Modeling of plant in vitro cultures – overview and estimation of biotechnological processes

Rüdiger W. Maschke, Katja Geipel, Thomas Bley

Institute of Food Technology and Bioprocess Engineering, Technische Universität Dresden, Dresden, Germany

Corresponding author:

Katja Geipel, Institute of Food Technology and Bioprocess Engineering, Technische Universität Dresden, Bergstraße 120, 01069 Dresden, Germany, phone 0049 351 463 39042, e-mail katja.geipel[at]tu-dresden.de

Running title:

Modeling in plant biotechnology

Abstract

Plant cell and tissue cultivations are of growing interest for the production of structurally complex and expensive plant-derived products, especially in pharmaceutical production. Problems with up-scaling, low yields and high-priced process conditions result in an increased demand for models to provide comprehension, simulation, and optimization of production processes.

In the last 25 years, many models have evolved in plant biotechnology; the majority of them are specialized models for a few selected products or nutritional conditions. In this article we review, delineate, and discuss the concepts and characteristics of the most commonly used models. Therefore, the authors focus on models for plant suspension and submerged hairy root cultures. The article includes a short overview of modeling and mathematics and integrated parameters, as well as the application scope for each model. The review is meant to help researchers better understand and utilize the numerous models published for plant cultures, and to select the most suitable model for their purposes.

Keywords

mathematical model; kinetic model; simulation; plant biotechnology; hairy root; cell suspension culture

What is plant biotechnology?

Plants produce a multifarious range of natural products which can be applied as active ingredients in pharmaceuticals, edibles, fragrances, flavors, and dyes (Georgiev et al., 2007; Rao and Ravishankar, 2002). The majority of these chemicals consist of small but complex molecules that are difficult to synthesize chemically while using economical methods. Most valuable phytochemicals are produced by means of secondary metabolism with highly branched and regulated pathways (Doran, 2009). Using plant biotechnology with optimized bioreactor systems offers an attractive alternative for agricultural and/or chemical production of bioactive plant ingredients (Kieran, 2001). Therewith, consistent high compound qualities and quantities can be ensured throughout the entire year. The utilization of harmful substances is not required. Due to accurately defined process steps and culture conditions, process and product accreditation will be facilitated. Thereby, process control and adjustment are easy to handle, enabling a production according to industry standards, e.g., good manufacturing practice. The yield can be increased with help of genetic modification, targeted elicitation, and optimization of cultivation parameters (Dörnenburg and Knorr, 1995). Several active plant ingredients are already synthesized by means of in vitro cultures. A general survey over production systems of bioactive plant metabolites using callus and suspension cultures can be found e.g., in Mulabagal and Tsay (2004).

The significance of modeling

Optimization procedures demand numerous experiments which are laborious and, hence, time and money consuming. Therefore, an initial theoretical process study followed by simulation and variation of parameters on the basis of existing data is mandatory. An exact knowledge about the influence of single variables as well as a detailed model with sufficient complexity are the main requirements for fast and easy forecasts of biotechnological processes (Dunn et al., 2003; Lee, 1992; Schügerl, 2001). A sensitivity analysis can be very effective for the identification of parameters with a strong influence on the cultivation outcome, thereby

limiting the number of experiments by varying only critical model parameters (Cloutier et al., 2008).

The investigated plant in vitro culture types

To date, different forms of plant in vitro cultures are in the interest of research investigations (Eibl and Eibl, 2008) and, in some cases, profitable industrial applications (Mulabagal and Tsay, 2004). Several publications deal with induction (Geipel et al., 2014; Mustafa et al., 2011), cultivation (Geipel et al., 2013; Haas et al., 2008), application for products (Kolewe et al., 2008; Weathers et al., 2010), and growth modeling (Bailey et al., 1985; Lenk et al., 2013) of plant in vitro cultures. Known culture types are callus, and associated plant cell suspensions (SU), hairy roots (HR), adventitious roots, and shoots, each with different advantages and disadvantages concerning handling or cultivation requirements (Rao and Ravishankar, 2002). On the basis of this abundance, the present review is focused on the most common and applied plant culture types: plant cell suspensions and hairy roots (Doran, 2009; Georgiev et al., 2009). Due to their broad significance for commercial processes, only liquid cultivation systems are considered.

General modeling concepts and mathematical background

The basic model requirements include: simplification of reality, reduction of data, identification of essential values with relevant influence, illustration of basic processes, and possibility of implementation and simulation (Dunn et al., 2003). Figure 1 depicts schematically the process of model development and evaluation.

There are many options for simplification: e.g., the assumptions of evenly distributed substrates or ideally constant temperature and pH throughout the cultivation progress. Commonly used is a classification into simple (unstructured and unsegregated) and complex (structured and/or segregated) models (Dunn et al., 2003). In a structured model, each cell is described as a multicomponent system; in a segregated model, the population is regarded as a heterogeneous system of distinguishable cells (Fredrickson et al., 1967). One of the easiest

and most widely used approaches is the Monod equation (Equation (1)) which is part of more than one third of all models investigated in this review. The Monod equation is applied for calculation of the specific growth rate μ (h^{-1}) in dependence of the substrate,

$$\mu = \mu_{max} \cdot \frac{c_S}{K_S + c_S} \quad (1)$$

whereas μ_{max} is the maximum specific growth rate (h^{-1}), c_S the substrate concentration (g L^{-1}), and K_S the affinity constant of the substrate (g L^{-1}) (Monod, 1959). For including substrate or product inhibition, Equation (1) can be extended analogously.

The product formation can be classified into three types, depending on the relation to the primary metabolism: direct (Type I), indirect (Type II) or not related (Type III) (Gaden, 1959). In the Luedeking-Piret approach (Equation (2)), the product formation is divided in a growth- and a non-growth-associated part (Luedeking and Piret, 1959).

$$\frac{dc_P}{dt} = \alpha \cdot \frac{dc_X}{dt} + \beta \cdot c_X \quad (2)$$

The change of product concentration $\frac{dc_P}{dt}$ (g L^{-1} h^{-1}) is calculated by means of the growth-associated product constant α (g g^{-1}), the change of biomass concentration $\frac{dc_X}{dt}$ (g L^{-1} h^{-1}), the non-growth-associated product constant β (g g^{-1} h^{-1}), and the biomass concentration c_X (g L^{-1}). With $\alpha = 0$ product formation (secondary metabolites without link to the energy metabolism, e.g., steroids, penicillin) is completely non-growth-associated (Type III). Completely growth-associated (Type I) product formation (primary metabolites e.g., ethanol, gluconic acid) occurs with $\beta = 0$. However, these are just simplifications for convenience. In practice, the constants have positive values greater than zero and typically below one. Hence, in not simplified cases or for the formation of products of Type II (intermediate metabolites, e.g., amino acids, citric acid) the product constants will be most likely unequal to zero. The

Luedeking-Piret approach is used unmodified or extended in about the half of the investigated publications with product formation.

Neither the Monod nor the Luedeking-Piret approach was originally designed for plant cell or tissue cultures, explaining their lack of accuracy without modifications. The primary use of both can be explained with the history of biotechnology and biotechnological modeling.

Chronological outline concerning biotechnological models

To make biotechnological processes more efficient, it is desirable to know as much as possible about all parameters and variables before starting optimization procedures. However, experimental elucidation of all conceivable settings is time, money, and resource consuming, but good models enable predictions of most states and simplify results. In addition to process research, investigations concerning modeling have played an increasing role in modern biotechnology since the mid-20th century. First models were simple in structure or segregation grade and dealt mainly with common microorganisms like bacteria and yeasts (Bailey, 1998). However, the efficacy of these simple approaches pertained only to small scale cultivations, while the population heterogeneity found in larger, industrial scale operations required the formation of dynamic models (Bley, 2011). With growing interest in fungi cultivation, biotechnological models became increasingly complex (Krull et al., 2013): segregated models are obligatory for fungi with hyphae growth and for growth of HR networks (Hjortso, 1997). In the late 1930s, the first plant in vitro cultures were established (Smetanska, 2008). Since that time, plant in vitro cultivations have advanced into the focus of research and industry, as well as that of model theoreticians. At the beginning simple model methods, e.g., originated by Monod, were applied. His fundamental approach is famous for simplicity, but it is deficient for most applications due to inadequate specialization. Further ideas led to the adaptation of models designed for fungi hyphae (Taya et al., 1989), adjusted to incorporate simulations of growth, branching, substrate consumption, and product formation of HR (Bastian et al., 2008). However, despite good conformity marks, direct

transference of that relatively complex modeling approaches originating from fungi cultures was not sufficient for most plant cultures (Bailey and Nicholson, 1989). Major problems exist e.g., concerning reproduction of lag and death phase. Fundamental models are still a common basis for modified plant in vitro culture approaches, but model theorists are increasingly turning to structured and segregated approaches or reducing their models for specific limiting conditions.

