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Scale-Up Can Reverse a Promising Fermentation Route: A Decision Model Linking Oxygen Transfer, Precursor Supply, Morphology, Product Toxicity, and Recovery Economics

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ABSTRACT

Industrial fermentation routes are commonly ranked from laboratory performance, yet scale-up changes the physical environment, the temporal exposure history of cells, and the economic consequences of upstream choices. This article develops an original, evidence-bounded bioprocess decision model for assessing when a route that appears superior at small scale may become less attractive after scale-dependent biological, engineering, and recovery constraints are considered. The analysis separates absolute performance loss from relative route reversal and integrates oxygen transfer, precursor and flux availability, morphology where biologically relevant, product or intermediate toxicity, robustness, and downstream recovery economics. The central proposed contribution is a comparative decision architecture in which each candidate route is assessed for scale-sensitive vulnerabilities, feasible mitigation, cross-domain interactions, and the possibility that one route incurs a larger scale penalty than another. The model does not assign universal weights or thresholds; instead, it identifies evidence states and discriminating experiments capable of changing route choice before major process commitment. Particular emphasis is placed on interactions, because oxygen limitation may alter precursor use, hydrodynamics may change morphology, toxicity may increase the value of in situ removal, and downstream burden may shift the economically preferred operating state. The framework is intended for structured precommitment reasoning rather than prospective prediction. Its principal limitations are the scarcity of matched head-to-head route comparisons across scales, imperfect fidelity of scale-down systems, model dependence in reactor simulations, host- and product-specific biology, and uncertainty in technoeconomic assumptions.

Keywords: Bioprocess scale-up, Fermentation, Oxygen transfer, Metabolic robustness, Downstream recovery, Technoeconomics

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Introduction

Industrial fermentation is commonly developed through a progression from strain construction and controlled small-scale cultivation toward progressively larger reactors. That sequence can encourage an implicit assumption that the route producing the highest titer, yield, or productivity under laboratory conditions will remain preferred after scale-up. Large-scale cultivation, however, exposes production organisms to mixing delays, fluctuating nutrient availability, oxygen limitation, stress, and other conditions that may be absent or attenuated in homogeneous small-scale systems [1]. The consequential question is therefore not simply whether scale reduces

performance. It is whether competing routes lose useful performance unequally enough for their relative ordering to change.

Industrial heterogeneity is structured rather than incidental. Mixing and mass-transfer limitations can generate spatial and temporal gradients in substrate, dissolved oxygen, pH, carbon dioxide, and temperature, with their magnitude and persistence determined by reactor geometry, operating conditions, rheology, and biological demand [2]. A vessel-average value can consequently obscure the sequence of environments encountered by individual cells. This distinction matters for route selection because two strains or biosynthetic strategies that appear comparable under steady conditions may differ substantially in sensitivity to transient depletion, substrate excess, redox disturbance, or local inhibition. Scale is therefore treated here as a change in the operating environment rather than as multiplication of vessel volume.

A second distinction concerns robustness. Engineering for maximal pathway output does not necessarily engineer the capacity to preserve that output during large-scale perturbation; production robustness constitutes a separate industrially relevant property [3]. A route can therefore be attractive because it reaches a high laboratory optimum, because it tolerates departures from that optimum, or because it combines both properties. Those states should not be conflated. The route that offers the highest laboratory value may require narrow environmental control, whereas a nominally lower-performing alternative may retain more of its performance across heterogeneous operating conditions.

This article consequently proposes a scale-aware decision model that compares candidate fermentation routes across biological constraints, engineering exposures, their interactions, and recovery economics before a route is treated as industrially preferred. The model is deliberately nonquantitative: it does not prescribe universal weights, thresholds, or a validated prediction score. Instead, it asks where route-specific vulnerabilities occur, whether they can be mitigated, whether mitigation creates new dependencies, and which unresolved comparison could plausibly change the decision. In this framing, route reversal means a change in relative preference after scale-relevant constraints are incorporated. It does not imply that reversal is inevitable, frequent across all processes, or prospectively predictable from the present framework.

