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Combination of limited measurement information and multidimensional population balance models

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Abstract:
Cell to cell variability with respect to intracellular composition as measured by flow cytometry is a core feature of many bioprocesses.
Models of the underlying processes are often available from small scale or single cell observations but generally fail to account for heterogeneity within the cell culture. This heterogeneity can be incorporated using population balance equations that have to be adapted to the flow cytometric measurements.
However, in general not the whole intracellular composition can be determined simultaneously for technical or economical reasons with flow cytometry. Furthermore, in many cases only integral quantities of certain compounds are measurable (e.g. overall amount of biomass in a cell culture).
In this contribution we will present a procedure for combination of these limited measurement informations with complex models. Application will be shown for production of biopolymer poly(3-hydroxybutyrate) (PHB) in bacteria cultures. Starting from a single cell description a corresponding population balance model which describes the heterogeneity on a macroscopic scale will be derived. Afterwards an efficient approximate moment method and the Method of Characteristics will be used to combine population balance models and limited measurement data.

Keywords: Cell to cell variability, Population Balance Modeling, Method of Moments, Biopolymer production

1. INTRODUCTION

Cell to cell variability plays an important role in many applications from bioprocess engineering (e.g. vaccine production in mammalian cell cultures as presented by Müller et al. (2013)) and systems biology (e.g. stem cell differentiation as presented by Glauche et al. (2010)).

Flow cytometry is frequently used to determine the heterogeneity within cell cultures (Srienc, 1999). Here the cells are stained with fluorescent dyes which bind to certain intracellular compounds that are of interest. The cells are passed along a beam of light and the corresponding fluorescences are measured. Cells of different fluorescence classes are counted or even sorted. Depending on the number of measured compounds a corresponding multi dimensional number density distribution n can be computed which gives a measure of the overall variance in the process.

However, often just a subset of intracellular compounds in which one is interested can be measured due to technical reasons (e.g. no adequate fluorescent dye is available) or economic considerations (e.g. a specific dye is to expensive). In result, only a projected number density $\widetilde{n}$ is available (see Fig.1 for a simple example). Furthermore, for some compounds only integral measurement are available on a macroscopic scale (e.g. the overall amount of a certain protein in the cell culture).

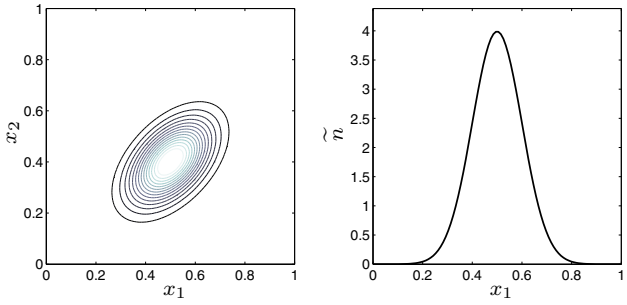

Fig. 1. *Schematic representation of full (left) number density distribution and corresponding projected number density distribution (right)*

This brings up the question how to combine these limited measurements and complex models of the underlying processes. The latter are often available from small scale experiments but generally fail to account for cell to cell variability on a macroscopic scale. Population Balance

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Modeling represents a suitable framework for the description of this heterogeneity.

In this contribution we will present a methodology to incorporate limited data from cell culture experiments and complex models based on small scale data. This algorithm is based on an efficient moment approximation algorithm and numerical solution via the Method of Characteristics (MOC) of a corresponding Population Balance Equation (PBE). Application will be demonstrated for biopolymer production in bacteria cultures.

2. POPULATION BALANCE MODELING

The dynamics of heterogeneous processes can be described within the framework of population balance modeling (Ramkrishna, 2000). The general PBE characterizes the dynamics of the cell cultures number density distribution $n(t, \mathbf{x})$ with respect to the vector of intracellular compounds $\mathbf{x} = \left[x_1, \dots, x_{N_{d_i}}\right]$ and is given by

$$\frac{\partial n(t,\mathbf{x})}{\partial t} + \sum_{k=1}^{N_{d_i}} \frac{\partial}{\partial x_k} \left\{h_k(t,\mathbf{x},\mathbf{c})\, n(t,\mathbf{x})\right\} = B(t,n,\mathbf{x},\mathbf{c}) - D(\mathbf{x},\mathbf{c},t)\, n(t,\mathbf{x})\,. \tag{1}$$

