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Review

Integration of microbial kinetics and fluid dynamics toward model-driven scale-up of industrial bioprocesses

Scale-up of bioprocesses is hampered by open questions, mostly related to poor mixing and mass transfer limitations. Concentration gradients of substrate, carbon dioxide, and oxygen in time and space, especially in large-scale high-cell density fed-batch processes, are likely induced as the mixing time of the fermentor is usually longer than the relevant cellular reaction time. Cells in the fermentor are therefore repeatedly exposed to dynamic environments or perturbations. As a consequence, the heterogeneity in industrial practices often decreases either yield, titer, or productivity, or combinations thereof and increases by-product formation as compared to well-mixed small-scale bioreactors, which is summarized as scale-up effects. Identification of response mechanisms of the microorganism to various external perturbations is of great importance for pinpointing metabolic bottlenecks and targets for metabolic engineering. In this review, pulse response experimentation is proposed as an ideal way of obtaining kinetic information in combination with scale-down approaches for in-depth understanding of dynamic response mechanisms. As an emerging tool, computational fluid dynamics is able to draw a holistic picture of the fluid flow and concentration fields in the fermentor and finds its use in the optimization of fermentor design and process strategy. In the future, directed strain improvement and fermentor redesign are expected to largely depend on models, in which both microbial kinetics and fluid dynamics are thoroughly integrated.

Keywords: Computational fluid dynamics / Heterogeneity / Mathematical model / Scale down / Scale up

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1 Introduction

Over the last decades, large-scale industrial fermentations using a wide range of microorganisms, plant cells, and mammalian cells have been extensively employed for the production of commercial items such as food ingredients, nutraceuticals, biomaterials, vaccines, pharmaceuticals, biofuels, and pigments [1, 2]. A leading example has been the industrial penicillin manufacturing, which was initiated more than 70 years ago and the productivity of the current industrial strains is now 100,000 times higher than the original strain, by means of successive rounds of strain improvement, bioreactor design, and process optimization [3]. From stoichiometric point of view, the maximum theoretical yield of penicillin on glucose (mole/mole) was calculated as 0.18 by van Gulik et al. and 0.5 by Jørgensen et al. [4, 5]. However, the current yield of penicillin is still far away from either of those values. As an accepted fact, fermentation performance is largely driven by both strain characteristics and the environmental conditions of the cells. It is, therefore, common practice to engineer the strain and optimize the process, together. To pinpoint potential targets for metabolic engineering, however, becomes at some stage the limiting step for getting closer to the theoretical maximum product yield [6]. In this respect, it is of added value to get a thorough knowledge of pathway kinetics. Pulse response experiments as of now are a very fast and useful instrument to gain knowledge of in vivo microbial kinetics. It also aids in identification of response mechanisms as well as metabolic bottlenecks for metabolic engineering [7].

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Abbreviations:: **ATP**, adenosine triphosphate; **CFD**, computational fluid dynamics; **ED**, Entner–Doudoroff; **EMP**, Embden–Meyerhof–Parnas; **PBM**, population balance model; **PFR**, plug flow reactor; **STR**, stirred tank reactor; **TREH**, trehalose

It is known that time constants of enzyme-catalyzed reactions have nothing to do with the vessel size with which mixing time is positively correlated. In large-scale high-cell density fed-batch bioprocesses, environmental concentration gradients such as for substrate, pH, CO_2, and dissolved oxygen are prone to be induced, as long as the mixing time of the fermentor is longer than the relevant cellular reaction time. As a consequence, large-scale bioprocesses are often characterized by strong dynamics with which cells are forced to cope [8]. In many cases, substrate gradients lead to the formation of undesired byproducts, but in contrast other cases proved that external perturbations such as pulsed feed schemes caused a substantial increase of the productivity [9–11]. To understand these scale-up effects, the scale-down method is often advocated to efficiently take the local information of a large-scale bioprocess into consideration. After studies in the lab, an optimized scheme is then transferred to the production scale [12]. Computational fluid dynamics (CFD) is a powerful tool to simulate the detailed flow field of the fermentor and has already been applied for fermentor design and process optimization [13, 14]. As an example, in a 12 m^3 cephalosporin C production case, the effect of different impeller configurations on cell morphology and cephalosporin C production was simulated in detail using CFD. It revealed that a novel impeller combination decreased power consumption and enhanced mass transfer as compared to the conventional configuration [14]. The results of CFD simulation were also helpful in identifying the main cause for undesired scale-up effects. By using CFD for real-time fluid dynamic simulation of 50 L and 132 m^3 fermentors, applied for the erythromycin fermentation, it was found that the decrease of oxygen transfer rate largely impaired cellular metabolism and product formation [13]. The real challenge for the validation of this CFD model is the lack of information about gas bubble size distribution in the reactors. To overcome this, a dual probe has been developed and used to measure the local gas bubble size in a stirred tank reactor (STR) with multiple impellers [15,16]. Further, in the multiphase flow models, the description of the interactions between the gas phase, liquid phase, and biophase as well as the bubble coalescence and break-up model are also crucial for the accuracy of the simulation results [17, 18].

