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Microbial Natural-Product Production Fails at Different Bottlenecks Across Scale: A Constraint-Switching Architecture for Fermentation Monitoring, Feeding, Induction, and Product Recovery

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ABSTRACT

Microbial natural-product manufacturing is usually optimized as though one dominant limitation can be identified, corrected, and then managed through scale-up. That assumption is increasingly difficult to sustain because pathway capacity, precursor allocation, product or intermediate toxicity, oxygen and substrate delivery, mixing, energy demand, and recovery can become restrictive at different process states and reactor scales. This article develops an original bioprocess architecture in which fermentation failure is treated as a changing constraint-identification problem rather than a fixed bottleneck problem. The analysis integrates evidence from natural-product cell-factory engineering, large-scale gradient characterization, scale-down perturbation studies, dynamic metabolic control, process analytical technology, hybrid state estimation, predictive control, and in situ recovery. The central contribution is a proposed constraint-switching architecture that separates candidate constraint classes from their observables, infers an active constraint with explicit uncertainty, and links that diagnosis to distinct actions in feeding, induction, process operation, or recovery before re-evaluation. The architecture is intended to preserve biological and engineering distinctions that are often collapsed in conventional scale-up reasoning. It does not assume that every fermentation has one uniquely identifiable constraint, that switching is always beneficial, or that an intervention resolving one limitation improves total process performance. Its principal value is therefore organizational and testable: it defines what must be monitored, distinguished, challenged, and validated before adaptive control of microbial natural-product production can be claimed across scale.

Keywords: Microbial natural products, Fermentation scale-up, Constraint switching, Process monitoring, Dynamic control, Product recovery

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Introduction

Microbial synthesis of plant natural products requires more than transferring a biosynthetic pathway into a host. Enzyme expression, precursor supply, cofactor balance, transport, pathway regulation, and host physiology must be coordinated if heterologous chemistry is to become productive [1]. Manufacturing-oriented analyses of yeast systems extend this problem beyond pathway reconstruction by emphasizing host choice, genetic stability, process operation, and downstream considerations as coupled requirements rather than separable optimization tasks [2]. These observations establish a multilevel production problem: a strain can be chemically competent yet process-fragile, while a well-controlled reactor cannot compensate indefinitely for an intrinsically weak pathway.

Recent natural-product engineering has increased the range and sophistication of microbial production strategies, particularly in yeast [3]. Synthetic-biology approaches now span enzyme discovery and optimization, pathway assembly, precursor engineering, regulatory design, and host-level interventions across diverse natural-product classes [4]. Yet these advances do not imply that the dominant reason for lost productivity remains constant once a strain leaves the conditions under which it was constructed. A pathway-limited system at small scale may become oxygen-transfer limited, stress limited, substrate-gradient sensitive, or recovery limited as cultivation conditions, biomass concentration, product accumulation, and reactor geometry change.

This creates a mismatch between how strain improvement is often conceptualized and how production failure may emerge during operation. Improving the strongest known molecular weakness can increase flux until another restriction becomes consequential; increasing feed can relieve precursor scarcity while intensifying overflow, oxygen demand, or toxic accumulation; stronger induction can increase pathway activity while shifting burden toward growth or stress tolerance. These are not asserted here as universal sequences. Rather, they illustrate why optimization should distinguish a constraint's biochemical identity from its current process dominance. The relevant object of control is therefore conditional on state, not merely on the organism's engineered genotype.

The central problem is not only how to remove a bottleneck, but how to determine which bottleneck is operationally dominant at a particular time and scale. Here, “dominant constraint” denotes the limitation whose relaxation would be expected to produce the greatest near-term improvement in the selected process objective under the current state, while acknowledging that several constraints may coexist and interact. “Constraint migration” is proposed as the change in that practical dominance across physiological phases, environmental exposures, or scales. It is a process-level interpretation, not a claim that one molecular mechanism literally moves between scales.