Here the rates h_k define the change of internal coordinates as result of intracellular reactions. The right hand side represents the net rate of formation of new cells. Therein, B summarizes the production and loss of cells as an effect of cell division and D is the cell death rate. The dimension of n can be derived as

$$[n] = \frac{1}{[x_1]\ \dots\ [x_{N_{d_i}}]}\,. \tag{2}$$

Additionally, in many cases intracellular reactions depend on availability of substrates, e.g. a carbon source, which also undergo temporal evolution. Thus the PBE is coupled to a system of ordinary differential equations (ODEs) characterizing the dynamics of the vector of extracellular substrates $\mathbf{c} = \left[c_1, \dots, c_{N_{d_e}}\right]$

$$\frac{\mathrm{d}\mathbf{c}}{\mathrm{d}t} = P\,(\mathbf{c}_{in} - \mathbf{c}) + \mathbf{f}(\mathbf{c}, n)\,. \tag{3}$$

Here, P represents the dilution rate, $\mathbf{c}_{in}$ the concentration of substrates in the feed and $\mathbf{f}$ the integral exchange with the dispersed phase. The dimension of c_i is given by

$$[c_i] = \frac{mol}{l}\,. \tag{4}$$

3. EFFICIENT APPROXIMATION OF MOMENTS

As interpretation of the overall number density distribution is difficult, it is often advantageous to characterize the distribution by more seizable properties like mean, variance and skewness. Those integral quantities can be calculated from the moments of the distribution which are defined as

$$m_{l_1,\dots,l_d} = \int_{\mathbf{X}} x_1^{l_1} \dots x_d^{l_d}\, n(t,\mathbf{x})\, \mathrm{d}\mathbf{x}\,. \tag{5}$$

The overall cell number is characterized by $m_{\mathbf{0}}$. Furthermore, mean and variance with respect to one specific internal coordinate x_i are defined as

$$\mu_i = \frac{m_{\mathbf{1},i}}{m_{\mathbf{0}}}$$
$$\sigma_i^2 = \frac{m_{\mathbf{2},i} m_{\mathbf{0}} - m_{\mathbf{1},i}^2}{m_{\mathbf{0}}^2}\,. \tag{6}$$

The dynamic moment equations can be derived from the PBE easily

$$\frac{\mathrm{d}}{\mathrm{d}t} m_{l_1,\dots,l_d} = -\sum_{k=1}^{d} \int_{\mathbf{X}} x_1^{l_1} \dots x_{N_{d_i}}^{l_{N_{d_i}}} \frac{\partial}{\partial x_k} \left\{h_k\, n\right\} \mathrm{d}\,\mathbf{x} + \int_{\mathbf{X}} x_1^{l_1} \dots x_d^{l_d} (B - D\, n)\, \mathrm{d}\mathbf{x}\,. \tag{7}$$

Here the right hand side has to be expressed in terms of moments. As a nice byproduct of this method, instead of solving the full PBE representing a multi dimensional partial differential equation (PDE), a set of ODEs has to be solved. However, computation of the moments dynamics using a closed set of equations is only possible under strict assumptions. Nevertheless, an approximate closure can be found applying approximate moment methods like the Quadrature Method of Moments (QMOM) as presented by McGraw (1997) or the Direct Quadrature Method of Moments (DQMOM) as described by Marchisio and Fox (2005). Here, it is assumed that the moments and other more general integrals of the number density distribution can be approximated using a weighted sum of abscissas

$$\int_{\mathbf{X}} f(\mathbf{x})\, n\, \mathrm{d}\,\mathbf{x} \approx \sum_{\alpha=1}^{N_\alpha} w_\alpha(t)\, f(\mathbf{x}_\alpha(t)) = \sum_{\alpha=1}^{N_\alpha} w_\alpha\, f_\alpha \tag{8}$$

with weight w_α and the corresponding abscissa $\mathbf{x}_\alpha$. Thus moment (5) approximations are given by

$$m_{l_1,\dots,l_d} = \int_{\mathbf{X}} x_1^{l_1} \dots x_d^{l_d}\, n\, \mathrm{d}\mathbf{x} = \sum_{\alpha=1}^{N_\alpha} w_\alpha\, x_{1,\alpha}^{l_1} \dots x_{d,\alpha}^{l_d}\,. \tag{9}$$

As a consequence of intracellular reactions, the distribution and also its corresponding moments undergo changes during the process. Instead of describing the evolution of any moment the main idea of the DQMOM is to track the temporal evolution of abscissas and weights directly.

