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Dos and don'ts for scaling up gas fermentations

Lars Puiman[1,2,*], Carolin Bokelmann[3,*], Sean D Simpson[4], Alfred M Spormann[5,6] and Ralf Takors[3]

Gas fermentation processes (using CO_2, CO, H_2, CH_4) have gained significant research and commercial interest in the last years due to their potential for carbon capture and sequestration. The small economic margins of these processes necessitate the use of large-volume bioreactors. For cost-effective gas delivery, we advise using pneumatically agitated bioreactors, like bubble column reactors, compared to traditional stirred-tank reactors. Although scale-up is conventionally done on an empirical and rule-of-thumb basis, rational methods are currently available. The most important one is the knowledge-driven scaling-up approach, wherein (CFD-based) hydrodynamic and kinetic models of large-scale bioreactors guide the design of representative lab-scale experiments. We suggest several future research directions to enhance the predictive capacity of these models and thereby accelerate scaling-up gas fermentation processes.

Addresses
[1] Australian Institute for Bioengineering and Nanobiology, The University of Queensland, Brisbane, Australia
[2] Department of Biotechnology, Delft University of Technology, Delft, the Netherlands
[3] Institute of Biochemical Engineering, University of Stuttgart, Stuttgart, Germany
[4] LanzaTech Inc, USA
[5] Department of Chemical Engineering, Stanford University, Stanford, USA
[6] Novo Nordisk Foundation CO2 Research Center, Aarhus University, Aarhus, Denmark

Corresponding author: Takors, Ralf (takors@ibvt.uni-stuttgart.de)
* These authors contributed equally to this work.

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Introduction

Gas fermentation as a carbon capture and valorization process has gained significant research and commercial interest for its potential to reduce greenhouse gas emissions [1,2]. With rising CO_2 taxes and limited fossil resources, commercializing this process becomes increasingly attractive. It is a key enabler for circular economy by recycling recalcitrant waste streams originating from the gasification of solid (non-)biogenic materials, off-gases, biogas, or via dedicated processes such as water electrolysis. These gases contain mixtures or purified fractions of CO, CO_2, H_2, and CH_4.

Microbial gas valorization can employ several metabolic routes [3]. Carboxydotrophic bacteria anaerobically convert CO, CO_2, and/or H_2 to acetate or other short-chain organic acids during acidogenesis. These acids may be further converted to alcohols during solventogenesis [4], to recombinant products like acetone and isobutanol [1], or to more functionalized compounds using sequential bioprocesses in bioreactor cascades [5]. Next to intensive academic research, companies such as LanzaTech commercialized anaerobic gas fermentation.

The low-value product spectrum of anaerobic gas fermentations has led to the development of aerobic gas fermentations with hydrogenotrophic microorganisms (carboxydotrophic or knallgas-based [CO_2 + H_2 + O_2]). These processes offer improved ATP supply but require challenging safety measures due to the simultaneous use of H_2 and O_2 [6]. The valorization of biogenic waste streams for single-cell protein (SCP) production is enabled, thereby complementing the historic approach of converting fossil CH_4 to protein for feed and food. Now, biomethane is converted to SCP by methanotrophic bacteria (e.g. by UniBio) or into biohydrogen under anaerobic or microaerobic conditions [7]. Knallgas-based fermentations are currently applied for SCP (food and feed) production at a near-commercial scale (e.g. SolarFoods (20 m^3 [8]), NovoNutrients, Aerbio).

To be competitive, the typical low-value large-volume products of gas fermentations require economies of scale. High gas-liquid mass transfer rates are required for highly productive bioreactors. Their ability to obtain high mass transfer capacities at lower operational (OPEX) and capital expenses (CAPEX) favors pneumatically agitated bioreactors instead of stirred-tank reactors (STRs). Although pneumatic bioreactors have been used in industry — starting with the ICI SCP production process from methane [9], and there is excellent literature from the 1980s on bioprocess scale-up [10,11], the majority of industrial biotech applications still focus on stirred-tank bioreactors [12]. In companies,

related decisions might be supported by missing own hands-on experience. In academia, the stirred tank is the dominating lab bioreactor, which might direct the scale-up thinking to related large-scale settings. In consequence, there is a hidden portion of high-value bubble column know-how that needs to be revisited, upgraded, and complemented with modern studies. The latter shall provide essential information about highly resolved spatial resolution to incorporate the microbial perspective. We deem this basic knowledge as crucial to properly design and scale-up pneumatically agitated bioreactors for implementing a circular bioeconomy.