This article develops a proposed constraint-switching bioprocess architecture for microbial natural-product fermentation. It separates biological capacity from reactor-imposed exposure, distinguishes observation from causal diagnosis, and treats feeding, induction, operating conditions, and product recovery as non-equivalent actuator families. The framework is deliberately evidence-bounded: it uses established scale-up, monitoring, dynamic-control, and recovery principles to motivate a testable architecture, but does not claim that a universal switching rule, sensor set, or control threshold has already been validated.

Why the dominant fermentation constraint changes

Scale-up changes the environment experienced by cells even when nominal recipe, temperature, pH set point, and feed composition are conserved. Computational scale-up frameworks therefore emphasize coupling cellular response with reactor hydrodynamics and transport rather than treating scale as a geometric enlargement of a well-mixed vessel [5]. Industrial bioreactors can develop spatial and temporal heterogeneity in substrate, dissolved gases, pH, temperature, and related variables because mixing and mass transfer are finite [6]. Consequently, the process state observed by instrumentation at one location or as a bulk average need not equal the sequence of microenvironments experienced by individual cells.

Three distinctions follow. First, a gradient is a reactor property, whereas a constraint is a performance-limiting consequence; the former cannot be labeled the latter without biological or process-response evidence. Second, exposure magnitude and exposure history differ: repeated brief excursions may generate adaptation or stress patterns that a time-averaged concentration conceals. Third, scale can alter several variables simultaneously, so the most visible deviation need not be the variable whose relaxation most improves production. These distinctions motivate diagnosis at the reactor–cell interface rather than simple thresholding of one bulk process variable.

Dynamic compartment models make this distinction explicit by translating heterogeneous reactor regions into time-varying exposure histories [7]. Likewise, diffusion-based descriptions of large reactors show that mixing and axial dispersion can be represented as spatial processes rather than assumed to be instantaneous [8]. When the same framework is extended to substrate, oxygen, carbon dioxide, temperature, and pH, multiple gradients can coexist and differ in magnitude or location [9]. The practical implication is not that every gradient is limiting, but that several candidate constraints may become plausible as scale changes.

Cell-lifeline approaches sharpen the argument because they describe what cells repeatedly encounter while circulating through heterogeneous zones and use those histories to design scale-down perturbations [10]. This creates a mechanistic bridge between reactor structure and cellular response. The proposed constraint-switching view adds a further step: the relevant question is whether the resulting exposure causes pathway insufficiency,

resource reallocation, stress, transport limitation, or another restriction to become dominant. Scale therefore changes not only the intensity of a known bottleneck but potentially the ordering of competing constraints.

Classes of biological and engineering bottlenecks

The first constraint class is intracellular production capacity. Hierarchical metabolic engineering shows that flux control can arise at multiple regulatory and network levels, so pathway performance cannot be reduced to a single enzyme or precursor node [11]. A second class is chemical stress: desired products or pathway intermediates may inhibit growth, damage cellular functions, or trigger protective responses, making tolerance itself a production constraint [12]. These classes can interact, but they are analytically distinct because increasing pathway flux can worsen toxicity rather than relieve it.

A third class concerns resource allocation between growth, maintenance, and product synthesis. Strategies that balance growth with production are needed precisely because biomass formation and heterologous synthesis can compete for cellular resources [13]. Robustness introduces a related but different interface constraint: engineered chassis properties can determine whether a strain maintains useful performance when production conditions impose large-scale-relevant stresses [14]. In the proposed architecture, allocation and robustness should therefore not be merged into a generic "cell health" category; they imply different diagnostics and interventions.

Engineering constraints occupy a different analytical level. Mixing and mass transfer determine how feed, oxygen, heat, and other process variables are delivered to cells, while reactor geometry determines how heterogeneous those exposures may become [6]. Their effects are mediated through cellular physiology rather than being interchangeable with it. A low dissolved-oxygen region, for example, is an exposure condition; oxygen limitation becomes the operational constraint only if it materially restricts the selected production objective. This separation matters because an engineering intervention may alter a cellular constraint indirectly, and a strain intervention may change the process demand that created the engineering constraint.