Recently, we have presented an efficient method for the approximate calculation of multi dimensional moments (Dürr and Kienle, 2014; Dürr et al., 2015). It was shown that, under the assumption of neglible cell division ($B = 0$), the dynamics of the weights and abscissas are given by

$$\frac{\mathrm{d}w_\alpha}{\mathrm{d}t} = -w_\alpha D_\alpha \tag{10}$$

$$\frac{\mathrm{d}x_{i,\alpha}}{\mathrm{d}t} = h_{i,\alpha}\,. \tag{11}$$

By using both derived equations the temporal evolution of abscissas and weights can be calculated directly. The abscissas move along the characteristic curves of (1).

It is obvious that the choice of the initial abscissas is crucial for the numerical effort as well as the quality of the approximation. The approximation will be more accurate for a larger number of abscissas but the numerical effort will also increase. For this reason a good trade-off between the two has to be found. There are many rules to determine a suitable set of weights and abscissas, which are often referred to as cubature formulas. which

can be categorized roughly into Random Based Rules and Deterministic Rules. The latter can be further classified into product formulas and non-product formulas (see e.g. (Stroud, 1971) and (Davis and Rabinowitz, 1994)):

Product formulas are multidimensional extensions of one dimensional quadrature rules. Here, the weight and abscissa sets are constructed by "tensoring" of one dimensional quadratures weight/abscissa sets. The number of abscissas increases exponentially with dimension of the underlying problem. In contrast, when using non-product formulas the abscissa set is chosen directly in the multi dimensional space assuming a special shape of the initial distribution (e.g. symmetric distributions). For non-product rules the size of the abscissa set scales polynomially, in the best case linearly, with N_{d_i}. Thus they are favorable for high dimensional applications. In case the initial cell number density distribution can be represented as Gaussian distribution with mean μ and covariance $\mathbf{\Sigma}$

$$n(t=0,\mathbf{x}) = \mathcal{N}(\mu, \mathbf{\Sigma}) \tag{12}$$

a particularly efficient rule known as the Sigma-Point rule (e.g. van der Merwe (2004)) can be found. This third order monomial cubature rule is also used in the core routine of the Unscented Kalman Filter. The values for the weights and abscissas can be computed by

$$\begin{aligned} \mathbf{x}_0 &= \mu \\ \mathbf{x}_k &= \mu + \sqrt{\lambda + N_{d_i}}\sqrt{\mathbf{\Sigma}}_k \\ \mathbf{x}_{N_{d_i}+k} &= \mu - \sqrt{\lambda + N_{d_i}}\sqrt{\mathbf{\Sigma}}_k \\ w_0 &= \frac{\lambda}{\lambda + N_{d_i}} \\ w_k = w_{N_{d_i}+k} &= \frac{1}{2(\lambda + N_{d_i})} \end{aligned} \tag{13}$$

For this classical Sigma Point formula the number of abscissas scales linearly with dimension of the problem ($N_\alpha = 2\ N_{d_i} + 1$). In case the initial distribution is non-Gaussian two possible approaches can be applied. For special distributions (e.g. logarithmic normal distributions and gamma distributions) transformation formulas can be applied to the above presented standard Sigma Points Schenkendorf (2014). Alternatively, any type of distribution (including multi modal ones) can be approximated as a *Gaussian Mixed Density* (i.e. a superposition of Gaussians).

4. RECONSTRUCTION OF PROJECTED DISTRIBUTIONS

The PBE (1) represents a first order quasi linear PDE which can be solved with the Method of Characteristics (MOC). Instead of the full PDE, a set of ODEs, i.e. the characteristic system

$$\frac{\mathrm{d}t}{\mathrm{d}\theta} = 1\,, \quad \frac{\mathrm{d}\mathbf{x}}{\mathrm{d}\theta} = \mathbf{h}\,, \quad \frac{\mathrm{d}n}{\mathrm{d}\theta} = -\sum_{k=1}^{d} \frac{\mathrm{d}h_k}{\mathrm{d}x_k} n + B - D\ n \tag{14}$$

has to be solved. A detailed description can be found for example in Ramkrishna (2000). The idea is now to use the the following procedure:

I choose set of representative points from the initial condition $n(t=0,\mathbf{x})$
II solve (14) for those points
III for each point in time the points are classified in subclasses according to their intracellular states
IV based on the classifications the corresponding projected number density distributions can be computed.

