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Dynamic Control of Engineered Metabolic Pathways: Balancing Enzyme Capacity, Cellular Burden, and Production Stability

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Abstract

Engineered microbial pathways operate within cells whose metabolic resources, physiological states, and population compositions change during cultivation. A pathway that increases short-term product accumulation may simultaneously consume translation capacity, perturb precursor supply, or select for nonproducing variants. This conceptual synthesis asks when dynamic regulation can reconcile enzyme capacity, resource allocation, cellular burden, and sustained production, and when that expectation is unwarranted. Evidence from enzyme-constrained modeling, proteome allocation, metabolite-responsive circuits, growth-phase switches, and production-stability studies is interpreted through an explicit separation of measured signals, controlled variables, and unobserved states. Four conditional principles emerge: actuate at the actual limiting node; align sensing and actuation with disturbance timescales; account for the resources consumed by the controller itself; and evaluate performance over a defined production horizon, including population change. Qualitative operating scenarios indicate why an effective early-stage response may fail after substrate, oxygen, growth rate, or producer fraction changes. The argument is analytical rather than a report of experiments or simulations. A validation agenda pairs controlled perturbations with time-resolved flux, expression, burden, and population measurements. Dynamic control is most defensible when its sensing specificity, intervention capacity, timescale, and stability benefits are tested together rather than inferred from final titer alone.

Keywords: Dynamic metabolic regulation, Enzyme-constrained modeling, Cellular resource allocation, Metabolite biosensors, Production robustness, Evolutionary stability

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Introduction

Microbial biomanufacturing requires heterologous reactions to compete with indispensable cellular functions for carbon, redox cofactors, energy, ribosomes, and catalytic protein. Early engineering often maximizes promoter strength or removes competing reactions, yet such interventions can transfer the bottleneck rather than remove it. F. Zhang *et al*. (2012) demonstrated a fatty-acid-linked sensor-regulator system; Dahl *et al*. (2013) used stress-responsive promoters to manage a toxic isoprenoid intermediate. These experiments establish the feasibility of responsive regulation, but do not establish that feedback is invariably superior to an optimized static pathway.

The central difficulty is temporal. Biomass formation, enzyme accumulation, metabolite pools, dilution, process feeding, and selection among cells evolve on different clocks. Solomon *et al*. (2012) showed that a controlled glycolytic valve could redirect carbon, whereas Gupta *et al*. (2017) introduced a pathway-independent quorum-sensing switch. Such systems respond to different variables and therefore solve different problems. A cell-density signal is not direct evidence of metabolite sufficiency; a metabolite concentration is not itself a reaction flux. Reviews have organized the increasingly diverse control designs, but their categories should not be mistaken for transferable performance guarantees (Venayak *et al*., 2015; Hartline *et al*., 2021).

This article develops testable reasoning for choosing control architecture under limited cellular capacity and uncertain operating conditions. It distinguishes enzyme capacity from realized flux, physiological burden from chemical toxicity, and short-run titer from retention of production across generations. The proposed relationships are conceptual and conditional. They are not fitted laws, new simulations, or measurements from a common dataset.

A useful abstraction is to view production as a sequence of decisions made under partially observable conditions. The same promoter command may produce different active enzyme amounts depending on translation availability and dilution, while the same enzyme amount may support different fluxes at different feeding states. Jiang *et al*. (2023) distinguish control logics that can be used to organize such decisions. Here, the priority is not to rank the logics but to identify which underlying constraint each logic can plausibly correct and which unmeasured states could invalidate that choice.

Analytical Approach and Evidence Foundations

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This targeted theoretical synthesis draws from accessible journal records on microbial dynamic control, proteome economy, enzyme-constrained metabolic models, and production escape. Searches conducted on 1 November 2026 used combinations of "dynamic metabolic engineering," "biosensor feedback," "enzyme-constrained model," "resource competition," "quorum sensing production," and "genetic stability," supplemented by citation chaining. Publisher and PubMed records were consulted for bibliographic identity and abstracts; available open full texts informed closer interpretation. Selection was purposive rather than exhaustive, and no systematic-review flow count is claimed. Papers were retained for their distinct mechanistic, experimental, methodological, or critical role, not because their outcomes were favorable.

