Effects and Applications of pCO2 in Mammalian Cell Culture

Authors: Liu Xiaoshuang, Xu Hanmei, Xu Hanmei

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Abstract

Partial pressure of carbon dioxide (pCO2) is an important parameter in mammalian cell culture. Its primary sources include CO2 generated from cellular aerobic respiration, bicarbonate in the culture medium, and CO2 added for pH control. Although pCO2 is not precisely controlled in typical cell culture processes, maintaining pCO2 levels within a specific range (30–80 mmHg) is essential, as both excessively high (>80 mmHg) and low (<30 mmHg) pCO2 levels are detrimental to cell growth, metabolism, and protein production and quality control. High pCO2 issues are relatively common in large-scale high-density cell culture processes; therefore, differences in pCO2 levels across various culture scales must be considered during process scale-up. This review summarizes the effects of pCO2 on cell growth, metabolism, and protein production in mammalian cell culture, as well as methods for controlling pCO2 at large scale.

Full Text

The Effects and Applications of pCO2 in Mammalian Cell Culture

Xiao-Shuang Liu[1], Fei Chen[2], Meng-Jiang Zhao[2], Qiao-Qiao Hong[2], Han-Mei Xu[1]*

[1] College of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, China
[2] WuXi Biologics, Shanghai 200131, China

Abstract

Carbon dioxide partial pressure (pCO2) is an important parameter in mammalian cell culture, originating from cell respiration, bicarbonate in culture me-

dia, and CO2 added for pH control. While pCO2 is generally not precisely controlled in cell culture like pH, maintaining pCO2 levels within a reasonable range (30–80 mmHg) is crucial, as both high (>80 mmHg) and low (<30 mmHg) pCO2 levels are detrimental to cell growth, metabolism, productivity, and product quality. High pCO2 issues are particularly common in large-scale, high-density cell culture processes, making it essential to consider pCO2 differences across scales during process scale-up. This review illustrates the effects of pCO2 on cell growth, metabolism, and protein production, as well as strategies for controlling pCO2 in large-scale operations.

Keywords: CO2 partial pressure (pCO2); Mammalian cells; Cell culture process development; pCO2 control

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Monoclonal antibody therapeutics have brought new hope for treating critical diseases such as cancer and autoimmune disorders, becoming the fastest-growing class of biopharmaceuticals over the past two decades. Large-scale animal cell culture in bioreactors represents the core technology for industrial antibody production. Achieving more efficient and higher-quality antibody manufacturing is the development goal and core competitive technology for pharmaceutical companies and research institutions, with process consistency being a critical aspect of large-scale animal cell culture process development and optimization. However, elevated pCO2 levels frequently occur during large-scale production, contributing to performance inconsistencies between small- and large-scale cultures. Investigating pCO2 effects on cells and establishing control methods for pCO2 during scale-up are therefore of great significance. The following sections review the impacts of pCO2 on cell growth, metabolism, and protein production, methods for reducing high pCO2 in large-scale reactors, and the current status and future perspectives of pCO2 control in cell culture process scale-up.

1.1 The Role of CO2 in CHO Cell Metabolism

Carbon dioxide is a product of cellular metabolism. Over the past two decades, scientists have employed metabolic flux analysis (MFA) using isotopic tracers and mass spectrometry, combined with dynamic metabolic flux analysis, to investigate the material and energy metabolism of CHO-K1 cells at different growth stages [1, 2]. The characteristics of central metabolic pathways in CHO cells and the position of CO2 in cellular metabolism are illustrated in Figure 1 [Figure 1: see original paper].

During the exponential growth phase of CHO-K1 cells (days 0–4, with a specific growth rate of 0.033 h ^{1} from day 1 to day 2), glycolysis and anaplerosis are highly active, leading to rapid cell proliferation. In the anaplerotic pathway, CO2/HCO3 serves as a substrate that carboxylates pyruvate to produce oxaloacetate. Glutaminolysis generates -ketoglutarate, while malate is converted to pyruvate by malic enzyme (ME), replenishing TCA cycle intermediates. Therefore, during exponential growth, CO2 acts as both a reaction

substrate and a metabolic product. In the stationary phase (days 4–6), glycolysis and anaplerosis decrease significantly, while TCA cycle activity remains similar to that in the exponential phase. The oxidative pentose phosphate pathway exhibits high metabolic flux during this stage, becoming the primary route for ATP and reducing cofactors (NADH/NADPH) production to meet cellular energy demands and provide reducing power for oxidative stress [3]. Due to increasing cell density, substantial amounts of CO2 are produced during the stationary phase. In the death phase, despite nutrient depletion, cells maintain TCA cycle activity by efficiently utilizing lactate and glycine to replenish pyruvate, sustaining metabolism and heat production [2]. In summary, the exponential growth phase requires CO2/HCO3 as a substrate, while the stationary and death phases generate large quantities of CO2. As an important substrate and product in cellular metabolism, CO2 has become a parameter of significant interest in mammalian cell culture process development.