Hence, the need of more accurate models increased the demand of more process information (Becker and Krause, 2010; Sagmeister et al., 2013). For single cell analysis of plant cell cultures, the tool of flow cytometry became indispensable. Since its broad implementation in medicinal laboratories and incremental implementation in microbiological and biotechnological laboratories in the mid-1990s (Kottmeier et al., 2009), the flow cytometer is perfectly suited for process monitoring (e.g., cell number, viability, detection of inclusion bodies and expression markers like GFP (Krull et al., 2013; Müller and Bley, 2010)), and therefore offers a useful method for obtaining data to establish and maintain individual-based models, especially in dynamic populations (Bley, 2011). The applicability of flow cytometry to particle size measurements is limited and the size of plant cells as well as fact that they usually occur as aggregates render this approach not applicable for measurements of intact plant cells. Solely practicable are protoplast investigations or cell cycle analysis after disruption of cells and specific coloring of nuclei (Haas et al., 2008).

Criteria catalog

For ensuring comparability of the analyzed publications the following criteria catalog was used for model evaluation (Table S-I in the Supplementary Information Section). Underlined words/word groups are analogous to the column headings of Table I.

The first contents – author(s) (1) and year (2) – are the primary bibliographic information to ensure explicit correlation, followed by the model complexity (3) in terms of structure and/or segregation. The next six points contain the description of different nutrients used in the

models: substrate in general (4) confirms the calculation of total amount of carbon source(s), whereat multiple C sources (5) confirm(s) if sucrose, glucose, fructose, and others are calculated separately. Other nutrients like phosphate PO_4 (6), nitrogen N_2 (7), oxygen O_2 (8), and other substrates (9), (e.g., potassium K) are listed to provide a convenient overview of the used nutrients. The calculation of concentration of hormones (10) is also applied in some models. The calculation of product in general (11), like secondary metabolites, and the differentiation of the product storage location (12) illustrate the focus on products. The viability (13) indicates the separation between viable and non-viable cells with well-known different growth, substrate uptake, and product formation characteristics. The number of variables (14) (e.g., biomass concentration c_X) and the number of parameters & constants (15) (e.g., yield coefficient $Y_{X/S}$) illustrates the complexity and the capacity of the modeling systems. Variables include every (differential) equation solved by the system; parameters are all values gained experimentally or through literature review, needed to solve these equations. Operating mode (16), plant species (17), and culture type (18) represent details of the experimental conditions applied for obtaining the data, and assist in identifying a matching model for similar experimental data. The number of quotations (19) demonstrates the importance of investigated models for other (model-) publications and was investigated at the end of January 2014.

Models in plant biotechnology

The current review is focused on literature concerning model concepts for plant in vitro cultures. Due to the large number of articles related to this topic, the authors highlight only key papers published in the last 25 years (1989-2013). Models which are highly specialized for very intrinsic species, culture systems, or products and thereby not easily transferable to other systems are not addressed in this review. This main part of the review will give a chronological survey over models applicable or already applied in plant biotechnology.

Fig. S-1 in the Supplementary Information Section provides an overview of specifications that are useful for determining an appropriate model publication.

In addition to the tabular overview (Table IA, B), the following section provides further information concerning special features and focuses of the investigated modeling approaches. The section is divided according to the two main plant culture types herein investigated.

Models concerning plant suspension cultures (SU)

In 1989, Frazier developed a simple model, dealing mainly with growth of *Dioscorea deltoidea* (yam) cell aggregates and intermediate metabolites leaking into the medium (1989). The biomass formation is dependent on an essential intermediate, whereby it can be calculated with a Michaelis-Menten type kinetic. With the help of dimensionless quantities like the Reynolds (*Re*) and the Sherwood number (*Sh*), the diffusive mass transport of intermediate metabolites from cell aggregates into the medium can be calculated. The result is a model with 14 equations (four dimensionless variables and ten parameters) which can be used to determine biomass and, substrate, as well as intra- and extracellular metabolite concentrations in batch cultivations.

Meanwhile, Bailey and Nicholson invented a structured model with data from *Catharanthus roseus* (Madagascar rosy periwinkle) batch cultures (1989). This basic approach includes, inter alia, the calculation of fresh weight, viability, and an extended Luedeking-Piret product formation. Thereby, the prediction of the culture expansion phase is possible, and the death phase is described more precisely. The Luedeking-Piret formula (Equation (2)) is augmented with the viability V (g g^{-1}), considering the facts that only viable cells are able to produce and dead cells release vacuole-stored content(s) into the medium. This leads to the product formation by Bailey and Nicholson (Equation (3)), in which the viability is added as factor to the first two terms (only viable cells produce), and in the third term viability is added as differential equation factoring the actual intracellular product concentration c_P, representing the irrecoverable loss of product due to cell rupture.

$$\frac{dc_P}{dt} = \alpha \cdot V \cdot \frac{dc_X}{dt} + \beta \cdot V \cdot c_X + \frac{dV/dt \cdot c_P}{V} \quad (3)$$

The final model contains six equations and the parameters were estimated for two cultivations with different temperatures (20°C and 25°C).

One year later, Bailey and Nicholson extended their previous mathematical construct (same species and culture type) and added temperature optimization methods (1990). Using a quasi-Newton algorithm, the temperature for maximal product yield is calculated. Furthermore, the "specific optimal control" approach from King et al. (1974), suggesting one single temperature switching point between growth and product formation phase, is introduced. Through this, an increase in product yield of 22 % is shown and, in addition, the requirement of Bailey and Nicholson´s more complex structured model for improved predictability is justified.

The same year, de Gunst et al. developed a segregated, stochastic model for a *Nicotiana tabacum* (cultivated tobacco) batch culture (1990). In order to describe the heterogeneity of cells more accurately, a corpuscular (segregated) model with two types (dividing/non dividing) of cells is chosen, including an equation for the general hormone concentration. The ratio of dividing and differentiated cells depends on the substrate and hormone concentration. That is why the equations of these concentrations build the core of the model, and processes like product formation or substrate utilization for maintenance are neglected. Altogether, after de Gunst the suggested model explains the monitored population growth relatively well, links cell growth and differentiation with substrate and hormone concentration, and suggests the mathematical connection of cell state (differentiated/non differentiated) with the formation of secondary metabolites.

In the year 1991, two not complex models for batch fermentation of SU were introduced, both dealing with the effects of phosphate. Bramble et al. investigated the influence of calcium and phosphate on *Coffea arabica* (Arabica coffee) cultures, especially on biomass

and alkaloid formation (1991). For the purpose of immobilization, plant cells can be bound into calcium alginate gels in bioreactors. Due to releases from these gels, as well as calcium chloride supplements into the media (added to ensure the stability of the gel), concentrations of calcium in the culture are increased, which may inhibit plant growth (Hepler and Wayne, 1985). However, calcium can precipitate phosphate and thereby decrease the phosphate concentration in the medium. Despite being necessary for plant cell growth, phosphate may inhibit secondary metabolite formation (Knobloch et al., 1981), so the yield of secondary metabolites may increase with higher calcium concentrations. The effect of calcium was determined experimentally by Bramble et al. (1991), showing a decrease in cell growth and greater caffeine production with increasing calcium chloride concentration. Another set of experiments in the same study revealed the inverted correlation with increasing initial phosphate concentrations. The final model consists of differential equations for biomass, substrate (glucose), alkaloid as well as intra- and extracellular phosphate concentrations, in which the specific growth rate is calculated using Haldane type kinetics, shown in Equation (4).

$$\mu = \frac{\mu_{\max}}{1 + K_S \cdot \left[\frac{1}{i} + \frac{i^2}{K_S \cdot K_I{}^2}\right] \cdot \left[\frac{K_I}{K_I + i}\right]} \quad (4)$$

The formula consists of the (maximum) specific growth rates μ and $\mu_{\max}$ (h^{-1}) (see Equation (2)), the kinetic constants K_S, representing the affinity constant of the substrate and K_I, the inhibition constant, as well as *i*, the concentration of calcium as inhibitory substrate (all in mol L^{-1}). Experimentally determined biomass, glucose, phosphate, and product concentrations were accurately forecasted with Equation (4). However, the predictive power for the stationary phase, and especially the secondary metabolite concentration within this phase, showed the limits of the model.

Concurrently, Curtis et al. proposed a modeling approach for phosphate limited *Papaver somniferurn* (opium poppy) cultures (1991). Several experiments with different sucrose, phosphate, and salt concentrations were performed, suggesting that phosphate is the growth limiting factor in Murashige and Skoog medium (Murashige and Skoog, 1962). Therefore, the focus lies on the internal phosphate concentration, which is divided into a free kinetic pool, versus phosphate bound in structural components. Additionally, the intracellular phosphate concentration of various plant cell cultures in the stationary phase is described. The outcome is a package of experimental data and a packed model concerning the effects of phosphate on cell growth.

