(M(\xi^*_{\theta_{(j)}},\theta_{(j)}))}\Big|_{u=u_{(s)}} \ \Big| \ h_j \ge 0;\ \sum_{j=1}^{n_2} h_j = 1 \Big\}, \end{aligned} \tag{4.4}$$

where $\|\cdot\|$ denotes the Euclidean norm, we have used Conjecture 4.1 with $\Omega_0 = \Omega_0(\xi_{u_{(0)}}) = \{\theta_{(1)},\dots,\theta_{(n_2)}\}$ and the formula for the directional derivative of the minimum. In the next step we calculate

$$u_{(s+1)} = u_{(s+1)}(h_s) = (1-h_s)u_{(s)} + h_s\bar{u}_{(s)}, \tag{4.5}$$

where the weight h_s maximizes the minimum Φ-efficiency

$$\text{eff}_\Phi(\xi,\theta) = \frac{\Phi(M(\xi,\theta))}{\Phi(M(\xi^*_\theta,\theta))} . \tag{4.6}$$

among all designs of the form ξ_u with $u = u_{(s+1)}$ defined by (4.5), that is

$$h_s = \arg\max\{ \min_{\theta\in\Omega} \text{eff}_\Phi(\xi_{u_{(s+1)}(h)},\theta) \mid 0 \le h \le 1 \} .$$

Obviously we obtain

$$\min_{\theta\in\Omega} \text{eff}_\Phi(\xi_{u_{(s+1)}},\theta) \ \ge \ \min_{\theta\in\Omega} \text{eff}_\Phi(\xi_{u_{(s)}},\theta)$$

and in the case of equality the design $\xi_{u_{(s+1)}}$ is standardized maximin Φ-optimal in the class of all designs with n_1 support points, i.e.

$$\Xi_{n_1} = \{\xi_u \mid u \in \mathcal{U}_{n_1}\} . \tag{4.7}$$

Otherwise it follows by standard arguments that the sequence of designs $(\xi_{u_{(j)}})_{j\in\mathbb{N}_0}$ contains a weakly convergent subsequence with limit, say $\xi^*_{n_1}$, which is a standardized maximin Φ-optimal in the class Ξ_{n_1}. Note that in all cases considered in our study the sequence $(\xi_{u_{(j)}})_{j\in\mathbb{N}_0}$ was weakly convergent and it is usually not necessary to consider subsequences. We can now use the general equivalence theorem for standardized maximin Φ-optimality [see Dette, Haines and Imhof (2003), Theorem 3.3] to check if the design $\xi^*_{n_1}$ is standardized maximin Φ-optimal in the class of all approximate designs (for the standardized maximin D-optimality criterion the corresponding equivalence theorem is stated in the appendix in Lemma 6.1). Note that the application of the

equivalence theorem requires the specification of a least favourable distribution on the set $\Omega(\xi^*_{n_1})$, which was finite in all examples considered in our study [see also Conjecture 4.1].
Otherwise the procedure is continued with n_1 replaced by n_1+1 and an initial design in the class Ξ_{n_1+1} constructed as follows: we define

$$t^* = \arg\max_{t\in[0,T]} \min\Big\{ \sum_{j=1}^{n_2} h_j \frac{\partial}{\partial\alpha} \frac{\Phi(M((1-\alpha)\xi^*_{n_1}+\alpha\delta_t,\theta_{(j)}))}{\Phi(M(\xi^*_{\theta_{(j)}},\theta_{(j)}))}\Big|_{\alpha=0^+} \;\Big|\; h_j \geq 0;\; \sum_{j=1}^{n_2} h_j = 1\Big\}$$

where δ_t denotes the Dirac-measure at the point t and

$$\alpha^* = \arg\max_{\alpha\in[0,1]} \min\Big\{ \sum_{j=1}^{n_2} h_j \frac{\Phi(M((1-\alpha)\xi^*_{n_1}+\alpha\delta_{t^*},\theta_{(j)}))}{\Phi(M(\xi^*_{\theta_{(j)}},\theta_{(j)}))} \;\Big|\; h_j \geq 0;\; \sum_{j=1}^{n_2} h_j = 1\Big\}\ .$$

The initial design $\xi_{u_{(0)}}$ for the calculation of the standardized maximin Φ-optimal in the class Ξ_{n_1+1} is finally defined by the vector $u_{(0)} \in \mathcal{U}_{n_1+1}$, which is given by

$$u_{(0)} = (u_1^*,\dots,u_{n_1}^*, t^*, (1-\alpha^*)w_1^*, \dots (1-\alpha^*)w_{n_1}^*, \alpha^*),$$

where $u_1^*,\dots,u_{n_1}^*$ denote the support points of the design $\xi^*_{n_1}$ with corresponding weights $w_1^*,\dots w_{n_1}^*$. The first step of the procedure is now continued to obtain the standardized maximin Φ-optimal design in the class Ξ_{n_1+1}. If this design is not standardized maximin Φ-optimal in the class of all approximated designs the procedure is repeated increasing the number of support points by 1. The algorithm stops if the standardized maximin Φ-optimality of the calculated design has been confirmed by the corresponding equivalence theorem.
Note that the algorithm definitively terminates, because by Theorem 3.2 any standardized maximin D, E- or e_i-optimal design is supported at a finite number of points. Moreover, in our numerical study all iterations usually stopped after a few steps and the standardized maximin Φ-optimal could quickly be identified using the described procedure.