The unit of interpretation is an identifiable experiment, modeling analysis, or narrative synthesis, not a DOI detached from its context. For example, conclusions about enzyme usage from a yeast model are not automatically observations about bacterial fermentations. Sánchez *et al*. (2017) constrained yeast pathway fluxes using enzyme abundance and turnover assumptions, while Domenzain *et al*. (2022) expanded the GECKO approach across model organisms. Their predictions are relevant to capacity, but model assumptions and parameter coverage limit transfer to a particular bioreactor. Similarly, the computational treatment of host-circuit coupling by Melendez-Alvarez *et al*. (2023) supplies a plausible mechanism, not empirical proof of improved chemical production.

For the present analysis, an observable is a measured quantity such as fluorescence, extracellular product, or growth rate; a state is an internal quantity that may require inference, such as enzyme saturation or the fraction of producer cells; and an actuator is an intervention that changes expression, degradation, or flux allocation. Success is evaluated using specified titer, yield, volumetric productivity, and retention across cultivation time. These outputs answer different questions and must be reported separately. The evidence base includes mechanistically informative earlier studies because they establish premises that recent implementations inherit.

Enzyme Capacity and Cellular Resource Constraints

Capacity, Flux, and Resource Allocation

An enzyme's nominal catalytic capacity depends on its effective abundance and turnover under the relevant intracellular conditions; realized flux additionally depends on substrates, cofactors, thermodynamics, competition, and network coupling. A convenient conceptual inequality, $\mathbf{v_i \leq E_i k_{cat,i}}$, expresses an upper bound rather than a measured kinetic law for a particular fermentation. It is useful only when enzyme abundance, active fraction, and kinetic parameter applicability are credible. GECKO illustrates the value of these constraints relative to stoichiometry alone; the GECKO 3.0 protocol also documents the substantial parameter construction and tuning that can be involved (Chen *et al*., 2024).

Increasing enzyme expression can therefore stop increasing useful output for several reasons. The substrate may be depleted; a downstream conversion may saturate; cofactors may become limiting; or the extra protein may displace host functions. Scott *et al*. (2010) linked bacterial growth and gene expression through resource allocation. Hui *et al*. (2015) quantified major growth-associated proteome sectors in *Escherichia coli*, whereas Basan *et al*. (2015) related overflow metabolism to proteome economics. These findings argue against the assumption that increasing one pathway's measured protein abundance leaves the rest of the cell unchanged.

Genetic circuits share translation and transcription capacity even when they lack direct biochemical interactions. Gyorgy *et al*. (2015) experimentally described expression trade-offs as isocost relationships; Qian *et al*. (2017) showed that resource competition can alter circuit input-output behavior. The implication for pathway control is that both the sensor and the actuator impose demands on the same host they seek to optimize. A higher sensor gain may enhance detection but also reduce resources available to the product-forming enzymes. Consequently, expression level, inferred enzyme capacity, and product formation rate require separate measurements.

A minimum resource accounting should include both synthetic and native demands. In a mechanistic model, increased heterologous translation can redistribute ribosomes, ATP, and amino acids; reduced growth may then change protein dilution and measured expression even in the absence of deliberate feedback. Weiße *et al*. (2015) analyzed these connected processes, making clear why a steady-state enzyme measurement cannot be interpreted independently of the growth condition that produced it. For practical inference, paired proteomics and isotope-based flux measurements are preferable to treating a fluorescent reporter as a flux meter. Where direct measurement is impractical, uncertainty in enzyme activity and saturation should be retained rather than hidden in a single optimized parameter.

Burden, Toxicity, and Production Stability

Physiological burden refers here to the resource demand or cellular performance loss associated with engineered expression and pathway operation. Chemical toxicity is a narrower mechanism arising from damaging metabolites or products. Ceroni *et al*. (2015) presented a real-time cellular-capacity monitor and showed that construct designs with equivalent output could impose different burdens. Later, Ceroni *et al*. (2018) used a burden-responsive controller to preserve native expression capacity and improve batch protein yield. These findings motivate burden-aware regulation without implying that a generic burden reporter identifies a particular toxic intermediate.

Longer-term instability introduces a different causal process. Rugbjerg, Myling-Petersen, *et al*. (2018) detected genetic error modes and expansion of low-producing variants in engineered mevalonate populations. Rugbjerg, Sarup-Lytzen, *et al*. (2018) then examined synthetic addiction to extend productive population lifetime. Heieck *et al*. (2023) documented metabolic-stress-associated transposition in cysteine production. These studies caution that a dynamically regulated strain can look physiologically efficient at one time point yet lose productive

members during propagation. **Table 1** makes the distinct measurement targets explicit.