Two structured approaches, both dealing with *Nicotiana tabacum* SU cultures, followed during the next two years. First, Hooker and Lee published a model focused on metabolism pathways and product synthesis which also considered substrate uptake kinetic and cell respiration (1992). The proposed pathways are shown in Figure 3, demonstrating the substrate activation (sucrose hydrolysis), uptake (expressed as Michaelis-Menten kinetic), metabolism, and formation of the three process end products: structural components (insoluble cell parts), respiratory mass loss, and secondary metabolites. In contrast to the classical Luedeking-Piret equation, the product formation is divided into a growth-competitive (using the same intermediates as for cell growth) and a non-growth-competitive (components are formed out of the activated substrates) part. Finally, a model with eight differential equations is proposed, showing a good agreement with experimental data and illustrating novel mathematical pathways.

Afterwards, Shibasaki et al. extended the models of Bailey and Nicholson (1989; 1990) and adopted the new approach to their own experimental data (1993). In this variation, the plant cell is divided into a vacuole and a non-vacuole part (cell wall and cytoplasm) with defined water content. In addition, the product synthesis is changed from the Luedeking-Piret approach to a set of own equations, distinguishing into intracellular, vacuole-stored product

in viable cells, and extracellular product released through cell lyses. Transferred to the experimental data of Shibasaki et al., the correlation of this updated model with the determined values is high.

A year later, van Gulik et al. presented a structured model for the batch cultivation of *C. roseus* cells in carbon- and phosphate-limited batch or chemostat cultures (1993). This publication further addressed the challenge of determining the ideal phosphate concentration for cell growth and product formation. While low phosphate concentrations result in limited growth, there is an improvement of secondary metabolite production; the opposite is found with high phosphate (Bramble et al., 1991; Curtis et al., 1991). In addition to phosphate, the model provides a method to calculate the concentration of structural biomass, stored carbohydrates, phosphorylated precursors, and O_2 consumption (indirectly by carbon dioxide production). For model evaluation, a parameter sensitivity analysis was conducted and several simulations and predictions were performed.

In 1995, a not complex model that connects biomass with secondary metabolite formation was suggested by Guardiola et al. (1995). For model development, batch and semicontinuous shake flask cultivations with cells of *Vitis vinifera* (common grape vine) were performed. A direct correlation between substrate concentration and cell viability is proposed (viability as function of substrate), assuming that the main reason for cell decay is the lack of energy for maintenance. Accordingly, the viability in semicontinuous cultivations is very high, and progressively decreasing in batch cultures. Secondary metabolite formation is hypothesized to be non-growth-associated, so the Luedeking-Piret approach can be reduced. The product catabolism is assumed to be independent from biomass concentration but correlated to viability (and hence to the substrate concentration) and can be – competitively with the biomass degradation – used for sustaining cell maintenance.

Three years later, Glicklis et al. published a structural model with focus on polysaccharide production, approved with experimental data of a *Symphytum officinale* (common comfrey)

culture (1998). These empirical data were taken from shake flask experiments at four different temperatures; an exemplary implementation of this demonstrative model with cell dry weight c_X, substrate concentration c_S and viability V for 15°C and 25°C is shown in Figure 3. The model combines certain components, including the approach for expansion and lysis phase, from Bailey and Nicholson (1989; 1990) with a modified Monod kinetic and a growth associated product formation process. Therein, polysaccharides can be stored inside the cell, excreted to the media, or used as energy source for cell maintenance if the substrate suspended in the media is exhausted. The article concludes with a discussion about statistics focused on the confidence intervals of the experimental data.

At the same time, Takeda et al. developed a structured model for secondary metabolite production with *Carthamus tinctorius* (safflower) batch cultures (1998). The division into structural components, stored carbohydrates, and respiratory intermediates, as well as the key aspect of secondary metabolite formation, is similar to the approach from Hooker and Lee (1992). Though, the focus of this new approach is phosphate limitation and stored carbohydrates rather than pathway designation. Therefore, sugar and phosphate uptakes are calculated separately by two Michaelis-Menten type equations and the intracellular phosphate concentration is used for calculation of stored carbohydrates, respiratory intermediates, and indirectly for tocopherol synthesis. Additionally, secondary metabolite production depends on respiratory intermediates (including acetyl-CoA) and cykimic acid concentrations, both initial compounds of the tocopherol formation. Altogether, the correlation between respiratory intermediate, phosphate, and biomass concentration, as well as product formation, is clearly illustrated by this model.

Another structured model focused on secondary metabolite production and substrate limitations was presented by Choi et al. (1999). Here, the carbohydrate concentration (substrate) is calculated separately, including a competitive inhibition for the uptake of glucose and fructose. The calculation of berberine concentration is implemented by an

extended Luedeking-Piret equation, containing a third term describing segregation of berberine into the media in case of cell lysis. For parameter estimation, empirical data of a *Thalictrum rugosum* (meadow-rue) batch culture were applied. Finally, simulation and prediction of biomass and berberine concentrations were feasible and showed good accordance.

Schlatmann et al. proposed another structured model, dealing with biomass formation, maintenance and secondary metabolite production (1999). The model was verified with data from *C. roseus* cells, grown in a two-stage batch cultivation, including first biomass and then product formation. The basis of this approach is the simplified model from van Gulik et al. (1993), dividing the biomass in stored carbohydrates and active biomass only. Moreover, secondary metabolite (ajmalicine) formation has no mathematical influence in the carbohydrate balance, and ajmalicine concentration is assumed to give no feedback (e.g., inhibition). However, ajmalicine production is described more precisely and the influences of dissolved oxygen, gaseous metabolites, and substrate (glucose) concentrations are considered (Schlatmann et al. (1995a; 1995b; 1997)). The final model (Schlatmann et al., 1995b) fits the main experimental data quite accurately, but lag and lysis phase are described inchoately as a consequence of simplifications.

Two more models concerning cell growth, as well as carbon and phosphate limitations, were developed in 2000. Pires Cabral et al. suggested a structured model with experimental data from a *Cynara cardunculus* (cardoon) batch cultivation (2000). Related to the model of Schlatmann et al. (1999), the basis of this model is the approach of van Gulik et al. (1993) and in addition to growth limitations, secondary metabolite formation is the focal point. The phosphate and sucrose uptake rates are dependent on the concentrations in the media, and for sucrose additional from the intracellular stored carbohydrate and phosphate concentrations. For calculation of maintenance energy, two scenarios are possible: (1) extracellular sucrose level is adequately high and directly utilized, or, (2) sucrose is depleted and structural

biomass is used. In both cases the maintenance energy demand and the secondary product formation rate are proportional to the amount of structural biomass.

Afterwards, Sirois et al. constructed a segregated model for an *Eschscholzia californica* (California poppy) batch culture (2000). The model construction was done in two phases, starting with a not complex model, expanding to a segregated one by way of reduced overall error and improved lysis phase. In this concept, cells are divided into three groups: (1) small and expanding, (2) large and dividing, and (3) inactive cells. Each group has its own constants for growth, substrate uptake, and decay rate. Altogether, this matrix style model using the reduction of the overall failure as a benchmark delivers good results in simulation and prediction.

Two years later, an article dealing with intracellular phosphate concentration and a corresponding model was published by Zhang and Su (2002). It is assumed that one of the decisive problems of modeling the intracellular phosphate concentration is the analysis: either it is precise but destructive (e.g., chemical), or non-invasive but inaccurate (e.g., by nuclear magnetic resonance (NMR) spectroscopy). The authors proposed the use of NMR spectroscopy together with an extended Kalman filter (nonlinear statistical algorithm) for more accurate online monitoring of intercellular concentrations, especially phosphate concentration (Haseltine and Rawlings, 2005; Kalman, 1960). The Kalman filter works in two stages – prediction and correction – and delivered, together with a sensitivity analysis, good findings for the experimental data which were established with an *Anchusa officinalis* (common bugloss) batch culture.

In 2003, Li et al. proposed a structured model for *Taxus chinensis* (Chinese yew) with a focus on stored carbohydrates and oligosaccharide elicited paclitaxel formation (2003). Therein, carbon sources are divided in sucrose, fructose, and glucose in the media, as well as soluble sugars and starch in the cell. The final model contains twelve equations and can be used for calculation of intra- and extracellular secondary metabolite concentrations. However,

considering the influence of the elicitor, an effective coefficient must be added to the paclitaxel production and respiratory loss equations, representing the quotient of the empirical parameters with and without oligosaccharide addition. In the end, the model is able to simulate cell sugar as well as intra- and extracellular product concentration, with or without elicitation accurately. Though, it is not capable of describing paclitaxel decay.

Finally, Kolewe et al. introduced a segregated model concerning aggregation of *Taxus cuspidate* (Japanese yew) cells (2012). In this mathematical description, the cell aggregates can either grow (biomass formation) or break apart (formation of smaller aggregates). Together with an added mathematical term for cell death, the biomass concentration and distribution can be calculated. Furthermore, the product formation with different aggregate breaking rates (can be influenced, e.g., by varying shear stress with different agitation rates/modes/systems) is investigated. As distribution of biomass, and therewith cell aggregate size, can influence the paclitaxel accumulation (Kolewe et al., 2011), the proposed model can be used for an optimization of breaking rates and therefore an increased paclitaxel production.