This method is basically similar to the computation of single cell dynamics for different initial conditions. Obviously, the "sample size" has an significant effect on this procedure. To obtain a smooth projected number density distribution, the number of representative points has to be sufficiently large. In fact, a reasonable "sample size" can hardly be determined from the start and has to be chosen depending on the initial distribution and the dynamics of the individual process.

5. EXAMPLE: BIOPOLYMER PRODUCTION IN BACTERIA

One important and well known bacterial polymer is poly(3-hydroxybutyrate) (PHB) which is a cell internal carbon and energy reserve material. It is accumulated under unbalanced growth conditions, e.g. excess of carbon source or lack of growth essential nutrients, such as nitrogen. The cell can be viewed as consisting of two compartments, namely PHB and non-PHB biomass BIO. Three processes (rates) have to be considered (see Figure 2) according to Franz et al. (2011):

r_1 growth
r_2 PHB synthesis
r_3 PHB metabolization

The non-PHB biomass compartment BIO includes all the lipids, DNA, RNA and proteins and is therefore the catalytic active component. If carbon and all other growth essential nutrients are available, BIO will increase (r_1). Under lack of nitrogen or excess of carbon, the external carbon source will be stored into the internal carbon reserve material PHB (r_2). If nitrogen limitation is removed, PHB can be metabolized to BIO (r_3).

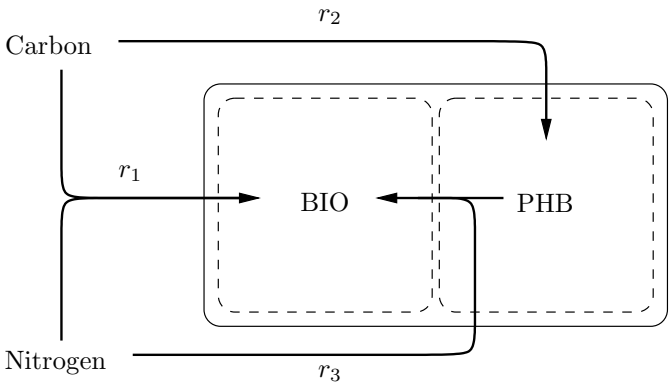

Fig. 2. *Basic reactions on the single cell level: (r_1) carbon and nitrogen are metabolized, BIO is produced, (r_2) Carbon is stored in form of* phb *, (r_3) conversion of PHB to BIO*

The reaction rates (r_1, r_2, r_3) can be formulated with Monod type kinetics (Monod, 1949), where r_2 (PHB synthesis) is inhibited, when nitrogen is available:

$$
\begin{aligned}
r_1 =& k_1 \frac{s_\mathrm{C}}{K_\mathrm{C}+s_\mathrm{C}} \frac{s_\mathrm{N}}{K_\mathrm{N}+s_\mathrm{N}} \quad , \\
r_2 =& k_2 \frac{s_\mathrm{C}}{K_\mathrm{C}+s_\mathrm{C}+K_\mathrm{I} s_\mathrm{N}^2} \quad , \\
r_3 =& k_3 \frac{m_\mathrm{phb}}{K_\mathrm{phb}+m_\mathrm{phb}} \frac{s_\mathrm{N}}{K_\mathrm{N}+s_\mathrm{N}} \quad .
\end{aligned} \tag{15}
$$

The parameters k_i are the rate constants, K_C and K_N are saturation constants, K_I is the inhibition constant, s_C and s_N are the substrate concentrations of carbon and nitrogen source and m_phb is the cell internal mass of PHB.

Each reaction is catalyzed by enzymes e_i^rel which also undergo temporal evolution. Hence, balancing of the intracellular compounds yields the single cell dynamics (Villadsen et al., 2011):

$$
\begin{aligned}
\frac{\mathrm{d}m_\mathrm{bio}}{\mathrm{d}t} =& r_1 e_1^\mathrm{rel} m_\mathrm{bio} + r_3 e_3^\mathrm{rel} m_\mathrm{bio} \quad , \\
\frac{\mathrm{d}m_\mathrm{phb}}{\mathrm{d}t} =& r_2 e_2^\mathrm{rel} m_\mathrm{bio} - \frac{1}{Y_\mathrm{bio/phb}} r_3 e_3^\mathrm{rel} m_\mathrm{bio} \quad , \\
\frac{\mathrm{d}e_i^\mathrm{rel}}{\mathrm{d}t} =& \alpha_i + r_{\mathrm{E},i} - (\beta_i + \mu) e_i^\mathrm{rel} \quad (i=[1,2,3]) \, .
\end{aligned} \tag{16}
$$

