Mathematical Model of Cholesterol Removal by Probiotics

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Abstract

Probiotics are living microorganisms which have beneficial effects and which can promote good health. While bacterial population dynamics is one of the classical and oldest areas of mathematical biology, it appears that the cholesterol assimilation phenomena by probiotics and its implications for health effects were so far ignored in modeling studies. In this paper, a dynamic model based on a qualitative-phenomenological description of cholesterol assimilation by probiotics is presented. The model consists of three autonomous differential equations and is able to describe the reduction of cholesterol by 11 different strains of lactobacilli. The model was solved numerically and was validated against an existing set of experimental observations. An optimization scheme that can perform parameter estimation using a multi-start approach was prepared and used to find the set of parameters that gives a best fit between the experimental observations and the model predictions. An important feature of this implementation is that the dynamic microbial model was introduced as a constraint in the optimization problem and this allows for the use of efficient differential equations solvers. The model proved to offer robust predictions for cholesterol assimilation by strains of lactobacilli and was also able to capture a number of experimental observations including that microbial growth is enhanced by the presence of cholesterol. This work is a first effort in modeling such phenomena and the ability of the model to represent different strains will allow for future work on optimizing an ecological mix of strains that will be able to reduce cholesterol levels in an efficient manner.

Keywords
Cholesterol, Optimization, Parameter estimation, Probiotic, Ordinary Differential Equations.

1 Introduction

Probiotics are microorganisms able to benefit health if administered in sufficient quantities (Frece et al., 2005). Probiotics have been consumed as functional foods and nutraceuticals for several decades, most notably together with dairy products. There has been recently a resurgence of interest in the potential of probiotics for medical and clinical purposes (Chen et al., 2012; Ooi and Liong, 2010; Sanders, 2000). Several factors prompted this development, but the most significant is the surging level of multi-drug resistance among pathogens in hospitals. This has been accompanied by an increasing emergence of scientific and clinical evidence for the efficacy and effectiveness of some probiotic strains (Reid et al., 2003).

A number of review articles have appeared over the years summarizing the health benefits of probiotics and their clinical applications (Anila et al., 2016; Kumar et al., 2013; Sanders, 2000; Mattila et al., 2002; Reid et al., 2003; Santosa et al., 2006). Probably the most understood and powerful clinical application of probiotics is in the treatment of enteric infection and diarrhea. Among the bacterial pathogens that have been fought with probiotics like *Bifidobacterium bifidum* and *Lactobacillus GG* are *Escherichia coli*, *Salmonella spp*, *Shigella spp*, and *Clostridium*

difficile} (Bezkorovainy, 2001). Inflammatory disease and bowel syndromes such as pouchitis and Crohn's disease are further promising areas in probiotic theory. Reid et al. (2003) report that for these diseases cases combinations of probiotic strains may be more effective. This poses the additional challenge of finding the right ecological mix, instead of identifying a single agent. Probiotics have also been found to reduce the risk of colon cancer (Liong, 2008; Sanders, 2000; Santosa et al., 2006). Further potential health benefits of probiotics reported in the literature are: lowering blood pressure and cholesterol; increased immune response; alleviation of lactose intolerance; treatment of urogenital infections; re-establishment of the natural microflora subsequent to a conventional antibiotic therapy.

Several studies have shown that high concentrations of cholesterol in the blood for long periods cause coronary heart disease (Akalin et al., 1997; Anderson and Gilliland, 1999). This disease is known to be a leading cause of death, similar to cancer (Aloglu and Oner, 2006). A number of studies stressed on the use of the chemical β −cyclodextrin to reduce cholesterol (Lee et al., 1999), however because of the high cost of this chemical combined with a loss of aroma made the practice of its use not preferred (Aloglu and Oner, 2006). On the other hand, the use of probiotics for reducing cholesterol has been shown to be an effective alternative (Liong and Shah, 2005), for instance, studied the effect of eleven strains of lactobacillion on cholesterol removal and presented growth curves that showed that all strains were able to assimilate cholesterol at varying levels ranging from *12.03* to *32.25* $\mu g/ml$. Furthermore, most strains considered exhibited better growth in the presence of cholesterol indicating that cholesterol has stimulated their growth.

In this paper, a mathematical model that is able to present the phenomena of cholesterol assimilation is attempted. The mathematical modeling of microbial populations has been a central issue for many groups that work in the general field of biology. The use of models describing the growth of microorganisms has been reported by many researchers (e.g. Rogers and Reardon, 2000; Arvin et al., 2000; Roberts and Baranyi, 1994; Swinnen et al., 2004) presented, for instance a common growth model that consisted of an adjustment function which enables the transition from the lag phase to the experimental phase. Rogers and Reardon (2000) presented models that take into account the interactions between microbial species during biodegradation. Wang et al (2006) presented a model that describes the interactions and coexistence of three microorganisms (filamentous bacteria, floc-forming bacteria, and protozoa) within mixed activated sludge systems. Rijgersberg et al. (2013) proposed a model that determines the maximum population density of a pathogenic microorganism (Salmonella) on alfalfa as a function of initial contamination level, the total count of the indigenous microbial population, the pathogen growth rate, and the microbial population density.

In the last few years, there has been a growing interest in preparing predictive microbiology models that can describe other phenomena. Poscet et al. (2004) proposed a model that is able to explain the transition from the exponential growth phase to the stationary phase due to substrate depletion. Dens et al. (1999) presented a model that can take into account the influence of a background of microflora in a food product and hence consider the growth difference of microorganism while in a pure culture and while in a mixed culture. McKellar (1997), and Dens and Van Impe (2001) presented models that take into account the variability of microbial growth with respect to space. The McKellar model (1997) is a two-compartmental model in which the cell population is distributed between a non-growing and a growing compartment. The Dens and Van Impe (2001) model introduces space as an extra dimension by assuming that the evolution of the microorganisms is location dependent. Recently Fgaier et al. (2008, 2009, 2010, 2011) presented mathematical models that can describe pseudomonas growth under conditions of iron limitation and discussed the potential for many medical applications. These included the design of drugs that can target iron overload and toxicity for the treatment of cancer and for the prevention of heart and other organ damage caused by iron overload.

A comprehensive literature survey yielded no model that can incorporate the mechanism of cholesterol removal by probiotics. There is therefore clearly a need for a suitable prediction model for such a phenomena. This paper is our first attempt to prepare a model in terms of a set of ordinary differential equations that can offer good predictions of cholesterol removal by probiotics. The variables of the model as well as the model parameters and conditions will be explained in details. A number of simulations were carried out in order to study the behavior of the model and the effect of the different parameters and initial conditions. The model is validated by comparing its predictions against

existing experimental measurements of eleven different probiotic strains. It was found that the model is able not only to provide the right qualitative behavior but also to give good predictions of the phenomena under study.

2 Mathematical Model

We formulate a mathematical model that describes the mechanism of cholesterol removal by strains of lactobacilly. The removal of cholesterol is due to its assimilation during bacteria growth at varying levels.