Environmental delivery forms another class. Repetitive substrate dynamics can induce adaptation and metabolic trade-offs that steady conditions do not reproduce [15]. Combined glucose and oxygen oscillations can also shift transcriptional and physiological state in ways that make single-variable interpretation incomplete [16]. These results support separating reactor-generated exposure from intracellular capacity, even though the two are coupled.

For this article, the working classification is therefore functional rather than taxonomic: pathway/network capacity, toxicity, allocation, robustness, and environmental delivery are candidate biological or interface families, while mixing, transfer, and other reactor restrictions remain engineering families. Their purpose is to organize diagnosis and intervention, not to assert that every fermentation exhibits all classes or that one can always be uniquely isolated.

Evidence for constraint migration

Evidence for changing constraint relevance is strongest when dynamic perturbations alter cellular state differently from steady or differently structured exposures. In an industrially oriented scale-down system, periodic dissolved-oxygen feast–famine cycles changed the metabolic response of *Penicillium chrysogenum* [17]. A subsequent study comparing mimicked local and global substrate and oxygen perturbations showed that physiological responses depended on how the disturbance was configured, not merely on whether a nominal gradient existed [18]. These observations are consistent with constraint migration because the process consequence of a stressor depends on exposure context, but they do not directly identify a universal dominant constraint.

At the reactor scale, spatially resolved simulation of a 600-m^3 bubble-column fermentation demonstrated how local process conditions can reveal optimization opportunities that bulk descriptions obscure [19]. A recent synthesis similarly connects bioreactor heterogeneity with multiscale modeling, sensing, and increasingly autonomous control strategies [20]. Together, these works strengthen the case for diagnosing process state across levels, while also exposing an identifiability problem: correlated gradients can produce similar phenotypes, and a model-predicted limitation is not automatically a measured causal bottleneck.

Constraint migration should therefore be treated as a falsifiable process hypothesis rather than inferred whenever performance changes with scale. Stronger evidence would require showing that the response to relaxing one candidate restriction diminishes while the response to relaxing another increases across state or scale, with alternative causes challenged. A shift in metabolite profile, stress marker, or bulk productivity alone is insufficient

because each can arise from several coupled disturbances. The architecture developed here consequently separates observed deviation, inferred active constraint, and intervention response as three different evidential stages.

Engineering trade-offs further complicate dominance. Mechanistic modeling of industrial precision fermentation shows that improvements in mixing or transfer must be considered alongside power demand [21]. Gas fermentation represents an especially stringent boundary case because gas–liquid transfer, reactor configuration, and biological conversion impose coupled scale-up constraints [22]. Thus, the "active" constraint may shift between cellular, transport, energetic, and recovery domains depending on state and process design.

This evidence supports a multilevel architecture in which scale-dependent exposures and candidate bottleneck classes remain distinct until monitoring supports a constraint diagnosis; **Figure 1** shows that evidence-to-proposal boundary and the recursive logic required after intervention.

