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Coupling of Oxygen Transfer Limitation, Substrate Gradients and Acetate Overflow in High-Cell-Density Fed-Batch Cultivation of Escherichia coli

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Abstract. High-cell-density fed-batch cultivation of Escherichia coli can achieve high volumetric productivity, but scale-up creates a growing mismatch between cellular demand and reactor transport capacity. This review integrates oxygen-transfer limitation, substrate gradients and acetate overflow within one multiscale framework. As biomass rises, oxygen uptake can approach the achievable oxygen transfer rate, while slower mixing and localized feeding expose circulating cells to repeated high-glucose/low-oxygen and low-glucose/higher-oxygen zones. These trajectories promote rapid switching between respiratory and fermentative states, redirect carbon toward acetate and reduce biomass or recombinant-product yield. Scale-down evidence shows that exposure amplitude, duration and frequency are more informative than bulk averages alone. The available control options therefore have complementary roles: reactor and gas-delivery design improve transfer, adaptive or distributed feeding limits local carbon excess, soft sensors and model-predictive control coordinate constrained actuators, and strain engineering can improve robustness to transient gradients. Linking computational fluid dynamics to cell trajectories and reduced metabolic models provides a practical basis for scale-down validation and digital-twin development. Acetate accumulation is best interpreted as a transport-metabolism mismatch rather than a single pathway defect, so reliable scale-up requires coordinated process, equipment and host design.

Keywords: Escherichia coli, fed-batch cultivation, oxygen transfer, substrate gradients, acetate overflow

1. Introduction

Escherichia coli remains a major host for recombinant proteins, enzymes and biobased chemicals because it grows rapidly, has well-characterized genetics and supports established expression systems. High-cell-density cultivation (HCDC), particularly fed-batch operation, increases reactor productivity by limiting instantaneous carbon supply while biomass accumulates [1, 2]. The operating window nevertheless narrows as cell density rises. Oxygen demand, heat release and

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carbon dioxide evolution increase, while gas-liquid transfer, agitation, cooling and foam control remain bounded by the equipment.

The central scale-up problem is the difference between cellular reaction time and reactor transport time. Oxygen uptake rate (OUR) increases with biomass and specific metabolic activity, whereas oxygen transfer rate (OTR) depends on gas-liquid driving force, the volumetric mass-transfer coefficient, broth properties and operating limits [3-5]. Mixing also becomes slower relative to glucose uptake. A feed stream can therefore create a substrate-rich plume even when the measured residual glucose concentration is low. Cells circulating through the vessel experience local conditions that differ from the bulk dissolved-oxygen (DO) or substrate signal [6, 7].

These gradients have direct metabolic consequences. Short exposure to high glucose can raise glycolytic flux and oxygen demand, while a following oxygen-poor interval restricts respiratory oxidation. Scale-down studies show that repeated transitions reduce yield and increase by-product formation or stress responses [8, 9]. Acetate is especially important because carbon overflow and respiratory restriction both redirect acetyl-CoA away from complete oxidation. Accumulated acetate wastes carbon, increases the energetic cost of pH homeostasis and can impair recombinant-protein formation [10-12]. This review therefore treats oxygen limitation, substrate gradients and acetate overflow as one coupled transport-metabolism problem and evaluates the engineering interventions that act at different levels.

2. Coupled transport and metabolic mechanisms

2.1. Oxygen transfer and cellular demand

For an aerobic stirred tank, OTR is governed by kLa and the difference between the equilibrium and liquid-phase oxygen concentrations, while OUR can be represented by the product of biomass concentration and specific oxygen uptake. The apparent simplicity of these relationships conceals strong process dependence. Agitation, airflow, oxygen fraction, pressure, impeller and sparger geometry, bubble coalescence, viscosity and antifoam all affect transfer [4, 5]. At high biomass, the broth can become less favourable for transfer at the same time that OUR rises. A DO cascade can delay limitation by increasing agitation, gas flow or oxygen enrichment, but once its final actuator saturates, additional feed converts a transfer constraint into a metabolic disturbance.

A bulk DO set point does not establish a uniform cellular environment. One probe samples one location and has finite response time. A value recorded near an aerated or well-mixed region can conceal oxygen-poor zones elsewhere. The critical oxygen level is also response-specific: a concentration that sustains growth may not preserve the same product yield or expression stability. Transfer capacity should therefore be interpreted together with off-gas oxygen and carbon dioxide, actuator position, estimated OUR and the available margin before saturation.

