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Multiscale modeling: A mathematical-based tool for bioprocess design, control and scale up/down

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Abstract

Performance of an industrial microbial strain can be determined not only by its genome but also by the environment surrounding it. Typically, microorganisms are isolated from natural environments where they have evolved to cope with environmental restrictions and interactions. However, when a microorganism is subject to a synthetically designed environment, like in industrial bioreactors, natural environmental pressures may not be present, forcing the microbe to behave differently. Hence, a microorganism needs to adapt expressing certain metabolic phenotypes that in its turn may alter the environment. Given this double-way conditioning, it is necessary to understand how microorganisms perform in a particular isolated environment through a multiscale modeling approach. At the macro scale, bioreactor modeling, which frequently assumes well mixed and homogeneous vessels, may not be sufficient to properly describe bioreactor's performance. On the other hand, addressing the culture by only modeling cells via metabolic models may result in a limited perspective at the micro scale. In this work, a mathematical tool is used to recreate observed metabolic behavior as a response to a highly dynamic environment in a bioreactor. Two mathematical models at different scales are coupled via specific q-rates to produce a tool that can be used to understand two-way interactions leading to improved bioreactor design or control but also for scaling purposes. The model at the macro scale follows a compartment-based approach by which hydrodynamics and substrate concentration gradients are recreated. These concentration gradients impose a metabolic response on the cells, which is modeled by a kinetic metabolic model.

Keywords

Bioprocess dynamics, Multiscale modeling, characteristic times, industrial bioreactors, metabolic response

Introduction

A deep understanding is often needed to design, control and scale up/down bioprocesses. Typically, the so-called chemical engineering approach is used for these purposes based on strong mathematical foundations [1-3]. However, this approach frequently tends to neglect the two-way interactions taking place in every industrial bioprocess [4]. A particular environment can exert certain constraints to biological systems, for instance, operation conditions could induce a distinctive microbial performance since the complex metabolic networks of intracellular reactions that define a phenotype are usually sensitive to them [5]. On the other hand, it is also true that robust regulatory mechanisms inside a cell could condition extracellular concentrations and other physicochemical properties of the surrounding liquid (rheology, temperature, acidity, etc.) [6-8]

Undoubtedly, chemical engineering principles consisting of mass, energy and momentum balances supported by sound mathematical modeling helps in understanding how complex living systems work in an industrial environment. Nevertheless, for a mathematical model to be somewhat descriptive it is required that most phenomena, both biological and physicochemical, can be described fulfilling physical laws and biological mechanisms (mostly regulatory) [4,8-10]. Unfortunately, industrial bioprocesses involve the interplay of many complex phenomena for some of which it is common not to have well-established thermodynamic laws [10-12].

Even though literature is rich in describing bioprocess modeling efforts, there is still room for new contributions towards a more comprehensive modeling approach [3,13,14]. Morchain et al. [9] redrew a schematic representation of modeling biological bioprocesses (Figure 1). In short, the problem to be described by means of a mathematical model consists of two main components: the biotic phase and the bioreactor phase. When modeling the biotic phase, living organisms can be described as behaving like an average or considering cell heterogeneity via population balances. More recent approaches have focused on modeling single cells given the vast amount of data available regarding genomics, transcriptomics, proteomics and metabolomics [15-18]. Nevertheless, according

to Morchain [9], constructing a comprehensive mathematical model over a bioreactor should involve topics like hydrodynamics, mass transfer expressions, population balance and reactions of the biotic phase (e.g., a particular set of metabolic reactions for a given microorganism). All those reactions regarding material transport across cell membranes and thermodynamic principles supporting technical feasibility should also be included. In principle, a comprehensive mathematical model for a bioprocess is a collection and coupling of different submodels that increase the complexity due to variations in scale and characteristic times [9]. Tackling these challenges is where multiscale modeling plays a critical role.

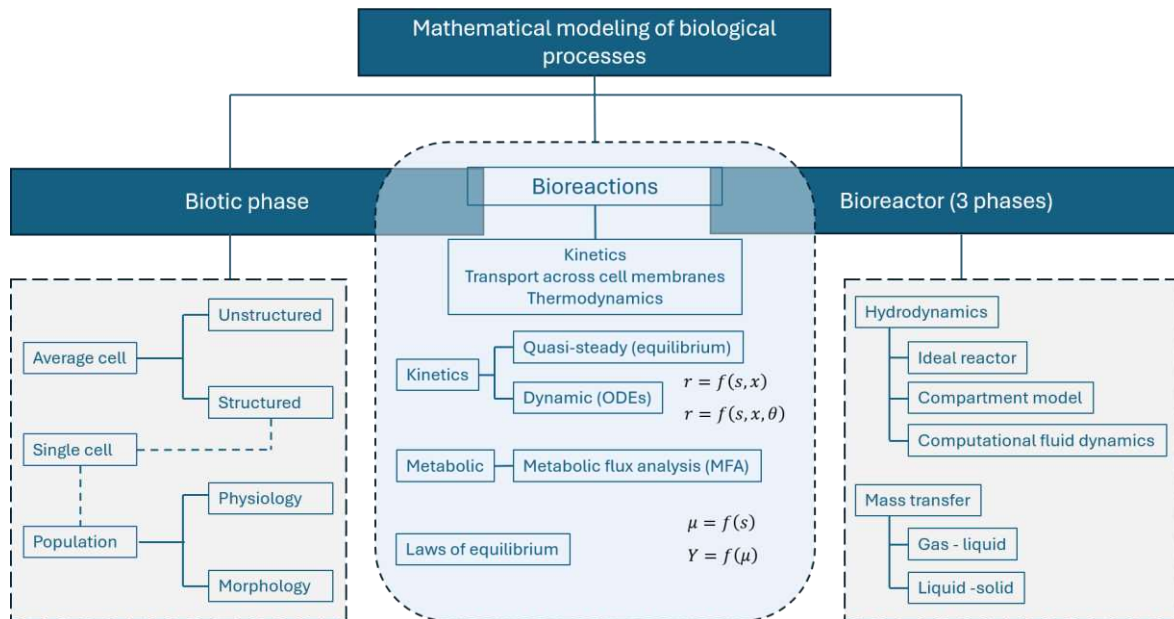


Figure 1. Main aspects to consider when modeling a bioprocess. Adapted from [9].

Furthermore, at the microbial level and to improve model outcomes, the use of *in vivo* dynamic metabolic data is mandatory in modeling metabolism since it allows for a more comprehensive understanding of the metabolic performance exhibited by different phenotypes encountered in industrial bioreactors. These phenotypes are the result of complex kinetic interactions such as allosteric control mechanisms that are usually described by *in vitro* assays limiting the capability for describing cell metabolism under non optimal conditions [19]. In this work, we address the metabolic performance of a microbial culture in industrial bioreactors by coupling two models, one at the mesoscale over the

bioreactor and the other at the microscale over the cell following a multiscale approach. Here, average non structured cells are considered to undergo dynamic perturbations in bioreactors, and their metabolic response is analyzed as two-direction interactions in large scale bioreactors.

Material and methods

Bioreactor modeling. In this work, we adapted the compartment model proposed by Vrábel et al. [21-22] to characterize an industrial bioreactor having 22 m³ of working volume. The complete set of equations can be found in the supplementary material, bioreactor modeling section. This model has also been previously used by other authors like Pigou and Morchain [4]. The model describes the geometry of an existing reactor (Figure 2) of a cylindrical shape with 4 Rushton turbines evenly placed along the axis (ratio of the distance between impellers to the impeller diameter is $1.46/0.7 = 2.086$). Vrábel's approach considers an axial symmetry; hence, the volume used for calculations is 11 m³, which is divided into 5 zones and 70 compartments (Figures 3a and 3b). Four of the zones are defined by the 4 impellers moving the fluid radially towards the vessel's wall. The fifth zone describes a region at the top of the liquid where a loop for air injection is considered (zone A). Air is directly sparged at the bottom of the vessel (compartment 66), hence, the design equations for the bottom compartments are adjusted correspondingly. Out of the 70 compartments, 15 are allocated to each of the four zones (60 in total) and 10 to zone A to account for the presence of superficial air.

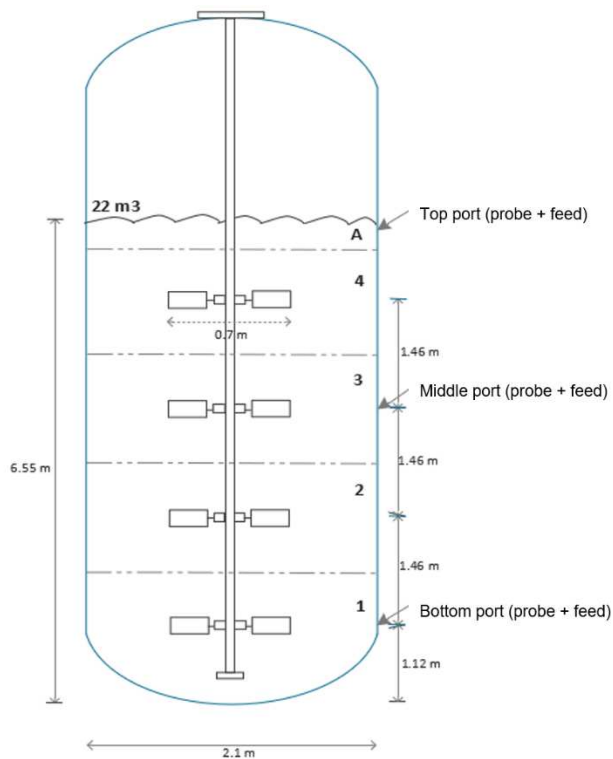


Figure 2. Bioreactor description

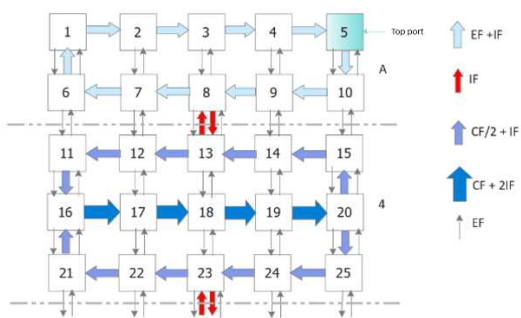


Figure 3a. Compartment scheme for the top part of the liquid (zone 4 and A)