5 A comparison of standardized maximin and uniform designs

Note that the discussion in Section 2 shows that the design problem in the Monod model on the interval $[0,T]$ corresponds to a design problem in the linear model (2.17) on the induced design space $\mathcal{X} = [\eta_0, \bar{c}]$ defined in (2.9), where $\eta_0 = \eta(0,\theta)$ and $\bar{c} = \eta(T,\theta)$. Moreover, for $T\to\infty$ we obtain $\bar{c}\to c$ and the design problem for the regression model (2.17) can also be considered on the interval $[\eta_0, c]$. By the transformation (2.11) this design corresponds to a design in the Monod model on the design space $[0,\infty]$. In other words because of the compactness of the induced design space there also exist locally and standardized maximin optimal designs for the Monod model on the infinite design space $[0,\infty]$.
For the sake of brevity we will restrict the calculation of standardized maximin optimal designs to a procedure, which uses the optimal designs from the infinite design space $[0,\infty]$. As a consequence we only have to tabulate designs for one design space, namely $[0,\infty]$. Moreover, the consideration of an infinite design space is justified by the following observations. First, it was demonstrated by Dette et al. (2003) that efficient locally optimal designs on a finite design space can easily be obtained from the designs on an infinite design space using the following method. If

$$\xi^*_\theta = \begin{pmatrix} t_1^* & t_2^* & \infty \\ w_1^* & w_2^* & w_3^* \end{pmatrix} \qquad (5.1)$$

denotes a locally D-, $E-$ or e_i-optimal design for the Monod model on the design space $[0,\infty]$ and the right boundary of the design space $[0,T]$ satisfies $T \geq 1.5 t_2^*$, then the design

$$(5.2) \qquad \tilde{\xi}_\theta^* = \begin{pmatrix} t_1^* & t_2^* & T \\ w_1^* & w_2^* & w_3^* \end{pmatrix}$$

on the finite design space has at least Φ-efficiency 0.98, where the Φ-efficiency is defined by (4.6). Similarly, it was observed in our numerical study that if

$$(5.3) \qquad \xi^* = \begin{pmatrix} t_1^* & \dots & t_{n-1}^* & \infty \\ w_1^* & \dots & w_{n-1}^* & w_n^* \end{pmatrix}$$

denotes a standardized maximin D-, $E-$ or e_i-optimal design for the Monod model on the design space $[0,\infty]$ and $T \geq 2t_{n-1}^*$, then the design

$$(5.4) \qquad \tilde{\xi}^* = \begin{pmatrix} t_1^* & \dots & t_{n-1}^* & T \\ w_1^* & \dots & w_{n-1}^* & w_n^* \end{pmatrix}$$

has at least maximin-efficiency 0.98, where the maximin-efficiency is defined by

$$(5.5) \qquad \mathrm{eff}_{\Psi_\Omega}(\xi) \;=\; \frac{\Psi_\Omega(\xi)}{\sup_\eta \Psi_\Omega(\eta)}$$

and the robust optimality criterion $\Psi_\Omega(\xi)$ is given by (2.5). Secondly we note that in microbiological studies the length of the design interval $[0,T]$ can often be chosen by the experimenter.

Table 5.1: *Standardized maximin D-optimal designs in Monod model for various regions* $\Omega = [z_{1,L}, z_{1,U}] \times [z_{2,L}, z_{2,U}] \times [z_{3,L}, z_{3,U}]$.