Here m_bio is the cell internal mass of BIO and the relative enzyme concentrations are defined as

$$
\begin{aligned}
e_i^\mathrm{rel} &= \frac{e_i}{e_i^{max}} \in [0..1] \\
e_i^\mathrm{max} &= \frac{\alpha_i + k_{\mathrm{E},i}}{\beta_i + k_i}
\end{aligned} \tag{17}
$$

with e_i being the specific enzyme concentration (Baloo and Ramkrishna, 1991). Furthermore, $Y_\mathrm{bio/phb}$ is a yield coefficient, α_i are constitutive enzyme synthesis rates, β_i are enzyme degradation constants and

$$
\mu = \frac{1}{m_\mathrm{bio}+m_\mathrm{phb}} \left(\frac{\mathrm{d}m_\mathrm{bio}}{\mathrm{d}t} + \frac{\mathrm{d}m_\mathrm{phb}}{\mathrm{d}t} \right) \tag{18}
$$

is the growth rate of the total cell mass ($m_\mathrm{bio}+m_\mathrm{phb}$). The enzyme synthesis rates ($r_{\mathrm{E},1}$, $r_{\mathrm{E},2}$, $r_{\mathrm{E},3}$) are also formulated with Monod type kinetics:

$$
\begin{aligned}
r_{\mathrm{E},1} =& k_{\mathrm{E},1} \frac{s_\mathrm{C}}{K_{\mathrm{E,C}}+s_\mathrm{C}} \frac{s_\mathrm{N}}{K_{\mathrm{E,N}}+s_\mathrm{N}} \quad , \\
r_{\mathrm{E},2} =& k_{\mathrm{E},2} \frac{s_\mathrm{C}}{K_{\mathrm{E,C}}+s_\mathrm{C}+K_\mathrm{I} s_\mathrm{N}^2} \quad , \\
r_{\mathrm{E},3} =& k_{\mathrm{E},3} \frac{m_\mathrm{phb}}{K_{\mathrm{E,phb}}+m_\mathrm{phb}} \frac{s_\mathrm{N}}{K_{\mathrm{E,N}}+s_\mathrm{N}} \quad .
\end{aligned} \tag{19}
$$

The above model formulation is not able to account for cell to cell variability as indicated by flow cytometric measurements of the intracellular amounts of PHB (Franz et al., 2014). To improve the situation a heterogeneity can be characterized with a correponding PBE. The intracellular states

$$
\mathbf{x} = \left(m_\mathrm{bio},\, m_\mathrm{phb},\, e_1^\mathrm{rel},\, e_2^\mathrm{rel},\, e_3^\mathrm{rel} \right)^\mathrm{T} \tag{20}
$$

directly translate to internal coordinates of the number density distribution. Neglecting cell division and cell death the corresponding PBE is given by

$$
\frac{\partial n(t,\mathbf{x})}{\partial t} = -\sum_{k=1}^{5} \frac{\partial}{\partial x_k} \left\{ h_k(t,\mathbf{x},\, s_\mathrm{C},\, s_\mathrm{N})\, n(t,\mathbf{x}) \right\} \, . \tag{21}
$$

The convection velocities h_k for the internal coordinates are given by the dynamics of the single cell model

$$
\mathbf{h} = \frac{\mathrm{d}}{\mathrm{d}t} \begin{bmatrix} m_\mathrm{bio} \\ m_\mathrm{phb} \\ e_1^\mathrm{rel} \\ e_2^\mathrm{rel} \\ e_3^\mathrm{rel} \end{bmatrix} \, . \tag{22}
$$

As mentioned previously, in result of the intracellular reactions the extracellular substrates s_C and s_N are consumed. In case of a batch experiment ($P=0$), balancing yields the dynamics of the substrates

$$
\begin{aligned}
\frac{\mathrm{d}s_\mathrm{C}}{\mathrm{d}t} &= -\int_\mathbf{X} \Pi_\mathrm{C}^1 r_1 \; n(t,\mathbf{x})\mathrm{d}\mathbf{x} - \int_\mathbf{X} \Pi_\mathrm{C}^1 r_2 \; n(t,\mathbf{x})\mathrm{d}\mathbf{x} \, , \\
\frac{\mathrm{d}s_\mathrm{N}}{\mathrm{d}t} &= -\int_\mathbf{X} \Pi_\mathrm{N}^1 r_1 \; n(t,\mathbf{x})\mathrm{d}\mathbf{x} - \int_\mathbf{X} \Pi_\mathrm{N}^3 r_3 \; n(t,\mathbf{x})\mathrm{d}\mathbf{x} \, ,
\end{aligned} \tag{23}
$$

where Π_i^k characterize the stochiometry of the reactions.