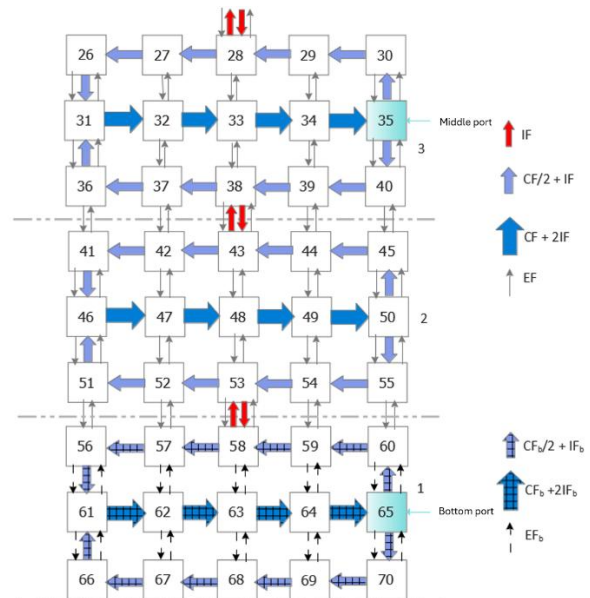


Figure 3b. Compartment scheme for the middle and bottom part of the liquid (zones 1 to 3)

The model description, which considers three fluxes for characterizing hydrodynamics (see the bioreactor modeling section in the supplementary material for a more detailed description of the model) is completed with the mass balance for component i in each compartment (Equations 1a and 1b.) Equation 1a includes a reactive term, R_i , and a term for gas-liquid mass transfer, T_i [4]. These two terms are replaced by an uptake/export term and the classical Fick's law model for mass transfer, respectively (Equation 1b). The reactive term depends on the amount of biomass present in the vessel (i.e., each compartment) while the transfer term depends on the actual concentration of either substrates or products in the vessel.

$$V_n \frac{\partial C_i^n}{\partial t} = R_i + T_i + \sum_{m=1}^N (M_{m,n}^f C_i^m) - C_i^n \sum_{m=1}^N (M_{n,m}^f) \quad (1a)$$

$$\frac{\partial C_i^n}{\partial t} = q_i^n X^n + k_l a (C_i^* - C_i^n) + \frac{1}{V_n} \sum_{m=1}^N (M_{m,n}^f C_i^m) - \frac{C_i^n}{V_n} \sum_{m=1}^N (M_{n,m}^f) \quad (1b)$$

In addition, for mass balance there is a flux matrix, M^f , with dimension $N \times N$, where N is the total number of compartments considered in the model, 70 in this work. Thus, $M_{m,n}^f$ represents the total flux transferred from the m -th to the n -th compartment. Each compartment volume, V_n , is assumed to be equal and constant.

gPROMS® software, a trademark of PSE-SIEMENS, was used for scripting not only the compartment model, but also the kinetic model, which it would be later coupled to. This software was also used for the corresponding parameter estimations using the embedded feature *Maximum Likelihood Estimator -MLE-*, and for subsequent simulations run for bioreactor characterization. The gPROMS® MLE method compares an experimental value with a predicted value for a given set of parameters; hence it searches for a set of parameters where the difference is minimized using Equation 2.

$$\varphi = \frac{N_d}{2} \ln(2\pi) + \frac{1}{2} \min \theta \left\{ \sum_{i=1}^{N_E} \sum_{j=1}^{N_{V_i}} \sum_{k=1}^{N_{M_{ij}}} \left[\ln(\sigma_{ijk}^2) + \frac{(\bar{z}_{ijk} - z_{ijk})^2}{\sigma_{ijk}^2} \right] \right\} \quad (2)$$

Where:

φ : objective function; N_d : total number of experimental data ; θ : parameter set to be estimated

N_E : Total number of experiments performed; N_{V_i} : total number of variables in experiment i
 NM_{ij} : Total number of experimental data of variable j in experiment i ; σ_{ijk}^2 : Variance of the k
 – th
 datum of variable j experiment i
 $\bar{z}_{ijk}$: dato exp. k de la variable j en experimento i ; z_{ijk} : predicción del modelo de la
 variable j en experimento i

Bioreactor characterization. The model was initially used to characterize the bioreactor by inspecting the mixing time. To do so, an *in-silico* addition of a tracer to the vessel was simulated implementing three different addition ports and three different probe locations (Figure 2 above). For these simulations neither a reactive term nor a gas-liquid transfer term was considered in the material balance for any of the compartments. The mixing time corresponds to the time elapsed to reach a stable concentration equal to 95% of the concentration value at infinite time. In addition, the time profile for dissolved oxygen is analyzed to consider the characteristic time for the liquid to get fully saturated with oxygen under the effects of hydrostatic pressure due to the water column in the reactor.

Following the hydrodynamic characterization, a preliminary characterization for the reactive system was performed to identify the concentration gradients and their influence on growth rate and biomass production throughout the reactive liquid using simple Monod's kinetics, assuming $\mu_{max} = 0.42 \text{ h}^{-1}$ and $Y_{xs} = 0.4853$ for estimating q_s by equation 3.

$$q_s = -\frac{1}{Y_{xs}} \mu; \quad Y_{xs} [=] \frac{g_X}{g_{glu}} \quad (3)$$

A simulation was run during a time window of 30 h following a glucose pulse perturbation to a chemostat running at a dilution rate of 0.1 h^{-1} and an assumed steady state glucose concentration equal to 0.017 g/L just before the pulse as reported by Suarez-Mendez et al. [23]. The simulation time window was enough to achieve the steady state concentration again.

Biotic space characterization. With the reactive term applied on the bioreactor side using q_s as the key variable for linking glucose uptake (it can also be a production flux expressed by any other q-rate function) to a metabolic kinetic model, which includes the glycolytic pathway, the pentose phosphate pathway and some pathways for the metabolism of reserve carbohydrates, such as glycogen and trehalose, following the study of Suarez-Mendez et al. [24]. Hence, the kinetic model starts with a kinetic expression for a hyperbolic glucose uptake (Equation 4) following the traditional hyperbolic mechanism. To differentiate between extra- and intracellular glucose the term «ex» is used for the former.

$$q_s = \frac{q_{s,max}[Glc_{ex}]}{K_s + [Glc_{ex}]} \quad (4)$$

$q_{s,max}$: Maximal specific rate for glucose uptake; K_s : Affinity constant
 $[Glc_{ex}]$: Extracellular glucose concentration.

Kinetic expressions for intracellular reactions were adapted from literature, mainly those considered by Smallbone et al. [25] and Messiha et al. [26] due to their comprehensiveness including metabolic regulations and kinetic mechanisms for the different enzymatic reactions. Reactions for the intracellular cycling of reserve carbohydrates (i.e., glucose) were developed based on those following the reported in the study of Suarez-Mendez et al. [24, 27]. For the complete kinetic model with a detailed description the reader can visit the corresponding supplementary material, modeling cellular metabolism section.

Performance of the metabolic model was first assessed by using experimental data from [23] where periodic pulses of glucose were given to a 4L-growth of *Saccharomyces cerevisiae*. The data set allows for a comprehensive dynamic characterization of a reactive system in a submerged fermentation. Once the set of kinetic parameters was estimated to reproduce the metabolic behavior extensively, the model was coupled to the bioreactor model, and a scaled-up perturbation was simulated to observe the possible implications of different operational bioreactor conditions.

A sensitivity analysis was performed by changing agitation speed (60, 120, 180 and 240 rpm) and air flowrate ($1 \text{ m}^3\text{min}^{-1}$ and $3 \text{ m}^3\text{min}^{-1}$) for repetitive perturbation cycles (30.05 min each) according to [23] where pulse addition lasted 0.55 min and broth withdrawal 5 min. To assess the repetitive perturbations as in [23] but at a larger scale, the simulation included three cycles at 0, 30.05 and 60.10 min, all of them having the same settings as for the individual perturbation. Results from simulations are used for analysis of characteristic times of several subprocesses (mixing, substrate addition, substrate uptake, biomass growth, dissolved oxygen). Matlab software was used for data visualization and additional analysis.

Results and discussion

Characterization of the extracellular space in bioreactors. This characterization is done *in silico* by applying hydrodynamic and substrate uptake models with and without the reactive term. For a 22 m^3 bioreactor, simulations of a pulse perturbation given to a chemostat would result in a local transient increase of the tracer concentration of about 60 times. When an *in-silico* probe is placed at the top of the liquid (See figure 2 above) it is expected to observe a slightly larger concentration in comparison to middle or bottom probes because of the mixing capacity that plays a role in distributing the tracer throughout the liquid phase. A limited mixing capacity would create a temporary overshoot in concentration whenever the time constant for mixing is smaller than the time constant for other subprocesses, in this case, the addition of the pulse, 33 s, and the flowrate at the outlet stream, 300 s. Clearly, one can notice that the fast addition of the tracer cannot be buffered by both a slower mixing and transport of the liquid outside the vessel, hence there is a rapid increase in concentration in about 1 second (Figure 4).

