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Enhanced γ -Decalactone Production from Castor Oil by UV-Mutated *Sporidiobolus salmonicolor*

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Abstract

Background: γ -Decalactone (GDL; $C_{10}H_{18}O_2$) is a peach-like aroma compound that can be produced by microbial conversion of ricinoleic acid-rich substrates. **Objective:** This rewritten study evaluates UV-derived isolates of *Sporidiobolus salmonicolor* MTCC 485 for improved GDL accumulation from castor-oil-derived substrate and places the reported results in the context of biotechnology advances through 2024. **Methods:** The source study exposed yeast cultures to ultraviolet radiation, screened four isolates (UV-1 to UV-4), and conducted batch biotransformation in a 5 L stirred bioreactor with a 2 L working volume. Product concentration was reported by gas chromatography, and the influence of cultivation time and medium pH was assessed. **Results:** The wild type reached 62.2 mg L⁻¹ GDL at 96 h. UV-3 reached 81.9 mg L⁻¹ at the same time point, corresponding to a recalculated improvement of 31.7%. UV-1 and UV-2 produced only small gains, whereas UV-4 performed below the wild type. Among the tested pH values, pH 4 gave the highest reported concentration for both the wild type and UV-3. **Interpretation:** UV mutagenesis produced one promising isolate, but the absence of a documented UV dose, biological replicate count, inferential statistics, genetic stability testing, and fully validated quantitative analytical details prevents a definitive claim of industrial readiness. Current evidence indicates that controlled oxygen transfer, statistical process optimization, immobilization or in situ product removal, metabolic engineering, and integrated downstream processing are the most relevant next steps.

Keywords: γ -decalactone, castor oil, ricinoleic acid, UV mutagenesis; *Sporidiobolus salmonicolor*, biotransformation, β -oxidation, bioprocess optimization.

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1. Introduction

γ -Decalactone is a ten-carbon γ -lactone with a strong peach-like, creamy-fruity aroma. The U.S. Food and Drug Administration's Substances Added to Food inventory lists γ -decalactone as a flavoring agent or adjuvant under 21 CFR 172.515, and the NIST Chemistry WebBook records the molecular formula $C_{10}H_{18}O_2$ and molecular mass 170.2487 g mol⁻¹ [2,3]. In food and fragrance biotechnology, the compound is valued because microbial conversion can provide a route to an aroma ingredient associated with natural-source processing, subject to applicable jurisdictional and labeling requirements.

Castor oil is especially attractive as a feedstock because its fatty-acid fraction is rich in ricinoleic acid. In suitable yeasts, hydroxy-fatty-acid intermediates enter β -oxidation,

are shortened to a C_{10} intermediate, and form γ -decalactone through lactonization [4,5]. The process is nevertheless difficult to optimize: the oily substrate has limited dispersion in aqueous broth; oxygen demand is high; product and substrate can inhibit cells; and continued metabolism can consume the desired lactone after its concentration peaks [6,7].

The 2016 source study addressed low GDL accumulation by applying random UV mutagenesis to *S. salmonicolor* MTCC 485 and screening four derived isolates [1]. UV mutagenesis is inexpensive and can generate useful phenotypes, but its effects are untargeted. A high-performing isolate therefore requires confirmation of phenotype stability, dose-response, genome-level changes, and fermentation reproducibility before process development.

Since the original work, the field has advanced from single-factor optimization toward designed experiments, cell immobilization, substrate-adsorption systems, oxygen-transfer control, continuous operation, integrated purification, and pathway engineering [11–20]. These developments do not invalidate the original dataset;

instead, they clarify which claims are supported, which comparisons are inappropriate, and what experiments would convert a preliminary mutant-screening result into a robust biotechnology process.

This rewritten paper has four aims: (i) present the original methods and results in clear scientific English; (ii) correct derived calculations without altering primary measurements; (iii) compare the findings cautiously with later work; and (iv) define a reproducible validation and scale-up roadmap for *S. salmonicolor* MTCC 485.

2. Biochemical and Bioprocess Basis

2.1 Conversion pathway

The substrate-to-product route begins with hydrolysis or cellular uptake of castor-oil-derived ricinoleate. After activation to a CoA thioester, repeated rounds of β -oxidation shorten the C_{18} hydroxy-fatty-acid chain to a C_{10} hydroxyacyl intermediate. Hydrolysis and intramolecular esterification then yield γ -decalactone. Foundational studies in *Sporidiobolus* and *Yarrowia* linked GDL formation to β -oxidation and showed that pathway activity beyond the desired C_{10} intermediate can reduce product accumulation [4,5].