In this review, single and multiple pulse response experimentations are presented as powerful instruments to generate in vivo kinetic properties of cellular metabolism and to better understand dynamic response mechanisms. CFD can interpret flow dynamics of the fermentor and has been widely used in scale-up of large-scale bioprocesses. For the future, it is essential that microbial kinetics should be coupled to the CFD model for detailed description and prediction of the entire bioprocess. Using this approach, both the strain and fermentor can be rationally designed to serve their purpose [19]. Challenges and implications for the future are also addressed.

2 Environmental gradients: Causes and consequences

The production of secondary metabolites, including antibiotics, is often repressed in the presence of excess glucose, which is called catabolite repression. A fed-batch mode is thus introduced as an effective way to bypass this obstacle and is also applied to avoid oxygen limitation in large-scale high-cell density fed-batch processes. The feed nutrient, often glucose, is then supplied at a growth limiting rate. To minimize dilution of the broth, this feed solution is fed in a highly concentrated form. Scale-up at constant variables such as volumetric mass transfer coefficient is performed, leading to heterogeneities at industrial scales [20]. Alternatively, if the liquid circulation time at production scales is kept the same as for the bench scale, an incredible power input would be required [8]. Therefore, it is rather difficult to realize successful scale-up due to limitations of power input at industrial scales and a lack of knowledge about cellular response mechanisms. As is shown in Tables 1 and 2, in large-scale bioprocesses, the mixing time is tens or even hundreds of seconds [8, 21–36]. This is longer than the relevant cellular reaction time; especially with respect to the intermediates of central metabolism, having turnover times in the order of seconds [37]. As a result, heterogeneities in the system inevitably occur. Previous studies revealed that substrate concentrations in the feed zone can reach values of dozens to even hundreds times higher than in the low-concentration zones, and this difference may increase with higher biomass concentration [10, 38]. In this case, cells are periodically forced to circulate through famine and feast regions where substrate availability changes lead to rapid metabolite, flux, and growth rate responses. Amounts, qualities, and yields of biomass and products will be severely affected and stress responses will also be induced [39].

To effectively study the influence of environmental gradients, scale-down of industrial conditions is highly advocated for its convenience and efficiency [12]. As is shown in Table 3, a vast majority of scale-down studies has dealt with microbial fermentation [40–45]. Generally, scale-down of industrial practices is performed either through a combination of scale-down bioreactors or a special feed regime. With respect to scale-down devices, one-compartment (STR) and two-compartment systems (STR-STR/PFR, where PFR is plug flow reactor) are of great use in investigating the influence of external stimuli mimicked from those at production scales [46]. As a case in point, using a two-compartment system (STR-PFR), oscillating dissolved oxygen and substrate concentration at the production-scale fermentations with *Bacillus subtilis* were simulated [43]. The results indicated that the decrease of amino acid synthesis was due in large part to a metabolic shift toward ethanol formation. Käβ et al. [47] used a two-compartment scale-down system to study the influence of oxygen supply and substrate oscillations on cell metabolism. Broth cycling from an aerobic STR to an anaerobic PFR can be employed to simulate oxygen feast and famine zones at large scales and the residence time in the PFR can be adapted to simulate circulation times of large fermentors. It was found that oscillations within a minute range exerted insignificant impact on the metabolism of *Corynebacterium glutamicum*. In this strain, a futile cycle of side products formation and subsequent consumption was found to enhance its metabolic robustness against process inhomogeneities, which facilitated its use in large-scale fed-batch applications [47]. Apart from scale-down devices, applying a cyclic feed regime is frequently used for scale-down studies of large-scale gradients. As an example, the influence of a substrate concentration gradient on penicillin

Table 1. Reported mixing times in bioreactors.