Here, we will outline our perspective on scaling-up more reliably, by rationalizing the reactor choice, reviewing available tools for scale-up, and summarizing available experimental data and models. Furthermore, future steps that must be taken in the upcoming years will be presented.

Bioreactor choice

We identified the reactor design objectives for gas fermentors (Table 1) to select the best reactor type. The two most common reactors for aerobic processes at scale are mechanically agitated bioreactors (STR) and pneumatically agitated bioreactors [13,14]. The latter category encompasses bubble column reactors (BCRs) and internal- and external-loop gas-lift reactors [10], as used by LanzaTech [15]. These three reactors offer similar characteristics, although loop reactors offer better mixing and worse mass transfer capacities than the BCR [16]. For simplicity, we group the pneumatically agitated bioreactors as BCRs.

Gas fermentors should provide high mass transfer rates at the lowest possible costs. The cost of gaseous substrates requires high single-pass conversion rates (> 90% [17]), and thus high gas–liquid mass transfer rates (> 250 mmol L^{-1} h^{-1} [15]). The low-value product spectrum of gas fermentations leaves little economic margin. Consequently, companies cannot afford bioreactors with high CAPEX and OPEX, which are related to size, power input, and maintenance costs, respectively [14].

The obvious choice is to employ BCRs for industrial-scale gas fermentation. From our comparison in Table 1, BCRs offer superior mass transfer rates at low power input and costs while offering decent performance in other categories. Humbird et al. [14] support our observations and calculated that for aerobic bioprocesses, the cost of oxygen transfer is significantly lower in BCRs than in STRs, even for coalescent broths.

Table 1

Comparison of bubble column reactors and stirred-tank reactors for industrial-scale gas fermentation, based on reactor design objectives.

Design objective	Importance	Reasoning	BCR	STR
High mass transfer rates	High	Costly gaseous substrates require high conversion	*High* mass transfer rates in non-coalescent broths; k_La = 0.157 s^{-1}	*High* mass transfer rates in non-coalescent broths at high stirrer speed; k_La = 0.154 s^{-1}
Low power consumption	High	High power consumption increases operational costs	Only depends on *gas compression*; *P/V* = 275 W m^{-3}	Depends on *stirring* and *gas compression*; *P/V* = 4145 W m^{-3}
Low operational costs	High	Tiny economic margin	*Low OPEX* due to low power requirement and maintenance cost	*Higher OPEX* related to power consumption and reactor maintenance [14]
Fast mixing	Moderate	Gaseous substrates mix better than liquid-phase substrates	*Decent*, but better at higher gas flow rates; t_m = 180 s	High stirring speed required for shorter mixing time; t_m = 116 s
Low infection risk	Moderate	Low pH and low-energy substrates, but long-term cultivation and GMO use	*Decreased* infection risk	*Higher* risk due to rotating shaft, seals and bearings [10]
Low viscosity	Moderate	Low cell densities barely influence viscosity	Not to be used for high-viscous broths	Can handle high viscous broths [13]
No concentration fluctuations	Moderate	Smaller concentration gradient than liquid-phase substrates	Short, but frequent, concentration fluctuations [20,21]	*Longer* starvation times, potentially leading to physiological changes [21]
Low shear stress	Low	Bacteria are not that sensitive to high shear-zones	*Lower*, effect of bubble burst is unknown	*High* shear zones close to shaft and impellers [10]
High heat transfer rates	Low	Little heat production in gas fermentations compared to aerobic fermentations	Low power input requires less cooling; cooling via external loop	*Increased cooling* required due to high power input

Typical conditions for both types for gas fermentation were assumed (gas flow rate of 2.8 cm s^{-1}, height (25 m), diameter (5 m), 3 Rushton impellers (2.5 m) with gassed power number (1.5), ungassed power number (5), stirrer speed (100 rpm), 37°C, atmospheric pressure, non-coalescent broth). Correlations for k_La, t_m *and P/V were used according to* [11,18,19]. k_La in non-coalescent broth in BCR was assumed four times higher than in coalescent broth as in Ref. [15].