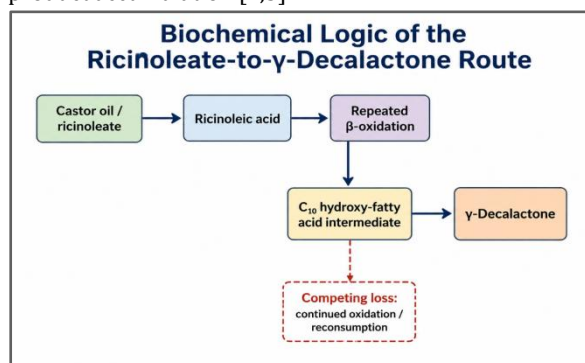


Figure 01: Simplified biochemical logic of γ -decalactone formation from castor-oil-derived ricinoleate. The scheme is interpretive and does not represent new experimental measurements.

2.2 Engineering constraints

Three linked constraints dominate GDL biotransformation. First, ricinoleate is hydrophobic, so its interfacial area and accessibility depend on mixing, emulsification, carrier materials, and cell-surface properties. Second, the oxidation pathway requires adequate oxygen transfer; agitation and aeration can therefore influence both biomass physiology and conversion rate. Third, GDL perturbs yeast membranes and may be further metabolized, creating a narrow operating window in which recovery at or near the peak is important [6].

The original study's maximum at 96 h followed by lower values at the next reported time point is consistent with product loss or reconsumption, but the dataset alone cannot distinguish biodegradation, volatilization, sampling variation, extraction loss, or analytical variation. A modern mass balance should quantify substrate, intermediate, product, off-gas loss, and residual broth phases.

3. Materials and Methods

3.1 Microorganism and maintenance

Sporidiobolus salmonicolor MTCC 485 was obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India. The culture was maintained on YM agar slants at 25 °C. Colonies grown for three days were stored under refrigeration until use.

3.2 Preculture and production medium

A loopful of yeast from a YM agar slant was transferred to 100 mL of glucose-based preculture medium in a 250 mL Erlenmeyer flask. The medium contained, per litre: glucose, 15 g; tryptone, 0.5 g; yeast extract, 1 g; malt extract, 1 g; casamino acids, 2 g; K_2HPO_4 , 2 g; $CaCl_2$, 0.13 g; $FeSO_4$, 0.01 g; and $MgSO_4$, 3 g. Precultures were incubated at 25 °C and 160 rpm. The source paper lists 16, 24, or 42 h in the culture-conditions section but 49 h in the mutagenesis section; this discrepancy could not be resolved from the supplied record.

For flask screening, stationary-phase cells were exposed to a castor-oil-derived substrate described in the source as 0.1% methyl ricinoleate (castor oil). Initial medium pH was adjusted with sterile 1 M HCl or 1 M NaOH. The exact identity and preparation of the substrate—whole castor oil, methyl ricinoleate, or a hydrolysate—should be clarified in any repeat experiment.

3.3 UV mutagenesis and isolate screening

Cells grown for 18 h at 25 °C were collected, suspended in sterile distilled water, counted, and spread onto YM agar. Plates were positioned 55 cm from a UV lamp and irradiated for 3, 5, 7, or 10 min. They were then held in darkness for 1 h and incubated at 25 °C for three days. Colony counts were used to assess survival, and four candidate isolates were designated UV-1, UV-2, UV-3, and UV-4. The source paper did not report UV wavelength, irradiance, delivered dose, survival fraction, isolate-to-exposure mapping, colony selection rule, or post-mutagenesis stability testing.

3.4 Batch biotransformation

A 2% (v/v) inoculum, described as approximately $1\text{--}10 \times 10^7$ cells mL^{-1} , was transferred to a 5 L stirred bioreactor containing 2 L of glucose medium. Agitation was 250 rpm and aeration was 1 vvm. The source reported a volumetric oxygen-transfer coefficient, kLa , of 90 h^{-1} . Castor oil or methyl ricinoleate was added at stationary phase to initiate biotransformation. Exhaust-gas oxygen and carbon dioxide were monitored using Servomex 1100 and URAS 3G analysers, respectively.