Ω	t_1	t_2	t_3	t_4	t_5	t_6	w_1	w_2	w_3	w_4	w_5	w_6
$[.24,.26]\times[.47,.53]\times[.24,.26]$	10.93	15.83	17.32	∞			.325	.223	.124	.328		
$[.23,.27]\times[.45,.55]\times[.24,.26]$	10.51	14.68	18.60	∞			.270	.244	.194	.292		
$[.23,.27]\times[.45,.55]\times[.23,.27]$	10.40	14.50	18.53	∞			.264	.241	.203	.292		
$[.23,.27]\times[.43,.57]\times[.24,.26]$	10.46	14.48	18.47	∞			.262	.243	.202	.293		
$[.23,.27]\times[.43,.57]\times[.23,.27]$	10.23	14.06	16.84	19.41	∞		.246	.219	.105	.149	.281	
$[.22,.28]\times[.45,.55]\times[.24,.26]$	10.22	14.08	17.12	19.69	∞		.244	.222	.106	.148	.280	
$[.22,.28]\times[.45,.55]\times[.22,.28]$	9.78	13.50	16.69	20.18	∞		.204	.229	.136	.162	.269	
$[.22,.28]\times[.43,.57]\times[.24,.26]$	10.01	13.65	16.79	20.13	∞		.216	.226	.135	.154	.268	
$[.22,.28]\times[.43,.57]\times[.22,.28]$	9.81	13.42	16.77	20.35	∞		.207	.233	.134	.166	.261	
$[.22,.28]\times[.41,.59]\times[.24,.26]$	9.96	13.58	16.91	20.42	∞		.214	.230	.141	.153	.263	
$[.22,.28]\times[.41,.59]\times[.22,.28]$	9.76	13.34	16.86	20.56	∞		.205	.237	.136	.164	.258	
$[.20,.30]\times[.41,.59]\times[.24,.26]$	9.19	12.72	15.69	18.58	22.00	∞	.159	.235	.101	.098	.161	.246
$[.20,.30]\times[.41,.59]\times[.20,.30]$	8.56	12.10	15.32	19.16	23.33	∞	.147	.218	.103	.128	.163	.241
$[.20,.30]\times[.40,.60]\times[.24,.26]$	9.16	12.64	15.59	18.60	22.08	∞	.159	.232	.099	.102	.163	.245
$[.20,.30]\times[.40,.60]\times[.20,.30]$	8.51	11.98	15.16	19.10	23.67	∞	.147	.212	.102	.138	.167	.235

In Table 5.1 and 5.2 we present some standardized maximin optimal designs for various regions of the parameter space Ω, where the design interval is given by $[0,\infty]$. A typical vector of parameters observed in studies of microbial growth is given by $\eta_0 = 0.03$, $s_0 = 1$, $\theta_1 = 0.25$, $\theta_2 = 0.5$ and $\theta_3 = 0.25$ [see Pirt (1975) or Blok (1994)] and for an illustration of the robustness and efficiency properties of the standardized maximin optimal designs we took this point as the center of the set Ω required for the definition of the standardized optimality criteria. In Table 5.1 we display standardized maximin D-optimal designs for the Monod model on the set $[0, \infty]$, while Table 5.2 contains the corresponding standardized maximin E-optimal designs. It is interesting to note that in all cases the standardized maximin optimal designs require at least four support points. Moreover, the number of support points is increasing with the size of the set Ω specified by the experimenter. This observation was also made by Dette and Biedermann (2003) for the Michaelis-Menten model. Note that according to Theorem 3.2 the standardized maximin optimal designs is always supported at a finite number of points and that in all cases considered in our study the optimal designs have at most 6 support points, including the right boundary point of the design space [see also our Conjecture 3.4]. As pointed out in the previous paragraph implementable and very efficient designs of the form (5.4) can be derived from the standardized maximin optimal designs on the infinite designs space, in the case $t^*_{n-1} < T$. In particular compared to the designs (5.1) on the infinite design space $[0, \infty]$ these designs have at least efficiency 0.98 provided that the point t^*_{n-1} satisfies $2t^*_{n-1} < T$.

Table 5.2: Standardized maximin E-optimal designs in Monod model for various regions $\Omega = [z_{1,L}, z_{1,U}] \times [z_{2,L}, z_{2,U}] \times [z_{3,L}, z_{3,U}]$.

Ω	t_1	t_2	t_3	t_4	t_5	t_6	w_1	w_2	w_3	w_4	w_5	w_6
$[.24, .26] \times [.47, .53] \times [.24, .26]$	9.47	15.58	17.52	∞			.302	.257	.189	.252		
$[.23, .27] \times [.45, .55] \times [.24, .26]$	9.19	14.77	18.34	∞			.273	.258	.245	.223		
$[.23, .27] \times [.45, .55] \times [.23, .27]$	9.15	14.63	18.28	∞			.271	.256	.252	.222		
$[.23, .27] \times [.43, .57] \times [.24, .26]$	9.25	14.60	18.23	∞			.270	.257	.254	.220		
$[.23, .27] \times [.43, .57] \times [.23, .27]$	8.99	14.03	16.72	19.31	∞		.261	.204	.166	.161	.207	
$[.22, .28] \times [.45, .55] \times [.24, .26]$	9.03	14.02	16.75	19.31	∞		.255	.206	.156	.173	.209	
$[.22, .28] \times [.45, .55] \times [.22, .28]$	8.78	13.48	16.55	19.91	∞		.235	.197	.200	.171	.197	
$[.22, .28] \times [.43, .57] \times [.24, .26]$	9.22	13.88	16.91	2.11	∞		.234	.220	.197	.153	.195	
$[.22, .28] \times [.43, .57] \times [.22, .28]$	8.86	13.45	16.60	2.09	∞		.230	.208	.206	.169	.188	
$[.22, .28] \times [.41, .59] \times [.24, .26]$	9.02	13.71	16.89	2.31	∞		.246	.213	.201	.154	.186	
$[.22, .28] \times [.41, .59] \times [.22, .28]$	8.62	12.86	15.38	17.71	2.73	∞	.218	.174	.148	.147	.137	.177
$[.20, .30] \times [.41, .59] \times [.24, .26]$	8.52	12.60	15.51	18.59	22.22	∞	.202	.180	.171	.158	.129	.161
$[.20, .30] \times [.41, .59] \times [.20, .30]$	8.24	12.16	15.08	18.24	22.19	∞	.182	.202	.164	.149	.152	.151
$[.20, .30] \times [.40, .60] \times [.24, .26]$	8.49	12.57	15.46	18.56	22.29	∞	.203	.182	.168	.159	.131	.158
$[.20, .30] \times [.40, .60] \times [.20, .30]$	8.20	12.10	15.02	18.26	22.20	∞	.176	.204	.168	.152	.156	.144