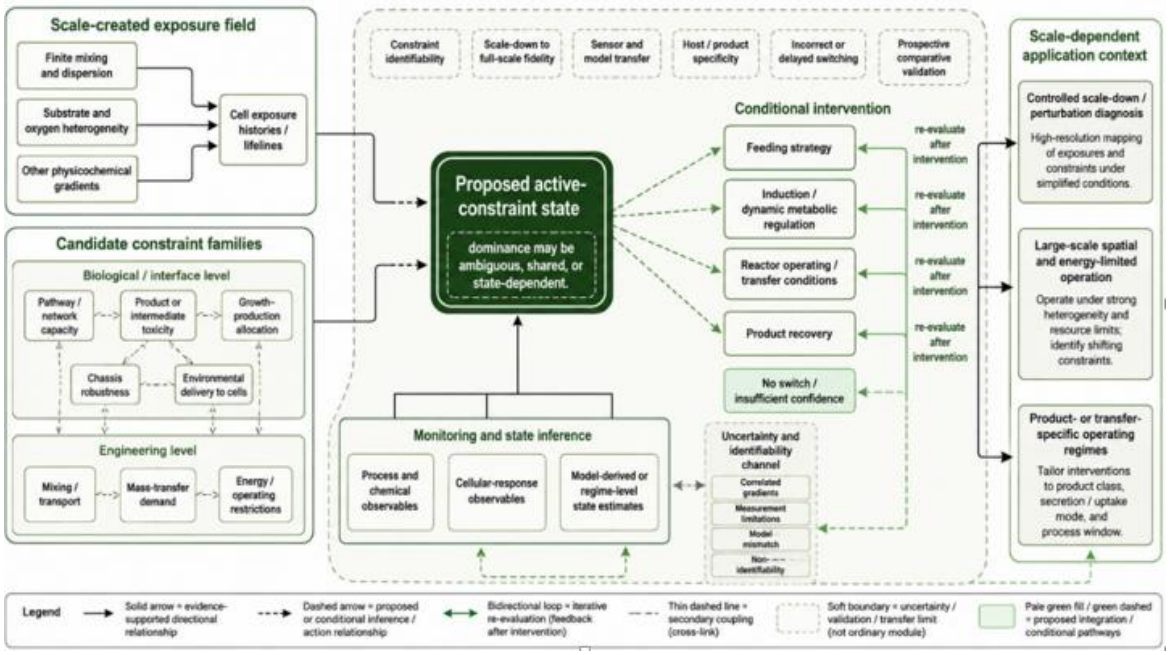

Figure 1. From scale-created heterogeneity to proposed active-constraint switching in microbial natural-product fermentation

A constraint-switching process architecture

The proposed architecture begins from a distinction between constraint class, constraint evidence, and constraint dominance. A pathway defect, toxic intermediate, oxygen-transfer restriction, or recovery limitation may exist without being the restriction that most strongly governs current process performance. The architecture therefore treats these mechanisms as competing hypotheses whose relevance must be updated as biomass, pathway activity, product accumulation, mixing environment, and operating regime change. This differs from conventional optimization in which a single bottleneck identified during strain construction or early process development remains the principal target throughout cultivation.

Dynamic process strategies provide important precedents. Two-stage metabolic deregulation has improved robustness and scalability in a defined engineered *Escherichia coli* system by separating physiological objectives across process phases [23]. More generally, two-stage fermentation is used to uncouple biomass formation from production when simultaneous maximization of both functions is unfavorable [24]. Dynamic metabolic control extends this concept by allowing pathway state to respond to biological or environmental signals rather than remaining fixed throughout cultivation [25]. These approaches establish that changing process objectives across state can be advantageous, but they do not establish which state transition should trigger a change in intervention for an arbitrary natural-product process.

The framework proposed here therefore replaces a predetermined phase schedule with a monitor–infer–act–reassess logic. Monitoring provides process, chemical, and cellular evidence. An inference layer evaluates which candidate constraint is most consistent with those observations while retaining uncertainty and competing explanations. The action layer selects a response only when the diagnosis is sufficiently discriminating: feed

modification for substrate or precursor delivery, induction adjustment for pathway allocation, reactor-operation changes for transfer or mixing limitations, and recovery intervention for product accumulation or compatible downstream removal. A low-confidence state can legitimately produce no switch.

Dynamic and two-stage regulation can reduce conflict between growth and production objectives by changing pathway control across physiological phases, although appropriate triggers and regulated nodes remain system dependent [23–25]. The article extends that established premise by proposing that the identity of the diagnosed active constraint, rather than cultivation time alone, should determine whether intervention changes. Because one action can expose another limitation, every switch returns the process to monitoring rather than terminating diagnosis.

Table 1 organizes the proposed constraint classes by what must be distinguished before an intervention is assigned.