Models concerning hairy root cultures (HR)

In 1989, Taya et al. described a segregated model for *Daucus carota* (wild carrot), *Armoracia lapathifolia* (horse radish), *Cassia torosa* (styptic weed), and *Ipomoea aquatica* (water spinach) batch cultures (1989). The approach is based upon the filamentous branching models used for the simulation of fungus cultivations and thereby utilized the linear growth law. Assuming a one-dimensional growth at the root tip meristem (growing point), a branching of growing points after a specific time, and the growing point decay caused by shear stress and other environmental influences, the model is able to simulate and characterize properties, like dry biomass and kinetic parameters of different HR cultivations. In addition to their mathematical approach, Taya et al. compared the specific growth rates and saturation constants of the four species and also compared the influence of different

fermentation systems (Erlenmeyer flask, turbine pump, airlift, rotating drum) on the decay rate constant of *D. carota* cultivations.

Five years later, Nakashimada et al. developed a segregated model concerning substrate utilization and influence of plant hormone 1-naphthalenacetic acid (NAA, auxin) on *A. lapathifolia* HR (1994). The experiments showed that NAA was quickly absorbed, and the number of root apical meristems increased while the root elongation rate decreased. However, with an optimal dosage of NAA the maximum specific growth rate can be increased. The kinetic model is an extension of the approach of Taya et al. (1989), adding both, the inhibitory and stimulating effects of NAA into the calculations. In addition, repeated batch cultivations were performed and the equations were fitted, generating accurate simulations and emphasizing the influence of NAA on the cultivation.

The next year, Uozumi et al. constructed a segregated model with focus on the implications of initial nutrient and hormone conditions for 20-hydroxyecdysone production (1995). Therefore, the *Ajuga reptans* (blue bugle) cultivation is divided into a biomass and a product formation phase with different phosphate and indoleacetic acid (IAA, plant hormone, auxin) concentrations. This was achieved by a two-stage fed batch cultivation, adding IAA in the biomass formation phase and reducing phosphates in the secondary metabolite production phase. The model is a continuation of approaches mentioned above (Nakashimada et al., 1994; Taya et al., 1989), altered for secondary metabolite production, phosphate accumulation, and elicitation with IAA.

In 2004, Han et al. invented a segregated model for *Helianthus annuus* (annual sunflower) HR (2004). Here, cells are classified into dividing tip cells (apical meristem) and the non-dividing residue. Moreover, a weight distribution function is used, describing the age of the branches at a certain time as well as an elongation (by cell division) and a splitting (by formation of new tips) function. Both, elongation and splitting are limited by a characteristic age above which the tip cells are unable to grow. The final model is fitted to experimental

data of *H. annuus* and six other datasets taken from literature, demonstrating the predictive capability of the model and comparing the characteristics of different HR.

Four years later, Cloutier et al. developed a structured model valid for HR **and** SU with focus of biomass formation and different nutrient limitations (2008). The approach contains twelve equations for the calculation of extracellular biomass, sucrose, sugars (sum of glucose and fructose), phosphate, nitrate, and ammonium concentration; intracellular phosphate, nitrogen, sugars, and stored carbohydrate concentration as well as specific growth rate and medium volume. Furthermore, a short comparison with seven other plant nutrition models is included, just as a sensitivity analysis for the identification of critical parameters. The required experimental data were gained from *C. roseus* and *D. carota* HR as well as *Eschscholzia californica* (California poppy) SU. Altogether, the proposed model is able to simulate and predict growth and substrate utilization of different plant species and culture types, and may be used especially for media optimization or process control strategies.

A not complex model, assuming non-limiting growth based on a proper feeding strategy, was suggested by Mairet et al. (2010). To accomplish this, the optimal feeding medium must be selected and a constant nutrient supply ensured. The concentrations of sucrose, nitrate, ammonium, phosphate, and potassium were measured and their influence on the specific growth rate was represented in a multiple Haldane type kinetic. Supposing the ideal nutrient concentrations are the initial ones, the objective is to keep levels constant. Therefore, it is important that the actual substrate concentrations be known at any time (online measurement) or appraised via simulations. However, if the estimated feeding rate is too high, overfeeding can occur. Hence, a safety coefficient is proposed, along with the use of online monitoring. The required parameters were estimated by data of a *Datura innoxia* (moonflower) cultivation.

Another not complex model was developed two years later by Palavalli et al. dealing mainly with oxygen transfer limitations in *Azadirachta indica* (neem) cultivations (2012). A major

challenge of process up-scaling is oxygen transport limitation, especially with dense root networks. Taking the dissolved oxygen concentration into account, an effectiveness factor (η) was introduced and added to the Monod style specific growth rate (Equation (1)). Targeting 100 % effectiveness, the agitation (which influences η in the mathematical model through *Re* and *Sh*) can be optimized and, the optimal oxygen supply can be assured.

Other models worth mentioning

Jolicoeur et al. proposed a structured model for a HR and fungus symbiosis system, focusing on nutrient (especially phosphate) concentrations and species interactions (2002). The publication of Omar et al. contained the comparison of different mathematical approaches (Monod, Logistic, and Gompertz) for *Centella asiatica* (Asiatic pennywort) SU in shake flasks and a stirred tank reactor (2006). Another model for *C. roseus* was invented by Leduc et al., containing a detailed analysis on metabolic regulation and cell nutrition (2006). Yan et al. focused on increased tanshinone yields in *Salvia miltiorrhiza* (Chinese sage) HR through elicitation and adsorption together with a corresponding model (2011). Finally, Lenk et al. published an article concerning 3D growth measurement and modeling of *Beta vulgaris* (beet) HR in Petri dishes with solid medium (2013).

Conclusion and prospects

As mentioned at the beginning, model development or selection should lead to the most simple but still suitable approach. However, it has to be considered that plenty of the proposed models are specialized for specific plant species, nutrition conditions etc. Thus generally, there is neither the best nor the worst model, only the most appropriate for the given case. Nevertheless, some of the publications will be highlighted in the following.

Bailey and Nicholson did a great leap forward with their structured model, being the basis for a lot of other approaches (1989; 1990). It may be too simple for specialized problems, but it is a great first attempt, particularly when product formation is important. Taya et al. published their branching growth model and several experimental data on three pages only

(1989), demonstrating plainness and efficiency. Clarifying the influence of different temperatures with an easy manageable set of equations, the model proposed by Glicklis et al. is a good choice for temperature and product (especially polysaccharide) optimization (1998). Provided that the very number of variables (26), parameters (26), and formulas (35) are indicators for model complexity, the work of van Gulik et al. is one of the most ambitious ones investigated in this review (1993), most suitable for problems with phosphate or glucose limitation. The model proposed by Cloutier et al. shows also great potential, being utilizable for both, HR and SU cultures (2008). Furthermore, the publication included plenty of supporting data, e.g., specifications of the media used, influence of initial nutrient concentrations and a comparison with other models.

Besides selecting the adequate model, suitable software has to be chosen. To sum up, the authors want to present three grades of software. A powerful numerical computing environment is MatLab® (The Mathworks, Inc., Natick, MA), e.g., used by Cloutier et al. (2008). However, the numerous possibilities may be overstraining and require a certain knowledge in programming. For solving a set of differential equations and displaying them graphically, Berkeley Madonna™ (Robert I. Macey & George F. Oster, University of California, CA) is a good and easy alternative and only basic programming skills are required. Some of the simplest models were implemented with standard spreadsheet applications, e.g., Microsoft® Excel® (Microsoft Corporation, Redmond, WA), whereby no additional software has to be acquired.

Finally, the current review gives a survey over models applied in plant biotechnology – including cell and tissue cultures – within the past 25 years. Using the provided tables and figures, a fast and convenient tool is supplied to support model theoreticians finding an appropriate model and at the same time highlight research needs for new model approaches.

Acknowledgments

The authors thank Leslie Sigmon, Katrin Pönisch-Pörschke, and Dr. Andreas Winkler for language support as well as Dr. Juliane Steingroewer, Maria Lisa Socher, Sibylle Kümmritz, and Christiane Haas for enriching comments and stimulating discussions. This work was financially supported by Central Innovation Program of the Federal Ministry of Economics and Technology (BMWi) (project number: KF2049811MD3).

Conflict of interest disclosure

The authors have declared no commercial, financial or other conflicts of interest.

Symbols and abbreviations

α, growth-associated product constant in $g \cdot g^{-1}$; β, non-growth-associated product constant in $g \cdot g^{-1} \cdot h^{-1}$; c_X, biomass concentration in $g \cdot L^{-1}$; c_P, product concentration in $g \cdot L^{-1}$; c_S, substrate concentration in $g \cdot L^{-1}$; i, concentration of inhibitory substrate (calcium) in $mol \cdot L^{-1}$; η, effectiveness factor; K_I, inhibition constant in $mol \cdot L^{-1}$; K_S, affinity constant of substrate in $g \cdot L^{-1}$ or $mol \cdot L^{-1}$; OUR, oxygen uptake rate in $mol \cdot (L \cdot h)^{-1}$; *Re*, Reynolds number; *Sh*, Sherwood number; V, viability in % or $g \cdot g^{-1}$; $Y_{X/S}$, yield of dry weight on substrate in $g \cdot g^{-1}$

F, fructose; G, glucose; HR, hairy root(s), hairy root culture(s); IAA, indoleacetic acid; NAA, 1-Naphthalenacetic acid; n.c., simple/not complex; NMR, nuclear magnetic resonance; S, sucrose; SU, plant suspension(s), plant suspension culture(s)

References

Allen MP, Tildesley DJ. 1989. Computer Simulation of Liquids. Oxford University Press.