An extensive literature lead us to conclude that there is currently no mathematical model that describes this phenomema. In this model, we distinguish between two fractions of cholesterol: cholesterol dissolved in the medium *C*, and the assimilated cholesterol *S* attached to the cells.

$$\frac{dC}{dt} = -aNC(k_2N - S) \quad (1)$$

$$\frac{dS}{dt} = aNC(k_2N - S) \quad (2)$$

Where the parameters (a, k_2) are positive constants. Equation (1) correctly states that the amount of cholesterol *C(t)* decreases over time. It assumes that the rate of cholesterol in the system is proportional to the microbial probiotic population *N(t)*, the amount of cholesterol in the system *C(t)*, and the population of free/ not assimilated cells (i.e. k_2N - *S).* Similarly in equation (2) and based on the principle of mass conservation, the assimilated cholesterol rate *dS/dt* is assumed to be identical in magnitude to the rate of cholesterol available in the system. The probiotic bacteria population is characterized by its population size *N* which is assumed to grow according to a logistic growth law, where η the growth rate is and $k_1$is the carrying capacity i.e.

$$\frac{dN}{dt} = \eta N(1 - \frac{N}{k_1}) \quad (3)$$

It was observed by Shah and co-workers (Liong and Shah, 2005) that adding probiotics to a solution containing cholesterol decreases gradually the amount of cholesterol in the medium. This growth was noticeable in the first hours in the experiments. This can be interpreted that bacteria take time to adapt and respond to new environmental conditions and factors. Therefore a mathematical factor which describes this adaptation phenomena must be introduced.

Following the lag model of Roberts and Baranyi (1994) and Fgaier et al. (2008) we introduce the following physiological state:

$$\alpha(t) = \frac{\varphi_0 e^{-\mu t}}{1 + \varphi_0 e^{-\mu t}} \quad (4)$$

The above adaptation function $\alpha(t)$ satisfies $0 \leq \alpha(t) \leq 1$ and $\frac{d\alpha}{dt} \geq 0$
We get a system of three autonomous nonlinear ordinary differential equations which is summarized by equations (1) and (2) above and a modified version of equation (3) that takes into account the lag phase as shown below:

$$\frac{dN}{dt} = \eta N\alpha(1 - \frac{N}{k_1}) \quad (5)$$

Adding equations (1) and (2) gives

$$\frac{dC}{dt} + \frac{dS}{dt} = 0 \quad (6)$$

Integrating equation (6) from 0 to *t* gives:

$$C(t) + S(t) = C(0) + S(0) = \gamma \quad (7)$$

Where γ is the initial amount of cholesterol in the system.
Based on equation (7), the amount of cholesterol *C(t)* is given by:

$$C(t) = \gamma - S(t) \tag{8}$$

Substituting equation (8) into equation (2) gives:

$$\frac{dS}{dt} = aN(\gamma - S)(k_2 N - S) \tag{9}$$

Our system therefore reduces to a system of two equations (equations (5) and (9)) in the two unknowns *S(t)* and *N*(t). Once *S* and *N* are analyzed and solved for, *C(t)* can be obtained from equation (8).

3 Qualitative Analysis

The solution to the system consisting of equations (5) and (9) is positive. We also note that $\frac{dN}{dt} > 0$, $\frac{dS}{dt} > 0$, and $\frac{dC}{dt} < 0$ (since the amount of cholesterol *C* is decreasing).

Theorem: Let $N_0 \leq k_1$ and $S \leq C(0)$ *and* $S(0) < \gamma N(0)$. The system {(5), (9)} associated with the initial conditions (S(0), N(0)) has a unique non negative bounded solution. Furthermore, the solution *N(t)* is monotonically increasing and $N(t) \to N_\infty = k_1 > 0$ *as* $t \to \infty$, *S(t)* is monotonically increasing $S(t) \to k_2 N_\infty = k_1 k_2 > 0$ *as* $t \to \infty$.

Proof*:* Let's first denote the RHS of *(5 and (9) by F(N, S) and let* $K = \{N \geq 0, S \geq 0\}$ be the positive cone. Then the vector function F is continuously differentiable and thus satisfies a Lipschitz condition. We also note that for the outer normal vectors *n (N, S)* to the boundary *of K* the tangent condition $n(N,S)^T F(N,S) \leq 0$ is satisfied. Therefore, the Invariance Theorem (Walter) is therefore satisfied and the solution to the model *(5)* and *(9*) is unique and remain in *K.*
Since *S* is positive, it is also bounded by a constan*t* γ which depends on the initial data.

The solution to the system *(5)* and *(9)* is bounded in positive time. Assuming α is nearly equal to 1 *and solving for N* we get

$$N(t) = \frac{N_0 k_1}{N_0 + (k_1 - N_0)\, e^{-\eta t}} \tag{10}$$

We also note that equation (5) contains the equilibrium solutions *N= 0* and $N = k_1$ corresponding to the initial condition $N_0 = 0$ *and* $N_0 = k_1$ respectively. Th*us if* $N_0 > 0$ and if we let $t \to \infty$ *in* equation (10) then $\lim_{t \to \infty} N(t) = \frac{N_0 k_1}{N_0} = k_1$. We can then conclude that for each *N>0* the solution approaches the solution $N = k_1$ asymtotically *as* $t \to \infty$. We can conclude that *N* is bounded by k_1 even when we include α in the model since α as given by equation (4) is always positive and less than one.

4 Quantitative Analysis

4.1 Description of dada:

The presented model will be evaluated using the existing experimental data of Liong and Shah (2005) who studied cholesterol removal by eleven different strains of lactobacilli. They investigated cholesterol assimilation by determining the difference of cholesterol content in the medium before and after incubation of the eleven strains, seven strains were of the Lactobacillus Casei type and four of the Lactobacillus acidophilus type. Growth curves of all eleven Lactobacilli were presented along with the cholesterol assimilated. Two replicates were performed with two measurements per replicates. The mean of the repeated measurement reported by the authors along with standard errors of means (error bars) ranging from ± 0.13 to ± 0.40.

In the next section, we will conduct a parameter estimation approach based on these experiments. The model we presented in section *4.2* will be validated against this available experimental data sets. We will also compare the qualitative prediction of the model to the observations of Liong and Shah (2005). Liong and Shah(2005) observed that: (i) Cholesterol removal is due to its assimilation during growth, incorporation into the membrane of the cells, and binding into the membrane cells, (ii) Cholesterol removal is associated with growth of cultures, (iii) cholesterol removal also by dead and resting cells, (iv) Most strains exhibit higher growth in the presence of cholesterol, and (v) Most strains show gradual growth for the first 12 to 18 h followed by rapid growth followed by rapid growth thereafter.

4.2 Parameter Estimation:

We will use here an optimization technique called sequential quadratic programming (SQP) in order to estimate the parameters of the model described by equations (5) and (9). We will use the experimental data discussed in the previous section in the implementation of SQP. Since equation (5) is decoupled from equation (9), the parameters η, φ_0 and k_1 in equation (5) will be first estimated independently and the remaining parameters appearing in equation (9) will then be estimated.

The estimation of unknown parameters in our model will be based on the measurement of Liong and Shah (2005) about the dynamic system. Numerous parameter estimation techniques in dynamic models are available in the literature. These are based on nonlinear regression algorithms such as Marquardt Newton and Gauss- Newton (Bard 1974). The parameter estimation problem can be regarded as the inverse of simulation (Wiesbaden/ Braunschweig Vieweg 1993). In simulation, the set of parameters in the model is assumed to be known and the model is used to offer predictions about the biological phenomena as a function of time. In parameter estimation, some observation about the phenomena are known at different times while the parameters themselves are unknown. The estimation of the model outputs and the measured data are minimized. The most used objective function in such minimization is based on the sum of squared differences between the measurements and the model predictions.