Type of reactor	Cell line	Mixing time (s)	References
Cell culture			
5-L STR	CHO	2–5	[21]
8.5-L STR	Plant cells	3.6	[22]
11-L STR Helical ribbon	Plant cells	18–25	[23]
20-L STR	CHO	20–80	[21]
8 m^3 STR	Namalwa cells	40–200	[24]
10 m^3 STR[a)]	Plant cells	20–200	[25]
12 m^3 STR	Mammalian cells	120–360	[26]
10-L STR with spin filter Hydrofoil impeller, 20 rpm	CHO	120	[27]
250-L STR with spin filter Hydrofoil impeller, 80 rpm	CHO	120	[27]
250-L STR with spin filter Pitched blade impeller, 80 rpm	CHO	1620	[27]
1 m^3 STR with spin filter Hydrofoil impeller/mixing through spin filter	CHO	3120	[27]
15-L bubble column	Plant roots	2400	[28]
10 m^3 Airlift[a)]	Plant cells	200–1000	[25]
Microbial cultures			
12 m^3 STR, equipped with 3 Rushton-type impellers	Microorganisms	10–50	[29]
12 m^3 STR, equipped with 3 Scaba-type impellers	Microorganisms	10–30	[29]
30 m^3 STR, equipped with 3 Rushton-type impellers	Microorganisms	125–250	[29]
30 m^3 STR, equipped with 3 Scaba-type impellers	Microorganisms	70–110	[29]
2 m^3 Bubble column	Microorganisms	18	[30]
2 m^3 Airlift	Microorganisms	80	[30]
4 m^3 Airlift tower loop	Baker's yeast	100–175	[30]
40 m^3 Bubble column	Microorganisms	80	[30]
40 m^3 Airlift	Microorganisms	101	[30]
150 m^3 Bubble column	Baker's yeast	10–1000	[31]

Adapted from [8] with permission from Springer-Verlag.
[a)] Estimated.

formation was investigated via a 6-min on–off feed cycle in a chemostat cultivation system. de Jonge et al. imposed this intermittent feed regime to study the influence of substrate gradients on process performance and cell metabolism. It was found that penicillin production was almost reduced by a factor of 2 because of fluctuations in energy levels in response to the glucose perturbations as compared with constant feed cultivations [48].

It is the in vivo kinetics of relevant enzymes that determine which parameters need to be altered to obtain a desired change in a system [49]. It is, therefore, necessary to understand the mechanism of strain responses upon an external stimulus, in order to find potential targets for metabolic engineering. It is highly recommended that dynamic strategies such as shifts, ramps, pulses, and oscillations should be used for fast characterization of cell metabolism and process optimization [7]. Pulse response experiments are typically carried out in a time frame of seconds to a few minutes. In this short time window, it can be assumed that the enzyme levels do not change. Therefore, changes in both intra- and extracellular metabolites are ascribed to rapid enzyme–metabolite interactions only. Carrying out pulse response experiments is thus an efficient and effective approach for obtaining in vivo kinetic information and estimating kinetic parameters for kinetic metabolic models [50].

3 Generation of in vivo kinetic properties of cellular metabolism by pulse response experiments

There has been a surge of the application of models in industrial bioprocesses to improve the understanding of the cellular metabolism and to identify genetic engineering targets to reach a desired product yield. The establishment of kinetic models of a cell factory is the first step for the integration of fluid dynamics and microbial kinetics. The fundamental workflow for the establishment of kinetic models has been excellently reviewed by Almquist et al. [51]. However, the establishment of metabolic models is often hampered by the lack of information on the in vivo enzyme kinetic properties of the metabolic reactions. Toward this end, it has been well proposed that in vivo studies using pulse response experiments could contribute to parameter estimation of enzyme kinetics [50]. In parallel, the number of kinetic parameters should be reduced as much as possible by using approximative kinetic formats such as the linear-logarithmic approach [52]. A successful kinetic model should be simple but yet complete enough to describe sufficient aspects of the dynamic reaction kinetics and also convenient to be integrated into CFD models.