For this reason we will now assume that the microbiological experiments can be carried out over a sufficiently long time T such that these strategies of design construction are applicable and compare the standardized maximin optimal designs with some uniform designs, which provide an alternative design of experiment if there is only very vague prior information regarding the unknown parameter. All our efficiency considerations are restricted to designs obtained from the optimal designs on an infinite design space $[0, \infty]$ by the procedure explained by (5.1) - (5.4). The efficiencies of the "true" standardized maximin optimal designs on the interval $[0, T]$ are slightly larger, but the additional effort of calculating these designs for any interval $[0, T]$ under

consideration is only justified if $T < 2t^*_{n-1}$.
For an illustration we consider the problem of designing an experiment for the Monod model with design space $[0, T] = [0, 40]$. For the uniform design, we chose the uniform distribution on 20 points in the interval $[0, 40]$, that is the design $\xi_{\mathcal{U}(20),40}$ defined in (2.7) for $N = 20$ and $\bar{T} = 40$. Note that it follows from Figure 1 that for $\eta_0 = 0.03$, $s_0 = 1$, $\theta_1 = 0.25$, $\theta_2 = 0.5$ and $\theta_3 = 0.25$ the locally D-optimal uniform design is the uniform distribution on the interval $[0, 32]$ and that $\xi_{\mathcal{U}(20),40}$ could be considered as an approximation to the locally D-optimal uniform design, which takes into account that the parameters required for the construction of the locally D-optimal uniform design have been misspecified. Moreover, we also observe from Figure 1 that for the point $\eta_0 = 0.03$, $s_0 = 1$, $\theta_1 = 0.25$, $\theta_2 = 0.5$ and $\theta_3 = 0.25$ the uniform design $\xi_{\mathcal{U}(20),40}$ is only slightly less efficient compared to the locally D-optimal uniform design $\xi_{\mathcal{U}(20),32}$. Note that Figure 1 refers to the D-criterion, but the situation for the other criteria is similar. In Table 5.3 we compare this uniform design with the standardized maximin D-optimal designs derived from Table 5.1 and the procedure described by (5.3) and (5.4). The comparison is performed by considering the ratios

$$C_D(\xi, \theta) = \left(\frac{\det M(\xi, \theta)}{\det M(\xi_{\mathcal{U}(20),40}, \theta)} \right)^{\frac{1}{3}} \tag{5.6}$$

$$C_i(\xi, \theta) = \frac{(e_i^T M^{-1}(\xi, \theta) e_i)^{-1}}{(e_i^T M^{-1}(\xi_{\mathcal{U}(20),40}, \theta) e_i)^{-1}} \qquad i = 1, 2, 3 \tag{5.7}$$

$$C_E(\xi, \theta) = \frac{\lambda_{\min} M(\xi, \theta)}{\lambda_{\min}(M(\xi_{\mathcal{U}(20),40}, \theta))} \tag{5.8}$$

of the corresponding optimality criteria. Note that these ratios depend on the parameter θ and that for a given $\theta \in \Omega$ a larger value than 100% indicates that the design ξ is more efficient than the uniform design $\xi_{\mathcal{U}(20),40}$ with respect to the corresponding optimality criterion. For the sake of brevity Table 5.1 contains the maximum, minimum and averaged values of these ratios, which are indicated by the symbols "max", "min" and "average" respectively. For example, in the column with the label C_D and "min" the reader finds the minimum ratio

$$\min_{\theta \in \Omega} C_D(\tilde{\xi}^*_D, \theta) = \min_{\theta \in \Omega} \left(\frac{\det M(\tilde{\xi}^*_D, \theta)}{\det M(\xi_{\mathcal{U}(20),40}, \theta)} \right)^{\frac{1}{3}}$$

taken over the set Ω, while in the column with the label C_D and "average" the corresponding integrated values with respect to the uniform distribution can be found, that is

$$\int_\Omega C_D(\tilde{\xi}^*_D, \theta) d\theta$$

[recall that the design $\tilde{\xi}^*_D$ on the interval $[0, T]$ is obtained from the standardized maximin D-optimal design ξ^*_D on the infinite design space in Table 5.1 by changing the design in (5.3) to the design in (5.4)].