Challenges for scale-up

Gradients in large-scale bioreactors

Typical operational challenges in bioprocesses at scale are related to diluted feedstocks, diluted products, slow reactions [22], and low biomass concentrations. The large reactor size typically leads to gradients (e.g. in pH or substrate concentration) when transport of a substance is slower than its uptake [23,24] and consequentially to decreased performance (e.g. biomass yield and productivity). Generally, gradients in gas-based fermentations are smaller than in sugar-based fermentations (c.f. [25,26]) due to the more uniform distribution of bubbles in the tank, while liquid (or solid) substrates are normally added at a single point [23].

The gradients may induce stress on cells, as they frequently experience changing environmental conditions. Cell physiology alters, transcriptome and proteome are influenced, finally leading to population heterogeneity [27]. This affects cell growth and increases maintenance requirements, which in turn influences product formation yield [23]. As the effects are strain-specific, reliable data on short- and long-term responses of the relevant microorganisms are required but still missing [28].

In gas fermentations, the low gas solubility and gas conversion enhance the occurrence of gradients. Interestingly, limited substrate access might be beneficial as high CO concentrations can inhibit cell growth and the gradients may enhance ethanol production [25].

Approaches for scaling

Scaling-up is still largely guided by empirical guidelines, rules, and dimensional analysis [29]. A common scaling strategy is to keep one important parameter constant, for example, geometrical ratios, mass transfer coefficient (k_La), mixing time (t_m), volumetric power input (P/V) or, for BCRs, gas holdup, fluid dynamics, and bubble characteristics [24]. Such scale-up criteria are rooted in the physical analysis of the reactor and neglect the microbial perspective.

For BCRs, it is generally accepted that scales are comparable in terms of gas holdup when three criteria are met:

i) a diameter exceeding 0.15 m, *ii)* sparger orifices above 1–2 mm, and *iii)* an aspect ratio above 5 [30]. This simplifies scaling up BCRs from the hydrodynamic perspective. However, it is unknown whether these criteria still hold for gas fermentation broths.

To account for the microbial perspective, insights into the large-scale processes are required *a priori*. In essence, this is the basis of the knowledge-driven scale-down approach (Figure 1) that mimics large-scale conditions at the laboratory scale [27] to understand the metabolic response to substrate gradients. As the interactions between operational conditions and microbial physiology are diverse, highly complex, and non-linear [31], modeling became essential to identify the bioreactor performance and the microorganisms' experience.

Tools for scale-up

Model-based scaling of gas fermentation processes

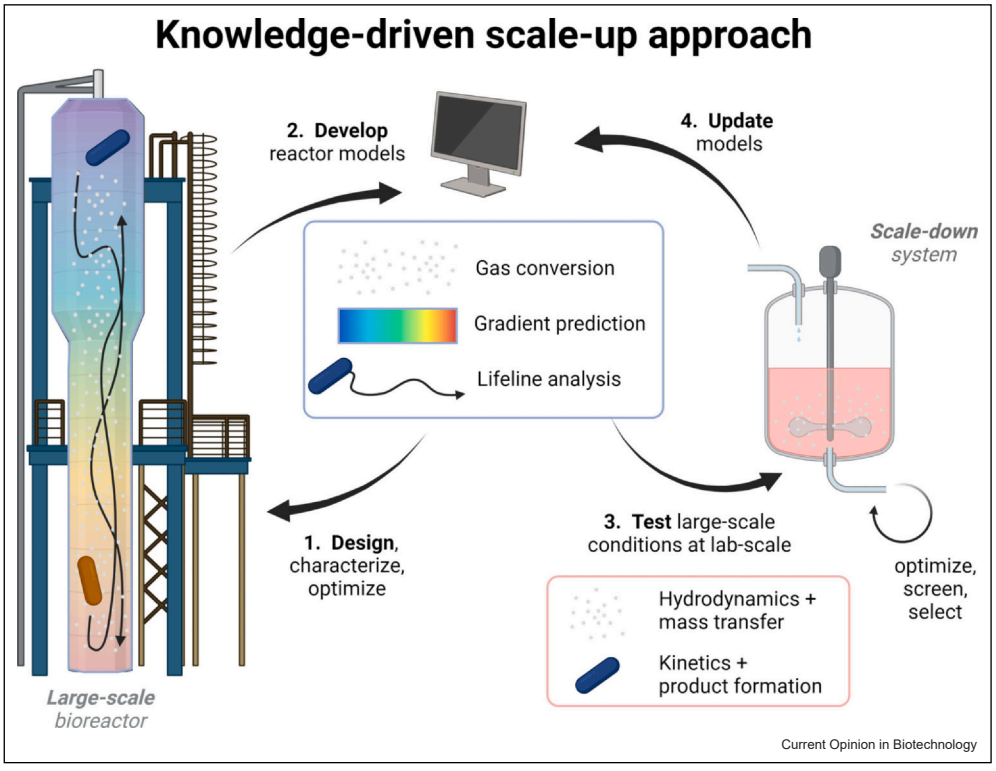
The available tools for bioprocess modeling are diverse due to the complex and strongly interconnected transport phenomena [32]. Both hydrodynamic and metabolic models have been developed with varying levels of complexity (Figure 2, Table 2).