Table 1. Operational distinctions required to diagnose a pathway-control failure

Quantity	What it indicates	Representative measurement and boundary	Interpretive risk
Effective enzyme capacity	Upper bound on reaction rate under stated assumptions	Proteomics plus kinetic parameterization; Sánchez *et al*. (2017)	Does not equal realized flux
Resource burden	Competition with native biosynthesis and growth	Capacity reporter and matched growth assay; Ceroni *et al.* (2015)	May not identify the damaging mechanism
Intermediate toxicity	Harm associated with accumulated pathway species	Targeted metabolomics and stress response; Dahl *et al.* (2013)	Pool size alone is not toxicity
Physiological stability	Retention of growth and metabolic activity	Time series of growth, uptake, secretion; Basan *et al*. (2015)	Short assays miss adaptation
Genetic/population stability	Retention of producer genotype and fraction	Longitudinal sequencing and production; Rugbjerg, Myling-Petersen, *et al*. (2018)	Stable mean titer can mask rare escape

Note. Entries synthesize cited experiments, models or clearly labeled proposals; measurement conditions are system-specific.

Importantly, an apparent drop in titer accumulation can reflect depletion, declining specific productivity, or an increased proportion of nonproducers. Without time-resolved concentration, biomass, and producer-fraction information, these explanations remain confounded. A useful controller diagnosis must first identify which instability is actually present.

Dynamic Control Principles and Assumptions

Sensing, Actuation, and Timescale Alignment

A metabolite sensor measures a pool that may imperfectly represent the regulated flux. D. Liu *et al*. (2015) implemented a malonyl-CoA-responsive negative-feedback circuit that adjusted acetyl-CoA carboxylase expression. Xu *et al*. (2020) built pyruvate-responsive circuits for dual regulation of central metabolism in *Bacillus subtilis*. In each case, engineering a response to a biologically meaningful signal was useful, but the outcome remained specific to that host, product, sensing range, and actuator coupling. A sensor whose output saturates outside the process's operating range cannot distinguish materially different high-concentration states.

Time alignment matters as much as specificity. Transcriptional regulation takes time to alter enzyme abundance, and enzymes already present may continue catalysis after repression. By contrast, a process disturbance may alter metabolite pools within a much shorter interval. A feedback loop acting after toxic accumulation may be protective only if the state remains reversible during the delay. Shen *et al*. (2016) combined coordination of a mevalonate pathway with metabolite-responsive regulation in zeaxanthin-producing *E. coli*, illustrating that pathway organization and dynamic actuation are intertwined. However, results from a particular flask or fed-batch configuration cannot determine general controller response limits.

Population sensing introduces another boundary. Dinh and Prather (2019) used an autonomous bifunctional quorum-sensing circuit to modulate metabolic flux; Gu *et al*. (2020) demonstrated a dual-function QS switch in engineered product pathways. Their switching logic can coordinate growth and production phases, yet signal accumulation depends on population density and medium behavior, not solely on precursor sufficiency. Thus a density-triggered transition should be treated as a proxy whose correlation with the desired state must be revalidated when feed profiles or inoculation conditions change.

Other endogenous population-linked cues may offer alternative switches but bring their own signal chemistry. H. Liu *et al*. (2019) used endogenous sulfide chemistry for a synthetic quorum-related circuit in *E. coli*. Such a demonstration broadens the potential input repertoire, but diffusible cues can depend on mixing, chemistry, transport, and growth history. A robust comparison should therefore record the actual input profile, promoter response, enzyme response, and final product output. Merely identifying an inducible promoter is insufficient evidence that its closed-loop behavior remains stable when transferred between culture scales.

Feedback, Adaptation, and Competing Objectives

The proposed controller selection principle is to match the measured disturbance to the limiting node and the action to a reversible lever. When metabolite toxicity dominates, regulate the relevant production-consumption balance. When expression burden dominates, constrain demand for scarce cellular resources. When biomass must first accumulate, a scheduled or population-triggered growth-to-production transition may outperform constant expression. Yet an added control circuit may create a fresh bottleneck, and the same intervention may have opposite effects before and after growth slows.