Pulse or stimulus-response experiments are an ideal tool to obtain understanding of the in vivo regulation mechanisms of

Table 2. Intracellular metabolite concentrations and turnover time in glucose-limited aerobic cultures of several organisms (*Saccharomyces cerevisiae* from [32], *Penicillium chrysogenum* from [33, 34], and *Escherichia coli* from [35]).

Metabolites	Intracellular level (μmol/gDW)			Turnover time (s)		
	P. chrysogenum	*S. cerevisiae*	*E. coli*	*P. chrysogenum*	*S. cerevisiae*	*E. coli*
Central metabolites						
G6P	4.64	5.2	1.42	23.3	17	3.6
F6P	0.71	1.4	0.38	5.7	7.3	1.2
T6P	0.55		0.13	47.8		NA
M6P	1.95		0.48			NA
6PG	0.25	0.48	0.10	3.7	4.5	1.1
Mannitol-1P			0.99			NA
G3P		0.13	0.17		57	13.1
FBP	0.9	0.64	0.82	7.2	3.2	2.5
F2, 6bP	0.01		0.35			NA
2PG+3PG	0.59	2.8	1.65	2.3	6.6	2.5
PEP	0.24	2.3	1.61	0.9	5.7	2.7
Pyruvate	0.22	1.1	0.75	0.9	1.7	1.5
α-Ketoglutarate	2.05		0.31	22.1		0.6
Succinate	0.23	4.0	2.65	3.3	20	8.9
Fumarate	0.65	0.85	0.22	13.0	4.1	0.7
Malate	3.33	7.3	0.94	19.0	30	2.8
Amino acids						
Alanine	21.7	32	1.34	269	3268	76.7
Asparagine	1.5	4.7	0.58	459	1142	81.7
Aspartate	16.3	21	2.57	717	577	35.0
Glutamate	53.0	170	74.69	658	1112	229.0
Glutamine	28.7	64	6.14	1243	2401	80.0
Glycine	2.1	2.9	1.51	244	247	31.0
Histidine	0.72	6.0	0.15	432	3141	53.8
Isoleucine	0.33	1.6	0.11	111	140	12.9
Leucine	0.73	1.0	0.36	131	125	27.1
Methionine	0.14	0.20	0.05	58.8	66	10.5
Phenylalanine	0.19	1.6	0.13	61.2	430	23.8
Proline	0.95	3.9	0.66	206	925	101.4
Serine	5.7		0.53	453		8.0
Threonine	5.9	4.0	0.47	758	220	29.3
Tryptophan	0.11	0.51	0.02	130	788	11.9
Tyrosine	0.26	1.6	0.18	145	832	44.3
Valine	2.1	10	0.51	243	490	40.9
Ornithine		4.1	0.49		502	49.1
Adenine nucleotides						
ATP	7.39	7.0	5.95	NA	1.4	2.0
ADP	1.03	1.3	2.31	NA	1.4	2.0
AMP	0.27	0.6	0.91	NA	3.1	9.4

Adapted from [36] with permission from Springer-Verlag.
G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; T6P, trehalose-6-phosphate; FBP, fructose-1, 6-biphosphate; M6P, mannose-6-phosphate; 6PG, 6-phosphogluconic acid; G3P, glycerol-3-phosphate; F2,6bP, fructose-2,6-biphosphate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate.