We observe that the standardized maximin D-optimal design is always better than the uniform design $\xi_{\mathcal{U}(20),40}$, if the D-, $E-$, e_1 and e_2-criterion are used for comparing competing designs, because the corresponding minimum values are larger than 100%. The improvement by using a

Table 5.3: *Comparison of standardized maximin D-optimal design with uniform designs of the form (2.7) ($N = 20$, $\bar{T} = 40$) on the design space $[0, T] = [0, 40]$. The table shows the minimum, maximum and average values of the ratios defined by (5.6), (5.7) and (5.8) in %.*

	min					max					average				
Ω	C_D	C_1	C_2	C_3	C_E	C_D	C_1	C_2	C_3	C_E	C_D	C_1	C_2	C_3	C_E
$[.24, .26] \times [.47, .53] \times [.24, .26]$	140	168	175	64	175	154	220	226	75	226	151	207	213	68	213
$[.23, .27] \times [.45, .55] \times [.24, .26]$	133	157	155	65	155	148	190	183	74	183	140	172	166	67	167
$[.23, .27] \times [.45, .55] \times [.23, .27]$	132	154	153	66	153	148	191	181	75	181	139	169	163	67	163
$[.23, .27] \times [.43, .57] \times [.24, .26]$	131	152	149	64	149	149	195	184	76	184	140	171	163	67	163
$[.23, .27] \times [.43, .57] \times [.23, .27]$	130	147	149	65	149	148	195	180	75	180	138	164	157	67	157
$[.22, .28] \times [.45, .55] \times [.24, .26]$	129	146	148	66	148	147	193	177	76	180	137	161	155	69	155
$[.22, .28] \times [.45, .55] \times [.22, .28]$	127	140	138	66	138	144	205	177	79	180	134	156	146	69	146
$[.22, .28] \times [.43, .57] \times [.24, .26]$	127	141	142	65	142	147	206	181	78	180	136	158	149	69	149
$[.22, .28] \times [.43, .57] \times [.22, .28]$	126	136	134	64	134	146	212	181	80	181	134	152	143	69	143
$[.22, .28] \times [.41, .59] \times [.24, .26]$	126	138	139	65	139	149	213	186	80	186	135	156	147	69	147
$[.22, .28] \times [.41, .59] \times [.22, .28]$	124	132	131	65	131	148	221	188	81	188	133	149	141	70	141
$[.20, .30] \times [.41, .59] \times [.24, .26]$	121	116	111	67	117	146	238	198	89	199	129	143	132	75	132
$[.20, .30] \times [.41, .59] \times [.20, .30]$	118	106	109	73	109	142	230	193	98	193	125	128	123	81	123
$[.20, .30] \times [.40, .60] \times [.24, .26]$	120	115	116	68	116	147	239	200	91	200	129	141	131	76	131
$[.20, .30] \times [.40, .60] \times [.20, .30]$	117	105	108	74	108	143	229	191	98	192	125	126	123	82	123

Table 5.4: *Comparison of standardized maximin E-optimal design with uniform designs of the form (2.7) ($N = 20$, $\bar{T} = 40$) on the design space $[0, T] = [0, 40]$. The table shows the minimum, maximum and average values of the ratios defined by (5.6), (5.7) and (5.8) in %.*

	min					max					average				
Ω	C_D	C_1	C_2	C_3	C_E	C_D	C_1	C_2	C_3	C_E	C_D	C_1	C_2	C_3	C_E
$[.24, .26] \times [.47, .53] \times [.24, .26]$	130	157	187	52	187	143	231	246	58	246	140	212	234	54	234
$[.23, .27] \times [.45, .55] \times [.24, .26]$	124	159	160	51	160	143	186	211	62	211	133	180	187	53	187
$[.23, .27] \times [.45, .55] \times [.23, .27]$	123	155	155	50	155	143	187	210	63	210	133	178	183	53	183
$[.23, .27] \times [.43, .57] \times [.24, .26]$	121	159	152	49	152	145	187	213	63	212	134	179	184	53	184
$[.23, .27] \times [.43, .57] \times [.23, .27]$	119	154	148	49	148	143	188	203	66	203	132	171	175	53	175
$[.22, .28] \times [.45, .55] \times [.24, .26]$	119	153	147	50	147	143	180	200	67	200	132	169	173	54	173
$[.22, .28] \times [.45, .55] \times [.22, .28]$	116	142	141	49	141	142	181	192	71	192	130	164	165	54	164
$[.22, .28] \times [.43, .57] \times [.24, .26]$	118	143	141	49	141	146	190	202	70	202	132	169	168	54	167
$[.22, .28] \times [.43, .57] \times [.22, .28]$	115	135	136	47	136	145	188	198	72	198	130	163	163	54	163
$[.22, .28] \times [.41, .59] \times [.24, .26]$	115	137	138	48	137	148	189	207	72	207	131	163	166	54	165
$[.22, .28] \times [.41, .59] \times [.22, .28]$	112	129	132	46	132	144	188	194	75	194	129	159	159	53	159
$[.20, .30] \times [.41, .59] \times [.24, .26]$	107	119	126	47	126	146	189	197	81	197	128	152	152	58	152
$[.20, .30] \times [.41, .59] \times [.20, .30]$	101	113	120	44	120	147	211	203	81	203	127	147	145	57	145
$[.20, .30] \times [.40, .60] \times [.24, .26]$	106	118	125	47	125	147	191	201	81	201	128	151	152	57	152
$[.20, .30] \times [.40, .60] \times [.20, .30]$	100	112	119	43	119	147	216	206	82	206	127	147	144	56	144