1.2 CO2 Sources and Stripping in Cell Culture

The pCO2 in cell culture represents the pressure exerted by dissolved CO2 in the medium. In mammalian cell culture, CO2 originates from three primary sources: CO2 produced by cellular respiration, decomposition of NaHCO3 in the culture medium, and CO2 added for pH control. Most animal cell cultures utilize the carbonic acid-bicarbonate (H2CO3-HCO3) buffer system. Higher bicarbonate concentrations in the medium result in higher pCO2 at the same pH, as shown in Figure 2 [Figure 2: see original paper]. At 37°C, the equilibrium system follows [7]:

$$pH = pK_2 + \log\left(\frac{HCO_3^-}{H_2CO_3}\right) \tag{1}$$

According to Henry' s law:

$$H_2CO_3 = 0.0307 \times pCO_2 \tag{2}$$

Combining equations (1) and (2) yields:

$$pCO_2 = GlucoseGlucoseGlucose{-}6{-}PPhosphoenolpyruvatePyruvateLactatePyruvateOxaloacetateMalate$$

From equation (3), the relationship between pCO2 and pH in buffer solutions with specific HCO3 concentrations can be plotted, as shown in Figure 2.

CO2 stripping refers to the process by which dissolved CO2 leaves the liquid phase and enters the gas phase. As illustrated in Figure 3 [Figure 3: see original paper], CO2 dissolution in culture medium involves several reactions, where K1 and K2 are equilibrium constants, CTR (carbon dioxide mass transfer rate) represents the CO2 transfer rate, CER (carbon dioxide evolution rate) denotes the

cellular CO2 production rate, and KLaCO2 is the CO2 mass transfer coefficient. The CO2 stripping rate in a culture system, typically expressed as pH change over time ($\Delta pH/\Delta t$), indicates how quickly CO2 escapes. Theoretically, when the CO2 stripping rate equals the CO2 production rate, all CO2 generated by cells can be completely removed without affecting pCO2 levels [9].

2. The Role and Effects of pCO2 in Cell Culture

2.1 Mechanism of Action

CO2 and its hydrated form HCO3 serve as substrates and products in intracellular carboxylation and decarboxylation reactions. Low HCO3 levels limit anaplerotic reactions, restricting oxaloacetate precursors in the TCA cycle and consequently affecting cell growth and metabolite production [4]. Mammalian cells possess effective pH regulation mechanisms, yet excessively high HCO3 concentrations can disrupt these systems [10]. When CO2 enters the cytoplasm and mitochondria, dissolved CO2 dissociates into hydrogen ions, lowering intracellular pH (pHi). This forces cells to upregulate Na /H and HCO3 /Cl antiporter activity to restore pHi, thereby disrupting normal pH regulation. CO2/HCO3 concentrations also affect intracellular enzyme activities; pHi changes can shift phosphofructokinase-catalyzed reactions away from their optimal pH range [11] and influence the activity of pH-sensitive sialyltransferases [12], ultimately impacting cell growth, metabolism, and protein glycosylation. High pCO2 levels reduce the concentration of CO2-generating enzymes (isocitrate dehydrogenase, -ketoglutarate dehydrogenase, malate dehydrogenase, phosphoenolpyruvate carboxylase), decreasing the specific CO2 production rate. Elevated pCO2 and HCO3 also affect the oxidative pentose phosphate pathway, leading to increased formation of highly reactive oxygen species—peroxymonocarbonate (HCO4). High HCO4 levels cause mitochondrial damage, impair cellular respiration, and reduce energy production during the stationary phase [13, 14].

The regulatory mechanisms by which CO2 and HCO3 control cellular metabolism remain incompletely understood, and their stimulatory effects may have been underestimated [4]. Research on high pCO2 effects in cell culture continues to expand, with new mechanisms of pCO2 influence being discovered.