There are two general approaches that can be employed to address the parameter estimation problem of dynamic systems. The first approach is based on converting the dynamic system into a set of algebraic equations which are then included in the optimization model for minimizing the errors between the model outputs and the experimental measurements. This approach is often referred to as simultaneous approach. The conversion can be done through the use of finite difference approximations, polynomial approximations, or collocation techniques Villadsen and Michelsen (1978) presented different polynomial approximations that can be used to solve differential equations. Van Den Brosch and Hellinckx (1974) used linearization techniques combined with collocation in order to solve problems where the differential equations are either first or second systems. Tjoa and Biegler (1991) used orthogonal collocation on finite elements.

The simultaneous approach for solving the parameter estimation problem of dynamic systems leads to large-scale nonlinear optimization problems which require special solution strategies (Betts and Frank 1994; Cervantes and Biegler 2000). Stability questions often accompany the transformed problems along with questions about the placement of the description points in order to maintain accuracy (Logsdon and Bieler 1989). Furthermore, the solutions of the resulting optimization problems are not always feasible.

The second approach for solving the parameter estimation problem of dynamic systems involves the use of numerical techniques for solving the differential equations using initial guesses of the parameters. The predictions of the model are then compared with the experimental measurements, and an optimization algorithm is used to determine a new set of parameter estimates. This approach is referred to as the sequential approach because the solution of differential equations and the optimization over the parameters are performed in a sequential manner, (Bellman et al. 1967; Luus 1998). The sequential approach results in smaller nonlinear optimization problems compared to the simultaneous approach. The solutions of the optimization problems are always feasible but the overall procedure is slower than the simultaneous approach because of the repeated numerical solutions of the differential equations.

In order to estimate the unknown parameters, an optimization criteria must be constructed. In this study, a least - square like objective function is used. Since we have experimental measurements of the 11 different strains at different times, the overall objective function is written as:

$$\min_{\theta} J = \sum ((N^{i,exp} - N^{i,mod})^2 \quad (11)$$

The constraints of the optimization problem are represented by the differential equation (5) for the problem P_1 of estimating the parameters η, φ_0 and k_1 and by the set of equations (5) and (9) for problem P_2 (below) for estimating the parameters a and k_2 The parameter γ (initial cholesterol content) was fixed mainly because it can be easily measured in an experimental settings. Furthermore, we observed that γ, a, and k_2 are correlated and multiple sets can leads to an efficient solution methodology. Fixing γ reduces the search space for the parameters a and k_2 tremendously.
The parameter estimation problems can be written as:

(P1) : $\min_{\theta=(\eta,\varphi_0,k_1)} J = \sum (N^{i,exp} - N^{i,mod})^2 \quad (12)$

s.t.

$$\frac{dN}{dt} = \eta N \alpha (1 - \frac{N}{k_1}) \quad (5)$$

and:

(P2) $\min_{\theta=(a,k_2)} J = \sum (N^{i,exp} - N^{i,mod})^2 \quad (13)$

s.t.

$$\frac{dN}{dt} = \eta N \alpha (1 - \frac{N}{k_1}) \quad (5)$$

$$\frac{dS}{dt} = aN(\gamma - S)(k_2 N - S) \quad (9)$$

The above problems are solved through a sequential approach by coupling the solutions of the differential equations with the optimization problem. The differential equations are solved by a stiff solver as we did in our previous work (Fgaier et al. 2008)} and the optimization problem is solved through a SQP technique.

The SQP method is due to the original work of Schittowowski (1985) and has been shown to outperform other nonlinear optimization methods in terms of efficiency and accuracy over a wide range of test problems.

However it is often very likely that the solution obtained will be a local one (Tawarmalani and Sahinidis 2002). In order to overcome this limitation, we decided to employ a multi-start strategy where we used the SQP algorithm for estimating the parameters repeatedly from different initial estimates. The approach proved to be robust as will be discussed in the results section (*section 4.3*) and different starting points lead to different optimal parameter values. More than *1000* starting points were obtained from a random generation scheme combined with an order of magnitude analysis that provided good starting bounding intervals.

Furthermore, we employed an interval analysis scheme in order to enhance our search for better estimates. In this scheme, the boundary intervals for the parameters were divided into 1000 subintervals each, and simulations were conducted with the parameters taking values the endpoints of each subintervals. A total of 10^9 simulations were conducted for estimating the parameters η, φ_0 and k_2 and 10^6 simulations for estimating the parameters a and k_2

.Contour plots were analyzed in order to identify regions of optimality and the reduced subintervals for the different parameters. The multi-start SQP strategy was then employed on the subintervals.

4.3 Results

In order to check the model validity, the parameter estimation procedure discussed in the previous section was employed. As we indicated earlier, we considered first the parameters η, φ_0 and k_1, that appear in equation (5) which gives the growth of a certain strain. Following the bounding step of the parameters and the interval estimation step, a multi-start SQP strategy was employed as discussed in the previous section. Different random initial starting points for the parameters η, φ_0 and k_1 were generated based on routine that randomly generates numbers (rand) in the interval [0, 1]. These random numbers are then converted to the desired ranges through the equation:

$$(P^U - P^L).rand + P^L \quad (14)$$

Where P^L and P^U represent lower and upper bounds for the parameters, respectively. These upper and lower values were obtained from the 10^9 simulations aimed at identifying regions of optimality (*section 4.2*). The estimation results for the eleven different strains are given in Table1.

A comparison of the model predictions and the experimental data is given in Figure 1. It can be seen from the figure that the model provides acceptable predictions. Table 1 gives also non-graphical statistics of the goodness of the fit. The sum of squares error (SSE) in the table is a measure of the overall deviation of the predicted values from the experimental observations (Freud, 1992):

$$SSE = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \quad (15)$$

The statistic R^2 shown in the table measures how successful is the fit in explaining the variation of the data. It is the square of the correlation between the observed experimental values and the predicted values from the model. It was calculated based on the equation:

$$R^2 = \frac{SSR}{SST} = 1 - \frac{SSE}{SST} \quad (16)$$

Where *SSR* is the regression sum of squares and is given by:

$$SSR = \sum_{i=1}^{n} (\hat{y}_i - \bar{y}_i)^2 \quad (17)$$

and **SST** is the total corrected sum of squares. i.e.

$$SST = \sum_{i=1}^{n} (y_i - \bar{y}_i)^2 \quad (18)$$

$\hat{y}_i$ in the above equations represents the predictions of the model, while y_i represents the experimental obsevations. $\bar{y}$ is the mean of all $y_i$values. As can be seen from Table 1, the $R^2$is more than *90%* for most strains and therefore the model with the estimated parameters η, φ_0 and k_1 explains more than 90% of the total variations in the experimental data of the growth of the strains. Figure 2 provides cross plots for the eleven different strains. As can be seen, the plots fall on the 45° pareto lines and this indicates again the goodness of the model predictions.

Having estimated the parameters associated with equation (5), we moved then to estimating the parameters *a* and k_2 that appear in equation (9). We proceeded in the same fashion as we did previously. The first step is an order of magnitude consideration. In order to have a robust starting interval for the parameters *a* and k_2 equation (9) is rewritten as:

$$\frac{dS}{dt} = b_1 n(t)(1 - b_2 S)(n(t) - b_3 S) \quad (19)$$

Where $n(t) = \frac{N(t)}{k_1}$ is a sort of normalized population size,$b_1 = \frac{a}{\gamma k_1^2 k_2}$, $b_2 = \frac{1}{\gamma}$ and $b_3 = \frac{1}{k_1 k_2}$
and we make use of the following observations:

(i) parameters a in equation (9) is much smaller than 1(, ii) parameter b_1 in equation (19) is expected to be between 0.1 and 10 and (iii) parameter b_1 in equation (19) is expected to be between 0.1 and 10.