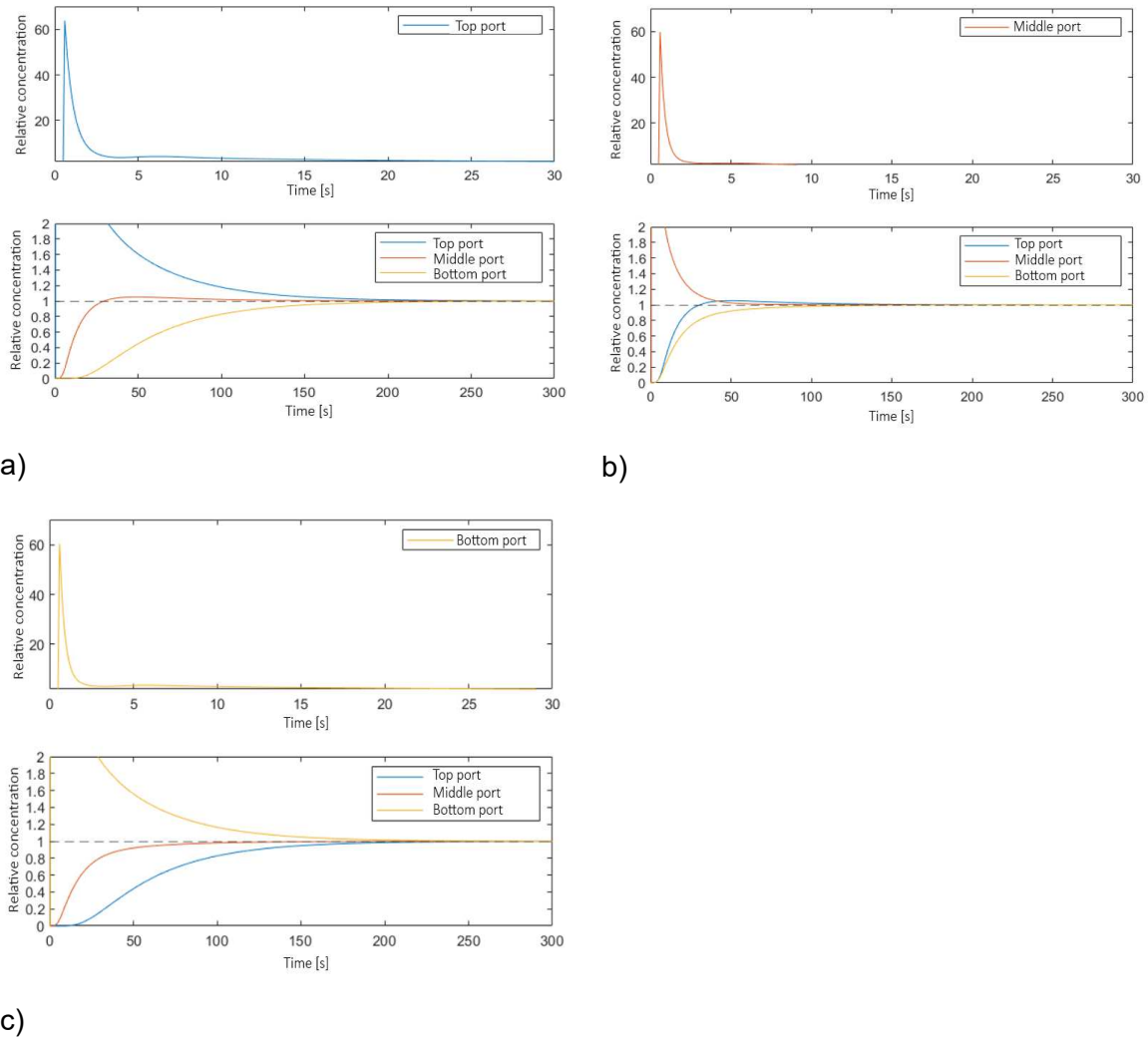


Figure 4. Mixing time in bioreactor. Tracer injected at: a) top port, b) middle port, c) bottom port. Trace pulse is given at 0.5s. Tracer concentration detected either at top, middle or bottom port. Stirring speed: 60 rpm

The mixing time, as it is typically determined [28], depends on both the location where the pulse is added and where the probe is located (top, mid or bottom sections of the vessel). The shortest mixing time (τ about 60 s) takes place when the tracer's point of addition and the probe are both located at the middle section of the vessel (Figure 4b) while the longest (τ about 170 s) occurs when probe and port of addition are placed in the most extreme positions (top vs bottom or vice versa). Typically, feed addition in large industrial scale bioreactors occurs at the top while the probes are located somewhere near the bottom impeller. One can figure out that uncoupling these subprocesses would produce concentration gradients of substrate throughout the vessel that would later result in varying

substrate uptake fluxes. This scenario would create a distribution of multiple metabolic rates inside the cells flowing across different compartments. Hence, time constant estimations become critical to induce a particular performance in bioreactors when a reaction term is considered.

In silico analysis of the dissolved oxygen shows that the characteristic time for the liquid to get fully saturated (τ_{DO}) is about 170s after starting aeration (Figure 5), although the dissolved oxygen reaches 90% of the saturation in less than 100 s. Apparently, for this bioreactor configuration there should not be major concerns with oxygen availability since τ_{DO} is in the same range of magnitude as mixing time (τ about 60-170 s). However, if the reactive term is considered, glucose and oxygen consumption by the cells will be present and these two should preferably be synchronized as well. No significant differences can be observed for dissolved oxygen throughout the liquid in the vessel, except for compartment 70 where the liquid is withdrawn from the bioreactor. This observation can be due to a modelling artifact rather than the creation of dead zones at the bioreactor bottom around compartment 70, which is located at the farthest point from the compartment at which the air sparger is located (compartment 66).

When the reactive term is added to the simulation, it is observed that substrate accumulation leads to a mini batch-like behavior with multiple transient growth rates at different compartments of the vessel (Figure 6). Here, the dilution rate was 0.1 h^{-1} which means a time constant of 10 h (36,000 s) for the steady state growth rate. Due to the different characteristic times of the several subprocesses involved it is possible to observe a distinguishable dynamic pattern in the vessel following the pulse perturbation of glucose ($\tau = 33 \text{ s}$), which is distributed in the liquid by mixing ($\tau = 60 \text{ s}$) before being transformed into new biomass ($10.2 < \tau < 4.7 \text{ h}$ corresponding to $0.098 < \mu < 0.211$ provided that $q_S = -\frac{1}{Y_{XS}} \cdot \mu$). Note that 300 s are needed to withdraw broth, substrate and some cells from the bioreactor, just after the beginning of the pulse. This highly dynamic regime shows that the reactive system may not be homogeneous throughout the vessel causing numerous potential phenotypes and therefore localized behaviors, some of which could be unwanted.

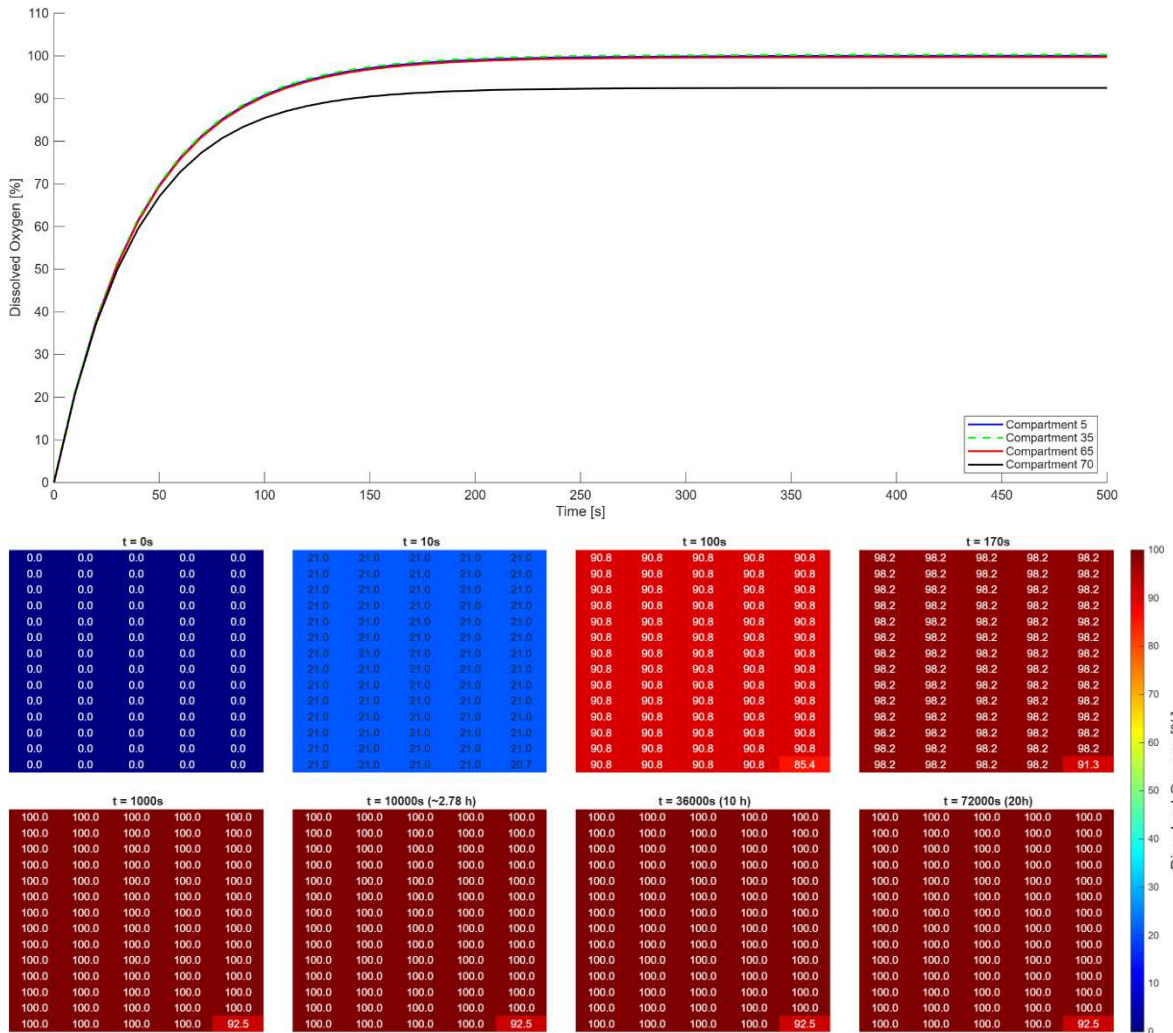


Figure 5. Bioreactor characterization: based on dissolved oxygen when aeration starts at 0s.

The average growth rate over all the compartments remains close to the steady state dilution rate, $\mu = 0.107 \text{ h}^{-1}$, after 20 h following the perturbation. The growth rate never reaches its maximum value, $\mu = 0.420 \text{ h}^{-1}$, since substrate concentration in the added solution (15 gL^{-1}) is rapidly distributed over the compartments. However, this scenario might happen in situations where the feeding port is placed in a less convenient location in the bioreactor or more concentrated feed solutions are used.