standardized maximin optimal design instead of the uniform design with respect to these criteria can be substantial. For example, consider the set $\Omega = [.20, .30] \times [.40, .60] \times [.20, .30]$ corresponding to a situation, where only vague prior information regarding the unknown parameters in the Monod model is available. In this case the minimum gain in D-efficiency by the standardized maximin D-optimal design is approximately 17%, the maximum is 43% and in the average we have 25% improvement compared to the uniform design $\xi_{\mathcal{U}(20),40}$. The performance of the standardized maximin D-optimal design would be even better, if the parameter space Ω could be specified more precisely. The advantages with respect to the E-, e_1- and e_2-criterion are even larger. On the other hand the uniform design $\xi_{\mathcal{U}(20),40}$ is more efficient for the estimation of the parameter θ_3. However, as pointed out by Dette et al. (2003) the efficient estimation of θ_1 and θ_2 is usually more important for the Monod model, because in realistic situations [see Pirt (1975) or Blok (1994)] the parameter θ_3 can be estimated with much higher precision than the parameters θ_1 and θ_2 [see Dette et al. (2003), Table 3]. The situation for the E-optimality criterion is very similar. The standardized maximin E-optimal design should be preferred in all cases, except if the primary goal of the experiment is the parameter θ_3 and all other parameters are not of interest for the experimenter.

It might also be of interest to compare the standardized maximin optimal designs with some robust designs proposed by Dette et al. (2003). For a rectangular parameter space Ω of the form (2.6) these authors suggest to use the locally optimal design for the parameter $\theta^0 = (z_{1,U}, z_{2,U}, z_{3,L})$ as a robust design for the Monod model. In Table 5.5 we compare for the E-optimality criterion this design with the uniform design obtained by the procedure described in the previous paragraph. These results correspond to the situation considered in Table 5.4. The results for the D-optimality criterion are very similar and not displayed for the sake of brevity. We observe that the locally E-optimal design $\xi^*_{\theta^0}$ yields substantially higher values for the maximum of $C_D(\xi,\theta)$, $C_i(\xi,\theta)$ and $C_E(\xi,\theta)$ than the standardized maximin E-optimal design. This corresponds to intuition, because the point θ^0 is an element of the set Ω, and for this point the design proposed by Dette et al. (2003) is in fact locally E-optimal. On the other hand the minimal values of $C_E(\xi,\theta)$ can be very small. In many cases, there exist parameters $\theta \in \Omega$, where the uniform design is at least 10 times more efficient than the locally E-optimal design $\xi^*_{\theta^0}$. For the averaged efficiencies the differences between the standardized maximin E-optimal and the locally E-optimal design $\xi^*_{\theta^0}$ are less substantial. For narrow parameter spaces Ω we observe some advantages of the designs $\xi^*_{\theta^0}$ which confirms the results in Dette et al. (2003). On the other hand, if the experimenter has less precise information regarding the location of the parameters the standardized maximin E-optimal designs yield better results. Summarizing this comparison we conclude that the locally E-optimal design $\xi^*_{\theta^0}$ proposed by Dette et al. (2003) should only be used for "small" parameter spaces Ω. Otherwise, there may exist parameter combinations in Ω such that this design is substantially less inefficient than a uniform design. The robust designs derived in this paper do not have the drawback of being completely inefficient for certain parameters and are usually preferable to the locally E-optimal design $\xi^*_{\theta^0}$.

We finally point out that a frequently used argument in favour of uniform designs is that these designs allow for the possibility of checking the model assumptions by means of a goodness-of-fit test, because they advise the experimenter to take observations at a large number of different experimental conditions. The design $\xi^*_{\theta^0}$ proposed by Dette et al. (2003) as a robust design for the Monod model has only three support points (because it is locally optimal for the parameter θ^0) and therefore does not allow model checking. However, the standardized maximin D- and E-optimal designs determined in this paper have at least four support points (in many cases they advise the experimenter to take observations at even more different points). As a consequence these designs