organisms to cope with external perturbations. In general, an external stimulus is imposed on a steady-state chemostat system whereupon the perturbed metabolome is quantitatively acquired via well-established fast sampling and quenching protocols [53, 54]. The BioScope devised by the technical university Delft group is a very helpful tool in pulse response experiments and is used as a special PFR in which an external perturbing agent such as the growth-limiting substrate, often glucose, can be pulsed. For example, in a glucose-limited chemostat cultivation of *Saccharomyces cerevisiae*, Visser et al. conducted pulse response experiments using the BioScope with both glucose and ethanol as the perturbing agents. By tracing the concentration change of intracellular and extracellular metabolites as well as energy and reducing equivalents, it was suggested that fructose-1, 6-biphosphate stimulated pyruvate kinase and PEP did not function in the allosteric regulation of phosphofructokinase. Further, the ethanol pulse might perturb the metabolism in an indirect way, which may be caused by the ratio variation of nicotinamide adenine dinucleotide (NAD) to NADH [50, 55]. Pulse response experiments are very helpful as well in understanding the robustness

Table 3. Environmental heterogeneity in bioprocess development.

Organism	Gradients of	Compartment system	References
Penicillium chrysogenum	Substrate	STR	[48] [60]
Corynebacterium glutamicum	Substrate/oxygen	STR–PFR	[47]
Saccharomyces cerevisiae	Substrate	STR/STR–PFR	[62] [63]
Pichia pastoris	Oxygen	STR–STR	[44]
Escherichia coli	Oxygen/carbon dioxide	STR–STR	[41] [42]
Bacillus subtilis	Substrate/oxygen/pH	STR–PFR	[40] [43]
CHO cells	Fluid dynamic stress	STR–PFR	[45]

of organisms. Robustness, the ability to maintain a balanced or functional state in coping with environmental changes or perturbations is the fundamental property of life [56]. Organisms in general have a specific robustness to survive unfavorable environments. Upon an external perturbation, different organisms may have similar but not the same responses. For instance, in the same steady-state chemostat cultivation systems, *Penicillium chrysogenum* and *S. cerevisiae* were transiently exposed to the same glucose pulse, but a more pronounced change of the glycolytic flux was observed in *S. cerevisiae* [34, 57].

4 Pulse response experiments combined with scale-down approaches for improved understanding of response mechanisms

According to literature, the Entner–Doudoroff (ED) pathway is very common in facultative anaerobes and aerobes. In comparison to the Embden–Meyerhof–Parnas (EMP) pathway, it seems that the ED pathway is not an efficient pathway of catabolizing glucose as it only produces one adenosine triphosphate (ATP) per glucose consumed. However, it was recently argued that the ED pathway struck a tradeoff between energy yield and the amount of enzymatic protein invested in the pathway, which seemed superior to the EMP pathway in terms of thermodynamics and kinetics [58]. Therefore, the ED pathway is still kept in the genome of a variety of organisms in case it needs to be used for survival. Similarly, microorganisms usually have a certain content of storage compounds such as trehalose (TREH), glycogen, as well as other reduced sugars to help to survive the dynamic conditions of undernutrition and overnutrition. On a steady-state chemostat system with a 6-min on–off feed regime, de Jonge et al. observed a high turnover rate of both internal and external storage metabolisms in *P. chrysogenum*. Storage turnover, referred to as a futile cycling, contributed to a large amount of ATP loss and observed reduction of penicillin formation [59, 60]. Nasution et al. found that in a steady-state chemostat system the absence of penicillin production may stimulate TREH metabolism [61]. It is conceivable that storage turnover plays an important role in maintaining cellular homeostasis [62]. However, less information is available with respect to operating mechanism in organisms. A recent study disclosed that TREH

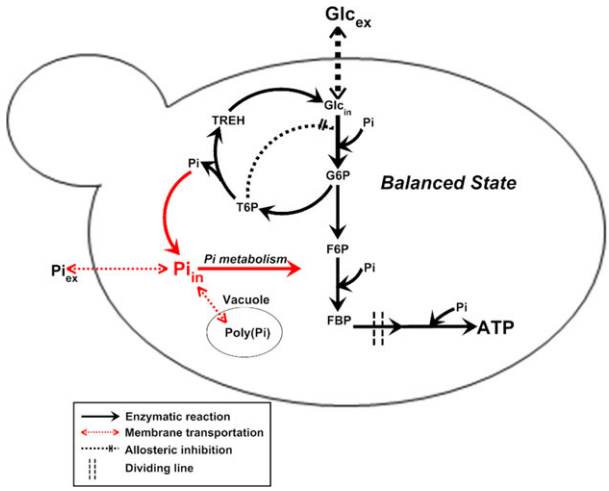

Figure 1. Overview of the trehalose cycle functioning in phosphate balance. Glc_{in}, intracellular glucose; Glc_{ex}, extracellular glucose.