Table 1. Proposed classification of active fermentation constraints and their diagnostic implications

Proposed constraint class	Distinguishing process question	Important confounder	Conditional intervention family
Pathway/network capacity	Is biosynthetic capacity limiting despite adequate delivery?	Regulatory or cofactor imbalance	Pathway or induction adjustment
Toxicity/stress	Does accumulation impair cellular function?	Nutrient or oxygen stress	Tolerance, induction, or recovery
Growth–production allocation	Are resources diverted from the selected objective?	General loss of viability	Feed or dynamic regulation
Environmental delivery	Are cells receiving substrate/oxygen unevenly?	Intrinsic metabolic limitation	Feed or reactor operation
Transfer/operating constraint	Is reactor transport restricting cellular supply?	Increased biological demand	Mixing, aeration, operating policy
Recovery constraint	Does product accumulation or removal limit continued production?	Cellular toxicity or pathway saturation	Compatible in situ recovery

Note: The classification is proposed as a diagnostic organization, not an established universal taxonomy. Classes may coexist or exchange dominance.

Figure 2 translates that classification into an operational decision architecture while retaining explicit uncertainty and scale-transfer boundaries.

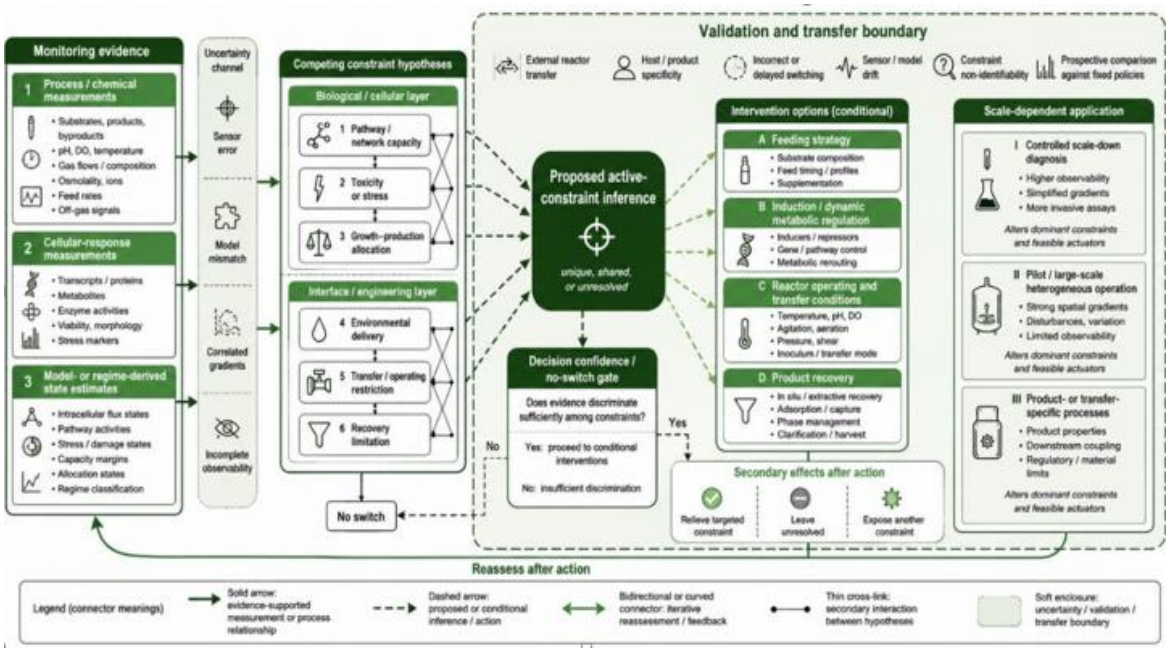

Figure 2. Proposed monitor–infer–act architecture for switching fermentation interventions when constraint dominance changes

Monitoring the active constraint

The architecture depends on recognizing that an active constraint is generally inferred, not directly measured. Process variables such as dissolved oxygen, feed rate, gas composition, or temperature describe the extracellular environment, whereas spectroscopy, metabolite measurements, stress markers, and physiological data describe different consequences of that environment. A diagnostic layer must therefore distinguish a measured deviation from the mechanism limiting the selected production objective.