Why route ranking changes with scale

A laboratory ranking becomes vulnerable when the comparison environment omits perturbations that matter industrially. Scale-down systems are useful because they can recreate selected features of large-scale operation, but their relevance depends on reproducing industrially meaningful exposure patterns rather than merely operating a smaller vessel [4]. For comparative route assessment, the important question is whether the selected challenge can reveal differential sensitivity. A scale-down experiment in which both candidates remain outside their consequential stress regimes may preserve the original ranking while providing weak evidence that the ranking will remain stable industrially.

Exposure is also temporal. The lifeline concept represents the changing environmental history of cells moving through heterogeneous reactor zones and thereby distinguishes cell-level trajectories from vessel-average conditions [5]. This supports a proposed construct used here: the differential scale penalty. A candidate incurs such a penalty when scale-relevant exposure reduces its biologically or economically useful performance more strongly than that of a competing route. The construct is explicitly comparative. Both candidates may lose performance while their order remains unchanged, or one may retain sufficient robustness for the relative preference to switch.

Industrial-scale analyses illustrate why penalties need not be uniform. In a 600-m^3 phenylalanine fermentation, spatially resolved simulation associated local glucose and oxygen conditions with different patterns of growth and product formation, demonstrating that feed placement and reactor transport can affect biological objectives differently [6]. Large-scale environments can also contain simultaneous gradients in substrate, oxygen, temperature, carbon dioxide, and pH rather than a single isolated oxygen-transfer limitation [7]. Route ranking should therefore be challenged against the combinations and temporal structures of exposure most capable of discriminating candidates. These analyses support the plausibility of differential effects but do not establish universal gradient magnitudes or guarantee that modeled heterogeneity will produce route reversal.

Biological constraints during scale-up

Biological scale sensitivity begins with the cellular response to changing exposure. In *Penicillium chrysogenum*, mimicked local and global perturbations in substrate and dissolved oxygen produced measurable physiological

responses, supporting the premise that coupled environmental fluctuations can matter beyond either variable considered independently [8].

Precursor supply is likewise dynamic rather than a fixed pathway attribute. Experimentally informed kinetic modeling of yeast glycolysis demonstrated time-dependent metabolic responses to glucose pulses [9]. The proposed model therefore distinguishes nominal biosynthetic capacity from scale-available precursor supply: whether precursor and cofactor provision remain adequate under the exposure history expected during operation. High production flux can also impose resource and metabolic burdens that compromise host fitness or productive robustness [10]. For complex products, coordinated enzyme, pathway, and host engineering is often required to establish adequate biosynthetic supply [11]. Precursor provision, pathway demand, and host capacity should therefore be treated as coupled constraints rather than assuming that greater pathway expression necessarily produces greater scalable output.

Growth and production demands can sometimes be separated through dynamic metabolic control [12]. Such control is a potential mitigation strategy rather than a universal solution. A route that depends on carefully timed switching may exchange a metabolic limitation for an additional process-control dependency.

Product or intermediate accumulation creates another route-specific constraint because toxic compounds can impair cellular function and production [13]. Stress-tolerance engineering offers several strategies for increasing resistance, but tolerance mechanisms are condition-specific and do not automatically confer broad process robustness [14]. Product toxicity and tolerance reserve should consequently remain analytically distinct.

For filamentous organisms, morphology introduces a scale-sensitive level absent from many unicellular systems. Pellet size, density, and structure can influence transport, broth properties, and productivity [15]. Morphology is therefore included in the proposed model only when host biology makes it mechanistically relevant.

Hydrodynamic conditions can alter that morphology. Controlled low-shear cultivation of *Aspergillus niger* showed that the reactor environment can modify macromorphological state [16]. Morphology observed during early screening should therefore not automatically be treated as invariant during scale translation.

Analysis of airlift cultivation further emphasizes interactions among fungal form, mixing, shear, and mass transfer [17]. The relevant decision is not which morphology is universally optimal, but whether the morphology adopted by a particular route remains compatible with the hydrodynamic regime needed at the intended scale.

The resulting biological distinctions are organized in **Table 1** to show which constraints should remain separate during route comparison and how each can alter the interpretation of apparent laboratory superiority.