Han and Zhang (2020) describe overlapping trade-offs among precursors, ribosomes, growth, and product. Ni *et al*. (2021) distinguish stimulus-responsive regulation from the metabolic nodes it controls; Dinh and Prather (2020) show why layered, multi-input architectures can be attractive. More inputs do not guarantee better control: each extra sensor increases calibration demands, leakiness opportunities, and the burden of circuit maintenance. A simple feedforward schedule may be more robust than feedback when the process trajectory is predictable and the chosen sensing variable is noisy or delayed.

The nonlinear relationship between burden and gene-circuit behavior further undermines universal prescriptions. The theoretical analysis by Melendez-Alvarez *et al*. (2023) illustrates that growth feedback can sometimes modify the competitive

interactions between circuit modules. This is a warning against treating hidden host-circuit links as either always harmful or automatically beneficial. **Table 2** contrasts operational strategies at the level of observability, actuation, and failure condition.

Table 2. Control options compared by what is sensed, changed, and potentially missed

Strategy	Observable / trigger	Actuation and lag	Objective conflict / failure condition
Metabolite feedback	Pathway intermediate; D. Liu *et al*. (2015)	Expression change; transcription-to-enzyme delay	Sensor saturation or late toxic response
Burden feedback	Cellular capacity signal; Ceroni *et al*. (2018)	Repress expression when burden rises	Global burden signal lacks pathway specificity
Population switch	Cell density / quorum cue; Gupta *et al*. (2017)	Growth-to-production reallocation	Density misaligns with physiological readiness
Bifunctional control	Paired up/down targets; Gu *et al*. (2020)	Coordinated promoters; multiple delays	Circuit cost and interference grow
Open-loop schedule	Time or process phase; author proposal	Preset expression trajectory	Fails when disturbances shift process phase

Note. Entries synthesize cited experiments, models or clearly labeled proposals; measurement conditions are system-specific.

The nested network in **Figure 1** connects three levels that should not be collapsed into a single arrows-only account: pathway conversion and enzyme capacity, host resource allocation and physiological responses, and process signals and population composition. Relationships are shown as supported dependencies or conditional proposals, not as universally quantified causal effects.