Data-driven regime recognition offers one way to organize multivariate operating states without assuming beforehand that one signal defines the process condition [26]. Such regimes can indicate that observations cluster into distinct operational patterns, but the clusters themselves do not identify causal bottlenecks. Process analytical technology provides another layer: in-line Raman spectroscopy combined with indirect hard modeling can extract process-relevant chemical information suitable for monitoring and control [27]. Its diagnostic value nevertheless depends on calibration, matrix effects, analyte observability, and transfer across changing process conditions.

Hybrid modeling is attractive because mechanistic structure can constrain interpretation while data-driven components accommodate relationships that are difficult to describe mechanistically [28]. Real-time hybrid state estimation has demonstrated that online process measurements can be transformed into richer inferred states than direct measurements alone provide [29]. For natural-product fermentation, however, an inferred state should not automatically be labeled “pathway limited,” “oxygen limited,” or “toxicity limited.” Several mechanisms may generate similar external signatures.

The proposed monitoring layer therefore retains competing constraint hypotheses until evidence distinguishes them sufficiently for action. Confidence should be expressed qualitatively or quantitatively only when supported by a validated diagnostic model; this article proposes no universal threshold. Where observability is weak, the appropriate output may be uncertainty rather than a forced constraint assignment. Monitoring becomes valuable not because it makes every limitation visible, but because it structures what is known, inferred, unresolved, and worth perturbing experimentally.

Adapting feeding, induction, and recovery

Once a constraint hypothesis is sufficiently supported, the intervention should match its mechanism rather than simply intensify the current operating policy. Closed-loop cell–machine control provides proof that microbial stress responses can be measured and manipulated under imposed process perturbations [30]. Model-based optimization and predictive-control formulations for fed-batch metabolic cybergenetics likewise demonstrate that control actions can be selected from an evolving process model rather than a fixed time schedule [31]. These studies establish feasibility of adaptive control concepts, not validated natural-product constraint switching.

Feeding is appropriate when delivery or precursor availability is implicated, but more feed is not automatically corrective: it can increase oxygen demand, overflow, osmotic stress, or toxic pathway flux. Induction provides a different actuator because it changes pathway demand and cellular allocation. Modular nutrient-responsive regulators have demonstrated that biosynthesis can be coupled experimentally to feed-related environmental signals in engineered microbial hosts [32]. Such circuits provide a plausible sensing–actuation mechanism, although their reliability under heterogeneous large-scale conditions remains a separate validation problem.

Recovery forms a third, physically distinct intervention family. In situ distillation can remove suitable volatile fermentation products while cultivation proceeds [33]. Reactive extraction has likewise been integrated with fermentation in a compatible itaconic-acid system [34]. In situ recovery can therefore employ materially different operations whose applicability depends on product physicochemical properties and fermentation compatibility [33, 34]. Neither strategy should be generalized to natural products lacking the required volatility, partitioning behavior, or host tolerance.

The switching principle is consequently conditional: feed, induction, reactor operation, and recovery are not interchangeable optimization levers. After any intervention, the system returns to diagnosis because relaxing one restriction may reveal another. Failure to improve performance is itself informative if the intervention was sufficiently specific and measurable; it weakens the corresponding constraint hypothesis rather than proving that the process is intrinsically uncontrollable.

Scale-dependent applications

At laboratory scale, the architecture is most useful as a perturbation framework. Candidate constraints can be challenged deliberately by varying feed delivery, oxygen transfer, induction timing, or product removal while

monitoring the corresponding physiological and chemical response. Cell-lifeline-informed scale-down designs are especially relevant because they can reproduce selected exposure patterns derived from industrial heterogeneity [10]. Their role is diagnostic rather than representationally perfect: a scale-down system reproduces chosen disturbances, not the entirety of full-scale hydrodynamics and population history.