Samples were collected over cultivation. The methods list 24, 48, 72, 96, and 120 h, whereas the mutant-comparison table reports a final value at 100 h. Separate pH experiments were described for pH 3–7 in 500 mL culture flasks using 2.5 N NaOH or H_2SO_4 for adjustment. It is unclear whether pH was controlled continuously or only set initially.

3.5 Biomass and viability

For viable counts, 1 mL of culture was serially diluted in sterile 0.85% NaCl and 100 μ L aliquots were plated on agar medium. For dry-cell mass, 5 mL of culture was vacuum-filtered, washed first with ethanol/acetone (50:50, v/v) and then with distilled water, and dried at 70 $^{\circ}$ C to constant mass.

3.6 Extraction and gas-chromatographic analysis

The fermentation broth was microfiltered through a 0.2 μ m membrane. The retained phase containing biomass and product was extracted batchwise with ethanol. The extract was neutralized with aqueous NaOH, salted with NaCl, and extracted with methyl tert-butyl ether (MTBE). Following solvent evaporation, the source protocol described flash distillation under vacuum.

Volatile compounds were analysed using an Agilent 7890 gas chromatograph fitted with a DB-FFAP fused-silica capillary column (30 m $\times$ 0.32 mm internal diameter, 0.25 μ m film). A 1 μ L sample was injected with a 30 s splitless period. The oven temperature increased from 40 to 240 $^{\circ}$ C at 3 $^{\circ}$ C min $^{-1}$, and hydrogen carrier gas was operated at a reported linear velocity of 50 cm s $^{-1}$ at room temperature. Peaks were integrated using ChemStation software. The supplied paper did not report calibration standards, internal standard, detector configuration, calibration range, recovery, limit of detection, limit of quantification, or compound confirmation by GC-MS/chiral analysis.

4. Results

4.1 Growth of the wild-type strain

The source study reported that the wild-type population increased from approximately 4.3×10^4 CFU mL $^{-1}$ at inoculation to 1.7×10^8 CFU mL $^{-1}$ at 72 h, after which it remained near 1.8×10^8 CFU mL $^{-1}$ through 120 h. GDL accumulation increased after 24 h and reached 62.2 mg L $^{-1}$ at 96 h. The source narrative refers to a decline after approximately 110 h, while the table shows a decrease at 100 h; this timing conflict requires verification against primary laboratory records.

4.2 Effect of UV-derived isolates

Table 01: Reported γ -decalactone concentration during cultivation of the wild type and four UV-derived isolates (mg L $^{-1}$).

Time (h)	Wild type	UV-1	UV-2	UV-3	UV-4
0	0.0	0.0	0.0	0.0	0.0
24	10.8	10.6	11.9	13.2	8.4
48	31.5	32.9	33.0	36.8	10.8
72	42.8	43.9	45.6	52.3	13.2
96	62.2	63.2	64.0	81.9	16.8
100	43.9	44.1	45.2	49.6	15.1

Note. Values reproduce the source table. Numerical SD values and replicate-level observations were not supplied.

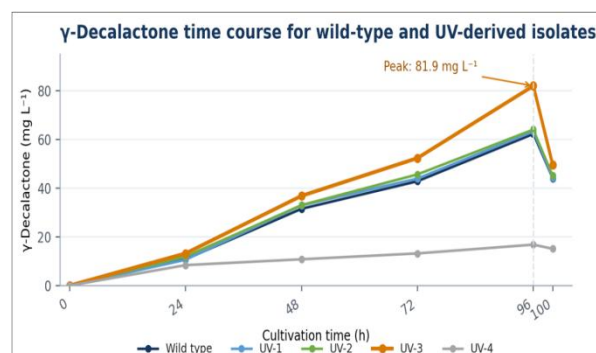


Figure 02: Time-course profiles reconstructed from Table 1. UV-3 showed the highest reported concentration at 96 h. Error bars are omitted because the underlying SD values were unavailable.

At 96 h, UV-1 and UV-2 exceeded the wild type by 1.6% and 2.9%, respectively. UV-3 produced 81.9 mg L $^{-1}$, 19.7 mg L $^{-1}$ above the wild-type value of 62.2 mg L $^{-1}$. This is a 31.7% relative improvement, not 33% as stated in the source paper. UV-4 reached only 16.8 mg L $^{-1}$ at 96 h, approximately 73.0% below the wild type.