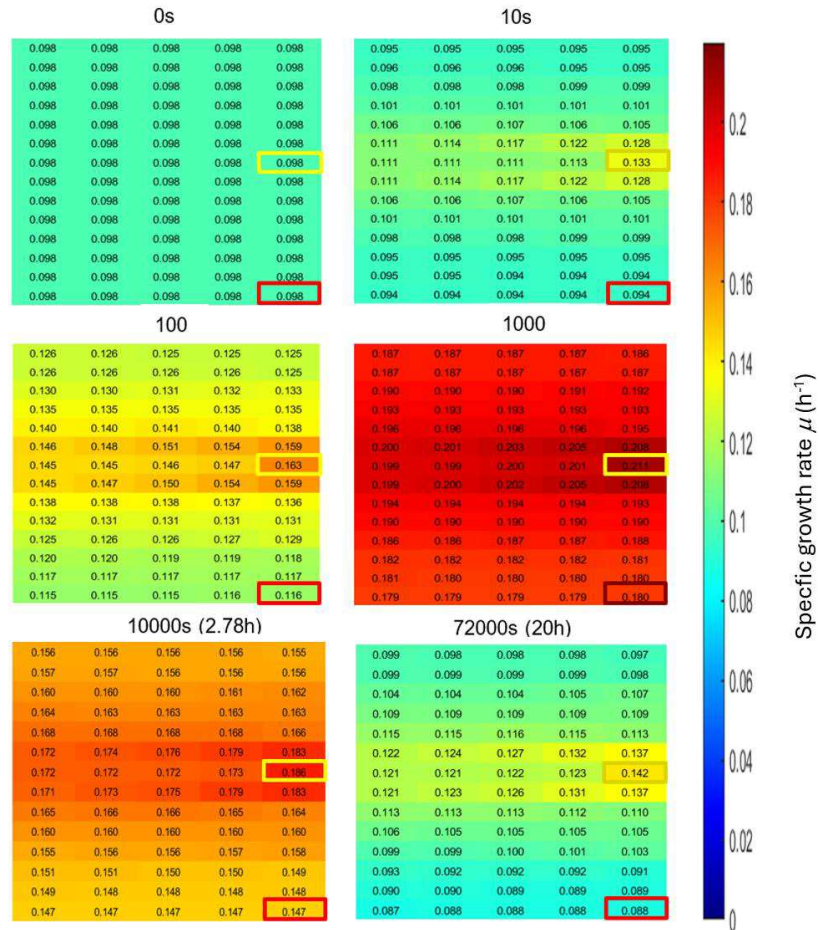


Figure 6. Heat map for specific growth rate, μ , at different times after the perturbation. Here, the pulse is added in the middle section (compartment 35) while outflow is withdrawn at compartment 70.

Characterization of the intracellular space. A kinetic model (Supplementary material – Modeling cellular metabolism section) is used to simulate the cell's metabolic response to a dynamic change in the bioreactor. The behavior of several intracellular metabolites is analyzed following a sudden perturbation in the bioreactor (extracellular space). As suggested above, the characteristic times of the different subprocesses running over the bioreactor can induce a temporary accumulation of substrate in the broth, which in turn would influence the substrate uptake rate and therefore the intracellular behavior of the different metabolic pools. Figure 7 shows the time profiles for metabolites of the upper glycolysis following a periodic perturbation according to [23] with the corresponding simulation of the metabolic kinetic model. Although modeling metabolic networks performance during transient regimes is a complex task, here we applied a sound kinetic

model capable of reproducing *in vivo* kinetics to an acceptable extent. Nevertheless, the main scope of this work is to apply a multiscale modeling approach for coupling kinetic and bioreactor models to study dynamic features of a microbial culture for design, control and scale up purposes. Exploring how bioreactor operation conditions can induce metabolic responses would help to foresee potential deviations in yields and titers from the desired operational set point but also to implement actions to either prevent or correct them.

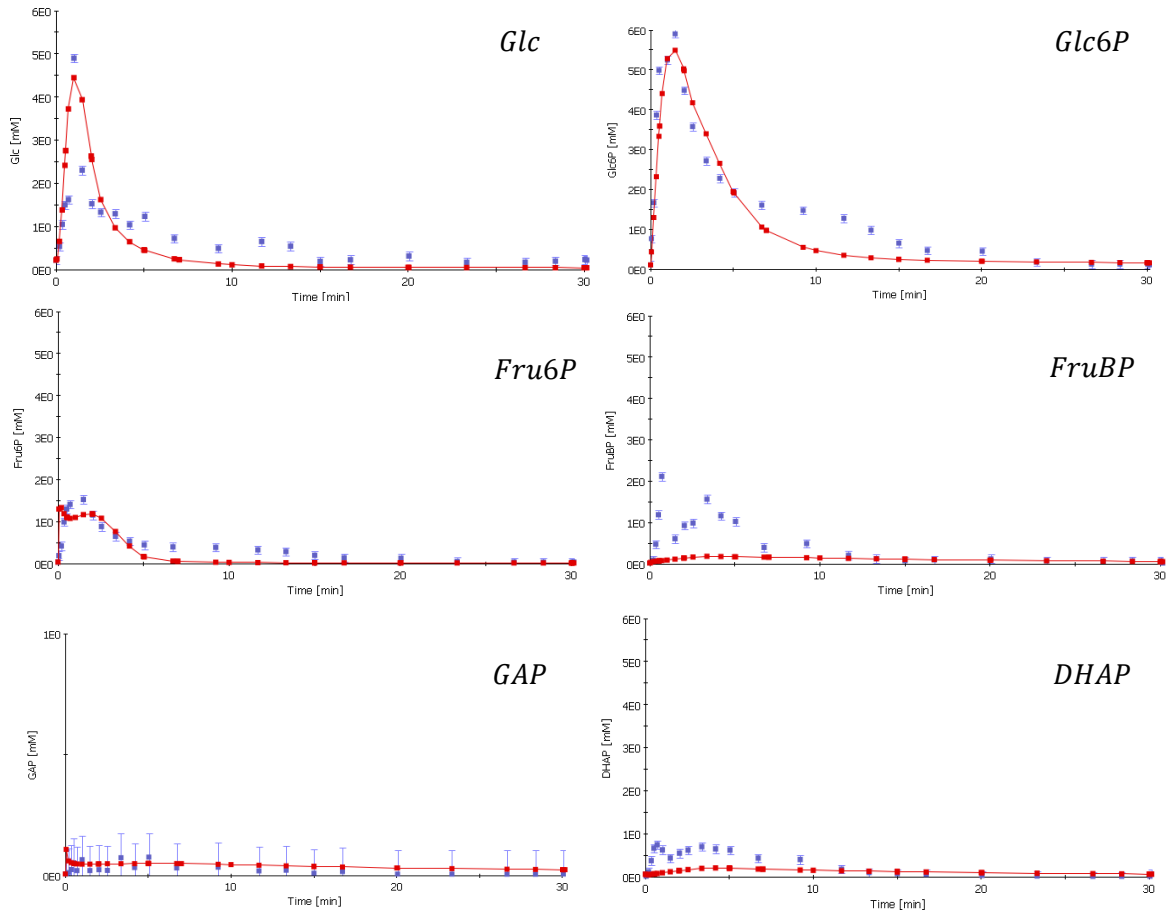


Figure 7. Concentration of upper glycolysis metabolites. Experimental data, blue-filled squares, Model simulation, red line and dots. Original data from [23] have been transformed into mM.

The intracellular transient response of metabolites in the upper glycolysis follows, to a large extent, that of the extracellular glucose, which was produced by periodic pulses of glucose to a 4 L-chemostat [29, 30]. Glucose transport across cell membranes that separate both intra and extracellular spaces, and therefore, its uptake fluxes (q_s), should

play an important role for the observed patterns, apart from being a common process on both spaces. Numerous kinetic expressions for glucose uptake in *S. cerevisiae* have been reported in literature, most of which consider the uptake as a function of glucose concentration in the extracellular space [31-34]. Although glucose uptake (limiting substrate) is critical to microbial phenotypes in bioreactors, all the other subprocesses running on both sides of the membrane should also influence the observed behavior. One can expect that metabolic responses in both upper and lower glycolysis, and some other pathways, will be subject to metabolic and genetic regulations, which might not be directly linked to changes in extracellular concentrations but more directly to the capacity of the cell to withstand a particular carbon flux that enters the cell, that is, a consequence for the cell trying to cope with a changing carbon flux through its metabolic networks.

As mentioned earlier in this article, we focus on coupling two physical compartments, the bioreactor's bulk and the intracellular space, and so, we limit the scope to the interaction between these two spaces at the level of glucose transport considering that this can be a two-way interaction. It has been reported that glucose uptake is mediated by facilitated diffusion [35-36] where concentration gradients on both sides of the membrane is the main driving force, however, the two concentration profiles do not need to be alike due to other different subprocesses acting on both sides. While the extracellular glucose concentration follows a typical batch-like pattern due to the pulse addition, mixing and uptake processes, the intracellular glucose concentration might be subject to other multiple metabolic regulations including glucose recycled by trehalose and glycogen pathways [24]. These subprocesses, different from transport, would define the observed concentration profiles in time. Since the nature of such processes differs on both sides, coupling two different models at the two different scales, meso and micro, could conveniently reproduce a particular phenotype. Glucose flux through the membrane (q_s) is only one no matter from what side (extra or intracellular) it is calculated, and thus, this becomes a convenient link between the two scales despite any small differences in glucose concentrations on both sides of the membrane.

Reproducing dynamic metabolic behavior in both extra and intracellular spaces by applying perturbations to a large bioreactor. By coupling both models while applying similar *in silico* perturbations to the reactive system, and considering the different scale, 22

m³ vs 4 L, we can analyze the metabolic behavior that cells would undergo in the large vessel when applying similar conditions as the ones tested at benchtop bioreactors. It is expected that hydrodynamic patterns at large industrial bioreactors would differ from the almost ideal mixed benchtop bioreactors and due to the different characteristic times of the subprocesses governing the overall process at both scales, cells would face different environmental conditions that might influence their metabolic performance. Figures 8 and 9 show how either the extracellular or intracellular glucose concentrations would behave after some time following a glucose perturbation given at time 0 min and depending on the location of the feeding port (compartments: 5, top; 35, middle; 65, bottom). The magnitude of the pulse (flow, 0.033 m³s⁻¹; volume, 1.0916 m³; glucose concentration, 15 g/L; time, 33 s), the agitation speed (60 rpm) and the gas flow rate (1 m³min⁻¹) were kept the same for the three scenarios tested as well as the location of the air sparger and port for broth withdrawal.