Table 1. Biological constraint classes that can destabilize fermentation-route rankings

Constraint class	Scale-sensitive manifestation	Proposed decision implication	Boundary condition
Precursor and flux availability	Transient substrate or cofactor supply changes effective pathway capacity	Compare scale-available supply rather than nominal pathway capacity	Flux responses remain host- and condition-specific
Metabolic burden	Production demand competes with cellular resources or robustness	Test whether high output persists during process perturbation	Burden is not the sole explanation for poor performance
Product/intermediate toxicity	Accumulation impairs cellular function	Separate toxicity load from tolerance reserve	Effects depend on compound identity, exposure, and host
Dynamic control	Growth and production requirements are temporally separated	Treat controllability as a mitigation option	Added control complexity can become a process dependency
Morphology	Scale-dependent form changes transfer, rheology, or productivity	Include morphology only where host biology makes it consequential	No universal optimal morphology is implied

Engineering constraints during scale-up

Engineering variables become decision-relevant when they change the exposure delivered to competing routes. Oxygen transfer is a prominent example, but it should be treated as a conditional requirement rather than a universally sufficient scale criterion. A kLa-based strategy supported transfer of *Sulfolobus acidocaldarius* cultivation from benchtop to pilot scale in the particular system studied [18]. This demonstrates that oxygen-

transfer matching can be informative when it captures the dominant requirement; it does not establish that constant kLa guarantees biological equivalence among organisms, products, or reactor configurations.

The delivery problem changes when the substrate itself is gaseous. Gas fermentation introduces gas–liquid mass-transfer, pressure, gas-composition, and reactor-operation constraints requiring scale-up reasoning appropriate to that class of process [19]. Oxygen transfer, liquid-feed distribution, and gaseous-substrate transfer should therefore not be collapsed into a single generic mass-transfer variable. Candidate routes may differ not only in metabolic demand but also in whether the reactor can physically deliver the required substrate or electron donor across the intended operating range.

Engineering assessment should also ask what is concealed by population averages. Microfluidic single-cell scale-down approaches can resolve heterogeneous responses to dynamic environmental changes that bulk measurements may obscure [20]. Their principal value for the present model is diagnostic: they can reveal whether instability is distributed throughout the population or concentrated in a vulnerable subpopulation. Device-level observations, however, do not constitute plant-scale validation, and their representativeness depends on which industrial exposures can actually be reproduced.

Finally, reactor modeling should guide the design of biological challenge experiments rather than substitute for them. CFD-guided downscaling can translate industrial mixing and circulation into representative exposure histories and thereby improve the rationale for scale-down experiments [21]. The resulting predictions remain contingent on reactor characterization, transport assumptions, and biological kinetics. Engineering evidence is consequently strongest when modeled exposure, experimentally observed biological response, and later scale confirmation are treated as complementary rather than interchangeable evidence states.

Table 2 compares the principal engineering representations by the physical feature that must be preserved, the simplification most likely to mislead route selection, and the comparative question that follows.

Table 2. Engineering representations required for comparative scale-up assessment

Engineering dimension	What must be represented	Misleading simplification	Route-comparison question
Oxygen transfer	Biological demand relative to achievable transfer and local exposure	Constant kLa as universal equivalence	Which route loses useful performance first as oxygen becomes limiting?
Feed and mixing	Spatial and temporal substrate exposure	Vessel-average concentration as complete environment	Are candidates differently sensitive to depletion or excess?
Gas-fed transfer	Gas–liquid delivery and operating constraints	Treating gaseous supply like liquid feeding	Does substrate-delivery physics change feasible productivity?
Cellular heterogeneity	Distribution of responses across individual cells	Population mean as complete phenotype	Is instability concentrated in a vulnerable subpopulation?
Scale-down representation	Reactor-specific exposure histories	Generic perturbation without industrial basis	Does the challenge reproduce the stress capable of changing route preference?

