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Bioprocess systems engineering: transferring traditional process engineering principles to industrial biotechnology

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Abstract: The complexity of the regulatory network and the interactions that occur in the intracellular environment of microorganisms highlight the importance in developing tractable mechanistic models of cellular functions and systematic approaches for modelling biological systems. To this end, the existing process systems engineering approaches can serve as a vehicle for understanding, integrating and designing biological systems and processes. Here, we review the application of a holistic approach for the development of mathematical models of biological systems, from the initial conception of the model to its final application in model-based control and optimisation. We also discuss the use of mechanistic models that account for gene regulation, in an attempt to advance the empirical expressions traditionally used to describe micro-organism growth kinetics, and we highlight current and future challenges in mathematical biology. The modelling research framework discussed herein could prove beneficial for the design of optimal bioprocesses, employing rational and feasible approaches towards the efficient production of chemicals and pharmaceuticals.

Review Article

Challenges in biotechnology

Biotechnology promises to deliver innovative and sustainable products and processes as solutions to various societal problems [1]. Important challenges in this field include sustainable production of biofuels from renewable sources as an alternative to fossil fuels, the exploitation of the metabolic capabilities of microorganisms towards the production of fine chemicals and pharmaceutical products, the construction of novel pathways for environmental bioremediation due to urban and industrial pollution, smooth transfer of lab scale experiments to pilot and production scale and the integration of biotechnology with chemical processes [2-4]. Recent advances in biotechnology have overcome several technological barriers encountered in industrial applications necessitating a deeper understanding of the underlying control mechanisms of cellular growth in order to achieve efficient and cost-effective bioprocesses.

Microorganisms hold great potential for industrial biotechnology, harbouring various metabolic pathways either for degradation of recalcitrant pollutants or the production of a series of compounds, which can be used as fuels, chemicals and pharmaceutical products [5]. However, the use of natural (wild type) microorganisms at large scale is usually hampered by sub-optimal bioprocesses in terms of yield, productivity and titre, along with the low tolerance of strains to process stresses, such as substrate and product toxicity, and fermentation inhibitors [6]. In order to improve the industrial efficacy of wild type microorganisms a variety of approaches have been proposed. Synthetic biology seeks optimal pathway configurations with the application of gene combinatorial methods to construct and consequently evaluate several metabolic pathways, combining genes from different sources. This *de novo* construction of artificial biological systems utilizes theoretical approaches for the design of modular system components [7-8]. Furthermore, systems biology methodologies attempt to use system-wide measurements obtained by high-throughput technologies in combination with mathematical methods for the elucidation and implementation of novel biosynthetic pathways and identification of genetic targets for modification [9]. Metabolic engineering also aims at the improvement of microbial strains for industrial application. Contrary to synthetic biology, metabolic engineering targets the optimisation of pathways by regulating the activity of intermediate reactions combining rational and combinatorial methods [10].

Mathematical models are increasingly becoming central to understanding and improving cellular based processes. However, with the field of biotechnology shifting from method development to application development [11], a systems biology approach of detailed, mechanistic modelling becomes problematic since modelling of complex biological systems inherently is an inverse problem that cannot be solved [12] and understanding of experimental information has lagged far behind data accumulation. Implementing microbial production on an industrial scale should focus towards bioprocess systems engineering strategies, which can ultimately enable control and optimisation at the bioprocess level [13].

Challenges in biological modelling

Despite the economic turmoil of the last few years, Thomson&Reuters concur that the bio-industry is a viable platform for low risk investments with a good profit margin [14]. Nonetheless, the bio-chemical industry requires improved process efficiency; alas, the sophisticated mathematical toolset that led to the explosive growth of manufacturing capacity in traditional chemical industries,

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known as Process Systems Engineering (PSE), is not readily applicable to the bio-industry. Obstacles hindering the adaptation of traditional PSE approaches to bio-processing include the complexity of the biological systems, the limited understanding of the biological processes, and the resulting lack of adequate process models. In the absence of model-based approaches, process optimisation in the bio-industry relies on extensive, and in certain cases unnecessary, experimentation.

The use of model-based techniques can facilitate the reduction of unnecessary experimentation by indicating the most informative experiments and providing strategies to optimise and automate the process at hand, resulting in a cost and time reduction. Mathematical models of biological systems developed in the past integrate various degrees of structure and mathematical complexity. Models of single cells, cell populations and cell cultures have been utilized in understanding and improving biological systems, as well as in the optimisation and control of bioprocesses [13]. Indicatively, mathematical models have been applied to various extents in the design of optimal media [15], the identification of previously ignored growth limiting factors [16], the optimization of culture growth and productivity [17, 18], and in the application of control approaches to cell culture processes [19].