Hydrodynamic models range from zero-dimensional (ideally mixed), over one-dimensional (gradients along one spatial coordinate) [33] to complex three-dimensional (3D) models [20,25,28]. The use of simple approaches allows for the combination of more complex metabolic models, which is restricted in high-dimensional hydrodynamic models.

Complex computational fluid dynamics (CFD) models with greater spatial resolution (e.g. [25]) allow for detailed predictions on the velocity and pressure fields, concentration gradients, and mixing and mass transfer phenomena. For gas fermenters, typically Euler-Euler models are employed that can capture the large gas holdup and high bubble numbers. Bubble coalescence and break-up may be simulated with population balance modeling. Via Lagrangian particles, one- or two-way coupling with metabolic models is possible, depending on the available computational resources.

The main limitations of CFD are related to high licensing and computational costs, the need for skilled personnel, and the model development and computation time. To overcome the latter, compartment models (CMs) were developed (e.g. [34]), wherein the reactor space is simplified into a network of ideally mixed compartments [35]. CFD results are used for compartment construction, either manually or automatically [35] or via unsupervised clustering algorithms [36]. The strong simplification of the system and hydrodynamics in CMs limits its accuracy but makes faster predictions possible. The high license fees and personnel costs for specialized CFD engineers for both CFD and CMs limit their use by small companies and start-ups. Nevertheless, we believe that CFD is key to scaling up bioreactors.

Figure 1



Conceptual representation of a knowledge-driven scale-up approach.

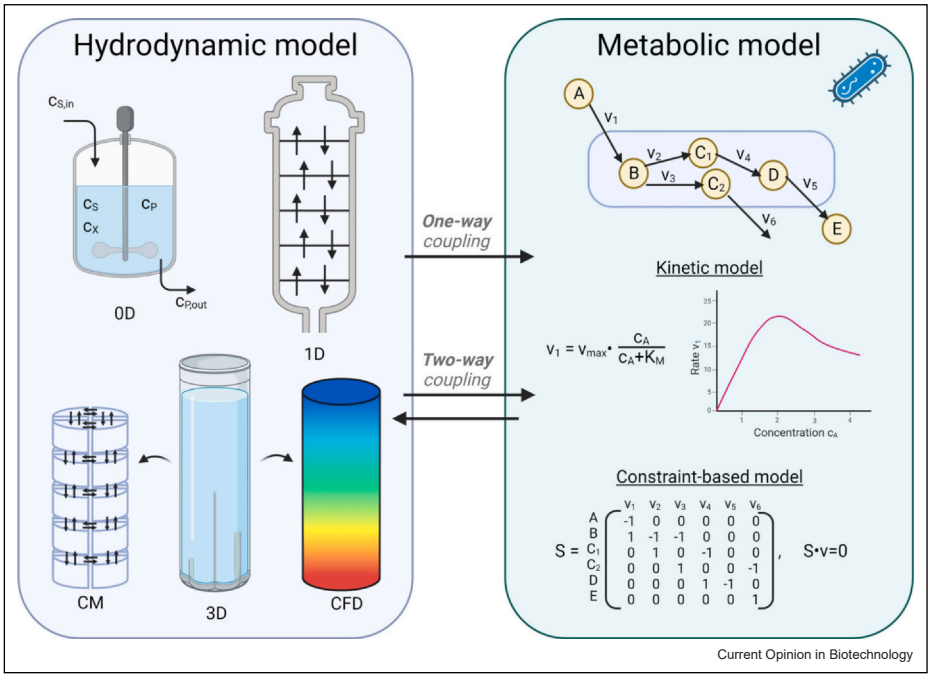
The metabolic submodels can generally be divided into constraint-based and dynamic models. The first class assumes a steady state inside cells, providing flux distributions by (dynamic) flux balance analysis. The latter incorporates kinetic information including detailed mechanisms and regulations to investigate the dynamic system changes. This results in enhanced predictive and extrapolation power but requires large datasets to ensure parameter identifiability. To combine metabolic models with hydrodynamics, simple model structures are favorable to reduce the computational burden. Simpler models can be developed by lumping reactions and metabolites into pools while containing regulatory mechanisms [37]. In 3D models, Lagrangian particles, which represent flow-following cells, can be tracked to obtain cellular experiences within the bioreactor. This approach is known as lifeline analysis [38].

