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| Scaling up challenges | Process strategy | | | References |
| Oxygen limitation | Increase oxygen supply | 1. Reactor design  2. Increase total operating gas pressure  3. Reduce dissolved oxygen set point to increase mass transfer driving force  4. Reduce temperature to increase oxygen solubility  5. Improve oxygen uptake rate through cell physiological modification | | (Alford, 2006; Gomes et al., 2018; Muniz et al., 2024; Noorman et al., 2018) |
| Reduce oxygen demand | 1. Reduce feed rate to maintain lower growth rate  2. Mixed feeding strategy  3. Intermittent feeding strategy  4. Lower temperature set point to reduce metabolic activity  5. Develop alternative promoter system | |
| Insufficient cooling capacity | Improve cooling efficiency | | 1. Improve temperature set point  2. Reactor design | (Alford, 2006; Muniz et al., 2024; Noorman et al., 2018) |
| Reduce heat generation | | 1. Reduce the working biomass concentration  2. Use mixed feeding strategy |
| Gradients generation (substrate、pH) | 1. Optimize reactor design to reduce gradients  2. Select robust strains to reduce the effects of gradients | | | (Gomes et al., 2018; Noorman et al., 2018; Park et al., 2019) |
| Low-quality or unstable protein | Reduce protein degradation | | 1. Lower the temperature to avoid cell lysis and prevent protease release into the medium  2. Lower the temperature to reduce protease activity  3. Lower the pH set point to reduce protease activity  4. Develop protease-deficient strains | (Boodhoo et al., 2022; Gomes et al., 2018; Haon et al., 2015; Muniz et al., 2024) |
| Reduce batch-to-batch variability | | 1. Implement a continuous culture strategy  2. Develop protease-deficient strains  3. Use an automated process with online monitoring and process control |