Bailey CM, Nicholson H. 1989. A new structured model for plant cell culture. Biotechnol. Bioeng. 34:1331-1336.

Bailey CM, Nicholson H. 1990. Optimal temperature control for a structured model of plant cell culture. Biotechnol. Bioeng. 35:252-259.

Bailey CM, Nicholson H, Morris P, Smart NJ. 1985. A simple model of growth and product formation in cell suspensions of *Catharanthus roseus* G. Don. Appl. Biochem. Biotechnol. 11:207-219.

Bailey JE. 1998. Mathematical modeling and analysis in biochemical engineering: past accomplishments and future opportunities. Biotechnol. Prog. 14:8-20.

Bastian P, Chavarría-Krauser A, Engwer C, Jäger W, Marnach S, Ptashnyk M. 2008. Modelling in vitro growth of dense root networks. J. Theor. Biol. 254:99-109.

Becker T, Krause D. 2010. Softsensorsysteme – Mathematik als Bindeglied zum Prozessgeschehen. Chem. Ing. Tech. 82:429-440.

Bley T. 2011. From Single Cells to Microbial Population Dynamics: Modelling in Biotechnology Based on Measurements of Individual Cells. In: Müller, S, Bley, T, editors. *High Resolut. Microb. Single Cell Anal.* Springer Berlin Heidelberg. Advances in Biochemical Engineering / Biotechnology 124:211-227.

Bramble JL, Graves DJ, Brodelius P. 1991. Calcium and phosphate effects on growth and alkaloid production in *Coffea arabica*: experimental results and mathematical model. Biotechnol. Bioeng. 37:859-868.

Choi J-W, Kim Y-K, Lee WH, Pedersen H, Chin C-K. 1999. Kinetic model of cell growth and secondary metabolite synthesis in plant cell culture of *Thalictrum rugosum*. Biotechnol. Bioprocess Eng. 4:129-137.

Cloutier M, Bouchard-Marchand É, Perrier M, Jolicoeur M. 2008. A predictive nutritional model for plant cells and hairy roots. Biotechnol. Bioeng. 99:189-200.

Curtis WR, Hasegawa PM, Emery AH. 1991. Modeling linear and variable growth in phosphate limited suspension cultures of Opium poppy. Biotechnol. Bioeng. 38:371-379.

Doran PM. 2009. Bioreactors, Stirred Tank for Culture of Plant Cells. In: Flickinger, MC, editor. *Encycl. Ind. Biotechnol.* Bioprocess, bioseparation, and cell technology. John Wiley & Sons, Inc.

Dörnenburg H, Knorr D. 1995. Strategies for the improvement of secondary metabolite production in plant cell cultures. Enzyme Microb. Technol. 17:674-684.

Dunn IJ, Heinzle E, Ingham J, Prenosil JE. 2003. Biological Reaction Engineering: Dynamic Modelling Fundamentals with Simulation Examples. 2nd edition. Wiley-VCH. p 524.

Eibl R, Eibl D. 2008. Design of bioreactors suitable for plant cell and tissue cultures. Phytochem. Rev. 7:593-598.

Frazier GC. 1989. A simple, leaky cell growth model for plant cell aggregates. Biotechnol. Bioeng. 33:313-320.

Fredrickson AG, Ramkrishna D, Tsuchiya HM. 1967. Statistics and dynamics of procaryotic cell populations. Math. Biosci. 1:327-374.

Gaden EL. 1959. Fermentation process kinetics. J. Biochem. Microbiol. Technol. Eng. 1:413-429.

Geipel K, Socher ML, Haas C, Bley T, Steingroewer J. 2013. Growth kinetics of a *Helianthus annuus* and a *Salvia fruticosa* suspension cell line: Shake flask cultivations with online monitoring system. Eng. Life Sci. 13:593-602.

Geipel K, Song X, Socher M, Kümmritz S, Püschel J, Bley T, Ludwig-Müller J, Steingroewer J. 2014. Induction of a photomixotrophic plant cell culture of

Helianthus annuus and optimization of culture conditions for improved α-tocopherol production. Appl. Microbiol. Biotechnol. 98:2029-2040.

Georgiev M, Pavlov A, Bley T. 2007. Hairy root type plant in vitro systems as sources of bioactive substances. Appl. Microbiol. Biotechnol. 74:1175-1185.

Georgiev M, Weber J, Maciuk A. 2009. Bioprocessing of plant cell cultures for mass production of targeted compounds. Appl. Microbiol. Biotechnol. 83:809-823.

Georgiev M, Eibl R, Zhong J-J. 2013. Hosting the plant cells in vitro: recent trends in bioreactors. *Appl. Microbiol. Biotechnol.* **97**:3787–3800.

Glicklis R, Mills D, Sitton D, Stortelder W, Merchuk JC. 1998. Polysaccharide production by plant cells in suspension: experiments and mathematical modeling. Biotechnol. Bioeng. 57:732-740.

Guardiola J, Iborra JL, Canovas M. 1995. A model that links growth and secondary metabolite production in plant cell suspension cultures. Biotechnol. Bioeng. 46:291-297.

Van Gulik WM, Ten Hoopen HJ, Heijnen JJ. 1993. A structured model describing carbon and phosphate limited growth of *Catharanthus roseus* plant cell suspensions in batch and chemostat culture. Biotechnol. Bioeng. 41:771-780.

De Gunst MCM, Harkes PAA, Val J, van Zwet WR, Libbenga KR. 1990. Modelling the growth of a batch culture of plant cells: A corpuscular approach. Enzyme Microb. Technol. 12:61-71.

Haas C, Weber J, Ludwig-Muller J, Deponte S, Bley T, Georgiev M. 2008. Flow cytometry and phytochemical analysis of a sunflower cell suspension culture in a 5-L bioreactor. Z. für Naturforschung C J. Biosci. 63:699-705.

Han B, Linden JC, Gujarathi NP, Wickramasinghe SR. 2004. Population Balance Approach to Modeling Hairy Root Growth. Biotechnol. Prog. 20:872-879.

Haseltine EL, Rawlings JB. 2005. Critical Evaluation of Extended Kalman Filtering and Moving-Horizon Estimation. Ind. Eng. Chem. Res. 44:2451-2460.

Hepler PK, Wayne RO. 1985. Calcium and Plant Development. Annu. Rev. Plant Physiol. 36:397-439.

Hjortso MA. 1997. Mathematical modeling of hairy root growth. In: Doran, PM, editor. *Hairy Roots Cult. Appl.* Harwood Academic Publishers: Amsterdam, The Netherlands, p 169-178.

Hooker BS, Lee JM. 1992. Application of a new structured model to tobacco cell cultures. Biotechnol. Bioeng. 39:765-774.

Jolicoeur M, Bouchard-Marchand E, Bécard G, Perrier M. 2002. Regulation of mycorrhizal symbiosis: development of a structured nutritional dual model. Ecol. Model. 158:121-142.

Kalman RE. 1960. A new approach to linear filtering and prediction problems. J. Basic Eng. 82:35-45.

Kieran PM. 2001. Bioreactor design for plant cell suspension cultures. New Jersey: Harwood Academic. Principles of multiphase reactor design.

King RE, Aragona J, Constantinides A. 1974. Specific optimal control of a batch fermentor. Int. J. Control 20:869-879.

Knobloch K-H, Beutnagel G, Berlin J. 1981. Influence of accumulated phosphate on culture growth and formation of cinnamoyl putrescines in medium-induced cell suspension cultures of *Nicotiana tabacum*. Planta 153:582-585.

Kolewe ME, Gaurav V, Roberts SC. 2008. Pharmaceutically Active Natural Product Synthesis and Supply via Plant Cell Culture Technology. Mol. Pharm. 5:243-256.

Kolewe ME, Henson MA, Roberts SC. 2011. Analysis of aggregate size as a process variable affecting paclitaxel accumulation in *Taxus* suspension cultures. Biotechnol. Prog. 27:1365-1372.

Kolewe ME, Roberts SC, Henson MA. 2012. A population balance equation model of aggregation dynamics in *Taxus* suspension cell cultures. Biotechnol. Bioeng. 109:472-482.

Kottmeier K, Weber J, Müller C, Bley T, Büchs J. 2009. Asymmetric division of *Hansenula polymorpha* reflected by a drop of light scatter intensity measured in batch microtiter plate cultivations at phosphate limitation. Biotechnol. Bioeng. 104:554-561.

Krull R, Wucherpfennig T, Esfandabadi ME, Walisko R, Melzer G, Hempel DC, Kampen I, Kwade A, Wittmann C. 2013. Characterization and control of fungal morphology for improved production performance in biotechnology. J. Biotechnol. 163:112-123.