2.2 Effects of pCO2 on Cell Culture

pCO2 influences cell growth, metabolism, protein productivity, and quality in mammalian cell culture, acting both independently and in combination with osmolality and pH [4]. Over two decades ago, researchers discovered that CO2 accumulation beyond certain concentrations becomes toxic to cells, and that appropriate pCO2 levels are essential for optimal cell growth and protein expression [15]. Further studies revealed that different cell lines exhibit varying tolerance to pCO2, and different growth phases show differential sensitivity to pCO2 fluctuations.

pCO2 affects intracellular lactate metabolism and the metabolic shift. The transition from lactate production to consumption is a common feature in mammalian cell culture that benefits cell viability and protein productivity [16]. In 2000 L single-use bioreactor (SUB) fed-batch cultures of CHO cells, high pCO2 caused lactate metabolism to shift from consumption back to production [17]. In continuous cultures of hybridoma cells [18] and glucose-limited CHO cells [19], high pCO2 increased specific lactate production rates. This phenomenon may occur because, in pH-controlled cultures, high pCO2 acidifies the medium, triggering base addition. Incomplete mixing in large-scale reactors creates localized high pH zones upon base addition, increasing the amount of base required for pH control and stimulating lactate re-production [17]. Additionally, base addition shifts the CO2 + H2O H + HCO3 equilibrium, causing HCO3 accumulation and increased osmolality, which affects lactate metabolism [20]. High pCO2, low pH, and high osmolality individually have minimal effects on lactate re-production; only their combined action induces this metabolic shift, which can be eliminated by enhancing CO2 stripping [17]. In 2000 L hybridoma cell cultures, high pCO2 increased specific lactate production rates, but increasing the aeration rate from 1 LPM (Liter Per Minute) to 2.5 LPM reduced lactate concentration by 50% [21]. Conversely, high pCO2 can also decrease lactate production rates. In perfusion cultures of Baby Hamster Kidney (BHK) cells, increasing pCO2 from approximately 84 mmHg to 230 mmHg decreased the specific lactate production rate [22]. In shake flask cultures of hybridoma [18, 23] and CHO cells [24, 25], specific lactate production rates decreased with increasing pCO2. Thus, pCO2 effects on lactate metabolism vary across different cell types and culture modes.

Brunner et al. [26] found that in CHO cell fed-batch cultures, high pCO2 inhibited the metabolic shift in lactate metabolism at the same pH level. Compared to the control group with lower pCO2 (12.5% CO2), the high pCO2 group (20% CO2) did not exhibit the transition from lactate production to consumption. Before the metabolic shift, the high pCO2 group showed reduced glycolysis, TCA cycle activity, and respiration compared to the control. After the shift, glycolytic flux remained higher in the high pCO2 group, while other metabolic fluxes matched the control. Ultimately, protein yield in the high pCO2 group decreased to 1.1 g/L compared to 2.0 g/L in the control.

pCO2 affects cell growth and protein productivity. In shake flask cultures of CHO cells, pCO2 levels of 76 mmHg and 152 mmHg severely inhibited cell growth compared to 38 mmHg, achieving only 0.5×10 cells/mL (from an inoculation density of 0.2×10 cells/mL). Zero pCO2 produced similar effects [15], indicating that initial cell growth requires a certain CO2 concentration, while both excessively high and low pCO2 prevent cell growth. In large-scale industrial cell cultures, pCO2 can reach 150–200 mmHg, significantly inhibiting cell growth and protein production [23]. Gray et al. [15] found that maximum protein productivity in CHO perfusion cultures was achieved at pCO2 levels of 30–76 mmHg, while higher pCO2 (>105 mmHg) inhibited cell growth and reduced protein yield. Increasing pCO2 from 38 mmHg to approximately 148 mmHg de-