(ii) Our understanding of the dynamics of the model leads us to conclude that in all 11 cases S must converge to $k_1 k_2$ as t becomes large (note: in some cases this convergence is observed in the data, in other cases, the experiments stop long before convergence is reached).

Following the above bounding phase, we performed a large number of simulations in order to pinpoint subintervals within which we look for optimal parameter values. The original intervals

$0 \leq k_2 \leq \frac{S_\infty}{k_1}$ and $\frac{0.1}{\gamma S_\infty k_{1k_2}^2} \leq \frac{10}{\gamma S_\infty k_{1k_2}^2}$ obtained from the above order of magnitude analysis were divided into 1000 subintervals each and 10^6 simulations were performed. Afterwards the multi-stat search was commenced. The parameter estimation results for the eleven strains are given in Table 2. A comparison of the model predictions of assimilated cholesterol and the experimental measurements is provided in Figure 3. As can be seen from the figure, the model predictions are good in this case too.
Given the variations in the experimental measurements, it can be concluded that the model output is within the error bars already present in the experimental data. The statistics measuring the goodness of fit are given in Table 2. The definitions of SSE and R^2 are the same as for the comparisons of growth data. Cross plots are shown in Figure 4 and the model predictions for cholesterol reduction in the system is given in Figure 5. The cross plots show again that the model offers good predictions within the measurement errors. No cholesterol measurement in the media are available, but Figure 5 shows the expected trend that cholesterol is reduced over time in an "exponential “fashion.

5 Conclusion

In this paper, a model that can describe the mechanism of cholesterol assimilation by different strains of lactobacilli was presented. Prior experimental observations on eleven different strains of lactobacilli showed that these strains are able to assimilate cholesterol and their growth gets actually enhenced by its presence. The presented model proved effective in representing experimental observations. The model consisted of a set of three autonomous differential equations. The qualitative properties of the model were studied using the theory of differential equations and were found to conform to the experimental observations. The parameters in the model were estimated using a robust multi-start sequential quadratic optimization approach. Eleven sets of parameters all in the same range were obtained for the different strains. The model was found to not only give the right qualitative behavior of the different experimental measurements but also give good quantitative predictions. These models will be used in the future to conduct a study on combining different strains with the objective of reducing the time it takes for cholesterol assimilation in an efficient manner.

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Biographies

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Table 1. Parameters estimated and statistical analysis for growth of the eleven strains

Strain	η	ϕ_0	k_1	SSE	R^2	Max error	Min error	Average error
1A	0.4753	0.0865	1.0883	0.0130	0.9918	0.0789	0	0.0276
1B	0.3808	0.1500	1.1535	0.0766	0.0766	0.1800	0	0.0689
1C	0.5728	0.1500	1.4004	0.0337	0.9870	0.1560	0	0.0410
1D	0.4036	0.1500	0.8049	0.0484	0.8385	0.1501	0	0.0512
2A	0.3798	0.1008	0.7137	0.0163	0.9701	0.0911	0	0.0329
2B	0.5010	0.0753	1.1184	0.0150	0.9912	0.0620	0	0.0327
2C	0.4533	0.1499	1.0601	0.0299	0.9800	0.1052	0	0.0388
2D	0.4551	0.1500	1.7877	0.0214	0.9949	0.0947	0	0.0332
2E	0.3649	0.1500	1.9186	0.0053	0.9988	0.0414	0	0.0192
2F	0.3758	0.1500	1.3407	0.0361	0.9833	0.1198	0	0.0447
2G	0.4428	0.0756	1.2727	0.0114	0.9946	0.0669	0	0.0254

Table 2. Parameters estimated and statistical analysis for cholesterol assimilated by the eleven strains.

Strain	a	k_2	SSE	R^2	Max error	Min error	Average error
1A	0.0210	12.4892	106.5129	0.7372	7.1297	0	2.5470
1B	0.0651	21.5529	93.0326	0.8880	7.2651	0	2.2292
1C	0.0209	13.3599	35.6473	0.9222	3.6657	0	1.4956
1D	0.0891	30.2181	124.0970	0.8503	8.8352	0	2.5389
2A	0.0232	42.5215	57.1318	0.9521	5.8646	0	1.7898
2B	0.0234	15.2044	128.9372	0.7330	8.0394	0	2.8910
2C	0.0423	10.4906	9.3141	0.9501	1.8178	0	0.8272
2D	0.0224	6.2116	35.1487	0.8131	3.4050	0	1.5897
2E	0.0309	14.4672	35.8475	0.9642	4.2894	0	1.3512
2F	0.0508	19.5227	118.7624	0.8724	8.5471	0	2.4817
2G	0.0684	15.0775	25.0938	0.9522	3.8730	0	1.1553