Recovery and process economics

Product-associated stress and engineered tolerance should therefore be treated as related but analytically distinct properties of a production route [13, 14]. Fermentation performance becomes commercially meaningful only when product can be recovered at an acceptable overall process burden. Titer, yield, and rate influence production cost through different mechanisms and should not be treated as interchangeable indicators of route quality [22]. A high-titer route may still consume expensive feedstock inefficiently or operate too slowly, whereas a lower-titer alternative can remain attractive if yield, cycle time, and downstream behavior are more favorable. The proposed model therefore treats economic performance as an emergent property of the complete route rather than a downstream adjustment to an upstream ranking.

Recovery can also modify the biological operating state. In situ recovery of volatile fermentation products can reduce inhibitory accumulation while introducing separation and energy requirements [23]. The model labels this conditional interaction recovery-enabled tolerance: product removal may relax an exposure constraint without changing the organism's intrinsic tolerance. That distinction prevents a favorable integrated process from being misinterpreted as a more robust cell factory.

Recovery architecture is necessarily product-specific. Computational solvent selection and process design for β-ionone illustrate how partition behavior and separation configuration can shape feasible recovery choices [24]. More generally, downstream operations for fermentation-derived fuels and chemicals are most informative when considered during process development rather than after the upstream configuration has been fixed [25]. Recovery can therefore alter which broth composition, product concentration, or operating mode is economically desirable. Evidence from integrated process development further shows why technical scalability and economic attractiveness are separate questions. Pilot-scale development coupled with technoeconomic analysis can expose cost consequences that laboratory fermentation metrics alone do not resolve [26]. Similarly, technoeconomic optimization of cellobiose-lipid production showed that fermentation and purification decisions can be evaluated together rather than assuming the fermentation optimum is the process optimum [27].

This changes the meaning of "scalable." A route is not scale-favored merely because the reactor can reproduce its upstream productivity; it must also deliver a broth and product state compatible with recovery at the intended throughput. Conversely, a route with a modest upstream penalty may become preferable if it simplifies isolation or permits inhibitory product to be removed during cultivation. These are scenario-dependent trade-offs, not general advantages. Recovery can form part of the fermentation strategy itself, but its usefulness remains conditional on product properties, broth composition, and separation-process requirements [23–25].

Interactions that produce route reversal

Route reversal becomes plausible when the scale penalty imposed on one candidate is larger, less mitigable, or more economically consequential than that imposed on another. Direct evidence that comparative performance can depend on the operating environment comes from octanoic-acid production with *Escherichia coli*: genome reduction improved production performance under intermittent carbon-starvation scale-down relative to the parental background [28]. This does not establish a general law of scale-up reversal, but it demonstrates the narrower point required by the model—candidate performance differences observed under homogeneous conditions need not remain unchanged under scale-relevant perturbation.

A complementary computational argument concerns the separation of biological and process optimization. Simultaneous optimization of fermentation and microbial design produced different solutions from sequential optimization in modeled *Escherichia coli* cases, supporting the possibility that a strain optimum and a process optimum are not independently separable [29]. The evidence is computational and does not prospectively validate the present decision model. It nevertheless supports evaluating candidate biology together with the environment in which that biology must operate.

The proposed interaction logic distinguishes three outcomes. First, a common scale penalty reduces both routes without changing preference. Second, a differential but recoverable penalty narrows or changes the ranking only after mitigation cost is included. Third, a coupled penalty propagates across domains: oxygen limitation may alter precursor availability; hydrodynamics may shift morphology; product accumulation may intensify toxicity; and recovery changes may alter the economically preferred fermentation state. Industrial scale exposes cells to heterogeneous conditions whose importance depends not only on spatial gradients but also on the temporal histories with which cells encounter them [1, 2, 5].

Table 3 converts these interactions into a decision workflow. It deliberately avoids numerical weights because the evidence does not justify universal thresholds.