creased protein yield by 70%, resulting from both reduced specific productivity (50% decrease) and lower cell density. Darja et al. [25] observed that CHO cells in exponential growth phase under combined stress of low temperature (33°C) and high pCO2 (20% CO2) preferentially consumed glucose to produce lactate, shifting metabolism toward inefficient anaerobic pathways, with upregulation of antioxidant-related proteins indicating oxidative stress and severe growth inhibition. Mostafa and Gu [27] reported that scaling CHO cell production of a therapeutic glycoprotein from 1.5 L to 1000 L pilot scale increased pCO2 from 68 mmHg to 179 mmHg, reducing specific protein productivity by 40%. Zhu et al. [28] found that high pCO2 (140–160 mmHg) and high osmolality (400–450 mOsm/kg) in large-scale CHO cultures inhibited cell growth and protein yield, while at laboratory scale under the same process, cell density decreased by only 18% with no effect on protein yield. Elevated pCO2 also affects other animal cell cultures. In myeloma cell batch cultures, high pCO2 caused excessive base addition, creating pH fluctuations that increased cell death [29, 30]. In hybridoma cultures, combined high pCO2 and high osmolality lowered intracellular pH, reduced glycolytic activity, decreased glutamine consumption, and inhibited cell growth without affecting specific antibody production rates [23]. Studies show that appropriate glucose limitation can compensate for cell growth inhibition caused by high pCO2 (190 mbar), as limiting glucose reduces lactate production, decreases base addition for pH control (and thus osmolality), and minimizes growth impacts [19]. Researchers have also proposed that for certain cell lines with strong lactate consumption capacity, using lactate instead of CO2 for pH control during the stationary phase can reduce pCO2 elevation while decreasing glycine utilization and mitigating ammonia accumulation [31].

pCO2 influences protein glycosylation. Zanghi et al. [32] found that high pCO2 alone had minimal impact on CHO cell protein glycosylation; rather, elevated HCO_3^- and osmolality under high pH and high pCO2 conditions were the root causes of reduced polysialylation in neural cell adhesion factor, with HCO_3^- having a greater effect than NaCl at equivalent osmolality changes. Interestingly, in CHO cell cultures, high pCO2 (140 mmHg) reduced the non-human sialic acid derivative (N-glycolylneuraminic acid) content in secreted glycoproteins by 46% compared to low pCO2 (20–80 mmHg) conditions. Since N-glycolylneuraminic acid can increase immunogenicity, this case demonstrates a beneficial effect of CO2 accumulation on protein quality.

3. Research and Application of pCO2 Control in Large-Scale Cell Culture

Due to differences in reactor design, increased liquid height and hydrostatic pressure in large-scale bioreactors enhance gas solubility, creating vertical gradients in pO2 (O2 partial pressure) and pCO2. Combined with metabolic activity, mass transfer limitations, and reduced mixing efficiency at large scale, these factors contribute to environmental heterogeneity [33]. Research on scale-down models simulating large-scale heterogeneity effects on cellular metabolism

has grown, particularly regarding substrate, pH, and dissolved oxygen (DO) gradients [34]. However, fluctuations in CO2/HCO3 concentrations, a typical phenomenon in large-scale reactors, have received limited attention due to two main challenges: the complex reactions involving multiple CO2 sources in culture medium, and the difficulty in controlling and measuring pCO2 across different scales where varying CO2 gas residence times affect mass transfer coefficient determination.

3.1 Causes of CO2 Accumulation in Large-Scale Cell Culture

CO2 accumulation occurs more readily in large-scale cultures for several reasons: (1) The surface area-to-volume ratio decreases in large-scale reactors, reducing surface CO2 transfer and overall CO2 stripping rates, leading to accumulation [8]; (2) Larger reactor height and liquid level increase hydrostatic pressure, enhancing CO2 solubility; (3) Mixing efficiency deteriorates at large scale. Researchers [35] measured mixing times in various scales of stirred single-use bioreactors (STR) at identical impeller tip speeds (0.6 m/s), finding mixing times of approximately 68, 60, 35, 35, and 19 seconds for 2000 L, 1000 L, 500 L, 200 L, and 50 L reactors, respectively, demonstrating increased mixing time with scale. Different reactor types also exhibit varying mixing times; at equivalent power input (20 W/m³), a 2000 L SUB showed approximately 100 s mixing time versus less than 10 s for a 3 L reactor [17]; (4) Process scale-up typically follows the constant kLa (oxygen mass transfer coefficient) principle, resulting in reduced aeration rates at large scale despite better oxygen transfer, which decreases CO2 stripping and increases accumulation; (5) In large-scale production, microsparging or increased oxygen content in the gas stream is often employed when cell density reaches certain levels to meet oxygen demand, both of which reduce overall gas flow and consequently decrease CO2 stripping rates [15].

3.2 Strategies for pCO2 Control in Large-Scale Cultures

pCO2 levels result from the balance between CO2 generation and stripping. With fixed cellular metabolism, pCO2 is primarily determined by medium properties and operating conditions. Strategies to reduce pCO2 can be categorized into two approaches.