6. SIMULATION RESULTS

It was mentioned in a prior contribution (Franz et al., 2014) that the flow cytometric measurement of m_bio is difficult. However, overall biomass of the cell culture can be determined indirectly e.g. by optical density analysis.

At first we consider that only integral measurements like mean and variance w.r.t. m_bio and m_phb are available. The initial number density distribution is assumed to be a Gaussian normal distribution. In this case, the mentioned efficient approximate moment method can be applied to compute the corresponding moments of the model. The dynamic equations for moments (10) have to be solved simultaneously to (23). As $N_\alpha = 2$ $N_{d_i}+1 = 11$ all in all a coupled system of $N_\mathrm{ODE} = (N_{d_i}+1)N_\alpha + 2 = 68$ ODEs has to be solved.

The simulation results are depicted in Fig. 3 and Fig. 4. In analogy to the experimental procedure which was reported in Franz et al. (2014) the simulation setup was chosen as follows: in the first part ($t=[0,\,6]$), both substrates are available and the cells preferentially increase there non-PHB biomass. For $t=[6,\,15]$ only substrate S_C is available and the cells store carbon in the form of PHB. At $t=50h$ nitrate is added to the reactor. In result PHB is metabolized to non-PHB biomass. It can be seen that the variance w.r.t. m_bio in the cell culture generally increases while variance w.r.t. m_phb increases only in the first part when PHB is produced and decreases in second part when PHB is metabolized after adding nitrate at $t=50h$. Those simulations can be used directly for adaption of the model to integral data obtained from cell culture experiments. Furthermore, the low computational costs of the approximate moment method are advantageous for possible online applications including model based process control.

However, if distributed measurements by means of projected number distributions are available, it is often advantageous to include them to the model adaption. They are of particular interest if the underlying models exhibit interesting dynamic effects like a bistability or switching behavior. On the macroscopic scale those effects may be observed in form of multi modal distributions. Those can not be analyzed effectively from integral properties, like mean and variance, alone.

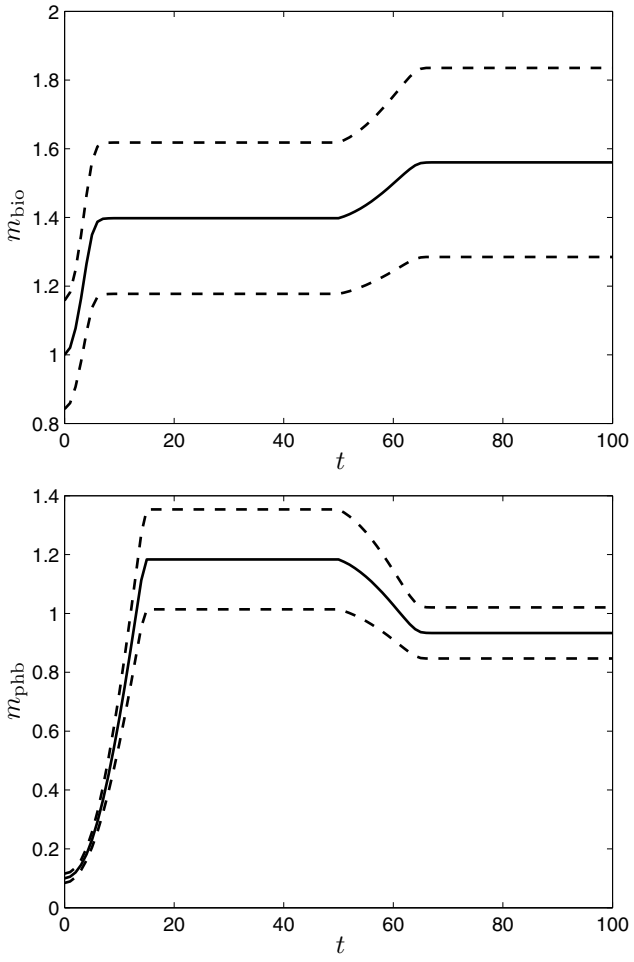

Fig. 3. *Mean (solid) and standard deviations (dashed) for m_{bio} and m_{phb}; while the variance of the cell population w.r.t. m_{bio} is increasing for the whole simulation setup, variance w.r.t. m_{phb} decreases in the case of PHB conversion to BIO*