Table 3. Proposed workflow for detecting scale-dependent route reversal before commitment

Decision step	Comparative question	Evidence sought	Decision state
Baseline ranking	Why is one route currently preferred?	Matched titer, yield, rate, robustness, and recovery assumptions	Provisional preference
Scale challenge	Which industrial exposure can discriminate candidates?	Reactor-informed oxygen, feed, shear, or temporal perturbation	Ranking stable or vulnerable
Biological diagnosis	What causes unequal response?	Flux, burden, toxicity, morphology, or response-distribution evidence	Candidate-specific penalty
Mitigation test	Can the penalty be reduced without creating a larger dependency?	Control, tolerance, transfer, feeding, or recovery response	Mitigable or persistent

Integrated comparison	Does mitigation alter downstream burden or economics?	Updated mass balance and process scenario	Preference retained, reversed, or unresolved
Escalation	Which remaining uncertainty could still change the choice?	Discriminating scale-down or pilot experiment	Commit or retain alternatives

The mature synthesis is shown in **Figure 1**, where scale-sensitive exposures remain separate from biological vulnerabilities and recovery consequences until their interactions are evaluated against an explicit evidence boundary.

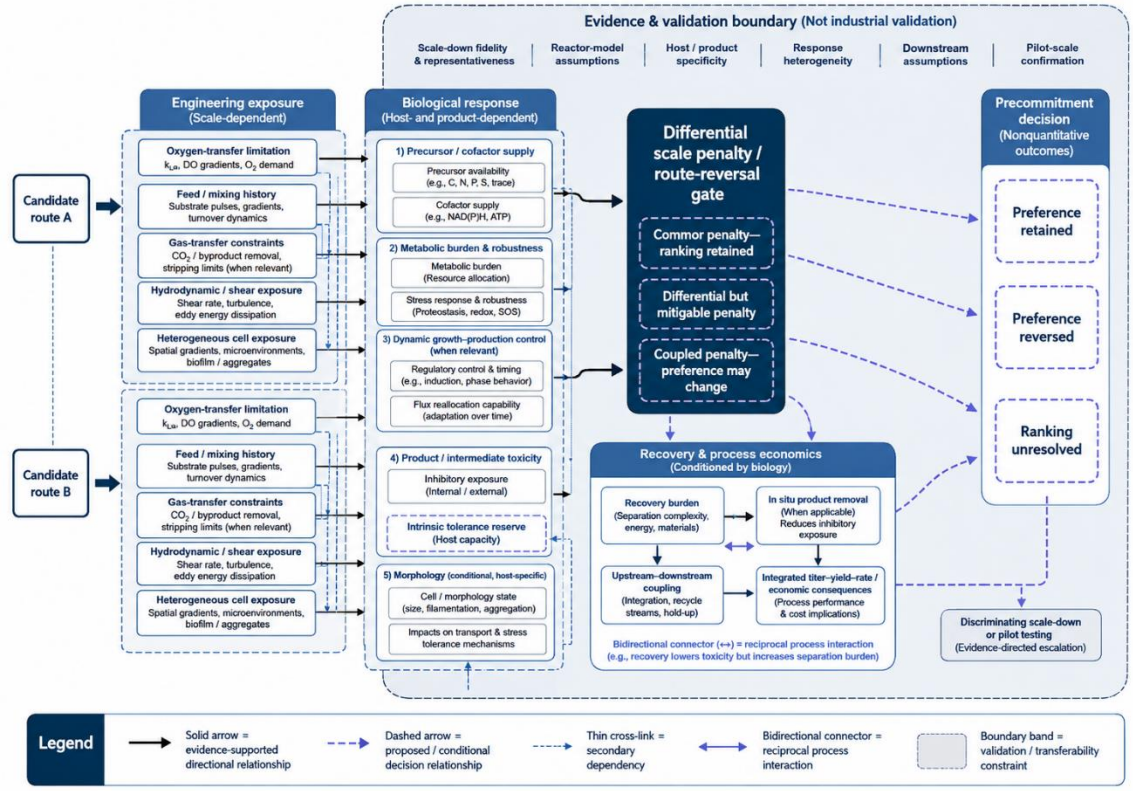

Figure 1. Proposed scale-aware architecture for detecting fermentation-route reversal

A scale-aware decision model

The proposed model begins with two or more candidate routes that have been compared under a common baseline. It does not replace conventional fermentation metrics; it asks whether their interpretation survives scale-relevant challenge. The first operation is therefore to state why the current leader is preferred and which assumptions make that preference possible. A ranking based on laboratory titer alone carries different uncertainty from one supported by yield, rate, robustness, and a plausible recovery route. Because industrial scale can create environmental exposures absent from homogeneous cultivation [1], baseline superiority is treated as provisional until consequential scale sensitivities are examined.