Leduc M, Tikhomiroff C, Cloutier M, Perrier M, Jolicoeur M. 2006. Development of a kinetic metabolic model: application to *Catharanthus roseus* hairy root. Bioprocess Biosyst. Eng. 28:295-313.

Lee JM. 1992. Biochemical engineering. Englewood Cliffs, NJ: Prentice Hall X, p 321.

Lenk F, Sürmann A, Oberthür P, Schneider M, Steingroewer J, Bley T. 2013. Modeling hairy root tissue growth in in vitro environments using an agent-based, structured growth model. Bioprocess Biosyst. Eng.1-12.

Li C, Yuan Y-J, Wu J-C, Hu Z-D. 2003. A structured kinetic model for suspension cultures of *Taxus chinensis* var. mairei induced by an oligosaccharide from *Fusarium oxysporum*. Biotechnol. Lett. 25:1335-1343.

Luedeking R, Piret EL. 1959. Transient and steady states in continuous fermentaion. Theory and experiment. J. Biochem. Microbiol. Technol. Eng. 1:431-459.

Mairet F, Villon P, Boitel-Conti M, Shakourzadeh K. 2010. Modeling and optimization of hairy root growth in fed-batch process. Biotechnol. Prog. 26:847-856.

Monod J. 1959. Recherche sur la croissance des cultures bactériennes. Hermann. Actualités scientifiques et industrielles.

Mulabagal V, Tsay H-S. 2004. Plant Cell Cultures - An Alternative and Efficient Source for the Production of Biologically Important Secondary Metabolites. Int. J. Appl. Sci. Eng. 29-48.

Müller S, Bley T. 2010. Flow cytometry. In: Flickinger, MC, Drew, S, editors. New York: Wiley. *Encycl. Ind. Biotechnol.* Bioprocess, bioseparation, and cell technology. 7, p 2451-2467.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Mustafa NR, de Winter W, van Iren F, Verpoorte R. 2011. Initiation, growth and cryopreservation of plant cell suspension cultures. Nat Protoc. 6:715-742.

Nakashimada Y, Uozumi N, Kobayashi T. 1994. Stimulation of emergence of root apical meristems in horseradish hairy root by auxin supplementation and its kinetic model. J. Ferment. Bioeng. 77:178-182.

Omar R, Abdullah MA, Hasan MA, Rosfarizan M, Marziah M. 2006. Kinetics and modelling of cell growth and substrate uptake in *Centella asiatica* cell culture. Biotechnol. Bioprocess Eng. 11:223-229.

Palavalli RR, Srivastava S, Srivastava AK. 2012. Development of a Mathematical Model for Growth and Oxygen Transfer in In Vitro Plant Hairy Root Cultivations. Appl. Biochem. Biotechnol. 1-14.

Pires Cabral PMNAC, Lima Costa ME, Cabral JMS. 2000. A structured growth model for *Cynara cardunculus* cell suspension. Bioprocess Eng. 23:199-203.

Rao SR, Ravishankar GA. 2002. Plant cell cultures: Chemical factories of secondary metabolites. Biotechnol. Adv. 20:101-153.

Sagmeister P, Wechselberger P, Jazini M, Meitz A, Langemann T, Herwig C. 2013. Soft sensor assisted dynamic bioprocess control: Efficient tools for bioprocess development. Chem. Eng. Sci. 96:190-198.

Schlatmann JE, Koolhaas CMA, Vinke JL, ten Hoopen HJG, Heijnen JJ. 1995a. The role of glucose in ajmalicine production by *Catharanthus roseus* cell cultures. Biotechnol. Bioeng. 47:525-534.

Schlatmann JE, Moreno PRH, Vinke JL, Ten Hoopen HJG, Verpoorte R, Heijnen JJ. 1997. Gaseous metabolites and the ajmalicine production rate in high density cell cultures of *Catharanthus roseus*. Enzyme Microb. Technol. 20:107-115.

Schlatmann JE, Ten Hoopen HJG, Heijnen JJ. 1999. A simple structured model for maintenance, biomass formation, and ajmalicine production by nondividing *Catharanthus roseus* cells. Biotechnol. Bioeng. 66:147-157.

Schlatmann JE, Vinke JL, ten Hoopen HJG, Heijnen JJ. 1995b. Relation between dissolved oxygen concentration and ajmalicine production rate in high-density cultures of *Catharanthus roseus*. Biotechnol. Bioeng. 45:435-439.

Schügerl K. 2001. Progress in monitoring, modeling and control of bioprocesses during the last 20 years. J. Biotechnol. **85**:149-173.

Shibasaki N, Yonemoto T, Tadaki T. 1993. Application of five-state structured models for growth and secondary metabolite production in cultures of *Nicotiana tabacum*. J. Chem. Technol. Biotechnol. 58:151-157.

Sirois J, Perrier M, Archambault J. 2000. Development of a two-step segregated model for the optimization of plant cell growth. Control Eng. Pract. 8:813-820.

Smetanska I. 2008. Production of secondary metabolites using plant cell cultures. In: Stahl, U, Donalies, UB, Nevoigt, E, editors. *Food Biotechnol.* Springer Berlin Heidelberg. Advances in Biochemical Engineering / Biotechnology, 111, p 187-228.

Takeda T, Takeuchi Y, Seki M, Furusaki S, Matsuoka H. 1998. Kinetic analysis of cell growth and vitamin E production in plant cell culture of *Carthamus tinctorius* using a structured model. Biochem. Eng. J. 1:233-242.

Taya M, Kino-oka M, Tone S, Kobayashi T. 1989. A Kinetic Model of Branching Growth of Plant Hairy Root. J. Chem. Eng. Jpn. 22:698-700.

Uozumi N, Makino S, Kobayashi T. 1995. 20-Hydroxyecdysone production in Ajuga hairy root controlling intracellular phosphate content based on kinetic model. J. Ferment. Bioeng. 80:362-368.

Weathers P, Towler M, Xu J. 2010. Bench to batch: advances in plant cell culture for producing useful products. Appl. Microbiol. Biotechnol. 85:1339-1351.

Yan Q, Wu J, Liu R. 2011. Modeling of tanshinone synthesis and phase distribution under the combined effect of elicitation and in situ adsorption in *Salvia miltiorrhiza* hairy root cultures. Biotechnol. Lett. 33:813-819.

Zhang J, Su WW. 2002. Estimation of intracellular phosphate content in plant cell cultures using an extended Kalman filter. J. Biosci. Bioeng. 94:8-14.

Figures

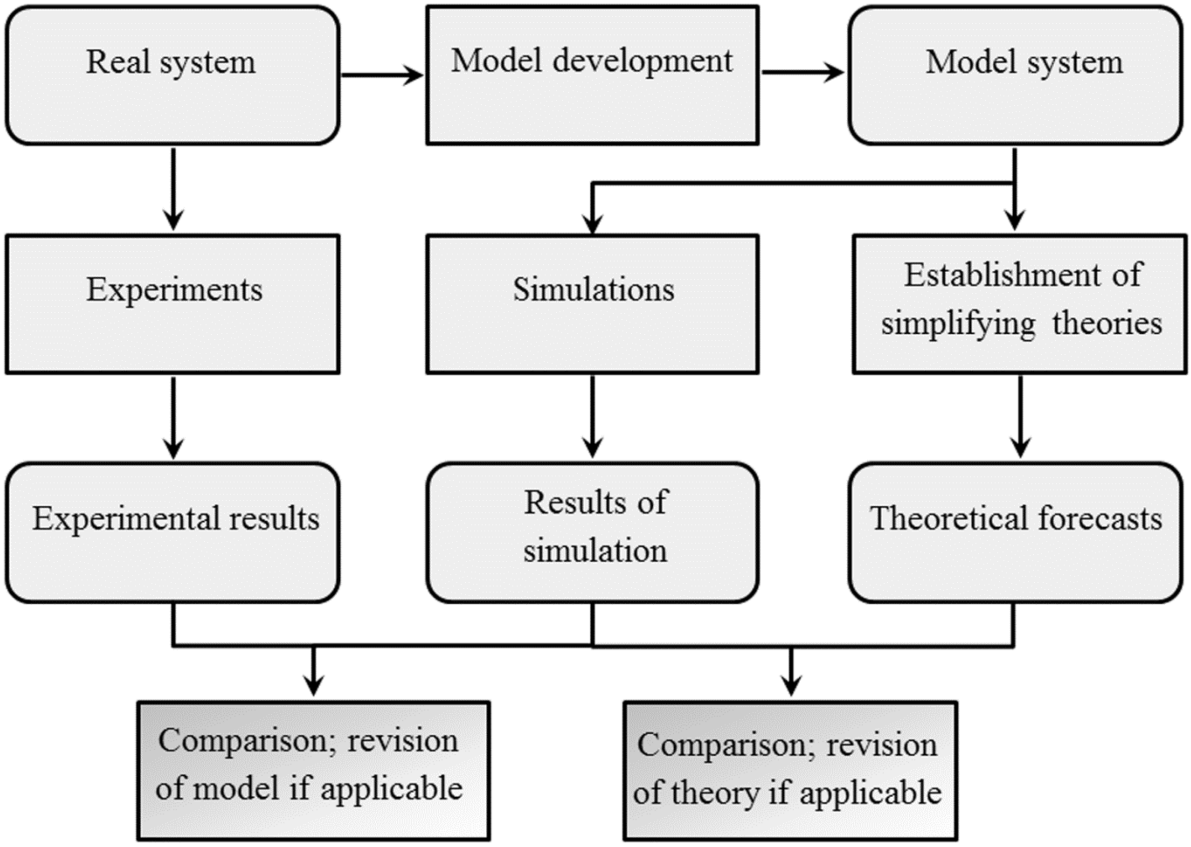

Figure 1: Scheme of model development and evaluation according Allen and Tildesley (1989).