At pilot and manufacturing scales, observability becomes more difficult while the engineering consequences become stronger. Spatial simulations of a 600-m³ fermentation illustrate how local process conditions can differ meaningfully from bulk measurements [19]. Power-aware mechanistic analysis further shows that improved mixing or transfer can conflict with energy objectives [21]. Thus, an intervention that is biologically attractive may be operationally unattractive, and constraint dominance should be defined relative to the actual process objective rather than titer alone.

Some processes require a different instantiation of the architecture. Gas fermentation emphasizes transfer and reactor-configuration constraints because substrate delivery depends directly on gas–liquid transport [22]. Recovery-intensive processes introduce product chemistry and solvent or separation compatibility into the active-constraint set, as demonstrated by integrated reactive extraction [34]. These cases should not be treated as exceptional failures of one universal model; they demonstrate why the same diagnostic categories can require different measurements and actions.

Accordingly, scale transfer should preserve decision logic, not necessarily sensors, thresholds, or controller parameters. The transferable proposition is that competing constraints should be distinguished, monitored, acted on conditionally, and reassessed. The identities of useful observables, acceptable uncertainty, intervention costs, and dominant constraints remain host-, product-, reactor-, and scale-dependent.

Validation needs

The proposed architecture requires validation that separates attractive explanation from useful diagnosis. First, challenge experiments should include combined and context-specific disturbances rather than relying only on isolated single-variable tests [16, 18]. Such designs should vary perturbation amplitude, duration, sequence, and location where feasible, because the same nominal stress can produce different cellular consequences when exposure histories differ. Orthogonal measurements are needed to determine whether apparently similar process responses arise from different underlying restrictions.

Second, prospective studies should compare constraint-switching policies with fixed policies under predeclared objectives. Relevant comparators could include fixed feeding, fixed induction timing, conventional two-stage operation, or fixed recovery schedules. The critical outcome is not whether a controller changes an observable, but whether identifying and acting on a different active constraint improves the selected process objective without unacceptable penalties elsewhere. Incorrect switches and delayed switches must be included as failure modes rather than removed from analysis.

Third, diagnostic models require external transfer tests. Hybrid models can fail when process structure changes or data move outside the development domain [28]. Closed-loop stress control and predictive metabolic control demonstrate useful engineering precedents [30, 31], but neither establishes cross-host or cross-product generalization. Validation should therefore span reactor scale, strain background, natural-product chemistry, and operating regime before claims of robustness are made.

Finally, the architecture must permit rejection. If competing constraints cannot be identified with adequate discrimination, if switching adds instability, or if fixed policies perform equivalently across relevant disturbances, then the proposed advantage is unsupported. Conversely, evidence would strengthen the architecture if interventions targeted to independently diagnosed constraints repeatedly outperform mismatched interventions and if the diagnosis remains calibrated after scale transfer. Until such tests are performed, the framework should be regarded as a structured hypothesis for bioprocess design and experimentation rather than an implementation-ready control standard.

Conclusion

Microbial natural-product production can fail for fundamentally different reasons at different process states and scales. Intracellular pathway capacity, toxicity, resource allocation, robustness, environmental delivery, reactor transport, operating costs, and product recovery should therefore not be collapsed into a single generic bottleneck

category. The architecture proposed here organizes these limitations as competing constraint classes whose practical dominance may change as cultivation conditions and scale change.

Its central distinction is between observing a process deviation, inferring the constraint that currently limits the selected objective, and choosing an intervention matched to that diagnosis. Feeding, induction, reactor operation, and recovery consequently enter the framework as mechanistically different action families rather than interchangeable optimization variables. Reassessment after intervention is essential because relieving one restriction may reveal another, leave the original limitation unresolved, or introduce a new trade-off.

The architecture remains deliberately bounded. Active constraints may be shared or non-identifiable; sensor and model errors can misclassify process state; scale-down systems only approximate selected full-scale exposures; and interventions validated in one host, reactor, or product class cannot be assumed to transfer unchanged. The framework therefore contributes a testable organization of fermentation reasoning rather than a validated universal controller. Its value will ultimately depend on prospective experiments showing that constraint-specific diagnosis improves decisions across realistic disturbances, scales, and natural-product production systems.

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