2.2. Substrate gradients and acetate overflow

Fed-batch cultivation reduces average glucose concentration but does not eliminate local carbon excess. When cellular uptake is faster than feed dispersion, the inlet region becomes glucose-rich and oxygen demand rises locally. A cell may then move through a glucose-depleted region before returning to the inlet. Its physiological input is a trajectory defined by amplitude, residence time and recurrence frequency rather than one average concentration [6, 7]. Repeated switching consumes regulatory resources and may produce population heterogeneity.

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Carbon flux from glycolysis forms pyruvate and acetyl-CoA. When uptake exceeds respiratory and biosynthetic capacity, E. coli directs acetyl-CoA toward acetate, principally through the phosphotransacetylase-acetate kinase route. This overflow can occur aerobically. Proteome-allocation analysis explains why a lower-yield fermentative route can support high flux with less enzyme investment than respiration [11]. Oxygen limitation intensifies the imbalance by restricting electron transport and redox regeneration. A large-scale trajectory may therefore alternate between aerobic overflow caused by carbon excess and fermentative metabolism caused by insufficient oxygen.

Acetate formation lowers carbon efficiency and can initiate process drift. If acetate or expression stress slows growth while the programmed feed remains unchanged, substrate delivery becomes excessive relative to biomass demand, which further increases overflow. Acetate may later be reassimilated under glucose limitation and adequate oxygen, but recovery does not restore the energy and production time already lost. The diagnostic relationships are summarized in Table 1.

Table 1. Coupled mechanisms and diagnostic implications

Mechanism	Process evidence	Engineering implication
Oxygen-transfer limitation	DO decline, cascade saturation, reduced oxygen in off-gas	Raise transfer capacity or constrain metabolic demand
Substrate gradient	Feed-zone excursion despite low bulk residual glucose	Improve mixing, feed position, dilution or distribution
Acetate overflow	Acetate rise with lower biomass yield and altered base demand	Limit uptake, expand oxidative capacity or improve reassimilation
Self-reinforcing drift	Growth decreases while feed remains excessive	Use adaptive state estimation and safe feed constraints

3. Scale-down and modelling approaches

Scale-down reactors convert an industrial heterogeneity problem into a laboratory experiment. A useful model should reproduce the distribution of peak concentration, minimum oxygen level, exposure duration and return frequency, not only the industrial mean. A single stirred tank with glucose or gas pulses is easy to operate but gives most cells a similar disturbance. A stirred-tank/plug-flow loop provides a defined transition through an oxygen-poor or substrate-rich section, while two stirred tanks can represent zones with independently controlled aeration and feeding. Multi-compartment systems approximate broader exposure distributions at the cost of greater parameter and sampling complexity [8, 9, 13]. Table 2 compares the principal configurations.

Table 2. Scale-down configurations for reactor heterogeneity

Configuration	Strength	Main limitation
Single tank with pulses	Simple screening of temporal disturbance	Does not reproduce spatial circulation
Stirred tank and plug-flow loop	Controls exposure duration and repeated return	Plug-flow and gas-transfer assumptions may differ from the plant
Two stirred tanks	Independent control of two metabolic zones	Represents continuous gradients as two discrete states
Multi-compartment system	Can use compartments derived from flow modelling	Higher complexity and parameter uncertainty

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Computational fluid dynamics (CFD) can estimate circulation, gas distribution and local concentration fields in a specific vessel. Converting these fields into Lagrangian cell trajectories produces time series that can define a scale-down experiment. Reduced kinetic or metabolic models then translate exposure into uptake, redox and acetate flux. For online use, a hybrid model can combine mass balances and off-gas measurements with a compact acetate-risk description. A digital twin becomes useful only when it is updated by process data and validated under disturbances; agreement with a homogeneous laboratory run is insufficient evidence for industrial prediction.

4. Engineering control strategies

4.1. Reactor, oxygen and feeding interventions

Reactor-level interventions address the physical origin of heterogeneity. Impeller selection and spacing, baffles, sparger design and liquid height determine gas dispersion and circulation. Multiple feed points can shorten the distance between addition and dispersion. Their evaluation should use representative broth because biomass, salts and antifoam can alter kLa relative to clean water. Operational oxygen strategies include greater agitation, airflow, oxygen enrichment and pressure. Each raises transfer differently and introduces limits involving power, heat, foaming, gas cost or equipment rating. Maintaining a deliberate transfer margin before induction is more robust than operating continuously at the final cascade limit.