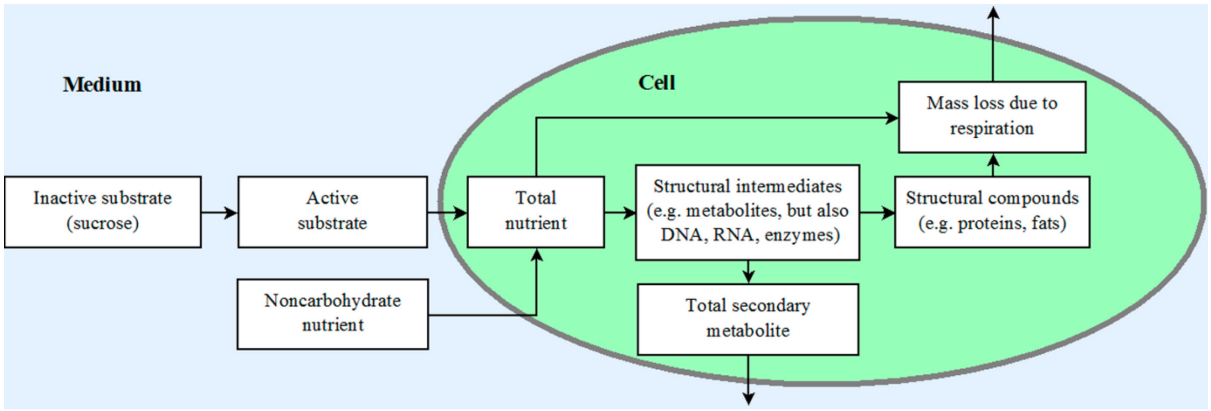

Figure 2: Kinetic pathways proposed by Hooker and Lee (1992).

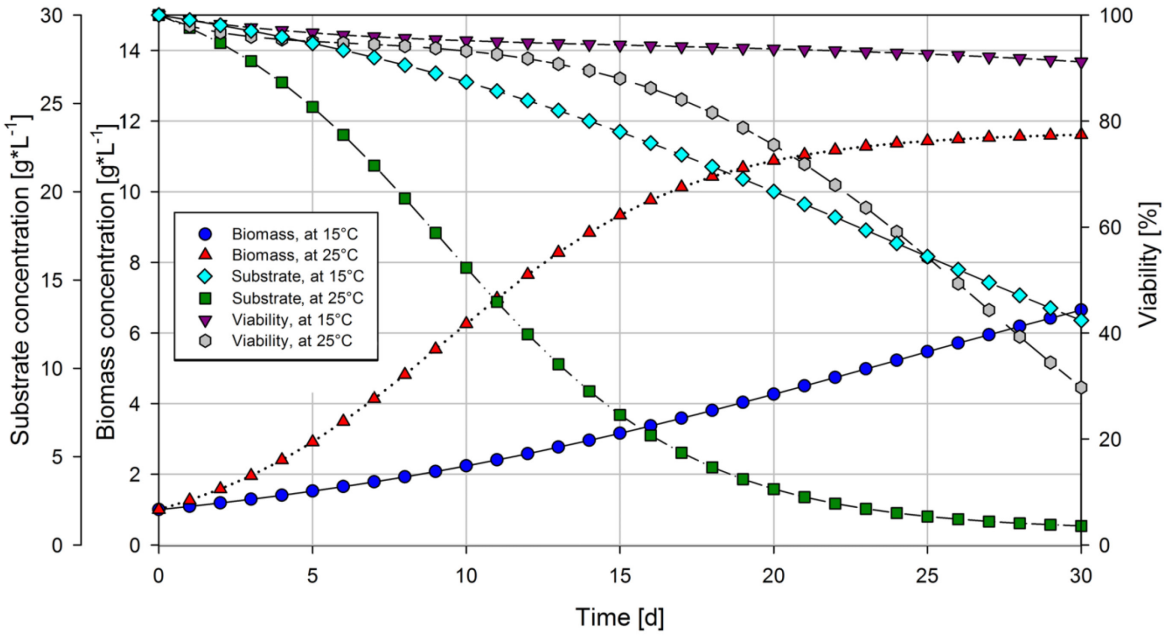

Figure 3: Simulated graphs for dry biomass and substrate concentration (left axis) and cell viability (right axis) each at 15°C respective 25°C, modeled with data of *S. officinale* batch cultures from Glicklis et al. (1998) simulated by the authors using Berkeley Madonna™.

Tables

Table IA: Survey of investigated modeling approaches; Models for SU are marked white, approaches for submerged HR are highlighted in light grey and approaches concerning both, SU and HR are marked dark grey.

	1	2	3	4	5	6	7	8	9	10
	Classification			**Nutrients**						**Additives**
No.	**Author(s)**	**Year**	**Model complexity**	**Substrate in general**	**Multiple C sources**	**PO_4**	**N_2**	**O_2**	**Other substrates**	**Hormones**
1	Frazier	(1989)	n.c.	Yes	Yes (Substrate intermediate)	No	No	No	No	No
2	Taya et al.	(1989)	Segregated	Yes	No	No	No	No	No	No
3	Bailey & Nicholson	(1989) (1990)	Structured	Simplified	No	No	No	No	No	No
4	De Gunst et al.	(1990)	Segregated/ corpuscular	Yes	No	No	No	No	No	Hormones in general
5	Bramble et al.	(1991)	n.c.	Yes	No	Yes	No	No	No	No
6	Curtis et al.	(1991)	n.c.	Yes	No	Yes	No	No	No	No

7	Hooker & Lee	(1992)	Structured	Yes	Indirect by substrate scission	No	No	No	Unspecified, no C source	No
8	Shibasaki et al.	(1993)	Structured	Yes	No	No	No	No	No	No
9	Van Gulik et al.	(1993)	Structured	Yes	No	Yes	No	Indirect by CO_2	No	No
10	Nakashimada	(1994)	Segregated	Yes	No	No	No	No	No	NAA
11	Guardiola et al.	(1995)	n.c.	Yes	No	No	No	No	No	No
12	Uozumi et al.	(1995)	Segregated	Yes	No	Yes	No	No	No	IAA
13	Glicklis et al.	(1998)	Structured	Yes	No	No	No	No	No	No
14	Takeda et al.	(1998)	Structured	Yes	No	Yes	No	No	Respiratory intermediated	No
15	Choi et al.	(1999)	Structured	Yes	Yes (S, G, F)	No	No	No	No	No
16	Schlatmann et al.	(1999)	Structured	Yes	No	No	No	Indirect by CO_2	No	No
17	Pires Cabral et al.	(2000)	Structured	Yes	No	Yes	No	No	No	No
18	Sirois et al.	(2000)	Segregated	Yes	No	Yes	No	No	No	No

19	Zhang & Su	(2002)	n.c.	Yes	No	Yes	No	By OUR	No	No
20	Li et al.	(2003)	Structured	Yes	Yes (S, G, F)	No	No	No	No	No
21	Han et al.	(2004)	Segregated	No	No	No	No	No	No	No
22	Cloutier et al.	(2008)	Structured	Yes	Yes (S, G+F)	Yes	NO_3, NH_4	No	No	No
23	Mairet et al.	(2010)	n.c.	Yes	No	Yes	NO_3, NH_4	No	K	No
24	Kolewe et al.	(2012)	Segregated	Yes	No	No	No	No	No	No
25	Palavalli et al.	(2012)	n.c.	Yes	No	No	No	Yes	No	No

F, fructose; G, glucose; HR, hairy root(s), hairy root culture(s); IAA, indoleacetic acid; NAA, 1-Naphthalenacetic acid; n.c., simple/not complex; S, sucrose; SU, plant suspension(s), plant suspension culture(s). * Please refer to table 1 in Georgiev et al. (2013) to gain more information about methods of feeding in plant biotechnology. ** Analysis carried out with help of "Web of Science" by Thomson Reuters™ (www.webofknowledge.com) on 01/28/2014

Table IB: Survey of investigated modeling approaches; Models for SU are marked white, approaches for submerged HR are highlighted in light grey and approaches concerning both, SU and HR are marked dark grey.