First, it is observed that adding a glucose pulse to a compartment located at the middle section of the vessel (compartment 35) would create extracellular concentration gradients which are less pronounced than if pulse is given either at the top (compartment 5) or at the bottom (compartment 65). At 0.55 min, when the pulse addition ceases, the average glucose concentration, considering all the 70 compartments, has increased up to 0.812 gL⁻¹ (4.509 mM) from the initial set point (0.09 gL⁻¹ or 0.5 mM at time 0 min), that is, 13 times higher concentration. Although the average concentration extensively agrees with experimental observations reported by Suarez-Mendez et al. [23] in benchtop bioreactors, there is a clear concentration gradient pattern throughout the vessel for the large industrial bioreactor.

At the feeding port (compartment 35) glucose concentration reaches 1.762 gL⁻¹ (9.79 mM), while at the top and bottom regions of the bioreactor (compartments 5 and 65) glucose concentrations are 0.528 gL⁻¹ (2.93 mM) and 0.424 gL⁻¹ (2.35 mM), respectively. These transient gradients in glucose concentration, as will be shown below, can produce even more significant differences in intracellular metabolites for the cells located at different compartments in the bioreactor. For an industrial bioreactor, like the one addressed in this work, having mixing times between 60 s and 170 s as described above, one can expect

glucose to accumulate in different compartments whenever the characteristic time for the pulse addition is shorter (33 s in this case).

Two minutes after starting the pulse, the observed difference in glucose concentrations between top and bottom compartments are somewhat homogeneous. While at compartment 5 cells would experience glucose concentrations of about 0.735 gL^{-1} (4.09 mM), at the bottom (compartment 65) it would be 0.689 gL^{-1} (3.83 mM). The almost homogenized glucose concentration calculated 120 s after the pulse has started can be explained by a combination of a mixing time of about 60 s (in the same order of magnitude as the characteristic time for pulse addition) and the characteristic time for the glucose uptake (771, 775 and 787 s in compartments 5, 35 and 65, respectively.) However, if glucose is added at the top of the vessel (as is usually done in industrial bioreactors) one can observe more significant glucose gradients from top to bottom since the mixing time will be longer (about 170 s). Thus, 2 min after starting the pulse it is expected to have a glucose concentration of 0.99 gL^{-1} (5.51 mM) at compartment 5 and 0.52 gL^{-1} (2.90 mM) at compartment 65. The almost double glucose concentration between top and bottom compartments would cause a varying profile in glucose uptake, and thus, a varying profile in intracellular metabolites.

When the glucose pulse addition is just finished ($t=0.55 \text{ min}$) in the extracellular space at the level of compartment 35, the intracellular glucose concentration has risen to 13.61 mM for those cells located in the same compartment (Figure 9, compartment 35, middle). This concentration is about 62 times higher than just before the pulse. For this setup, the time constant for the pulse addition is still 33 s, which is almost half the time constant for mixing ($\tau_{\text{mix}} \approx 60 \text{ s}$). One can expect that at slower mixing there will be a more drastic buildup in glucose concentration in the region near the feeding point. Hence, if glucose is added at the level of compartment 5 (Figure 9, top), the intracellular glucose concentration increases up to 22.57 mM just when the pulse addition is about to finish. This is about 100 times higher than just before the pulse, and up to 210 times higher two minutes after the pulse has started, changing from 0.22 to 46.38 mM. This remarkable intracellular glucose buildup experienced by the cells located at the same compartment where the pulse is added (compartment 5), a typical location for feeding ports in industrial bioreactors, can be

404 explained by the greater characteristic time for mixing ($\tau_{\text{mix}} \approx 170$ s in this case) and the
 405 characteristic time for the uptake (780 s).

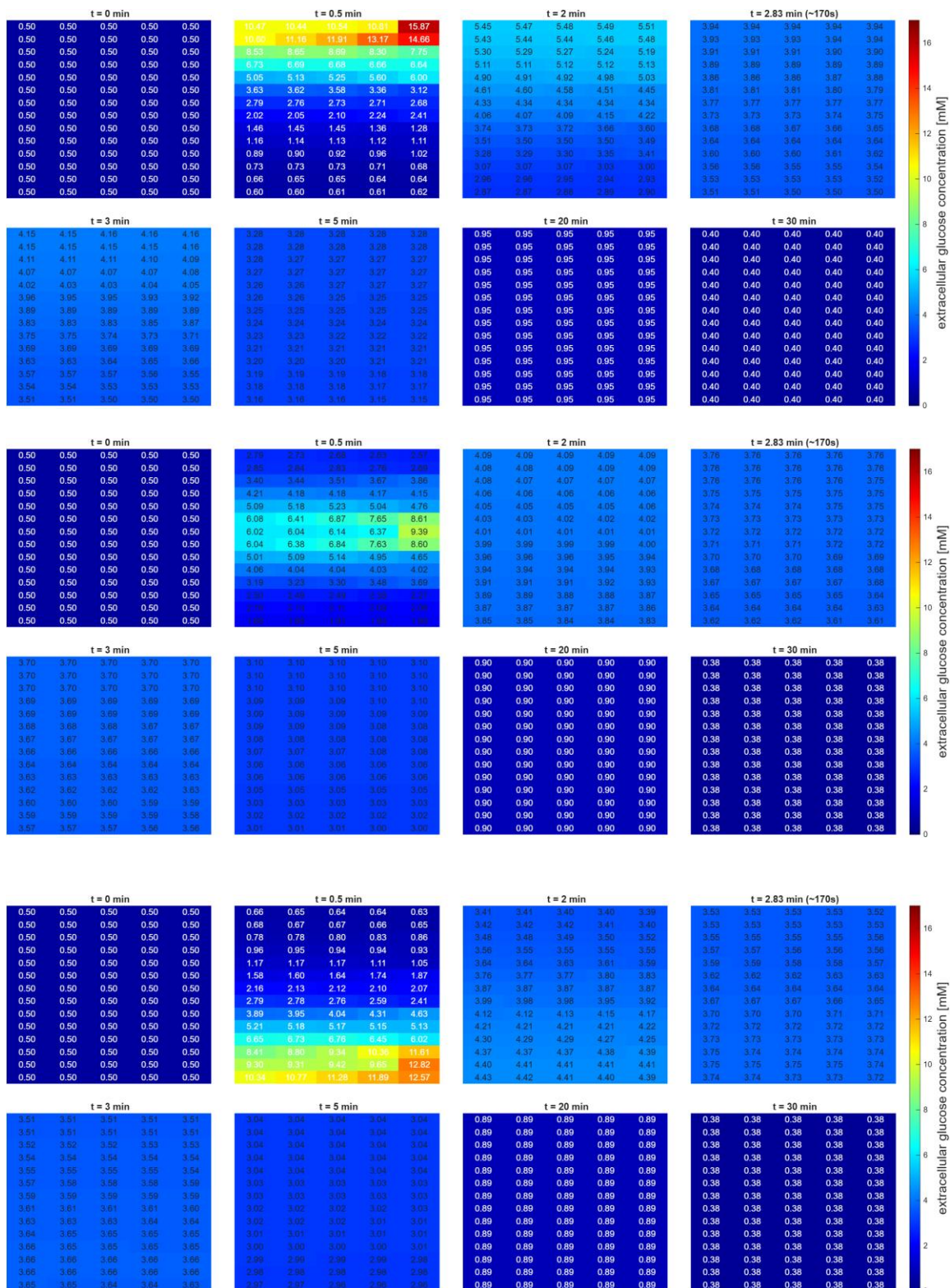


Figure 8. Time Course of the extracellular glucose concentration experienced by the cells located at different compartments for three different feeding ports: top (No. 5), middle (No. 35) and bottom (No. 65).

Moreover, the longer time required for complete mixing in the bioreactor will cause a more notorious difference in the intracellular glucose concentration, about 13 times higher in the cells at the top of the vessel than those at the bottom (46.38 vs. 3.56 mM, respectively). The intracellular glucose concentration depends on the extracellular glucose concentration via the uptake function, thus, differences in glucose uptake rates can be expected as well throughout the compartments of the vessel. Indeed, while at the top compartments, cells exhibit a glucose uptake of $0.011 \text{ gGlcgDW}^{-1}\text{min}^{-1}$, those at the bottom would experience smaller fluxes $0.0084 \text{ gGlcgDW}^{-1}\text{min}^{-1}$. This is 1.3 times lower. Even 5 minutes after the pulse, significant differences can be observed in concentration profiles among those cells at the top and those at the bottom, and eventually, the intracellular glucose concentration would return to the chemostat value. However, long transient times can be enough to cause distinctive metabolic behaviors in the culture forcing the cells to adjust their metabolic/regulatory machinery differently and so, creating a non-homogeneous culture that would result in different process yields with changing intracellular carbon fluxes. At 0.55 min, just after the pulse addition is complete, the extracellular glucose concentration and glucose uptake are maximal at the port of addition (Compartment 5), 16.6 mM and $0.032 \text{ gGlcgDW}^{-1}\text{min}^{-1}$, respectively. While at compartment 70 (bottom part of the bioreactor) the cells experience 0.69 mM and $0.0018 \text{ gGlcgDW}^{-1}\text{min}^{-1}$. At the same time, the characteristic times for glucose uptake are 985 s at port 5 and 728 s at port 70. These results suggest that for the complete uptake of the surrounding glucose, cells located at port 5 will need a longer time than those at the bottom, or in other words, cells at the bottom will deplete glucose faster than those at the top.