metabolism played a vital role in keeping a balanced metabolic state and how it worked in a dynamic situation. van Heerden et al. used two model strains of *S. cerevisiae*, one was the wild type and the other was a TREH mutant [63]. Upon glucose pulses in the BioScope, coupled to a glucose limited steady-state chemostat system, rapid snapshots of the perturbed metabolome were taken for comparison. It was found that in the TREH mutant strain, upon a glucose pulse, the influx of the upper EMP was faster than the outflow of the lower part, and fructose-1, 6-biphosphate was accumulated at constant low levels of ATP and inorganic phosphate. As a consequence, this TREH mutant strain cannot maintain its intracellular pH and a balanced metabolism, resulting in growth arrest. At the same time, in the wild type, a ^{13}C wash-in experiment using a mixed carbon source of glucose ([1–$^{13}C_1$] and [U-$^{13}C_6$]) and ethanol [U-$^{13}C_2$] and tracing the labeling enrichments of glycolytic metabolites confirmed TREH turnover and almost 30% of the pulsed glucose was transiently shuttled into TREH formation. In this respect, as shown in Fig. 1, this futile cycle functioned in the release of inorganic phosphate to keep the phosphate balance. It successfully helped the strain buffer the transient glucose excess and maintained the normal functional state [63]. Likewise, by conducting dynamic ^{13}C experiments, it was found that about 38% of the incoming glucose was channeled into storage metabolism, which partly accounted for almost 52% of the missing ATP and the twofold reduction of penicillin formation [59, 60]. However, related experiments are needed to test if in *P. chrysogenum*, turnover of storage metabolism functions in the same way as in *S. cerevisiae*.

In addition to single-pulse experiments, also the response to multiple, repeated disturbances needs to be studied, and this can very well be done via scale-down simulators. As shown in Fig. 2A, the circulation times of cells in large-scale fermentors are actually within a wide distribution rather than at a single, average time (t_{avg}). At the same time, cells in large vessels have different paths circulating the external environments. Under these circumstances, scale-down experiments incorporate circulation frequency and random trajectories for simulation of large-scale conditions. As a consequence, an on–off feed regime with