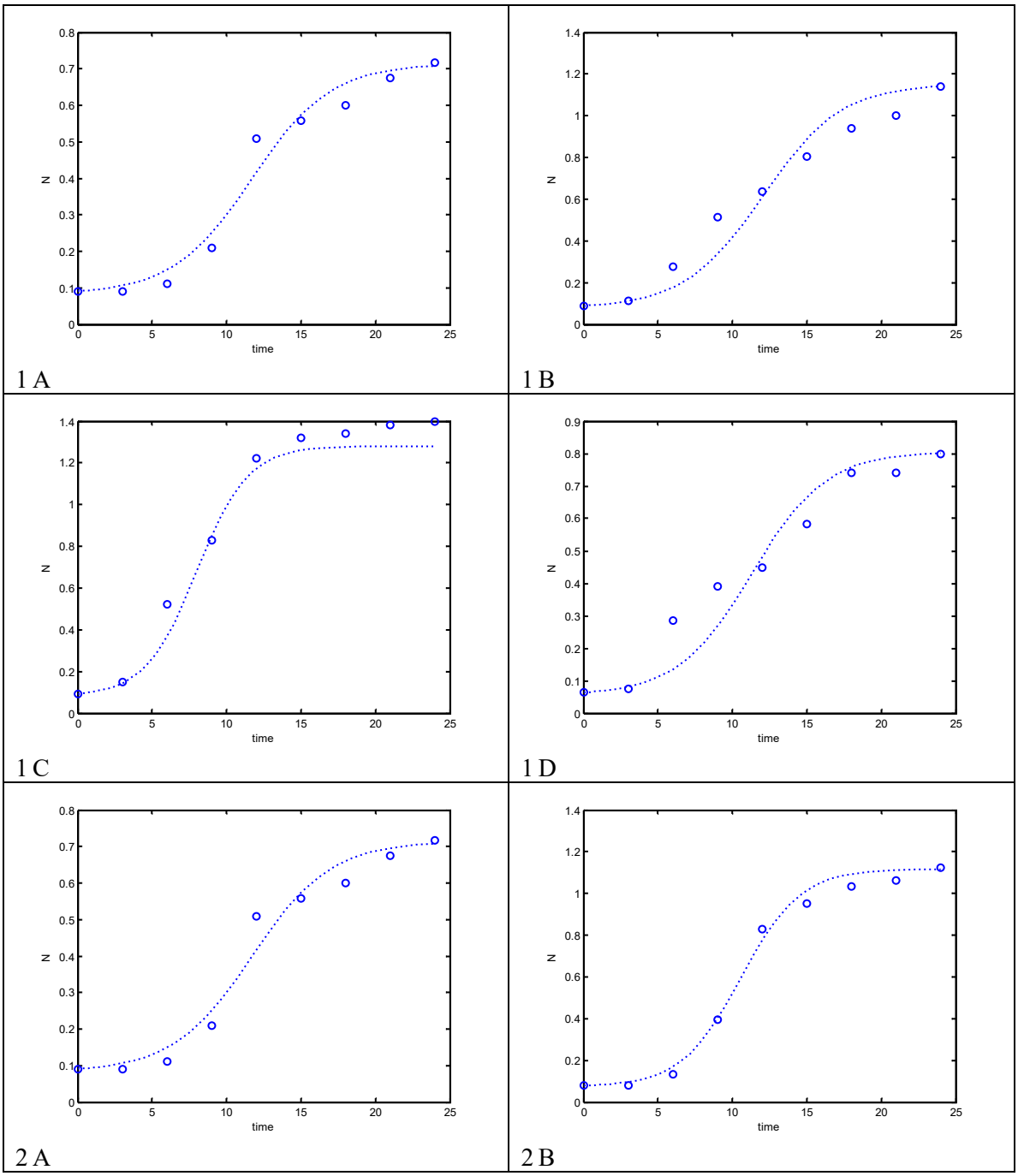

Figure 1. Comparison of model predictions for the growth of the eleven different strains with experimental data used in the parameter estimation. The continuous line represents the model predictions and the circles (o) represent the experimental measurements.

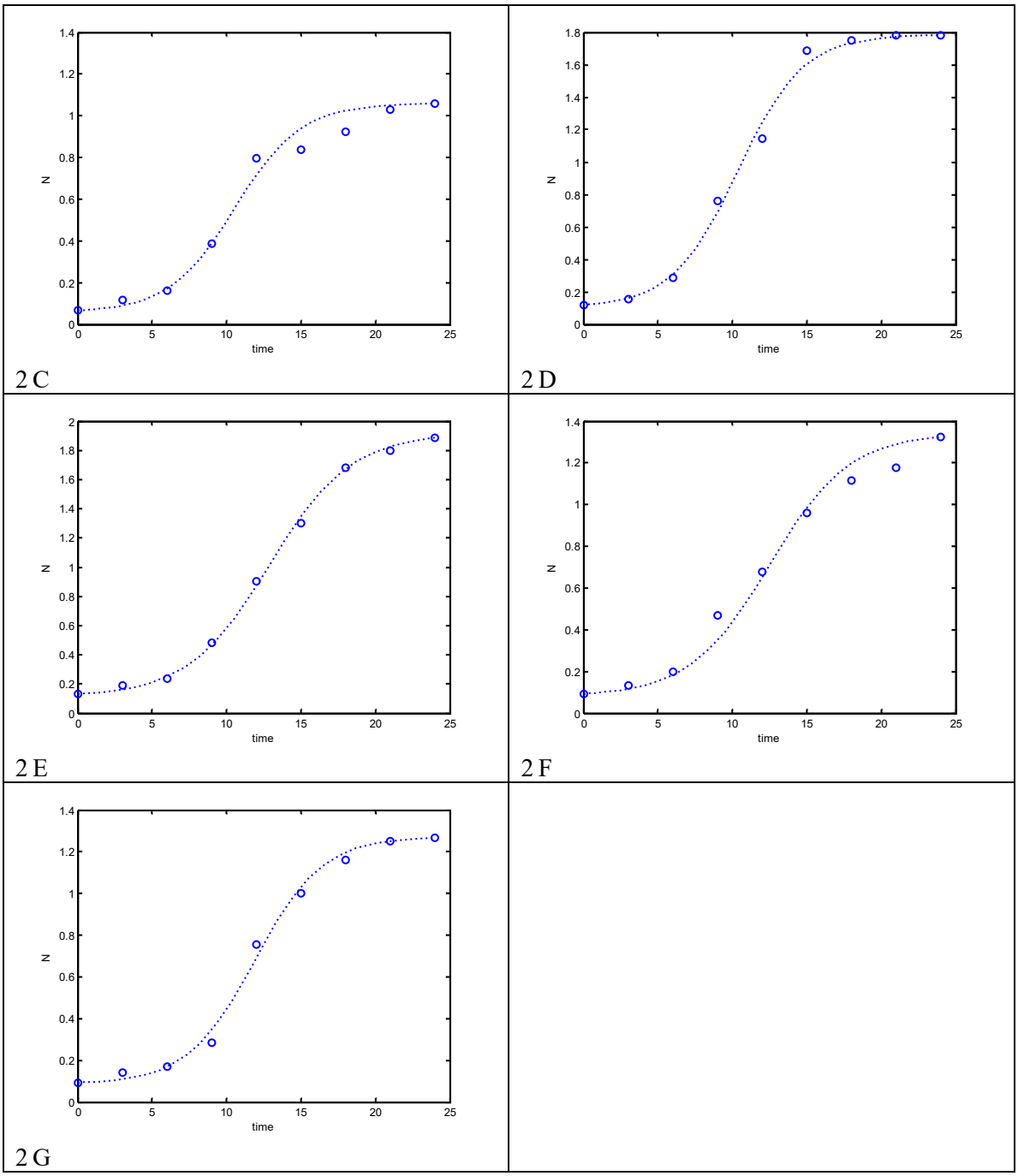

Figure 1. Continued - Comparison of model predictions for the growth of the eleven different strains with experimental data used in the parameter estimation. The continuous line represents the model predictions and the circles (o) represent the experimental measurements.