Between 96 and 100 h, the reported GDL concentration decreased from 62.2 to 43.9 mg L $^{-1}$ for the wild type and from 81.9 to 49.6 mg L $^{-1}$ for UV-3. The common decline supports harvesting near the observed peak, but mechanistic attribution would require time-resolved mass-balance and metabolite data.

4.3 Effect of culture-medium pH

Table 02: Reported γ -decalactone concentration for the wild type and UV-3 across tested pH conditions (mg L $^{-1}$).

pH	Wild type	UV-3
3	45.6	58.7
4	62.2	81.9
5	55.5	74.6
6	58.1	76.3
7	30.5	32.1

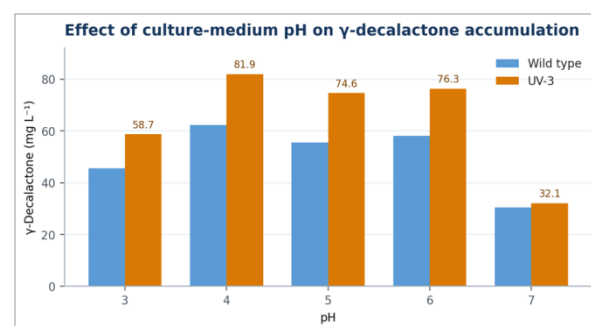


Figure 03: pH response reconstructed from Table 2. The highest reported value for both strains occurred at pH 4. Error bars are omitted because numerical SD values were not supplied.

The maximum for both strains occurred at pH 4: 62.2 mg L $^{-1}$ for the wild type and 81.9 mg L $^{-1}$ for UV-3. UV-3 outperformed the wild type at every tested pH, although the advantage at pH 7 was small (5.2%). Relative gains at pH 3, 4, 5, and 6 were 28.7%, 31.7%, 34.4%, and 31.3%, respectively. These descriptive differences should be

confirmed with independent biological replicates and a prespecified statistical analysis.

5. Discussion

5.1 Meaning of the UV-3 phenotype

The data identify UV-3 as the only isolate with a practically meaningful increase under the reported conditions. UV-1 and UV-2 were close to the wild type, while UV-4 was strongly impaired. This broad response is typical of random mutagenesis: most genetic changes are neutral or deleterious, whereas a minority may alter substrate uptake, lipid metabolism, β -oxidation control, stress tolerance, or product export in a favourable direction.

The result is best described as a promising phenotype rather than a confirmed superior production strain. Without whole-genome or targeted sequence analysis, the causal mutation is unknown. Without serial-passage testing, cryostock recovery tests, and replicate bioreactor runs, phenotype stability and process reproducibility remain undetermined. A modern strain-improvement program should preserve the UV-3 isolate, verify identity, and separate genetic effects from inoculum age or physiological adaptation.

5.2 pH and harvest-time control

In the source dataset, pH 4 was optimal among the tested values. A later study using the same MTCC 485 strain and response-surface/artificial-neural-network models reported an optimum near pH 5.32, 23.22 °C, 99.89 h, and 29.68% castor oil, with a predicted concentration of 72.73 mg L⁻¹ [12]. The different pH optima are not necessarily contradictory: substrate preparation, inoculum state, pH control mode, mixing, oxygen availability, and model design can shift an apparent optimum. The appropriate conclusion is that the productive region likely lies in the mildly acidic range and should be remapped using a designed experiment rather than a single-factor screen.

The decrease after 96 h indicates that harvest timing is a critical process variable. In situ adsorption, a second phase, or rapid broth clarification may reduce product toxicity and loss [7,15]. Online or at-line analytics would permit harvest based on product trajectory rather than a fixed clock time.

5.3 Position relative to recent biotechnology

Subsequent studies have achieved much higher titres or productivities in other yeasts, especially *Yarrowialipolytica* and *Candida parapsilosis*, by combining high cell density, optimized ricinoleic-acid supply, oxygen-transfer control, immobilization, adsorption-assisted delivery, continuous operation, and integrated purification [11,13,15–17]. These values are useful as engineering benchmarks, but they are not direct strain-to-strain comparisons because organism, substrate concentration, biomass loading, reactor mode, definition of yield, and analytical methods differ.

Table 03: Selected post-2016 advances relevant to γ -decalactone bioprocess development. Reported values are not directly comparable across platforms.