Yet when Pörtner and Schäfer [20] compared a selection of models for cell growth and metabolism of hybridoma cell lines through an analytic error and range of validity analysis, they found significant variations in the values of maximum growth rate, yield and Monod constants. They concluded that the model predictions involved significant errors, particularly due to the limited understanding of cellular metabolism and the narrow data ranges within which the models were valid. The observed discrepancies were partly attributed to the absence of a formalised approach for the proper identification of process parameters. It was concluded that specific process models should be used for the estimation of specific types of process parameters. For example, it was suggested that static batch cultures should be used for the determination of the maximum specific growth rate, but not for establishing a relationship between the growth rate and substrate concentration, whereas continuous cultures could yield reliable data due to the steady-state operation conditions. For very low substrate concentrations, they suggested using fed-batch cultures.

The large-scale generation of biological data obtained with a variety of high-throughput experimental technologies demand the development of integrated mathematical models of cellular processes [21]; alas, integration of mathematical modelling in bio-processing has proven to be challenging. The way biochemical engineers conceive and mathematically describe biological processes, by and large, is still defined by a mathematical formulation derived a century ago to describe enzyme kinetics [22]. Although the hypothetical system studied was the simplest possible, the conversion of one molecule of a given substrate to a product via a single enzymatic reaction, it has shaped the way we conceive kinetic rates in biology. Since then, the theory provided by Michaelis and Menten has evolved, being used as a starting point when attempting to describe much more complex systems, such as microbial growth [23]. Considering the developments in analytical and molecular biology over the past decades brings Bailey's [24] argument that the development of mathematically and computationally orientated research has failed to catch up with developments in biology. Mathematical biology today revolves around mathematical expressions developed a hundred years ago (Table 1).

Notable studies attempting to introduce a new approach to biological systems modelling include, but are not limited to, cybernetic modelling presented by Ramkrishna [25], the introduction of structure as defined by Fredrickson [26] and extended to the genetic level by Lee and Bailey [27, 28]. The concept behind cybernetic modelling is the adaptation of a mathematically simple description of a complex organism which is compensated for over-simplification by assigning an optimal control motive to its response [29]. Microbial cells growing in the presence of multiple substrates are assumed to follow an invariant strategy to optimise a certain goal by choosing which substrate to consume first. Thus, by assuming a multi substrate environment containing cells that follow different strategies of substrate consumption, those cells that choose to grow first on the fastest substrate available will grow much faster than cells that respond differently. After some time all the cells that remain in the environment will be those that have responded in the optimal manner.

Table 1. Enzyme and microbial growth kinetic expressions.

Name	Expression	Function
Michaelis-Menten	$V_0 = \frac{V_{max}[S]}{K_m + [S]}$	Describes the kinetics of the simple enzyme catalysed reaction: $E + S \xleftarrow{k_1 / k_{-1}} ES \xrightarrow{k_2} P$
Hill	$\theta = \frac{[L]^n}{(K_A)^n + [L]^n}$	Describes the fraction of the macromolecule saturated by ligand as a function of the ligand concentration.
Monod	$\mu = \mu_{max} \frac{[S]}{K_S + [S]}$	Describes microbial growth based on the consumption of one substrate.

θ: fraction of occupied ligand binding sites; μ: the specific growth rate of a microorganism; μ_{max}: the maximum specific growth rate of a microorganism; k_1: rate constant for association of substrate and enzyme; k_{-1}: rate constant for dissociation of unconverted substrate from the enzyme; k_2: rate constant for dissociation of product from the enzyme; K_A: ligand concentration producing half occupation, which is also the microscopic dissociation constant; K_m: Michaelis constant; K_s: Monod coefficient; $[L]$: ligand concentration; n: Hill coefficient designating cooperativity; $[S]$: substrate concentration; V_0: initial velocity of the enzymatic reaction; V_{max}: maximum velocity of the enzymatic reaction.

Lee and Bailey [27, 28], extended the concept of structure to the level of nucleotide sequences. They introduced an explicit connection between a particular nucleotide sequence and the affinity of a particular protein for that sequence, which in turn influences the corresponding transcription event, deriving a quantitative mapping from nucleotide sequence to overall phenotype. Even though in his detailed review, Bailey [24], predicted that this new "genetically structured model" would be widely embraced in the future, supported by the advancement of the "omics" techniques, little work has been done in that direction.

Savageau [30-33] was amongst the first to investigate metabolic pathway control from a mathematical analysis point of view. A few years later the work of Kacser and Burns [34] and Heinrich and Rapoport [35] defined the field of Metabolic Control Analysis (MCA), which quantitatively studies the degree of flux control that is applied on a metabolic pathway by various effectors, such as enzyme activities and metabolite concentrations. Papoutsakis [36] demonstrated that it was possible to formulate balance equations using a metabolic map, a concept which later evolved into Flux Balance Analysis (FBA) [37, 38]. The idea of controlling flux balance through a given metabolic pathway towards achieving a desired overall behaviour (e.g. maximisation of product formation) was shaped into