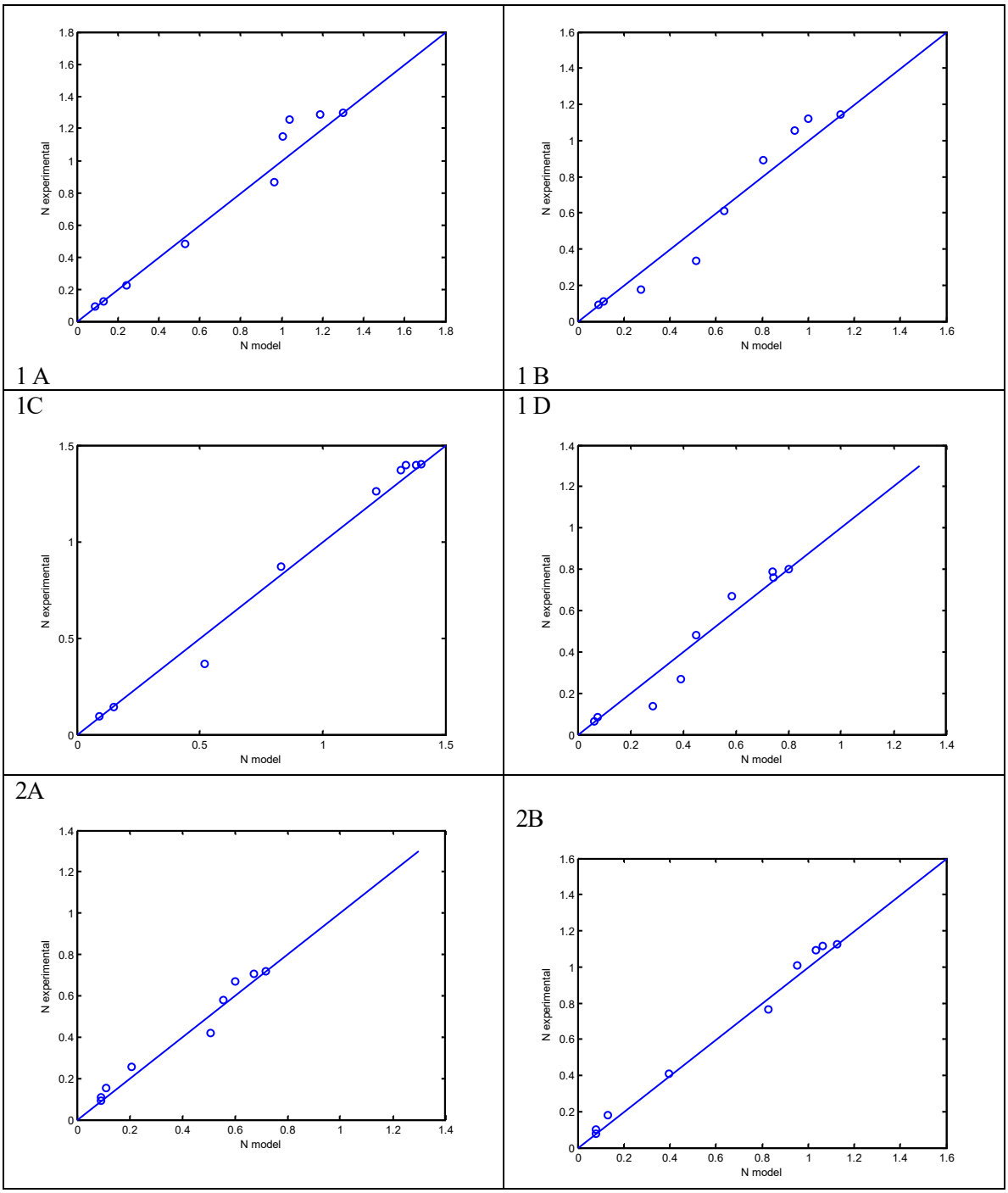

Figure 2. Cross plots for the growth of the eleven different strains.

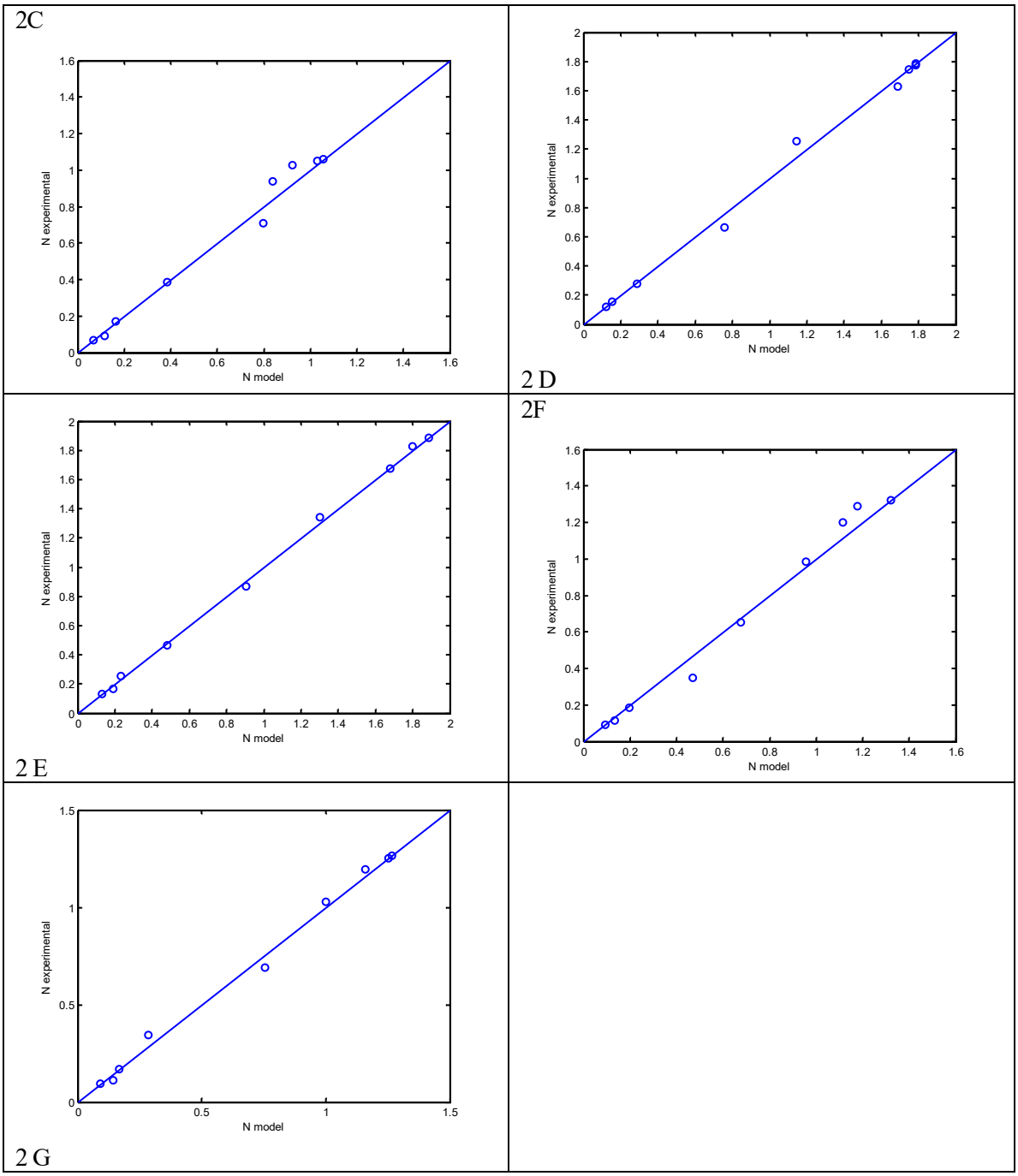

Figure 2. Continued - Cross plots for the growth of the eleven different strains.