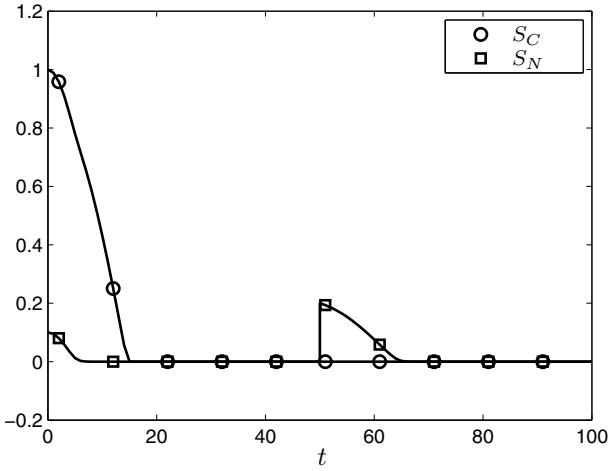

Fig. 4. *Dynamics of extracellular substrates, (I) preferential biomass production, (II) production of PHB, (III) PHB metabolization*

As mentioned previously the MOC was applied to 10^4 points, which had been randomly sampled from the initial distribution in order to obtain a sufficiently smooth projected number density distribution. Thus, a system of 60002 ODEs has to be solved. The simulation results

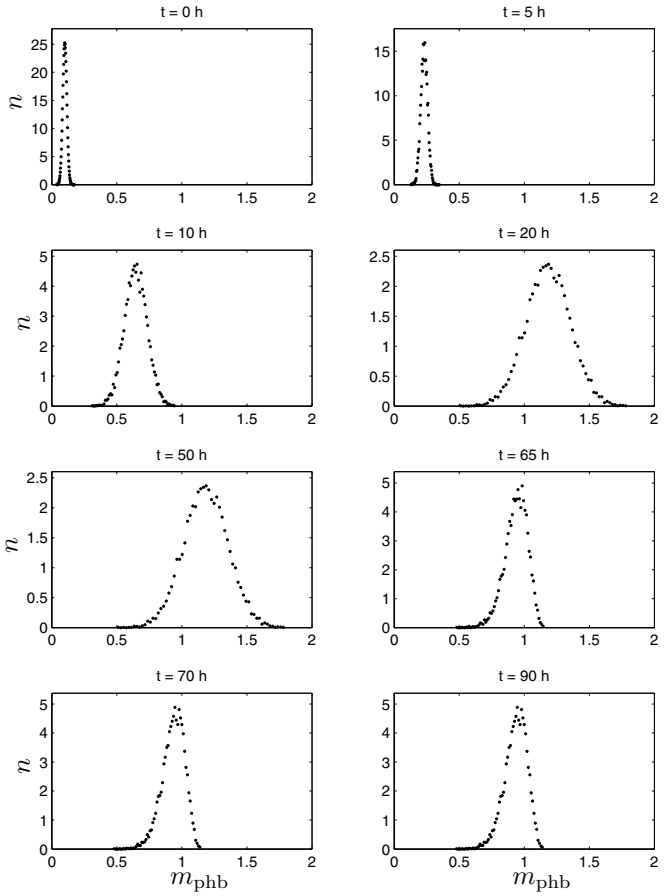

Fig. 5. *Snapshots of projected number density distributions; it can be seen, that the variance of the cells w.r.t. PHB significantly increases in the first part of the simulation setup; after adding nitrate at $t = 50h$ the variance decreases*

are depicted in Figure 5. The principle effects already seen from simulation of the moment dynamics can also be observed here: in presence of S_{N} and S_{C} the intracellular amount of m_{phb} increases only slightly as the cells use their capacity to increase m_{bio}. In the absence of nitrate the cells store carbon in form of m_{phb}. In addition, the cell-to-cell variability increases. In the last scenario the intracellular amount of PHB decreases as result of metabolization to non-PHB biomass m_{bio}.

7. SUMMARY AND OUTLOOK

In this contribution we presented a method to combine limited measurements from cell culture experiments and complex models characterizing the dynamics on the single cell level. In particular, the numerically efficient approximate moment method is promising for an application to model adaption and online applications. The method was applied to the example of biopolymer production in bacteria.

So far, the presented method is only applicable to bioprocesses in which cell division and cell death are negligible. Thus future focus will be on extension of this method

to cope with those effects. Furthermore, the method will be used to adopt a model for biopolymer production to experiments which are currently planned within our group.