Reducing HCO3 sources in the medium. In perfusion culture of Baby Hamster Kidney (BHK) cells at 20×10 cells/mL, contributions to pCO2 from cellular CO2 production, NaHCO3 in the medium, and NaHCO3 added for pH control were 25%, 35%, and 40%, respectively. Replacing the NaHCO3 buffer with MOPS-Histidine (3-(N-morpholino)propanesulfonic acid-histidine) buffer and using Na2CO3 instead of NaHCO3 for pH control reduced pCO2 from 225 mmHg to 68–73 mmHg (a 68–70% reduction) in 15 L BHK perfusion cultures, and from 238±16 mmHg to 84±7 mmHg (a 65% reduction) in 1000 L perfusion cultures [22]. This approach reduces pCO2 at the source without modifying reactor configuration or culture conditions, offering simplicity and operational advantages. Mostafa and Gu [27] attempted similar methods to reduce high pCO2

in late-stage 1000 L CHO fed-batch cultures, but 3 L small-scale experiments showed no significant effect of varying NaHCO3 concentrations (0.5 g/L vs. 2 g/L) on late-stage pCO2 levels. Moreover, low-NaHCO3 medium has reduced buffering capacity, which is disadvantageous for processes requiring narrow pH control ranges. In such cases, alternative buffer systems can be employed, along with strategies such as widening pH control limits (e.g., lowering the pH setpoint to reduce base addition) or adjusting feed pH (allowing feeds to contribute to pH control and reducing acid/base addition), which can effectively lower pCO2 in small-scale cultures (data not shown).

Enhancing CO2 mass transfer. The CO2 transfer rate is given by CTR = KLaCO2(C* - C), where KL is the volumetric mass transfer coefficient, a is the specific interfacial area, C is the dissolved CO2 concentration in solution, and C* is the CO2 concentration at the gas-liquid interface (i.e., in bubbles) [36]. KL is closely related to reactor configuration, medium properties, and operating conditions. Regarding reactor design, impeller position affects gas-liquid mixing and mass transfer; studies show that lowering the upper impeller position in dual-impeller systems enhances gas transfer [8]. Medium properties have minimal impact on CO2 mass transfer coefficients, as demonstrated in 1000 L large-scale cultures where HCO3 concentration did not significantly affect KLaCO2 [8]. Operating conditions such as surface aeration and agitation speed have limited effects on CO2 transfer in large-scale reactors. Although KLaCO2 increased with surface aeration rate (10× and 20× the bottom sparge rate) in 80 L and 500 L reactors, this effect diminished and even saturated in larger 2000 L and 10000 L reactors due to decreasing surface area-to-volume ratios [37]. Sieblist et al. [36] found that in large-scale reactors (400 L stainless steel), KLaCO2 increased with aeration rate (3.25, 4.5, 9 LPM) but was unaffected by increasing power input (38, 200, 300, 870 W/m^3), indicating that agitation speed has minimal impact on CO2 stripping. This was confirmed by Mostafa and Gu [27], who showed that CO2 stripping rates correlated strongly with aeration rate but weakly with agitation in both 1.5 L and 1000 L reactors, with higher aeration rates improving CO2 transfer. Although agitation speed minimally affects CO2 stripping, it directly influences bubble residence time; in a 200 L stirred-tank reactor, bubble residence time was less than 7 s at 200 rpm but increased to 11.3 s at 500 rpm, making agitation speed a critical parameter for oxygen transfer [36].

Gas flow rate and sparging mode are crucial factors affecting CO2 transfer and are most commonly considered in pCO2 control strategies. From a single-bubble perspective, O2 transfers from the bubble to the medium while CO2 transfers from the medium into the bubble. However, bubble CO2 content reaches saturation (matching medium CO2 levels) within seconds, after which bubbles no longer absorb CO2. Consequently, CO2 transfer in large-scale reactors occurs only during a limited period as bubbles pass through the medium [36]. Since bubble residence times in large-scale reactors exceed CO2 saturation times, bubbles entering the headspace can be considered CO2-saturated. Therefore, CO2 stripping in large-scale cultures can be simplified as the product of saturated

bubble CO2 content and bubble number, depending on total gas flow rather than KLaCO2 or bubble size [36]. At equivalent KLaCO2, open-pipe spargers achieve higher CO2 stripping rates than drilled-hole spargers, demonstrating that CO2 stripping is not solely determined by KLaCO2 [8]. Mostafa and Gu [8] controlled pCO2 below 61.2 mmHg using open-pipe sparging with minimal antifoam, solving a previous problem where pCO2 reached 180 mmHg. Studies showed minimal impact of bubble size on CO2 stripping in 2000 L reactors; varying bottom sparger pore sizes (10 m, 20 m, and 1 mm) did not affect KLaCO2 [37], further confirming that total gas flow is the key determinant of CO2 stripping in large-scale cultures.