Table 5.5: *Comparison of the locally E-optimal design ξ_{θ^0} with uniform designs of the form (2.7) ($N = 20$, $\bar{T} = 40$) on the design space $[0, T] = [0, 40]$, where $\theta^0 = (z_{1,U}, z_{2,U}, z_{3,L})$. The table shows the minimum, maximum and average values of the ratios defined by (5.6), (5.7) and (5.8) in %.*

	min					max					average				
Ω	C_D	C_1	C_2	C_3	C_E	C_D	C_1	C_2	C_3	C_E	C_D	C_1	C_2	C_3	C_E
$[.24, .26] \times [.47, .53] \times [.24, .26]$	121	171	149	52	149	141	269	278	63	278	137	246	246	57	246
$[.23, .27] \times [.45, .55] \times [.23, .27]$	89	63	50	49	51	142	282	289	71	289	129	228	202	58	203
$[.22, .28] \times [.45, .55] \times [.22, .28]$	60	15	16	47	16	144	299	301	78	301	116	182	152	58	152
$[.23, .27] \times [.43, .57] \times [.23, .27]$	89	64	51	49	51	140	284	287	74	288	130	224	211	58	212
$[.22, .28] \times [.43, .57] \times [.22, .28]$	61	16	16	47	16	142	299	298	82	299	119	190	164	59	164
$[.22, .28] \times [.41, .59] \times [.22, .28]$	61	16	16	46	16	141	299	297	86	297	120	193	173	59	173
$[.20, .30] \times [.41, .59] \times [.20, .30]$	26	1	2	44	2	145	328	323	114	324	96	126	107	61	107
$[.20, .30] \times [.40, .60] \times [.20, .30]$	26	1	2	43	2	144	338	323	118	323	98	129	111	61	111
$[.23, .27] \times [.45, .55] \times [.24, .26]$	98	94	70	50	70	142	275	284	70	284	131	231	216	58	217
$[.22, .28] \times [.45, .55] \times [.24, .26]$	76	34	31	48	31	143	281	293	75	293	124	201	180	58	180
$[.23, .27] \times [.43, .57] \times [.24, .26]$	98	92	70	49	70	140	277	283	72	283	132	224	222	58	222
$[.22, .28] \times [.43, .57] \times [.24, .26]$	77	35	31	48	31	142	287	292	78	292	125	203	188	58	189
$[.22, .28] \times [.41, .59] \times [.24, .26]$	77	36	32	47	32	140	288	289	82	289	126	201	193	59	193
$[.20, .30] \times [.41, .59] \times [.24, .26]$	43	6	7	45	7	144	295	305	101	305	111	154	144	60	144
$[.20, .30] \times [.40, .60] \times [.24, .26]$	44	6	7	45	7	143	299	306	104	306	111	155	146	61	146

can on the one hand be used for checking the assumption of the Monod model by a goodness of fit test and are on the other hand substantially more efficient for estimating the parameters in the Monod model than uniform designs. For these reasons we recommend the application of standardized maximin optimal designs for the analysis of microbial growth data with the Monod model.

Acknowledgements. The work H. Dette and V.B. Melas was partially supported by the Deutsche Forschungsgemeinschaft (SFB 475: Komplexitätsreduktion in multivariaten Datenstrukturen). The work of V.B. Melas was also partially supported by the Russian Foundation of Basic Research (project 04-01-00519). Parts of the paper were written during the first author's visit at the Department of Statistics of the University of Toulouse, and this author would like to thank the departement for its hospitality. The authors are also grateful to Isolde Gottschlich, who typed parts of this paper with considerable technical expertise

6 Appendix: Proof of technical results

6.1 Proof of Lemma 3.1.

Because all cases are proved similary, we restrict ourselves to the proof of part (i) of Lemma 3.1. For a fixed θ we define

$$M(\xi_T) = M(\xi_T, \theta), \;\; f(t) = f(t, \theta) = \frac{\partial}{\partial \theta} \eta(t, \theta).$$

Using the well known formula

$$\frac{\partial}{\partial \alpha} \ln \det B(\alpha) = \operatorname{tr}\Big\{B^{-1}(\alpha)\frac{\partial B}{\partial \alpha}\Big\}$$

we obtain

$$\begin{aligned}\frac{\partial}{\partial T} \ln \det M(\xi_T) &= \operatorname{tr}\Big\{M^{-1}(\xi_T)\Big[-\frac{1}{T}M(\xi_T) + \frac{1}{T}f(T)f^T(T)\Big]\Big\} \\ &= -\frac{3}{T} + \frac{1}{T}d(T),\end{aligned}$$

where the function d is defined by $d(T) = f^T(T)M^{-1}(\xi_T)f(T)$. Therefore the condition $d(T) = 3$ is a necessary condition for the local D-optimality of the uniform designs ξ_T.

6.2 Proof of Theorem 3.2.

We will only prove the statement for the D-optimality criterion. The results for E- and e_i -optimality criteria follow by similar arguments. The basic tool for proving Theorem 3.2 is an equivalence theorem for standardized maximin D-optimal designs. The proof and corresponding results for other optimality criteria can be found in Dette, Haines and Imhof (2003).

Lemma 6.1. *Assume that the set Ω in the standardized maximin optimality criterion is given by (2.6) with $z_{i,L} > 0$ $(i = 1, 2, 3)$.*

(i) There exists a standardized maximin D-optimal design for the Monod model (2.3).