Year	Platform / strategy	Selected reported outcome	Relevance to MTCC 485	Ref.
2020	<i>Y. lipolytica</i> ; BC-alginate immobilization	8.37 g L ⁻¹ in repeated-batch operation	Cell retention and carrier mechanics can support reuse	[11]
2022	<i>S. salmonicolor</i> MTCC 485; RSM/ANN	72.73 mg L ⁻¹ at modelled optimum	Map the acidic operating region with multivariable design	[12]
2022	<i>Y. lipolytica</i> NCIM 3590; continuous process	75% molar conversion; 200 mg L ⁻¹ h ⁻¹ productivity	Evaluate cell recycle and continuous conversion	[13]
2022	Adsorption-embedding systems	2.79 g L ⁻¹ ; up to 88.99% conversion in one system	Improve hydrophobic-substrate presentation	[15]

Two newer directions broaden the field. First, synthetic biology now enables de novo lactone formation from central metabolism rather than dependence on ricinoleate feeding. Muller, Xia, and Jones engineered a defined pathway in *Escherichia coli* and obtained 3.53 mg L⁻¹ γ -decalactone, a low titre but clear proof that chain-length and ring-position specificity can be designed [18]. Second, β -oxidation-pathway engineering in *Y. lipolytica* has delivered a greater than fivefold increase in GDL titre, illustrating the value of targeted pathway control [19]. Industrial translation increasingly requires more than high flask titre. A 2026 process-design study for de novo GDL production with *Ashbya gossypii* showed that downstream purity can approach 96% in simulation, but economics remain sensitive to fermentation titre and the cost of inputs, while environmental burdens are influenced by salts, steam, and resin use [20]. For MTCC 485, these findings argue for linking strain improvement to oxygen demand, solvent and resin recovery, water use, and product recovery from the beginning.

5.4 Regulatory and analytical wording

The original paper's phrase "FDA approved" is too broad. A more accurate statement is that the FDA Substances Added to Food inventory lists γ -decalactone as a flavoring agent or adjuvant under 21 CFR 172.515 [2]. Whether a specific fermentation-derived product can be marketed as "natural" depends on the production route, purity,

intended use, jurisdiction, and supporting documentation. Scientific manuscripts should therefore separate chemical identity, food-use status, and labeling claims.

For an aroma product intended for food applications, GC peak integration alone is insufficient for a complete quality claim. Recommended confirmation includes an authentic standard, internal-standard calibration, recovery studies, limits of detection and quantification, GC-MS identity confirmation, enantiomeric analysis where relevant, residual-solvent testing, and a documented mass balance. These measures are particularly important when comparing milligram-per-litre research runs with gram-per-litre process reports.

6. Limitations and Reporting Gaps

The following limitations materially affect interpretation of the source study:

- **Mutagenesis exposure was incompletely specified.** UV wavelength, irradiance, delivered dose, survival curves, isolate-to-dose mapping, and colony selection criteria were not reported.
- **Experimental replication was unclear.** The source captions mention standard-deviation error bars, but replicate counts, individual observations, SD values, and statistical tests were unavailable.
- **Timing was internally inconsistent.** The methods describe sampling to 120 h, the mutant table ends at 100 h, and the narrative refers to a decline after approximately 110 h.
- **Preculture duration was inconsistent.** Culture conditions list 16, 24, or 42 h, while the mutant protocol states 49 h.
- **Substrate identity and loading were ambiguous.** The source alternates among castor oil, methyl ricinoleate, hydrolysate, 0.1% substrate, and 0.06% ricinoleic acid without a complete preparation protocol.
- **Analytical validation was incomplete.** Detector type, calibration equation, internal standard, extraction recovery, LOD/LOQ, and structural or chiral confirmation were not reported.
- **Strain identity and stability were not demonstrated after mutation.** No molecular characterization, serial-passage stability, or independent bioreactor confirmation was provided.
- **No process mass balance was reported.** Substrate conversion, intermediate accumulation, volatilization, broth/oil phase partitioning, and downstream recovery were not quantified.

INTERPRETIVE BOUNDARY The dataset supports the descriptive conclusion that UV-3 produced the highest reported GDL concentration under the tested conditions. It does not yet support claims of statistical superiority, causal genetic mechanism, stable inheritance, or industrial-scale performance.

7. Recommended Validation and Development Plan

Table 04: Stage-gated roadmap for converting UV-3 from a screening hit into a biotechnology development candidate.