The second operation is domain separation. Engineering exposure, biological response, and recovery consequence are first examined independently so that unlike mechanisms are not hidden inside a composite score. Metabolic burden, for example, concerns cellular resource allocation rather than oxygen-transfer capacity [10]. Morphology is retained only for organisms in which form materially affects transport or productivity [15], whereas downstream burden remains a process-level property even when it feeds back on biological inhibition [25]. This separation prevents a favorable attribute in one domain from numerically cancelling an untested vulnerability in another.

Crucially, the domains are not converted into additive points. A severe but readily controlled oxygen constraint may be less decision-relevant than a moderate toxicity–recovery interaction that persists across feasible operating states. The proposed architecture therefore records vulnerability, evidence strength, mitigation feasibility, and downstream consequence separately before comparative judgment. This preserves mechanistic meaning and prevents an arbitrary total score from implying unsupported precision.

The third operation is interaction testing. Evidence of differential performance under scale-down supports asking whether a candidate-specific penalty is large enough to destabilize a ranking [28]. The proposed model classifies the answer as ranking retained, potentially reversed, or unresolved. These are decision states, not predicted probabilities. Integrated strain–process optimization provides a methodological rationale for avoiding rigid sequential optimization when biological and operating decisions are coupled [29], but the model does not assume that simultaneous optimization will always produce a superior industrial route.

The final operation is evidence-directed escalation. When the ranking depends on an untested interaction, the appropriate output is not a forced winner but the experiment most capable of resolving that interaction. Scale-down systems, mechanistic measurements, recovery scenarios, and pilot studies can thus be prioritized according to their ability to change the decision rather than accumulated indiscriminately.

Table 4 states the principal boundaries that must be crossed before a proposed route preference is interpreted more strongly.

Table 4. Boundary and validation requirements for the proposed scale-aware decision model

Boundary	What remains uncertain	Appropriate validation	Claim allowed after assessment
Exposure fidelity	Whether the challenge reproduces consequential industrial histories	Reactor-informed scale-down and trajectory comparison	Candidate response under the tested challenge
Biological attribution	Whether unequal performance arises from the proposed mechanism	Targeted flux, physiology, toxicity, or morphology measurements	Mechanistically constrained interpretation
Model dependence	Whether simulated gradients reflect the intended reactor	Model–measurement comparison and sensitivity analysis	Reactor-specific exposure estimate
Recovery coupling	Whether mitigation remains beneficial after separation burden	Integrated mass balance and recovery scenario	Process-specific feasibility
Transfer to scale	Whether comparative behavior persists beyond scale-down	Matched pilot or larger-scale confirmation	Evidence for the tested scale and configuration
Decision validity	Whether the model selects the better industrial route	Prospective head-to-head route evaluation	Validation only within the evaluated domain

Using the model before process commitment

The model is most useful before equipment, strain, and downstream choices become expensive to reverse. Application starts by defining a baseline preference and its evidence basis. The question is not "Which route is best?" in isolation, but "Under which assumptions is route A preferred to route B?" Those assumptions should include the fermentation metric driving the ranking, the expected reactor environment, the likely recovery configuration, and any biological feature—such as morphology or product toxicity—that could interact with scale.

Next, candidate-specific failure hypotheses are generated. A high-oxygen-demand route may be challenged against realistic transfer limitation; a route dependent on narrow precursor balance may be exposed to representative feed fluctuations; a filamentous route may require hydrodynamic conditions capable of changing morphology. Rational scale-down is valuable here because industrially relevant perturbations should be recreated deliberately rather than selected for convenience [4]. CFD-informed exposure analysis can help identify which histories deserve experimental reproduction, while remaining a model-based input rather than biological validation [21].