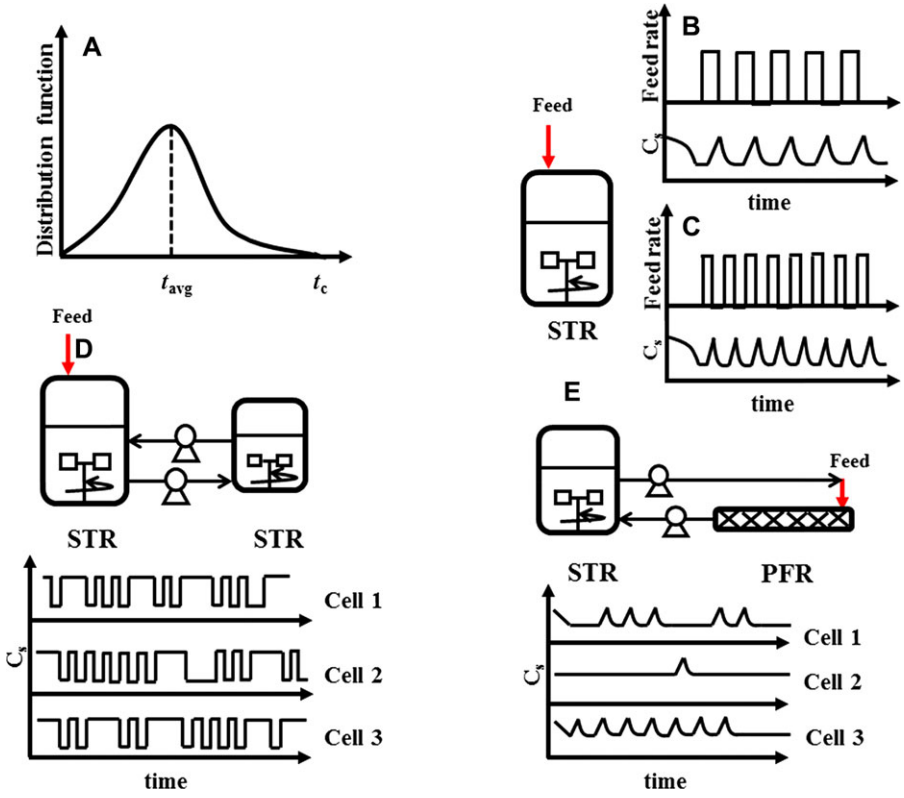

Figure 2. An overview of scale-down strategies in bioprocess development. (A) Circulation time distribution; (B) low-frequency feed cycle; (C) high-frequency feed cycle; (D) STR/STR; (E) STR/PFR.

constant frequency (Fig. 2B and C) is not sufficient to simulate the conditions in large-scale bioreactors. In a one-compartment system, all cells will respond in the same way which is not the reality on industrial scales. To address this issue, as shown in Fig. 2D and E, two-compartment systems are better suited to study the influence of the environmental heterogeneities. However, accurate mimicking of the large-scale conditions via down-scaling experiments still remains challenging now and in the future.

5 State of the art of integrating biotic and abiotic kinetic models

In the past few decades, unstructured metabolic models have been integrated with CFD models for simulation. In these approaches, the fluid phase was treated as continuum and the biophase was represented by a single chemical equation such as saturation-type kinetics of sugar uptake [38]. With the fast development of computer science and simulation algorithms, more detailed CFD models have already been established where Euler–Euler framing and Euler–Lagrange framing are the most frequently used approaches to describe the multiphase flow in bioreactors.

5.1 Euler–Euler frame

In the Euler–Euler approach, the fluid phase and biophase are treated as continuum, and described in terms of their volume fractions [64]. This approach has been applied in a number of bioreactors studies and also found wide applications in related fields [65–68]. For example, using segregated solutions of the Euler–Euler approach for the sake of less computational effort, the time course of production of gluconic acid was simulated and the numerical results showed satisfactory agreement with the experimental data. It was found that as the biomass density increased, the mass transfer coefficient decreased and the gluconic acid production rate was reduced. This allowed to better understand the performance of this important bioprocess at different scales [69]. Moilanen et al. investigated the xanthan fermentation process in aerated fermentors by coupling gas–liquid mass transfer, xanthan bioreaction kinetics, and non-Newtonian hydrodynamics with CFD. Gas–liquid hydrodynamics in xanthan fermentations including the bubble rise velocity and bubble size distribution was thus studied in detail [70]. In a cellulase production case, an integrated model combining both the biomass kinetics and multiphase Euler–Euler formulation was successfully used to predict dynamic profiles such as the distributions of oxygen, cellulose, and the shear stress within the fermentor [71].

It is well known that population balance model (PBM), capable of illustrating the population heterogeneities, is often integrated with the Euler–Euler approach. For example, using the specific growth rate as a criterion, Morchain et al. built a PBM to describe the heterogeneity of a cell population. It was shown that the model correctly represented the population growth rate dynamics. The model was used to predict the changes in the population growth rate as a response to the environmental change in the PFR [72]. Scale-up effects have also been explained in detail by the model combining the Euler–Euler approach, PBM, and

a kinetic model. Lab-scale and industrial-scale bioreactors were simulated by the model for comparison. It was confirmed that due to different time scales of mixing, mass transfer and chemical reactions in bioreactors scale-up at a constant operating variable likely resulted in formation of heterogeneities at a large scale [73]. The Euler–Euler approach is widely used, but there are several disadvantages associated with it. First, the biophase is treated as a continuum, and it is therefore unable to distinguish between individual cells. As a consequence, it is impossible to describe the trajectory of a single cell in the flow field, which makes this approach less suitable for simulating the performance of the cell population upon an oscillating environment. Second, the cell state cannot be described accurately enough by just one or two parameters that can be used to distinguish cell populations in PBM. Thus, a detailed description of the intracellular reaction network by PBM would lead to a high dimensional distribution function that is computationally intractable. As a consequence, such PBM has so far never been implemented [74, 75].