	11	12	13	14	15	16	17	18	19
	Products		**Mathematical & experimental parameters**						**Other**
No.	**Product in general**	**Product storage location**	**Viability**	**Variables**	**Parameters & constants**	**Operating mode***	**Plant species**	**Culture type**	**Number of quotations****
1	Inter-mediate	No	No	20	35	Batch	*Dioscorea deltoidea*	SU	15
2	No	No	No	9	15	Batch	*Daucus carota, Armoracia lapathifolia, Cassia torosa, Ipomoea aquatic*	HR	18
3	Yes	No	Yes	9	7	Batch	*Catharanthus roseus*	SU	(1989): 23 (1990): 17
4	No	No	No	10	7	Batch	*Nicotiana tabacum*	SU	13
5	Yes	No	No	7	11	Batch	*Coffea arabica*	SU	32
6	No	No	No	6	8	Batch	*Papaver somniferum*	SU	38

7	Yes	No	No	17	21	Batch	*Nicotiana tabacum*	SU	14
8	Yes	Yes	Yes	13	10	Batch	*Nicotiana tabacum*	SU	6
9	Yes	No	No	26	26	Batch, continuous	*Catharanthus roseus*	SU	35
10	No	No	No	13	24	Batch, repeated batch	*Armoracia rusticana*	HR	13
11	Yes	No	Yes	4	14	Batch, semi-continuous	*Vitis vinifera*	SU	12
12	Yes	No	No	16	30	Batch, fed-batch	*Ajuga reptans*	HR	12
13	Yes	Yes	Yes	8	15	Batch	*Symphytum officinale*	SU	8
14	Yes	Of product precursors	No	19	19	Batch	*Carthamus tinctorius*	SU	2
15	Yes	Yes	Yes	18	20	Batch	*Thalictrum rugosum*	SU	No spec.
16	Yes	No	No	19	11	Two stage batch	*Catharanthus roseus*	SU	5
17	Yes	No	No	17	14	Batch	*Cynara cardunculus*	SU	2

18	No	No	No	15	17	Batch	*Eschscholzia californica*	SU	2
19	No	No	No	8	14	Batch	*Anchusa officinalis*	SU	8
20	Yes	Yes	No	13	25	Batch	*Taxus chinensis*	SU	8
21	No	No	No	8	11	Batch, continuous	*Helianthus annuus*	HR	7
22	No	No	No	13	40	Batch	*Eschscholzia californica*	SU	12
							Catharanthus roseus, Daucus carota	HR	
23	No	No	No	20	27	Fed-batch	*Datura innoxia*	HR	2
24	No	No	No	12	7	Batch	*Taxus cuspidata*	SU	3
25	No	No	No	11	18	Batch	*Azadirachta indica*	HR	0

F, fructose; G, glucose; HR, hairy root(s), hairy root culture(s); IAA, indoleacetic acid; NAA, 1-Naphthalenacetic acid; n.c., simple/not complex; S, sucrose; SU, plant suspension(s), plant suspension culture(s). * Please refer to table 1 in Georgiev et al. (2013) to gain more information about methods of feeding in plant biotechnology. ** Analysis carried out with help of "Web of Science" by Thomson Reuters™ (www.webofknowledge.com) on 01/28/2014.

Supplementary Information Section

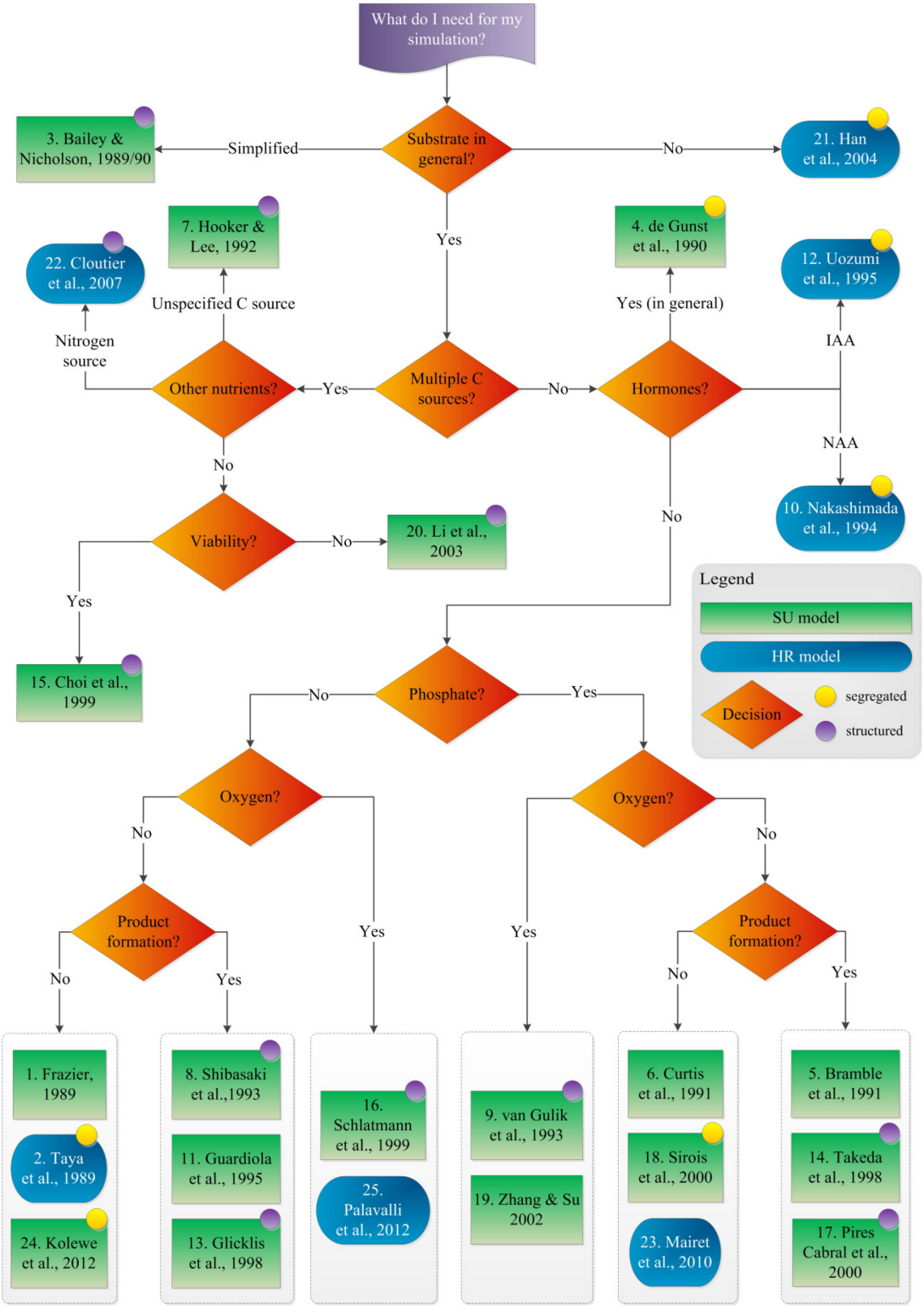

Figure S-1: Flowchart used in model selection for plant in vitro cultivation; selected publications.

Table S-I: Classification of investigated modeling approaches, explanations

No.	Classification		Description	Box content
	Rough	Explicit		
1	Classification	Author(s)	Publishing author(s)	Name(s)
2		Year	Publishing year	Date
3		Model complexity	Model structured, segregated or not complex (n.c.)?	Segregated/ structured/n.c.
4	Nutrients	Substrate in general	Concentration of substrate (carbon source) calculated?	Yes/no
5		Multiple C sources	Calculation based on more than one carbon source, e.g., G, F, S?	Yes (+ abbreviation of multiple C sources)/no
6		PO_4	Phosphate calculated?	Yes/no
7		N_2	Nitrogen source, e.g., NH_4 or NO_3 calculated?	Yes (+N source)/no
8		O_2	Oxygen as substrate or as limiting condition calculated?	Yes/no
9		Other substrates	Further substrates used for calculation (e.g., potassium (K))?	Yes (+substrate)/ no
10		Hormones	Hormone concentration calculated?	Yes (+hormone)/ no
11	Products	Product in general	Amount of product calculated?	Yes/no
12		Product storage location	Product storage divided into intracellular/extracellular?	Yes/no
13	Mathematical & experimental parameters	Viability	Cell viability used for calculation?	Yes/no
14		Variables	How many variables were calculated (including sum of variables)?	Number
15		Parameters & constants	How many parameters & constants (e.g., $Y_{X/S}$, k_s) – set or known from experiments – used?	Number
16		Operating mode*	Type of bioreactor operating mode considered?*	Batch, fed-batch, continuous, etc.*
17		Plant species	Plant(s)/biological systems used for experiments?	Plant species
18		Culture type	Culture types used for experiments?	SU/HR
19	Other	Number of quotations	Number of citations in subsequent publications?	Number

F, fructose; G, glucose; HR, hairy root(s), hairy root culture(s); n.c., simple/not complex; S, sucrose; SU, plant suspension(s), plant suspension culture(s). * Please refer to table 1 in Georgiev et al. (2013) to gain more information about methods of feeding in plant biotechnology.