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REFERENCES

Baloo, S. and Ramkrishna, D. (1991). Metabolic regulation in bacterial continuous cultures: Ii. *Biotechnology and Bioengineering*, 38(11), 1353–1363.

Davis, P.J. and Rabinowitz, P. (1994). *Methods of Numerical Integration*. Computer Science and Applied Mathematics. Academic Press, 2. ed., 4. print edition.

Dürr, R. and Kienle, A. (2014). An efficient method for calculating the moments of multidimensional growth processes in population balance systems. *The Canadian Journal of Chemical Engineering*, 92, 2088–2097.

Dürr, R., Müller, T., and Kienle, A. (2015). Efficient dqmom for multivariate population balance equations and application to virus replication in cell cultures. In *8th Vienna International Conference on Mathematical Modelling*, 29–34.

Franz, A., Dürr, R., and Kienle, A. (2014). Population balance modeling of biopolymer production in cellular systems. In *Proceedings to 19th IFAC WC - Cape Town*, 1705–1710.

Franz, A., Song, H.S., Ramkrishna, D., and Kienle, A. (2011). Experimental and theoretical analysis of poly(beta-hydroxybutyrate) formation and consumption in ralstonia eutropha. *Biochemical Engineering Journal*, 55(1), 49–58.

Glauche, I., Herberg, M., and Roeder, I. (2010). Nanog variability and pluripotency regulation of embryonic stem cells - insights from a mathematical model analysis. *PLoS ONE*, 5, e11238.

Marchisio, D.L. and Fox, R.O. (2005). Solution of population balance equations using the direct quadrature method of moments. *Journal of Aerosol Science*, 36(1), 43–73.

McGraw, R. (1997). Description of aerosol dynamics by the quadrature method of moments. *Aerosol Science and Technology*, 27(2), 255–265.

Monod, J. (1949). The growth of bacterial cultures. *Annual Review of Microbiology*, 3, 371–394.

Müller, T., Dürr, R., Isken, B., Schulze-Horsel, J., Reichl, U., and Kienle, A. (2013). Distributed modeling of human influenza a virus-host cell interactions during vaccine production. *Biotechnology and Bioengineering*, 110(8), 2252–2266.

Ramkrishna, D. (2000). *Population Balances: Theory and Applications to Particulate Systems in Engineering*. Academic Press, San Diego.

Schenkendorf, R. (2014). *Optimal experimental design for parameter identification and model selection*. Ph.D. thesis, Otto-von-Guericke University Magdeburg.

Srienc, F. (1999). Cytometric data as the basis for rigorous models of cell population dynamics. *Journal of Biotechnology*, 71(1–3), 233–238.

Stroud, A. (1971). *Approximate Calculation of Multiple Integrals*. Prentice-Hall, Englewood Cliffs, N.J., Englewood Cliffs, N.J.

van der Merwe, R. (2004). *Sigma-Point Kalman Filters for Probablilistic Inference in Dynamic State-Space Models*. Ph.D. thesis, Oregon Health & Science University.

Villadsen, J., Nielsen, J., and Liden, N. (2011). *Bioreaction Engineering Principles*. Springer Science & Business Media, Heidelberg, 3 edition.

Appendix A. SIMULATION PARAMETERS

Parameter	Value	Parameter	Value
k_1	0.2500	$k_{\mathrm{E},1}$	0.2574
k_2	0.1000	$k_{\mathrm{E},2}$	0.1089
k_3	0.3500	$k_{\mathrm{E},3}$	0.3564
K_C	0.01	$K_{\mathrm{E},C}$	0.01
K_N	0.03	$K_{\mathrm{E},N}$	0.03
K_I	10	$K_{\mathrm{E},I}$	10
K_{phb}	10	$K_{\mathrm{E,phb}}$	10
α_1	0.0026	β_1	0.01
α_2	0.0011	β_2	0.01
α_3	0.0036	β_3	0.01
Π_{C}^1	0.3333	Π_{N}^1	0.2500
Π_{C}^2	0.8000	Π_{N}^2	0.8000
$Y_{\mathrm{bio/phb}}$	0.65		

Appendix B. INITIAL CONDITIONS

State	Value
$S_C(t=0)$	1
$S_N(t=0)$	0.1
μ	[1, 0.1, 0.01, 0.01, 0.01]
$\mathbf{\Sigma}$	0.025 diag(μ^2)