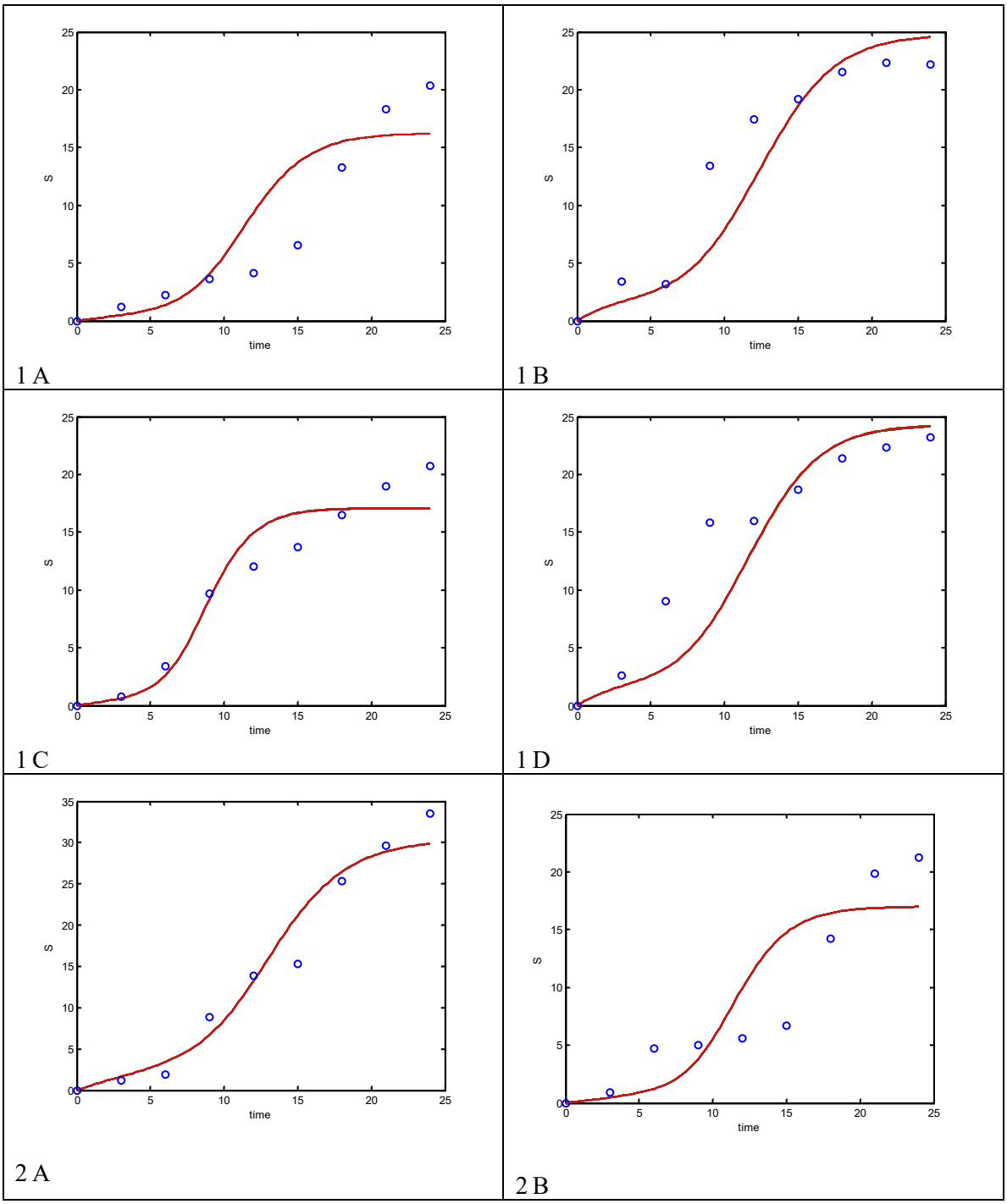

Figure 3. Comparison of model predictions for cholesterol assimilated by the eleven different strains with experimental data used in the parameter estimation. The continuous line represents the model predictions and the circles (o) represent the experimental measurements.

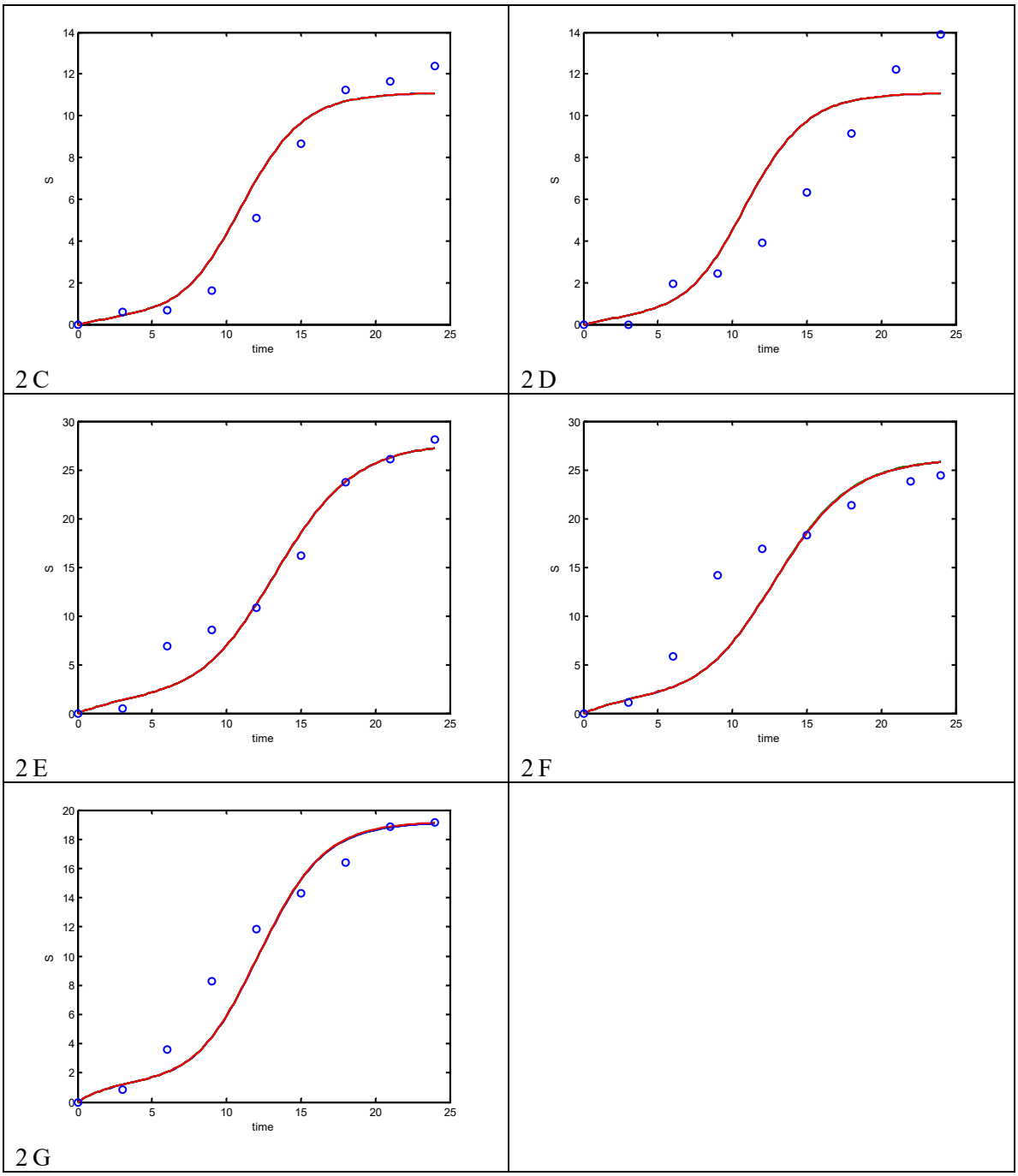

Figure 3. continued- Comparison of model predictions for cholesterol assimilated by the eleven different strains with experimental data used in the parameter estimation. The continuous line represents the model predictions and the circles (o) represent the experimental measurements