In summary, HCO3 concentration, surface aeration, impeller position, and agitation speed have minimal effects on CO2 stripping in large-scale reactors, whereas gas flow rate and sparging mode significantly impact CO2 removal. When bubble residence time far exceeds CO2 saturation time, stripping rate is determined by total gas flow, with bubble size being negligible. However, larger bubbles cause less cell damage, making them preferable when considering shear stress effects on animal cells, though they provide poorer oxygen transfer. This limitation is typically addressed by increasing oxygen proportion, which inevitably reduces total gas flow and promotes CO2 accumulation. While agitation speed minimally affects CO2 transfer, it influences bubble residence time, with higher speeds benefiting oxygen transfer. Therefore, practical applications must balance these interconnected parameters.

Conclusion and Outlook

CO2 serves as both a critical substrate and metabolic product in mammalian cell central metabolism, influencing cell growth, metabolism, protein productivity, and quality both independently and through interactions with pH, HCO3 , and osmolality. Based on CO2 sources and stripping characteristics, combined with cellular metabolism and mass transfer principles in large-scale reactors, common strategies for pCO2 reduction include decreasing HCO3 concentration and increasing gas flow and bubble size.

Given the large dosing requirements of biopharmaceuticals, process scale-up is essential. Parameter control during scale-up ensures consistent culture environments, yet while pCO2 is not precisely controlled at small scale, it often fluctuates and increases during scale-up, affecting process performance. Therefore, to ensure equivalent cell growth performance and consistent protein quality across scales, pCO2 levels and other operating parameters must be comprehensively evaluated during scale-up, minimizing pCO2 differences while maintaining critical quality attributes and operational simplicity [38].

CO2 stripping issues during scale-up have garnered increasing industry attention. Xu et al. [9] employed constant P/V and VVM (gas volume/working volume/min) scale-up strategies, using constant VVM during the exponential growth phase to enhance CO2 stripping, successfully maintaining pCO2 below

50 mmHg when scaling from 3 L to 500 L SUB. However, this method only applies to cultures with high pCO2 during the exponential phase. Xing et al. [39] developed a large-scale pCO2 prediction model based on bubble residence and saturation times, though the model involves complex calculations and assumes a respiratory quotient of 1, limiting its applicability to certain cell lines. Currently, no universal pCO2 prediction model exists to forecast large-scale pCO2 levels and guide scale-up parameter selection prior to implementation. Thus, balancing oxygen supply and CO2 removal remains a significant challenge in scale-up, particularly for high-density cell culture processes.

As a valuable parameter in process scale-up, pCO2 reflects not only reactor mixing and mass transfer performance but also potentially indicates complex intracellular metabolic activities. We propose the following approach for scale-up (Figure 4 [Figure 4: see original paper]): First, analyze pCO2 levels and parameters affecting CO2 transfer rates in small-scale experiments to calculate cellular CO2 metabolic rates. Then, assuming unchanged metabolic state after scale-up and incorporating aeration/agitation strategies based on general scale-up principles, estimate large-scale pCO2 levels and assess their potential impact on cell growth or protein quantity/quality. If significant effects are predicted, adjust aeration/agitation strategies based on the target maximum pCO2 level to minimize process impacts. If effects are negligible, simulate large-scale high pCO2 conditions in a scale-down model. Additionally, low predicted pCO2 indicates minimal high pCO2 risk. Current challenges include different CO2 stripping rate calculations between scales: small-scale rates rely on empirical mass transfer coefficient formulas [27, 40], while large-scale rates consider gas residence and saturation times [36, 39]. Accurate CO2 stripping rate calculation requires further investigation. Moreover, online pCO2 electrodes enabling real-time monitoring and feedback control will represent a future trend, allowing pCO2 to be controlled with the same precision as pH and DO.

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Note: Figure translations are in progress. See original paper for figures.