(ii) A design ξ^* is standardized maximin D-optimal for the Monod model (2.3) if and only if there exists a prior h on the set

$$\Omega_0 = \Omega_0(\xi^*) = \Big\{ \theta \;\Big|\; \theta = \arg\min_{\theta\in\Omega} \frac{\det M(\xi^*, \theta)}{\det M(\xi_\theta, \theta)} \Big\}.$$

such that the inequality

$$\int_{\Omega_0} \{f^T(t,\theta)M^{-1}(\xi^*, \theta)f(t, \theta) - 3\}dh(\theta) \;\le\; 0 \tag{6.1}$$

holds for all $t \in [0, T]$, where $f(t, \theta)$ denotes the gradient of the response function $\eta(t, \theta)$ with respect to the parameter θ. Moreover, there is equality in (6.1) for all support points of the standardized maximin D-optimal design ξ^*.

We now continue with the proof of Theorem 3.2 by first showing that any standardized maximin D-optimal design has a finite support and secondly proving that the largest point of the design space is always a support point of a standardized maximin D-optimal design.
Recall that it was proved in Dette et al. (2003) that the function $\eta(t, \theta)$ is strictly increasing for any fixed θ with positive coordinates and that the inverse function is given by (2.11), which is obviously a real analytic function whenever $\eta_0 \le x \le c = \lim_{t\to\infty} \eta(t, \theta)$, $\theta_1, \theta_2, \theta_3, \eta_0 > 0$. Therefore we obtain that for fixed θ the functions $\eta(t, \theta)$ and $f_i(t, \theta) = \frac{\partial}{\partial\theta_i}\eta(t, \theta)$, $i = 1, 2, 3$ are also real analytic on the interval $[0, T]$. Consequently the function

$$Q(t) = \int_{\Omega_0} \{f^T(t, \theta)M^{-1}(\xi^*, \theta)f(t, \theta) - 3\}dh(\theta)$$

defined by the left hand side of the inequality (6.1) is also real analytic on the interval $[0, T]$. Because $Q(0) = -3$ it follows that for sufficiently small $\varepsilon > 0$ there exists no local maximum of the function $Q(t)$ in the interval $[0, \varepsilon]$. Moreover, the function $Q'(t)$ is also real analytic on the interval $[0, \tilde{T}]$ for some $\tilde{T} > T$ and therefore either

$$Q'(t) \equiv 0, \ t \in [0, T] \ , \tag{6.2}$$

or the function $Q(t)$ has a finite number of local maxima. The identity (6.2) implies $Q(0) = Q(t) = -3$ for all $t \in [0, T]$, which yields a contradiction. Due to Lemma 6.1 all support points of the standardized maximin D-optimal design ξ^* are points where the function $Q(t)$ attains a local maximum. Consequently the standardized maximin D-optimal design ξ^* has finite support.

We finally prove that the right boundary point of the design interval T is a support point of the standardized maximin D-optimal design. For this we assume the contrary, that is $T \notin \text{supp}(\xi^*)$, and denote by

$$\xi^* = \begin{pmatrix} t_1 & \dots & t_{n_1} \\ w_1 & \dots & w_{n_1} \end{pmatrix}, \ t_{n_1} < T.$$

the standardized maximin D-optimal design, which has finite support by the first part of this proof. From the Cauchy-Binet formula we have

$$\det M(\xi^*, \theta) \ = \sum_{1 \le \alpha_1 < \alpha_2 < \alpha_3 \le n_1} \left(\det \left(f_i(t_{\alpha_j}, \theta) \right)_{i=1,2,3}^{j=1,2,3} \right)^2 \cdot w_{\alpha_1} w_{\alpha_2} w_{\alpha_3}.$$

Observing Lemma 2 in Dette et al. (2003) it follows that

$$\frac{\partial}{\partial t_{n_1}} \left(\det \left(f_i(t_{\alpha_j}, \theta) \right)_{i=1,2,3}^{j=1,2,3} \right)^2 \ > \ 0$$

for any $1 \le \alpha_1 < \alpha_2 < \alpha_3 = n_1$ and $\theta \in \Omega$. Consequently there exists $\varepsilon > 0$ such that the design

$$\bar{\xi} = \begin{pmatrix} t_1 & \dots & t_{n_1-1} & t_{n_1} + \varepsilon \\ \omega_1 & \dots & \omega_{n_1-1} & \omega_{n_1} \end{pmatrix}$$

satisfies $\det M(\bar{\xi}, \theta) > \det M(\xi^*, \theta)$ for any $\theta \in \Omega$. But this implies

$$\min_{\theta \in \Omega} \frac{\det M(\bar{\xi}, \theta)}{\det M(\xi_\theta^*, \theta)} > \min_{\theta \in \Omega} \frac{\det M(\xi^*, \theta)}{\det M(\xi_\theta^*, \theta)} \ ,$$

which contradicts the standardized maximin D-optimality of the design ξ^*.

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