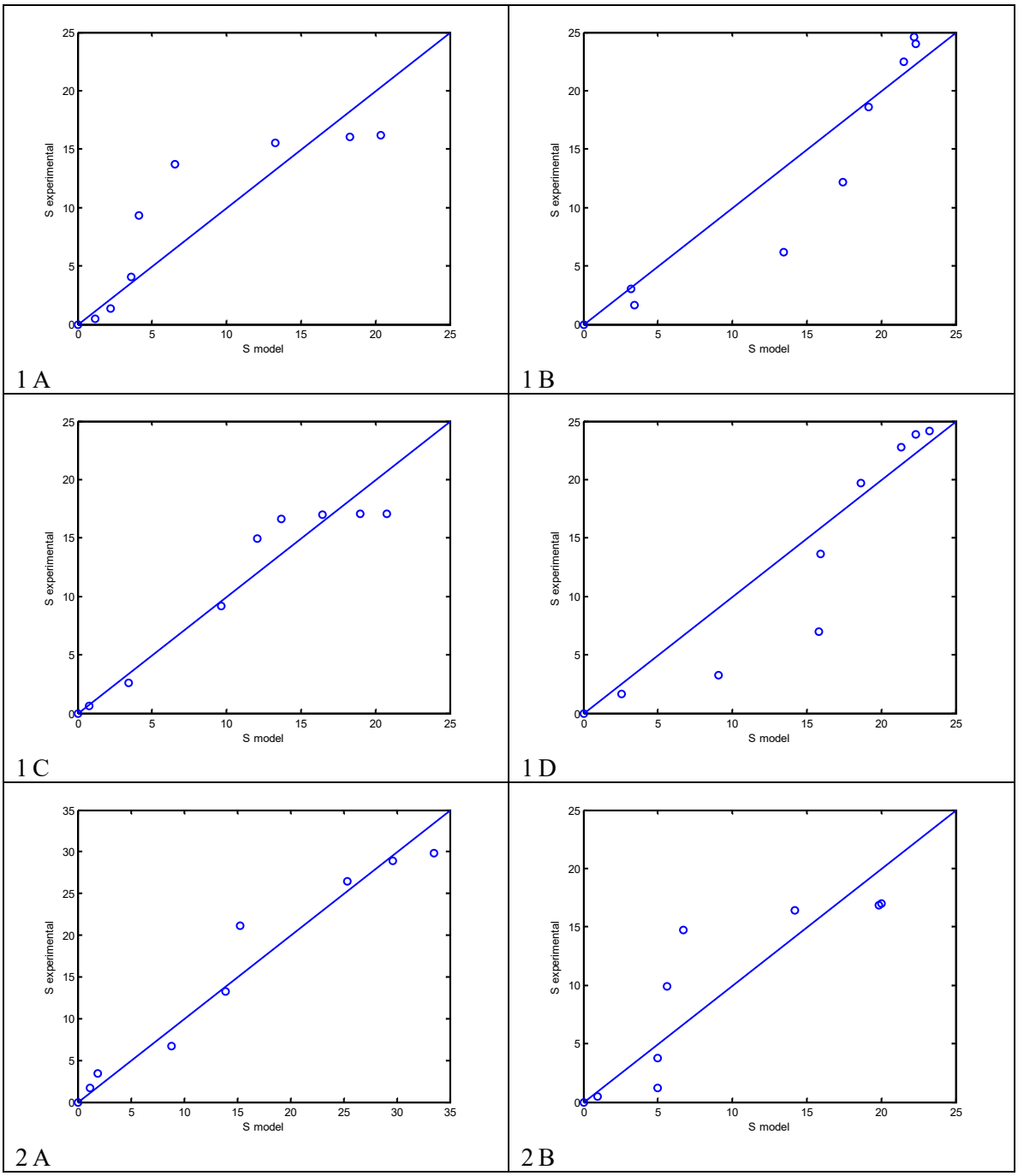

Figure 4. Cross plots for cholesterol assimilated by the eleven different strains.

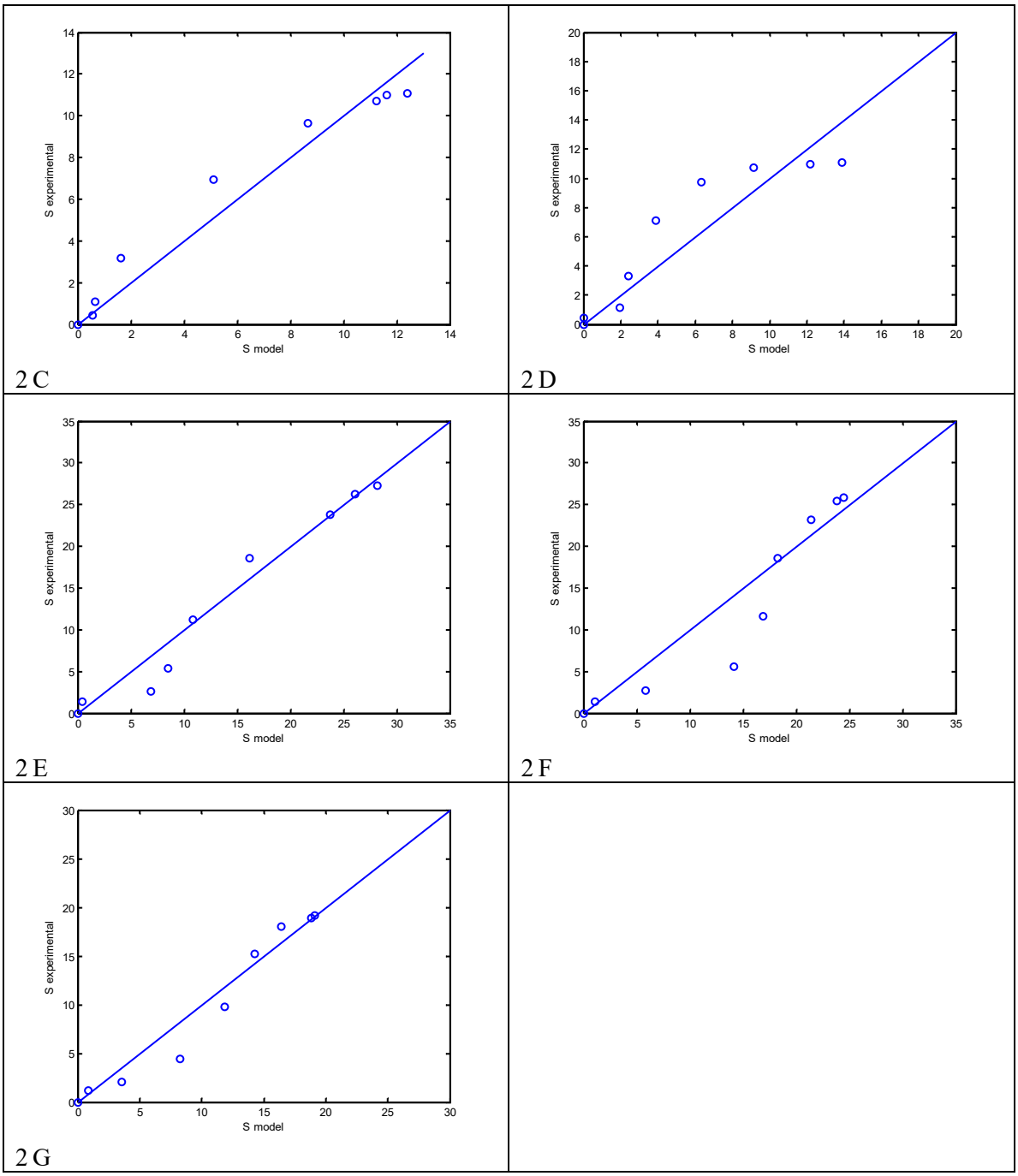

Figure 4. continued- Cross plots for cholesterol assimilated by the eleven different strains.