Stage	Decision question	Minimum evidence	Go/no-go criterion
1. Reproduce	Is the UV-3 advantage real?	≥3 independent cultures per strain; randomized runs; wild type, UV-1–UV-4; prespecified ANOVA/post-hoc analysis	UV-3 gain reproducible with acceptable variability
2. Stabilize	Is the phenotype heritable?	Identity check; ≥10 serial passages; cryostock recovery; titre and growth after each checkpoint	No material loss of titre or fitness
3. Optimize	Which operating window maximizes productivity?	DOE across pH 4–6, substrate loading/form, temperature, inoculum age, agitation, aeration, and feed timing	Validated model with confirmation runs
4. Scale	Can oxygen transfer and product recovery be maintained?	Parallel 2–10 L runs; matched <i>kLa</i> /OTR; off-gas analysis; adsorption or two-phase removal; mass balance	Comparable yield, productivity, and recovery
5. Translate	Is the route economically and environmentally credible?	Purity, residual solvents, solvent/resin recycle, utilities, TEA, LCA, food-safety documentation	Target cost, purity, safety, and impact thresholds met

7.1 Immediate experimental priorities

- Recover the wild type and UV-3 from authenticated stocks and repeat matched shake-flask and bioreactor experiments with biological replicates.
- Record UV source wavelength and calibrated dose; if the original isolate cannot be traced, repeat mutagenesis using survival-based dose selection.
- Use a central-composite or Box-Behnken design around pH 4–6, temperature 23–28 °C, inoculum age, and ricinoleate loading rather than varying one factor at a time.
- Measure dissolved oxygen, oxygen-transfer rate, carbon-dioxide evolution, biomass, residual ricinoleate, GDL, and major pathway intermediates at shorter intervals around 72–108 h.
- Test in situ adsorption or immobilized-cell operation to reduce product toxicity and permit cell reuse, guided by recent carrier and adsorption studies [11,15].
- Validate GC quantification and identity with authentic standards, GC-MS, recovery experiments, and enantiomeric analysis before making purity or food-use claims.

7.2 Longer-term strain and process engineering

If UV-3 is stable, whole-genome sequencing against the parental strain can identify candidate mutations. Transcriptomics or targeted enzyme assays during the productive window can then test whether changes involve ricinoleate uptake, lipase activity, acyl-CoA activation, β -oxidation, redox balance, membrane stress, or product export. Causal variants should be reconstructed where feasible; this converts an empirical mutant into a genetically understood production strain.

Process development should focus on volumetric productivity and recovered product, not titre alone. Fed-batch substrate delivery can limit hydrophobic-substrate toxicity; oxygen-transfer targets should be defined by OTR rather than only rpm or vvm; and in situ recovery can shift partitioning away from cells. The final optimization objective should combine GDL productivity, substrate conversion, selectivity, recovery, solvent consumption, and strain-reuse performance.

8. Conclusion

The supplied study demonstrates that random UV mutagenesis can generate divergent GDL-production phenotypes in *S. salmonicolor* MTCC 485. Among four screened isolates, UV-3 reached 81.9 mg L⁻¹ at 96 h compared with 62.2 mg L⁻¹ for the wild type, a recalculated increase of 31.7%. The best reported pH was 4 for both strains, and concentrations declined after the observed peak, emphasizing harvest-time control. The result is scientifically useful as a screening-level finding, but it is not yet an industrial process claim. Missing replicate data, incomplete UV-dose and analytical details, inconsistent timing, ambiguous substrate description, and absent genetic-stability testing limit certainty. Literature

published after 2016 shows that the strongest gains now come from combining strain engineering with controlled oxygen transfer, multivariable optimization, immobilization or adsorption-assisted conversion, rapid product removal, scale-up validation, and integrated purification. Applying those tools to a reproducibly verified UV-3 strain is the most credible next step.

9. Acknowledgements

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10. Data Provenance and Editorial Declaration

The numerical results in Tables 1 and 2 originate from Nama et al. (2016) [1] and were supplied in the attached manuscript. This document is a language, structure, calculation, and literature update; it does not represent a new experimental study. The only numerical derivations added are transparent arithmetic comparisons based on the reported values. Where the source contained conflicting or missing information, the issue is disclosed in the text.

11. Conflict of Interest

The authors declare that they have no conflict of interest.

12. Funding

The authors received no specific funding for this study.

13. Ethical Approval

Ethical approval was not required for this study as it did not involve human participants or animals.

14. Informed Consent

Informed consent was not applicable as the study did not involve human participants.

15. Institutional Review Board Statement

Institutional Review Board approval was not required for this study as it did not involve human participants or animals.

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