The importance of validation

To ensure the reliability and accuracy of the integrated models, thorough validation with experimental data is essential. Increased interest in gas fermentation in the last years has led to more experimental investigations at a lab scale. Growth on different substrates [42], novel analytical methods [48], important mechanisms in metabolism [42,49,50], and the genome of the associated microorganisms [51] was examined.

For commercial-scale processes, almost no experimental data are available due to confidentiality inside companies and insufficient access to industrial reactors for research endeavors [23], making validation extremely difficult. Evidence of model validity can be obtained from pilot-scale data [15], experimental evidence in other/similar setups [28], experimental studies done in aerobic bubble columns (e.g. [52]), and patents. Experimental observations like bubble size distributions, k_La-values, and mixing times would be a huge advantage, but performing these measurements at scale is not straightforward. Several values for mixing times might be observed depending on the tracer addition location [53], while multiple probes and sample ports (which are missing) are necessary for measuring gradients [23]. Flow-following sensors gained attention for the validation of lifeline analysis (e.g. [54]) but cannot measure horizontal positions, while high gas holdups influence the axial position of the probe [55]. For the validation of the bubble size distribution, image analysis methods can be used [56].

Figure 2



Overview of hydrodynamic and metabolic models of different complexity. Hydrodynamic models are distinguished into zero-dimensional (0D), one-dimensional (1D), and three-dimensional (3D) models. The latter consist of compartment models (CM) and computational fluid dynamics (CFD) models. These are one- or two-way coupled with kinetic or constraint-based metabolic models.

Several scale-down devices have been developed that mimic large-scale conditions at a lab scale. These include single vessel systems with special sampling devices, oscillatory feed profiles [57], inserted plates separating the tank into multiple compartments (Single multi-compartment bioreactor) [58], or combinations of multiple reactors (e.g. STR-STR, STR-plug flow reactor) [57]. A conceptual scale-down design for gas fermentation was recently presented [20]. Experimental insights into commercial-scale processes are, however, essential for making realistic designs of scale-down devices, which enable further research on process and strain optimization with the end goal of successful and risk-reduced commercialization in mind.

Research directions

In gas fermentation, successful collaboration between biochemical engineers and microbiologists is imperative for experimental design and result interpretation. To alleviate the considerable number of modeling

Table 2

Overview of some models of syngas fermentation in literature.

Model/reference	Hydrodynamics	Metabolic model	Strain
MetaCLAU [39]	0D	steady-state, genome-scale model (GSM)	*C. autoethanogenum*
iCLAU789 [40]	0D	steady-state, GSM	*C. ljungdahlii*
iHN637 [41]	0D	steady-state, GSM	*C. ljungdahlii*
Hermann et al. [42]	0D	steady-state, central metabolism model	*C. ljungdahlii*
Ruggiero et al. [43]	0D	dynamic kinetic model	*C. autoethanogenum*
Almeida Benalcázar et al. [44]	0D / 1D	dynamic, thermodynamics-based black-box model + 9 compartments (dispersion), steady-state	*C. ljungdahlii / C. autoethanogenum*
Chen et al. [33]	1D	GSM with multiphase transport equations	*C. ljungdahlii*
de Medeiros et al. [45]	1D	axial dispersion model + dynamic multi-response model	*C. ljungdahlii*
Li et al. [46]	1D	hydrodynamics (pseudo steady state) + GSM + multiphase convection-dispersion equations	*C. autoethanogenum*
Puiman et al. [25]	3D, CFD	dynamic, 12-pool model [47]	*C. autoethanogenum*
Puiman et al. [20]	3D, CFD	dynamic	*C. autoethanogenum*
Siebler et al. [28]	3D, CFD	dynamic, minimum stoichiometry model	*C. autoethanogenum*

assumptions, advances in hydrodynamic and kinetic models are necessary, requiring experimental datasets to be available.