Exponential feeding can target a selected specific growth rate when biomass yield and maintenance assumptions remain valid. Induction, acetate stress and oxygen limitation change those assumptions. DO-stat, pH-stat and DO-transient methods provide feedback, but bulk signals can miss localized production. DO-transient control has also shown limitations in complex media when acetate reoxidation or local formation is poorly represented [14]. A stronger architecture combines carbon and gas balances with soft sensors for biomass, OUR, carbon dioxide evolution and respiratory quotient. Feed constraints should respond when predicted demand approaches the validated transfer envelope.

4.2. Strain robustness and integrated control

Host engineering can reduce glucose uptake, limit acetate-forming pathways, increase tricarboxylic-acid-cycle capacity or improve acetate reassimilation. Each strategy changes the whole flux network. Deleting an acetate route can redirect carbon toward another by-product, while slower uptake may reduce maximum productivity. Recent comparison under glucose shock found that the effect depended on the specific modification and strain background; reduced uptake alone did not guarantee the lowest acetate, whereas combined changes that expanded oxidative capacity were more effective in the tested system [12]. Candidate strains should therefore be screened using exposure profiles from the intended scale.

Control logic should distinguish prevention from recovery. Preventive limits keep predicted OUR below transfer capacity and avoid feed changes that create large local pulses. Recovery logic reduces feed, preserves oxygen supply and monitors whether respiration and growth return. An increase in DO after a disturbance may indicate lower cellular activity rather than restored capacity, so the original feed should not restart automatically. Table 3 summarizes the roles and constraints of the major intervention layers.

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Table 3. Engineering interventions for coupled oxygen and acetate risk

Intervention	Primary benefit	Principal constraint
Reactor and gas-delivery design	Improves mixing and oxygen-transfer capacity	Capital cost and scale-specific hydrodynamics
Adaptive or distributed feeding	Limits local substrate excess and demand peaks	Depends on sensing and sterile feed distribution
Soft sensor and predictive control	Coordinates feed and oxygen under actuator limits	Model mismatch and sensor drift
Strain engineering	Improves tolerance or oxidative carbon processing	Condition-dependent trade-off with productivity

5. Scale-up implications and limitations

A practical workflow begins by defining the relationship among growth rate, OUR, acetate formation and product expression in a well-mixed reactor. Representative-broth measurements should then establish kLa, mixing time, gas and power limits, probe location and feed-zone risk. Flow modelling or plant data can define plausible cell trajectories, which are reproduced in scale-down tests to compare candidate strains and controllers. Scale-up should preserve an oxygen-transfer and control margin rather than merely match a laboratory set point.

The framework does not provide a universal numerical threshold. Published studies use different strains, media, products and reactor geometries. Scale-down devices simplify the full distribution of industrial conditions, and CFD depends on uncertain gas-dispersion and rheology inputs. Acetate is also not a unique diagnostic: it can reflect carbon overflow, oxygen limitation or both, while a DO rise can reflect substrate depletion or declining cellular activity. Recommendations should therefore be verified with aligned feed, gas, DO, off-gas, biomass, glucose, acetate and product data. The most defensible optimization is multi-criteria, balancing titre and yield against power, oxygen use, heat removal, product quality and robustness to disturbances.

6. Conclusion

Oxygen-transfer limitation, substrate gradients and acetate overflow form a coupled multiscale problem in high-cell-density E. coli fed-batch cultivation. Rising biomass increases oxygen demand, while broth properties and equipment constrain transfer. Localized feeding and slow mixing create trajectories that alternate between carbon excess, oxygen limitation and starvation, driving metabolic switching and carbon loss. No single intervention is universally sufficient. Reliable scale-up combines reactor and gas-delivery design, adaptive feeding, online state estimation, scale-down validation and strain robustness. CFD-derived trajectories linked to reduced metabolic models can support predictive control, provided that the model is validated under realistic disturbances. The design objective is to maintain compatibility among substrate delivery, oxygen-transfer capacity and cellular processing capacity throughout growth and production.

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