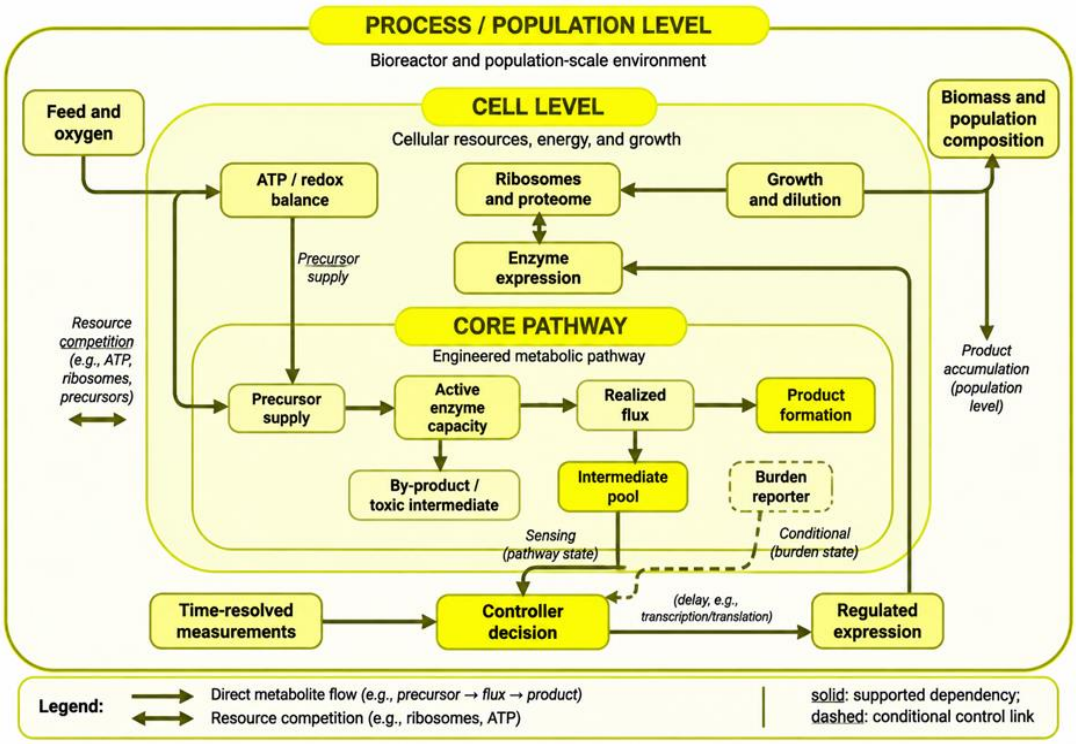


Figure 1. Nested pathway–cell–process dependencies in dynamic production control

Note. The diagram separates precursor conversion, enzyme capacity and realized flux from cellular resource competition and process-level biomass or population composition. Solid connectors denote supported dependencies, not automatically proven causation. Dashed connectors denote proposed burden-responsive regulation. Actuator delay, sensor dependence and state uncertainty are shown qualitatively; no values were fitted.

A practical decision rule is therefore conditional: choose feedback only when the monitored variable tracks the relevant constraint sufficiently, the actuator can change that constraint before unacceptable damage, and the resulting pathway remains productive over the intended horizon. A high final titer without matched time-series evidence does not test the entire rule.

Analytical Scenarios and Validation Agenda

Consider three hypothetical regimes; their boundaries are conceptual, not measured thresholds. First, enzyme abundance may be low while substrates and host resources remain available. Adding expression could increase flux, but capacity must be verified against metabolite consumption and product formation.

Second, protein synthesis may become resource-limited: stronger expression then reduces growth or other pathway activities without proportional production gain. Third, a process with satisfactory initial flux may become unstable as toxic intermediates rise or nonproducer lineages accumulate. A controller successful in the first regime can worsen the second and fail to prevent the third.

These regimes are not a fixed chronological sequence. A nutrient feed can move a culture toward precursor abundance while oxygen limitation pushes it toward cofactor or resource stress. Likewise, selection can alter population composition even when individual producer cells retain normal metabolism. Recent experimental applications include autonomous phosphoenolpyruvate-node control for muconic acid production (Yang *et al*., 2018), spatiotemporal cysteine-pathway regulation (B. Zhang *et al*., 2023), and central-metabolism sensing for trehalose and 4-hydroxycoumarin production (Zou *et al*., 2025). Their products, hosts, and interventions differ, supporting the existence of multiple workable designs rather than one validated regime map.

Table 3 translates the scenarios into prospective falsification tests. Each prediction should be evaluated against a matched static or open-loop comparator under the same cultivation history, alongside measurements that could refute the proposed limiting mechanism.

Table 3. Hypothetical regimes, falsification tests, and necessary observations

Conditional regime	Proposed response	Observation that would refute interpretation	Necessary paired measurements
Unused catalytic capacity	Raise limiting enzyme expression	More enzyme yields no flux change despite substrate supply	Enzyme amount, product rate, metabolite pools
Resource-limited expression	Reduce or redistribute synthetic demand	Lower demand does not restore host capacity	Growth, capacity reporter, proteome, product
Fast toxic intermediate accumulation	Increase consumption or reduce precursor entry	Toxic pool remains high after verified timely actuation	Intermediate time course, response delay, viability
Growth-to-production transition	Use proxy-informed conditional phase change	A matched scheduled switch is equally robust to disturbances	Phase signal, biomass, titer, rate
Population production escape	Pair pathway control with producer retention	Output declines without a demonstrable change in producer genotype or fraction	Sequencing, producer fraction, multi-generation output

Note. Entries synthesize cited experiments, models or clearly labeled proposals; measurement conditions are system-specific.

The proposed **Figure 2** is intended to depict conditional operating transitions without numerical axes or invented stable zones. A viable validation programme would perturb promoter strength, feed composition, and sensor threshold independently; monitor biomass, precursor pools, product, expression burden, and sequence stability over time; and compare responses under replicated production horizons. Such work would separate benefit attributable to feedback from benefit attributable to a better mean expression level.

A strong experiment should also distinguish feedforward scheduling from state-dependent feedback. First tune an open-loop schedule so that its total expression cost and average pathway activity resemble those of the candidate controller. Then change one disturbance at a time, such as carbon supply or oxygen transfer, while keeping sampling frequency sufficient to resolve the predicted response lag. If the feedback system performs no better under disturbances than the carefully matched schedule, its extra sensing circuitry may not be justified. Conversely, rapid recovery of a stressed culture without growth collapse would support the proposed timescale principle, subject to replication and direct confirmation of the regulated metabolite.