5.2 Euler–Lagrange frame

In contrast, the Euler–Lagrange approach still treats the fluid phases as a continuum but the dispersed biophase is then tracked by the Lagrangian approach [76]. As the biophase is present in the Lagrange frame, it becomes possible to track single cells and record the intracellular state as a function of time. As a result, the analysis of the lifelines of individual cells in space and time is possible. Until now, very few studies have been reported applying the Euler–Lagrange approach. By integrating a kinetic mode of the glycolysis of yeast with the CFD model, Lapin et al. were the first who described temporal oscillations in glycolytic metabolites at the single-cell level in the presence of a spatially heterogeneous glucose concentration field [77, 78]. To reduce the computational cost, the cell in this case represented a large collective of real cells. The simulation results showed that ideal mixing conditions contributed to synchronization of the individually autonomous oscillations at the population level, while in the presence of substrate gradients a dramatic loss of synchrony occurred. In order to further verify the integration method, a larger bioreactor was simulated with a more sophisticated cell kinetic model. The cell kinetic model contained a phosphotransferase system for the sugar uptake. The behavior of an *Escherichia coli* population in an oscillating environment was studied by integrating the CFD model with central carbon metabolism. Simulation results confirmed distinct differences in cell viability that had been observed experimentally at different operation scales [79, 80].

Compared to the Euler–Euler approach, the Euler–Lagrange approach is believed to be a more accurate method to describe the interaction between discrete cells and their environments. Cell population heterogeneity depends on the environments experienced at the single-cell level [81]. The unique trajectory of a single cell can be depicted since the Lagrangian approach tracks single cells along their paths in bioreactors. Meanwhile, the effect of temporal and spatial environmental changes along the trajectory of single cells can be introduced as well.

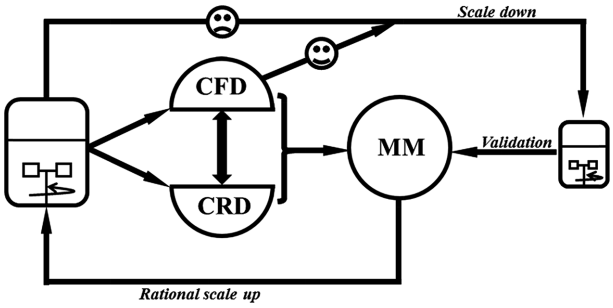

Figure 3. Schematic roadmap of rational scale-up in bioprocess development. CFD, computational fluid dynamics; CRD, chemical reaction dynamics; MM, mathematical model.

6 Concluding remarks

Understanding the effect of environmental heterogeneities on cells in bioreactors is of great importance for rational scale-up of industrial bioprocesses. Single pulse response experiments are an efficient tool in the identification of response mechanisms upon an external perturbation and facilitate the establishment of pathway kinetics. A couple of scale-down systems have been devised to study the effect of environmental gradients and multiple, prolonged perturbations in large-scale practices. In parallel, cell kinetic models still need to be reduced to be simple but yet efficient to describe the many types of cellular reactions. However, kinetic models alone are not capable of interpreting an oscillating environment. Therefore, an extensive understanding of a real bioprocess needs the information of the detailed flow field of the fermentor. As of now, the technology of CFD has been applied to represent the fluid and reaction dynamics as a result of the environmental changes in bioreactors. Two numerical approaches, the Euler–Lagrange approach and Euler–Euler approach, have been introduced to simulate the multiphase flow and reactions in bioreactors. In the Euler–Euler approach, the biophase is treated as a continuum, which is a gross simplification of reality. In contrast, the Euler–Lagrange approach is suitable for describing both continuum fluid environments and a discrete biophase. The huge consumption of computation time and space is, however, still a great hurdle for integration of complex models. In the future, it is highly advocated that more efficient numerical procedures should be established to enable the application of the Euler–Lagrange approach. As shown in Fig. 3, an integrated model combining both microbial kinetics and fluid dynamics will be essential to realize rational scale-up of industrial bioprocesses.

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