Milder concentration gradients are expected when the feeding port is located at the middle compartments, thus, intracellular glucose concentrations can get back to the set point faster. This behavior is most likely due to a shorter path length that a piece of fluid must travel to be distributed all over the vessel and shorter mixing times (τ about 1 min). For this case, the highest change in intracellular glucose concentration following two minutes after the pulse is about 77 times (17.31 vs 0.22 mM in cells located at port 35). Moreover,

440 the concentration difference between the middle port and the top and bottom ports is about
 441 1.9 and 2.6 times, respectively. This behavior would be most favorable for bioprocessing
 442 since cells would undergo less drastic changes.

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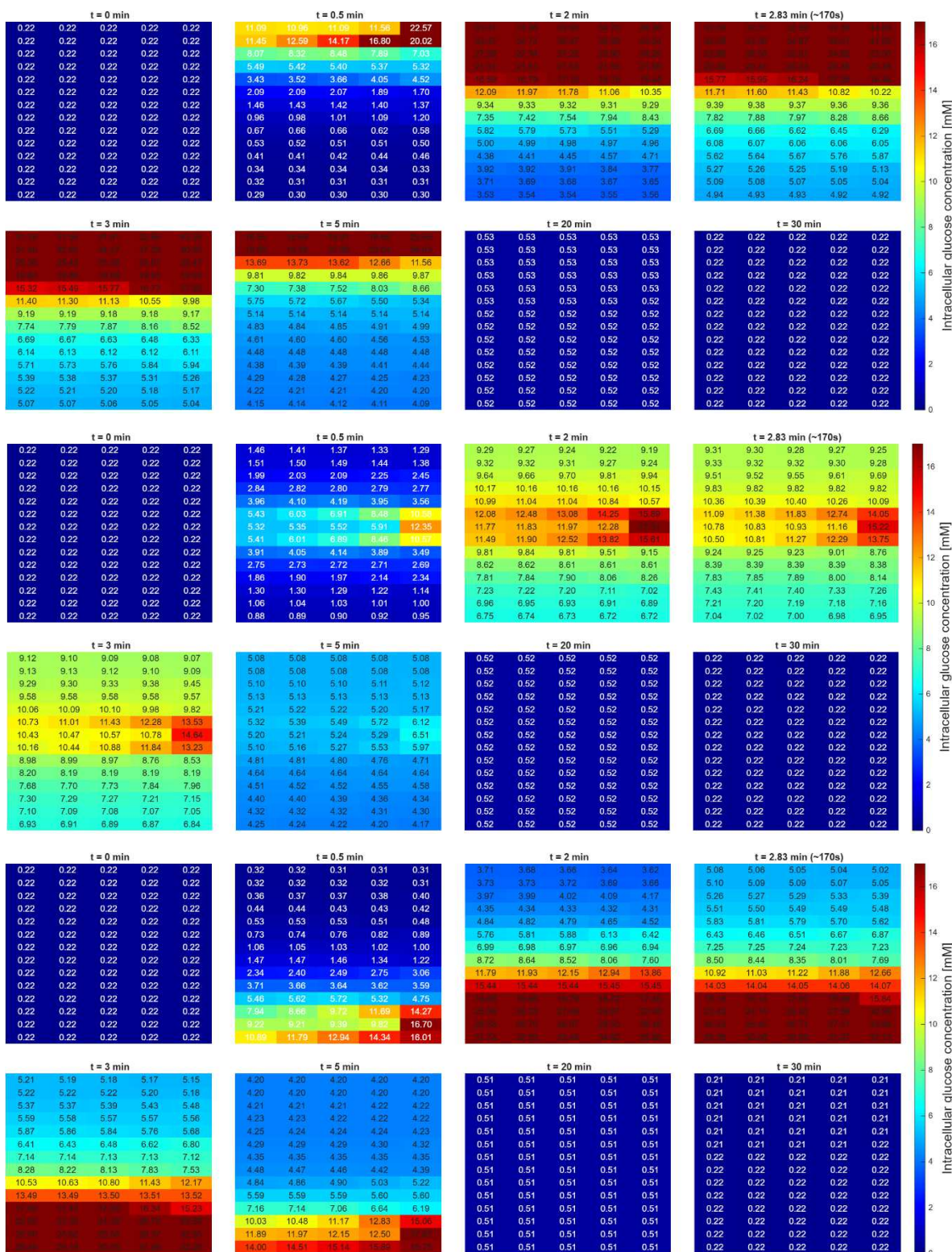


Figure 9. Time Course of the Intracellular glucose concentration experimented by the cells placed at different compartments for three different feeding ports: top (No. 5), middle (No. 35) and bottom (No. 65).

Based on both observations, extra- and intracellular glucose behaviors, one can expect that the intracellular metabolic pathways will react correspondingly (Figure 10), as has been previously reported for cultivations in benchtop bioreactors [23-24, 27, 29-30]. In addition, in case that glucose perturbations follow a periodic pattern like in small bioreactors, the metabolic system would also reach a new “dynamic steady state” in the industrial bioreactor (Figure 11).

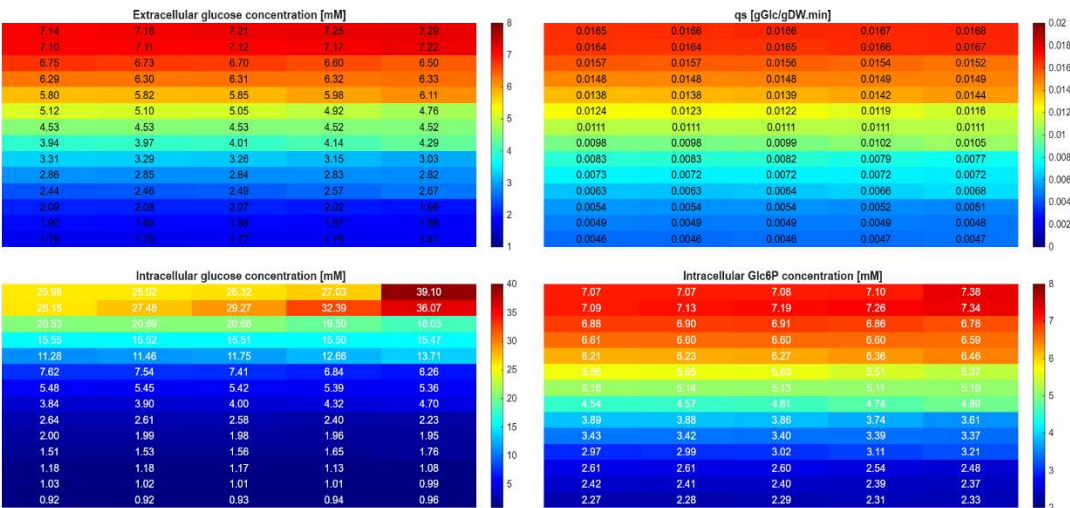


Figure 10. Concentration profiles of extracellular glucose, intracellular glucose, Glc6P and q_s at 1 min after the pulse starts. Glucose pulse fed at port 5 (top).

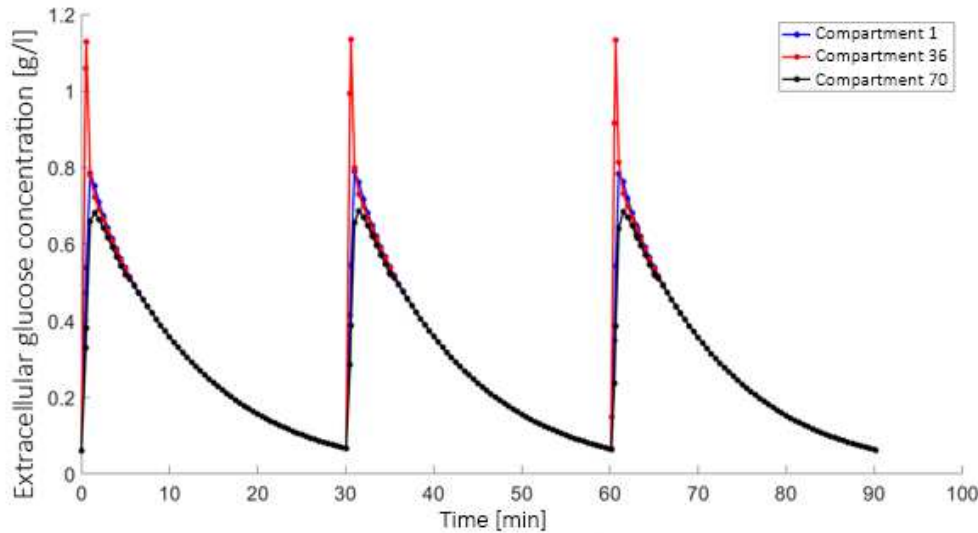
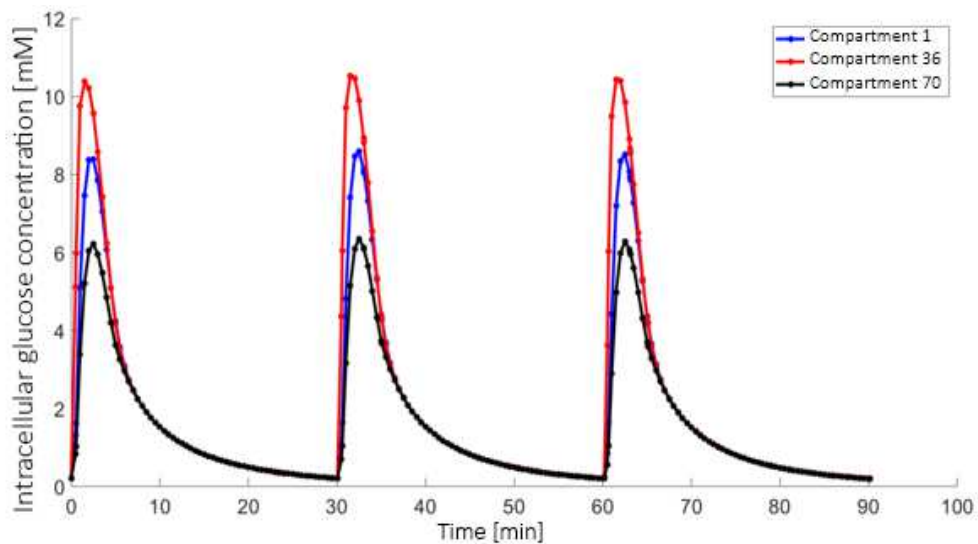


Figure 11. Extracellular and intracellular glucose concentration after three subsequent feed perturbations in the bioreactor. Glucose pulse fed at port 35 (middle).