The presence of biomass, products, salts, and surfactants significantly affects hydrodynamics and mass transfer rates (via bubble coalescence) in fermentation broths [59], for example, at high surfactant concentrations, an increased drag coefficient is needed to capture gas profiles well [60]. Only if bubble dynamics can be accurately described, their impact on mass transfer and mixing — crucial parameters in BCRs — may be well calculated. Studies with the final broth composition are necessary to identify mass transfer performance and guide the development of coalescence and break-up models [61].

Kinetic models to predict biomass-specific gas uptake rates are required for successful bioreactor design. However, public information on dynamic and steady-state kinetics of gas-fermenting bacteria is too limited, and the reliability of the developed models is modest [48,62] due to uncertainties in mass transfer rates and dissolved gas concentrations. Innovative methods should be designed to study kinetic parameters without prior knowledge of k_La or dissolved gas concentrations [48].

Mechanistic understanding of product inhibition — which may be substantial [63] — enables whole-process optimization as increased titers decrease productivity but improve separation efficiency [64]. Similarly, mechanistic models that relate acetic acid concentrations to increased ATP requirements for cellular maintenance [65] are unavailable.

Scale-down studies that resemble the industrial gradients with gas excess and shortage are required, as distinct and recurrent metabolic stalls or electron oversupply influence microbial metabolism [25,28]. These 'feast or famine' studies may reveal how concentration variations affect growth yield and whether translational changes occur that influence maintenance metabolism [66].

Additionally, coupling genome-scale metabolic models with bioreactor models is required to study how operational choices and extracellular conditions influence metabolism and product spectrum. High gas conversion rates challenge the ideal-mixing assumption and the estimation of gas solubility, while hydrophobic products increase k_La and mass transfer rates. Such nonlinearities necessitate careful interpretation of the impact of individual variables at the reactor level.

To further investigate gas fermentation processes, several analytical tools are lacking [62]. Recent advances in online measurement of dissolved gas concentrations (CO, H_2) are promising [67,68]. Meanwhile, rapid-sampling techniques in microfluidics might be interesting options to study short-term concentration fluctuations of dissolved gases on the cellular level [69]. Measuring ferredoxin concentration, which is expected to play an important role in the response to cyclic electron excess and shortage [25], is still extremely difficult [62].

Despite knowledge-driven scaling strategies, pilot-scale studies are still required. Piloting reveals how important variables like gas hold-up and k_La scale in fermentation broths, and can be used to validate hydrodynamic and mass transfer models [15]. Pilot and mobile gas fermentation laboratories [70] identify how real-life process variations (e.g. impurities in the inlet gases) affect product quality during long-term cultivation.

Outlook

Gas fermentation is a core platform for future bio- and Greentech technologies, covering various processes like protein production via aerobic carboxydothropic fermentation, knallgas fermentation, and bioreactor cascades. It enables the production of high-value chemicals via synthetic co-cultures, either suspended or via biofilms. H_2-coupled carboxydothropy may enhance the sustainability of CO fermentations, allowing storage of intermittent energy supplies. These approaches extend gas fermentation's potential beyond the current CO-based application and may lead to closed recycling loops for persistent compounds. Gas fermentation should not serve as an airy excuse for the continuing exploitation of replaceable fossil resources that may be recycled only partly. A truly circular economy should be targeted based on sustainable resources.

We notice a lack of courage in the industry to shift to pneumatically agitated bioreactors, despite their proven reliability. This is reflected in the absence of BCRs in commercial pilot and demonstration plants. Interestingly, companies that have decided to apply large-scale BCRs once continue to use the technology for novel applications. The high capital costs and small economic margins limit the commercialization of gas fermentation processes. However, CO_2 taxation may shift the playing field.

Bioreactor scale-up can be accelerated using the recently developed knowledge-driven scale-up approach. CFD models help predict mass transfer rates and concentration gradients and enable scale-down studies. Kinetic metabolic models provide information about how microorganisms react to concentration gradients. Research is needed to limit the number of assumptions in these models, increase process understanding, and enable commercialization.

CRediT authorship contribution statement

Lars Puiman: Conceptualization, Writing – original draft, Writing – review & editing. **Carolin Bokelmann:** Conceptualization, Writing – original draft, Writing – review & editing. **Séan D Simpson:** Writing – review & editing. **Alfred M Spormann:** Writing – review & editing. **Ralf Takors:** Conceptualization, Supervision, Writing – review & editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Séan Simpson is co-founder and shareholder of LanzaTech. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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