Results and Discussion

Dynamic metabolic control has accumulated credible demonstrations, but its reported improvements are not directly comparable without attention to the outcome denominator. For instance, a two-stage shift can improve eventual titer by sacrificing early product formation, while a feedback loop may increase production per cell without improving growth. The foundational observations of F. Zhang *et al*. (2012) and Dahl *et al*. (2013) concern metabolite-responsive control; the more recent *Pseudomonas putida* anthranilate strategy by Fernández-Cabezón *et al*. (2022) combines quorum sensing with conditional auxotrophy. These are different control questions, and an increase in one study's reported titer is not evidence of a generally superior control algorithm.

The analytical contribution is a measurement-to-action discipline. A pathway may have spare theoretical catalytic capacity but insufficient substrate; a controller directed at enzyme abundance would then be misplaced. Conversely, a controller targeting precursor supply may aggravate toxicity if downstream capacity is saturated. The experimentally grounded resource-allocation observations reviewed here also show why pathway control should be evaluated at the host level, including its sensing and regulatory hardware. Even an apparently orthogonal genetic module competes for cellular synthesis capacity.

Economic and process robustness remain separate issues. A strain may have high yield under a defined medium yet require costly induction, narrow oxygen control, or short propagation times. Toya and Shimizu (2020) emphasize central-carbon control under changing fermentation conditions. A transferable design should report the operating envelope, response latency, biomass trajectory, and longitudinal retention of the production phenotype rather than only a best-condition endpoint. A practical hierarchy is

to diagnose the constraint first, test the least burdensome intervention second, and increase controller complexity only after identifying a reproducible failure that demands it.

This hierarchy also guards against confusing construct stabilization with whole-process stability. Selection circuits can preserve producer lineages without eliminating oxygen gradients or nutrient transients; a well-tuned metabolite loop can buffer a pathway yet leave a segregationally unstable production plasmid untouched. Combining these mechanisms may be sensible, but their interactions should be established with factorial designs. A new design should therefore provide an explicit failure hypothesis, a controller-disabled comparison, and a measurement capable of contradicting the hypothesized benefit. This is especially important when an autonomous response looks attractive because it reduces operator intervention: autonomy shifts responsibility from external control to endogenous signals whose reliability must be demonstrated.

Limitations

This article is a purposive conceptual synthesis and cannot establish the frequency with which dynamic control outperforms static engineering. The publication set mixes experiments, model studies, methods papers, and reviews; it does not provide a common effect size or validated quantitative threshold. Abstract-only access for some sources limits inspection of methods and secondary outcomes.

The simple capacity bound assumes that an effective enzyme concentration and appropriate turnover value can be defined. Intracellular substrate channeling, enzyme complexes, compartmentalization, post-translational regulation, and poorly measured maintenance demands can undermine that description. Population models introduce further uncertainty about mutation rates, selection coefficients, and bottlenecks. Neither **Figure 2** nor the scenario table defines a universal stability boundary. No simulation, optimization, or new bench experiment has been performed here; proposals about controller performance remain falsifiable hypotheses.

The literature is also uneven across industrially relevant settings. Laboratory studies frequently assess limited host backgrounds and cultivation scales; single-cell heterogeneity, oxygen-transfer excursions, prolonged seed trains, and harvest-dependent product degradation may remain underreported. A fluorescence-based burden monitor is useful for detecting host expression stress but should not be equated with direct ATP accounting. Longitudinal producer-fraction assays may themselves miss rare escape variants unless sampling and sequencing depth are adequate. These are substantive constraints on the strength of the manuscript's proposed control principles.

Conclusion

Dynamic regulation is most likely to help engineered pathways when the relevant limitation can be observed, changed quickly enough, and relieved without introducing a larger cellular or evolutionary cost. Catalytic capacity, available resources, and realized production must be diagnosed separately. Physiological burden, metabolite toxicity, and population escape require different interventions and different measurements. The central test is whether an intervention maintains useful output over a defined operating horizon, not merely whether it raises a final concentration in one condition. Comparative time-series experiments combining enzyme abundance, flux, growth, burden, and producer fraction can determine when these conditional principles hold and where they fail.

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