How changing operation conditions in bioreactors can induce a particular metabolic response: a sensitivity analysis. The two mathematical models coupled can be used as a tool for investigating how microorganisms would perform under certain conditions. For instance, exploring scenarios with varying agitation speeds (60, 120, 180 and 240 rpm) shows that, at low values, variations in extracellular glucose concentrations tend to be more notorious than those at high agitation speeds because of poorer mixing (Figure 12). The maximal differences observed among different compartments suggest that larger concentration gradients could induce a metabolic performance deviating from the desired

point of operation and process yields. To mitigate differences in metabolic concentrations, at both extra- and intracellular spaces, an increase in agitation power can be applied at the expense of a higher and more costly energy consumption. Following the classical expression for power dissipation in agitated vessels $P = N_p \rho N^3 D^5$ one can expect that to avoid differences in concentrations at a given bioreactor region, let's say from 60 to 240 rpm as in figures 12 and 13, a 64-times higher power consumption would be required. A similar observation could be drawn for dissolved oxygen (Data not shown) where a similar increase in energy consumption would be needed to improve gas-liquid mass transfer and mixing, while shortening the corresponding characteristic times. Whether or not this is a convenient option during bioprocessing in large scale bioreactors would most likely depend on the process economy.

Some alternative options could involve better bioreactor designs such as changing locations of feeding ports, improving the way substrate is added to the vessel among others. All these features could be explored during early stages of the process design by extending the tool developed here with the corresponding functionalities. Moreover, this sort of approach can also help in evaluating potential phenotypic performances that would be beneficial for process economics while implementing simple solutions.

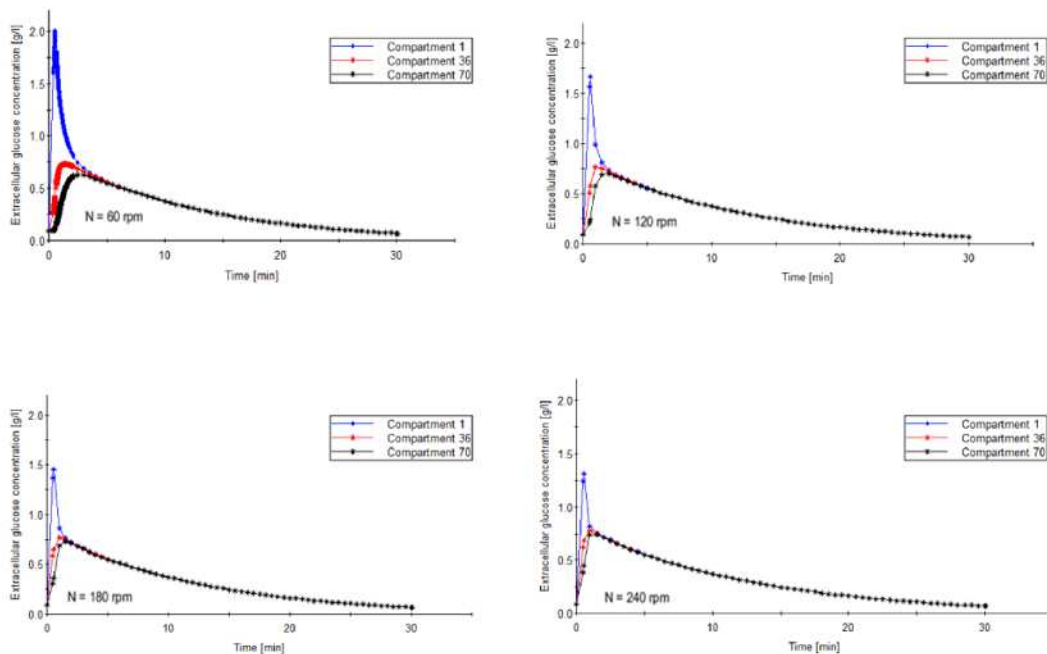


Figure 12. Extracellular glucose concentration at three different 3 compartments and 4 stirring speeds: $N = 60 \text{ rpm}$; $N = 120 \text{ rpm}$; $N = 180 \text{ rpm}$; $N = 240 \text{ rpm}$. Glucose pulse fed at port 5 (top).

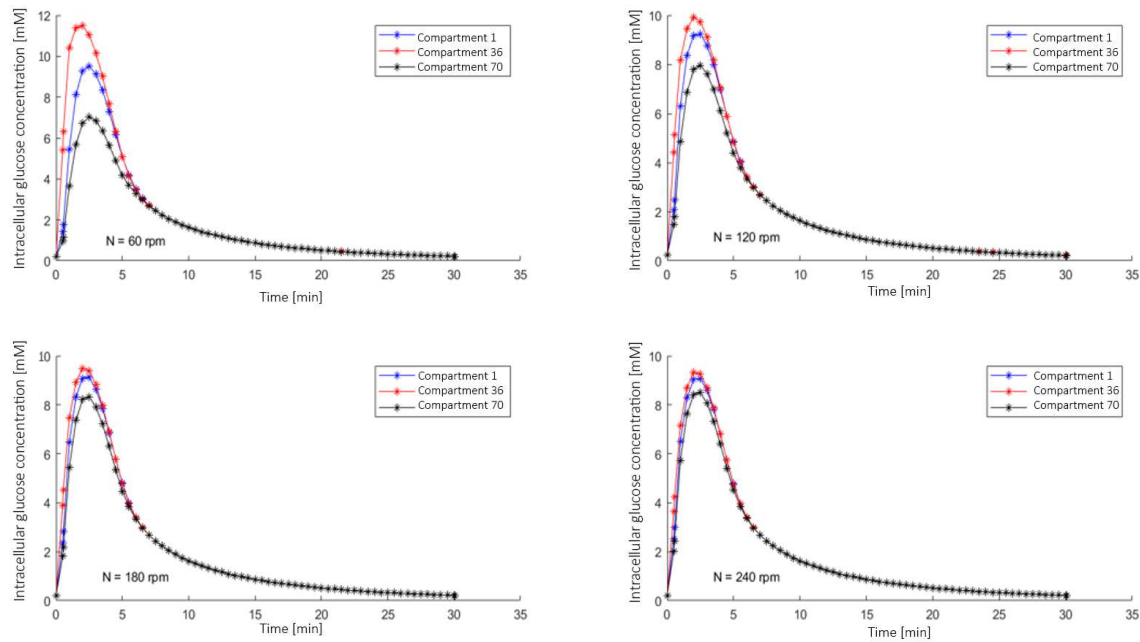


Figure 13. Intracellular glucose concentration at three different compartments and 4 stirring speeds: $N = 60 \text{ rpm}$; $N = 120 \text{ rpm}$; $N = 180 \text{ rpm}$; $N = 240 \text{ rpm}$. Glucose pulse fed at port 35 (middle).

Changes in gas flowrate (1 and $3 \text{ m}^3\text{min}^{-1}$) were also explored (data not shown). As a result, we found that the higher the gas flowrates the smaller the relative decrease in dissolved oxygen (relative to the initial oxygen concentration in a cycle), and the faster the recovery to the initial oxygen concentration. When the gas flowrate was $3 \text{ m}^3\text{min}^{-1}$, the maximal extracellular glucose concentration obtained at 0.55 min (when the pulse addition ends in compartment 5) was 90% of that obtained with $1 \text{ m}^3\text{min}^{-1}$, while q_s decreased by about 7%. In general, increasing the gas flowrate from 1 to $3 \text{ m}^3\text{min}^{-1}$ would cause a lower dissolved oxygen depletion, slower glucose uptake and lower maximal extra- and intracellular glucose concentration between 85 – 93% of the former value. Again, whether

increasing the gas flowrate is a convenient action to be implemented for a desired behavior would depend on the process economy.

Conclusions

In this work we explored a mathematical modeling tool for recreating the operation of large industrial bioreactors following a multiscale modeling approach. First, a previously known bioreactor model was used to characterize hydrodynamics in the industrial bioreactor following a compartment-based approach. Then, this model was coupled to a kinetic metabolic model, which has been developed based on highly dynamic metabolic data from an aerobic fermentation of *Saccharomyces cerevisiae* in a benchtop bioreactor. The two models were coupled via the specific substrate uptake rate to reproduce *in silico* the dynamic perturbations formerly applied to a 4L-bioreactor but now applied to a 22 m³ industrial bioreactor. Understanding how complex hydrodynamics in large reactors can affect the metabolic response of the cells throughout the vessel becomes a major part of this study. In this direction, we used an approach where characteristic times of different subprocesses were analyzed to show that concentration gradients impose different uptake rates and a varying behavior of the intracellular metabolic pools. Although the tool shows its potential for being applied for bioreactor design, control and scale up/down purposes, there is still room for making it more robust by implementing additional features like better *in vivo* kinetic expressions, additional transport phenomena, and control loops. Here, we show that nonhomogeneous mixing in large bioreactors can induce different biological phenotypes where performance of the cells located in different sections of the vessel can deviate up to 15% from the ideally mixed benchtop bioreactor.

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Author contributions

CSM conceived and designed the study. SMO, JERC and CSM performed simulations, CSM wrote the manuscript and provided scientific advice. All authors read and approved the final manuscript.