The comparison should then focus on discriminating responses. If both routes deteriorate similarly, the laboratory preference may remain informative. If one route deteriorates more strongly, mechanistic measurements should determine whether the penalty is persistent or mitigable. Mitigation must then be re-entered into the full process comparison. Product removal, for example, may reduce inhibitory exposure while adding separation demand; dynamic control may improve flux allocation while increasing operational dependency. The model therefore favors iteration rather than treating upstream optimization and downstream design as consecutive one-way stages.

A useful precommitment exercise should also define reversal criteria qualitatively before the challenge experiment is run. Investigators should specify what observation would justify abandoning the current leader, what result would merely trigger mitigation, and what result would leave the comparison indeterminate. Predefining these consequences reduces the tendency to reinterpret every adverse result as solvable after one route has accumulated substantial commitment.

An unresolved ranking is an acceptable output. Forcing a route choice when the preference depends on an untested coupled constraint converts uncertainty into hidden process risk. Comparative scale-down evidence demonstrates that strain performance can diverge under intermittent industrially relevant challenge [28], but such evidence remains system-specific. Before commitment, the strongest next experiment is consequently the one whose plausible outcomes would change the route decision, not necessarily the experiment that adds the largest volume of characterization.

Evidence needs and boundaries

The principal evidence limitation is fidelity. Scale-down systems can reproduce selected industrial perturbations, but they do not recreate an industrial reactor in full, and their interpretation depends on whether the relevant exposure history has been identified [4]. Lifeline approaches improve temporal representation, yet remain dependent on reactor characterization and the biological relevance of modeled trajectories [5]. Consequently, successful performance in one scale-down configuration should be interpreted as challenge-specific evidence rather than proof of scale readiness.

Computational tools have a similar boundary. CFD can identify reactor regions and exposure trajectories useful for experimental design [21], while simultaneous strain–process optimization can reveal consequences of coupled decision variables [29]. Neither establishes causal biological response without appropriate experiments. Models should therefore be evaluated for sensitivity to kinetic, transport, and process assumptions and anchored to measurements at the level required by the claim.

Biological domains also require conditional use. Product toxicity should be demonstrated rather than inferred from low titer [13], and morphology should enter the model only when host form affects process behavior [15]. The framework also separates absence of evidence from evidence of stability. If a candidate has not been tested under the exposure, morphology state, toxicity range, or recovery condition expected to discriminate it from its competitor, the appropriate designation is unresolved rather than robust.

Finally, prospective validation of route reversal requires matched candidate routes challenged under comparable scale-relevant conditions, followed where feasible by pilot-scale confirmation. Integrated pilot and economic assessment can strengthen process-specific conclusions [26], but no single case establishes universal transferability. The model should therefore be refined, rejected, or restricted when prospective comparisons fail to reproduce its proposed decision logic.

Conclusion

A promising fermentation route can become less attractive when scale changes the environment experienced by cells, the biological capacity to tolerate that environment, or the cost of recovering product from it. The central contribution of this article is a proposed scale-aware decision model that reframes scale-up as a comparative ranking problem rather than a uniform performance penalty. Oxygen transfer, feed and mixing history, precursor availability, metabolic burden, morphology where relevant, product toxicity, tolerance, and recovery economics remain analytically distinct until their interactions are examined.

The model's critical question is whether competing routes incur different scale penalties and whether mitigation preserves, reverses, or leaves their relative preference unresolved. This framing discourages premature commitment to the laboratory leader and makes uncertainty an explicit decision state rather than an inconvenience to be hidden inside an aggregate score. It also places recovery inside route selection, because a biologically superior operating state is not necessarily the economically preferred process state.

The framework is not a validated predictor of industrial success and supplies no universal weighting scheme, threshold, or optimal reactor strategy. Its value is instead methodological: it identifies which assumptions support a current route ranking and which scale-relevant experiment could overturn that ranking. Prospective head-to-head evaluation across routes, scale-down configurations, host classes, products, recovery architectures, and pilot systems is required to determine where the proposed model is useful, where its domains require modification, and where scale-aware reasoning does not materially change the original choice.

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