Declarations

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Conflict of interest. The authors declare that they have no competing interests

References

- [1] Charpentier JC (2009) Perspective on multiscale methodology for product design and engineering. *Computers and Chemical Engineering*, 33(5), 936–946. <https://doi.org/10.1016/j.compchemeng.2008.11.007>
- [2] Gelves R, Dietrich A & Takors R (2014) Modeling of gas-liquid mass transfer in a stirred tank bioreactor agitated by a Rushton turbine or a new pitched blade impeller. *Bioprocess and Biosystems Engineering*, 37(3), 365–375. <https://doi.org/10.1007/s00449-013-1001-8>
- [3] Luo Y, Kurian V, Ogunnaike B A (2021) Bioprocess systems analysis, modeling, estimation, and control. *Current Opinion in Chemical Engineering*, 33. <https://doi.org/10.1016/j.coche.2021.100705>
- [4] Pigou M, Morchain J (2015) Investigating the interactions between physical and biological heterogeneities in bioreactors using compartment, population balance and metabolic models. *Chemical Engineering Science*, 126, 267–282. <https://doi.org/10.1016/j.ces.2014.11.035>
- [5] Nielsen J, Villadsen (1994) *Bioreactor Modeling. Bioreaction Engineering Principles*. Springer Science. https://doi.org/10.1007/978-1-4757-4645-7_9
- [6] Haringa C, Tang W, Wang G, Deshmukh AT, van Winden WA, Chu J, van Gulik WM, Heijnen JJ, Mudde RF, Noorman HJ (2018) Computational fluid dynamics simulation of an industrial *P. chrysogenum* fermentation with a coupled 9-pool metabolic model: Towards rational scale-down and design optimization. *Chemical Engineering Science*, 175:12–24. <https://doi.org/10.1016/j.ces.2017.09.020>
- [7] Haringa C, Tang W, Deshmukh AT, Xia J, Reuss M, Heijnen JJ, Mudde R F, Noorman HJ (2016) Euler-Lagrange computational fluid dynamics for (bio)reactor scale down: An analysis of organism lifelines. *Engineering in Life Sciences*, 16(7): 652–663. <https://doi.org/10.1002/elsc.201600061>
- [8] Hahn J (2020) *From Parts to the Whole A Whole-Cell Model for Saccharomyces cerevisiae*. Dissertation Humboldt University Berlin
- [9] Morchain J (2017) Numerical Tools for Scaling Up Bioreactors. *Current Developments in Biotechnology and Bioengineering: Bioprocesses, Bioreactors and Controls*, 495–523. <https://doi.org/10.1016/B978-0-444-63663-8.00017-3>

- 588 [10] Noorman H J, Heijnen JJ (2017) Biochemical engineering's grand adventure.
589 Chemical Engineering Science, 170: 677–693.
590 <https://doi.org/10.1016/j.ces.2016.12.065>
- 591 [11] Kim OD, Rocha M and Maia P (2018) A Review of Dynamic Modeling Approaches
592 and Their Application in Computational Strain Optimization for Metabolic
593 Engineering. Front. Microbiol. 9. <https://doi.org/10.3389/fmicb.2018.01690>
- 594 [12] Mayr B, Horvat P, Nagy E, Moser A (1993) Mixing-models applied to industrial
595 batch bioreactors. Bioprocess Engineering, 9(1): 1–12.
596 <https://doi.org/10.1007/BF00389534>
- 597 [13] Jourdan N, Neveux T, Potier O, Kanniche M, Wicks J, Nopens I, Rehman U, Le
598 Moullec Y (2019) Compartmental Modelling in chemical engineering: A critical
599 review. Chemical Engineering Science, 210.
600 <https://doi.org/10.1016/j.ces.2019.115196>
- 601 [14] Nadal-Rey G, McClure DD, Kavanagh JM, Cornelissen S, Fletcher DF, Gernaey KV
602 (2021) Understanding gradients in industrial bioreactors. Biotechnology Advances,
603 46. <https://doi.org/10.1016/j.biotechadv.2020.107660>
- 604 [15] Gustafsson J, Anton M, Roshanzamir F, Jörnsten R, Kerkhoven EJ, Robinson JL,
605 Nielsen J (2023) Generation and analysis of context-specific genome-scale
606 metabolic models derived from single-cell RNA-Seq data, Proc. Natl. Acad. Sci.
607 U.S.A. 120. <https://doi.org/10.1073/pnas.2217868120>
- 608 [16] Hrovatin K, Fischer DS, Theis FJ (2022) Toward modeling metabolic state from
609 single-cell transcriptomics, Molecular Metabolism, 57.
610 <https://doi.org/10.1016/j.molmet.2021.101396>
- 611 [17] Baek S, Song K, Lee I (2025) Single-cell foundation models: bringing artificial
612 intelligence into cell biology. Exp Mol Med 57: 2169–2181.
613 <https://doi.org/10.1038/s12276-025-01547-5>
- 614 [18] Guo W, Chen Z, Gu J (2025) Computational modeling of single-cell dynamics data,
615 Briefings in Bioinformatics, Volume 26. <https://doi.org/10.1093/bib/bbaf305>
- 616 [19] Wu Y, Judge MT, Edison AS, Arnold J (2022) Uncovering in vivo biochemical
617 patterns from time-series metabolic dynamics. PLoS ONE 17(5).
618 <https://doi.org/10.1371/journal.pone.0268394>
- 619 [20] Vasilakou E, Machado D, Theorell A, Rocha I, Nöh K, Oldiges M, Wahl SA (2016)
620 Current state and challenges for dynamic metabolic modeling, Current Opinion in
621 Microbiology, Volume 33: 97-104, <https://doi.org/10.1016/j.mib.2016.07.008>
- 622 [21] Vrabel P, Van Der Lans RGJM, Cui YQ, Luyben, KCAM (1999). Compartment
623 model approach: Mixing in large scale aerated reactors with multiple impellers.
624 Chemical Engineering Research and Design, 77(4): 291–302.
625 <https://doi.org/10.1205/026387699526223>
- 626 [22] Vrabel, P, Van Der Lans, RGJM, Luyben KCAM, Boon L, Nienow AW (2000).
627 Mixing in large-scale vessels stirred with multiple radial or radial and axial up-

628 pumping impellers: Modelling and measurements. Chemical Engineering Science,
629 55(23): 5881–5896. [https://doi.org/10.1016/S0009-2509\(00\)00175-5](https://doi.org/10.1016/S0009-2509(00)00175-5)

630 [23] Suarez-Mendez CA (2015) Dynamics of Storage Carbohydrates Metabolism in
631 *Saccharomyces cerevisiae*: A Quantitative analysis. Dissertation Delft University of
632 Technology, Delft, The Netherlands

633 [24] Suarez-Mendez CA, Hanemaaijer M, ten Pierick A; Wolters JC, Heijnen JJ, Wahl
634 SA (2016). Interaction of storage carbohydrates and other cyclic fluxes with central
635 metabolism: A quantitative approach by non-stationary ¹³C metabolic flux analysis.
636 Metabolic Engineering Communications 3: 52-63.
637 <https://doi.org/10.1016/j.meten.2016.01.001>

638 [25] Smallbone K, Messiha HL, Carroll KM, Winder CL, Malys N, Dunn WB, Murabito E,
639 Swainston N, Dada JO, Khan F, Pir P, Simeonidis E, Spasić I, Wishart J, Weichart
640 D, Hayes NW, Jameson D, Broomhead DS, Oliver SG, Mendes P (2013) A model
641 of yeast glycolysis based on a consistent kinetic characterisation of all its enzymes.
642 FEBS Letters, 587(17): 2832–2841. <https://doi.org/10.1016/j.febslet.2013.06.043>

643 [26] Messiha H, Kent E, Malys N, Carroll K, Mendes P, Smallbone K (2014) Enzyme
644 characterisation and kinetic modelling of the pentose phosphate pathway in yeast.
645 PeerJ PrePrints. <https://doi.org/10.7287/peerj.preprints.146v4>

646 [27] Suarez-Mendez CA, Ras C, Wahl SA (2017) Metabolic adjustment upon repetitive
647 substrate perturbations using dynamic ¹³C-tracing in yeast. Microb Cell Fact.
648 16(1):161. <https://doi.org/10.1186/s12934-017-0778-6>

649 [28] Van 't Riet K, Tramper J (1991) Basic bioreactor design. Ed. Marcel Dekker.

650 [29] Suarez-Mendez CA, Sousa A, Heijnen JJ, Wahl SA (2014) Fast “feast/famine”
651 cycles for studying microbial physiology under dynamic conditions: a case study
652 with *Saccharomyces cerevisiae*. Metabolites 4: 347-372.
653 <https://doi.org/10.3390/metabo4020347>

654 [30] de Jonge L, Buijs NAA, Heijnen JJ, van Gulik WM, Abate A, Wahl SA, (2014). Flux
655 response of glycolysis and storage metabolism during rapid feast/famine conditions
656 in *Penicillium chrysogenum* using dynamic ¹³C labeling. Biotechnol. J. 9(3):372-85.
657 <https://doi.org/10.1002/biot.201200260>

658 [31] Youk H, Van Oudenaarden A (2009). Growth landscape formed by perception and
659 import of glucose in yeast. Nat. 462: 875-879. <https://doi.org/10.1038/nature08653>

660 [32] Van der Rest ME, Kamminga AH, Nakano A, Anraku Y, Poolman B, Konings WN
661 (1995) The plasma membrane of *Saccharomyces cerevisiae*: Structure, function,
662 and biogenesis. Microbiol. Rev. 59, 304-322. <https://doi.org/10.1128/mr.59.2.304-322.1995>

664 [33] Bertilsson, M., Andersson, J. & Lidén, G. Modeling simultaneous glucose and
665 xylose uptake in *Saccharomyces cerevisiae* from kinetics and gene expression of
666 sugar transporters. Bioprocess Biosyst Eng 31, 369–377 (2008).
667 <https://doi.org/10.1007/s00449-007-0169-1>

- 668 [34] Does AL, Bisson LF (1989) Comparison of glucose uptake kinetics in different
669 yeasts. J Bacteriol 171. <https://doi.org/10.1128/jb.171.3.1303-1308>
- 670 [35] Lagunas R (1993) Sugar transport in *Saccharomyces cerevisiae*. FEMS Microbiol
671 Rev. 10(3-4):229-42. [https://doi:10.1016/0378-1097\(93\)90598-v](https://doi:10.1016/0378-1097(93)90598-v)
- 672 [36] Maier A, Völker B, Boles E, Fuhrmann GF (2002) Characterisation of glucose
673 transport in *Saccharomyces cerevisiae* with plasma membrane vesicles
674 (countertransport) and intact cells (initial uptake) with single Hxt1, Hxt2, Hxt3, Hxt4,
675 Hxt6, Hxt7 or Gal2 transporters, FEMS Yeast Research 2(4): 539-550,
676 [https://doi.org/10.1016/S1567-1356\(02\)00140-X](https://doi.org/10.1016/S1567-1356(02)00140-X)

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