Microbial Violacein for Sustainable Textile Colouration: A Critical Review of Biosynthesis, Dye-fibre Interactions and Industrial Translation

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Abstract

Violacein is a blue-violet bisindole bacterial pigment whose microbial origin, strong chromophore, and reported antimicrobial activity have encouraged its consideration as a bio-based alternative to petrochemically derived textile colourants. Yet the sustainability and industrial readiness of violacein dyeing remain much less certain than the frequently invoked label of a natural pigment implies. This critical narrative review integrates evidence on violacein biosynthesis, fermentation engineering, textile application, dye-fibre interactions, fastness, functional performance, safety, and process translation. Literature was selected through live searches of multidisciplinary and life-science scholarly sources, with bibliographic identity, retraction status, and digital object identifiers verified before inclusion. The biosynthetic evidence is mechanistically mature: the VioA-VioE enzyme sequence converts L-tryptophan through oxidative coupling, rearrangement, and oxygenation steps, while producer physiology and pathway regulation strongly influence pigment composition and productivity. Production has advanced from native strains to heterologous hosts, fed-batch culture, and agro-industrial feedstocks, but reported titres remain difficult to compare because studies quantify different mixtures of violacein, deoxyviolacein, crude pigment, and purified product. Direct textile evidence is far narrower. Verified studies demonstrate colouration of polyamide, silk, wool, cotton, regenerated cellulose, polyester-containing fabrics, and related substrates, but only a small number quantify colour strength, process variables, fastness, or retained antimicrobial function. Polyamide currently has the strongest integrated evidence, including in-process dyeing during fermentation, whereas proposed molecular interactions with cellulose, protein fibres, and polyester are still based largely on chemical plausibility rather than direct adsorption or spectroscopic proof. Light stability, shade reproducibility, solvent-intensive recovery, leaching, toxicological uncertainty, and absent comparative life-cycle and techno-economic evidence are principal barriers. Violacein is therefore best regarded as a technically credible but conditionally sustainable textile colourant whose industrial case depends on standardised pigment analytics, solvent-minimised process integration, fibre-specific dyeing science, durability testing, safety assessment, and system-level environmental and economic validation.

Keywords: *Bacterial pigment; biogenic colourant; biosynthetic pathway; fermentation; natural dye; polyamide; textile fastness; sustainable colouration.*

1. Introduction

Textile colouration is chemically and operationally demanding because a commercially useful dye must combine reproducible hue, affinity for a defined fibre, controlled exhaustion or fixation, acceptable fastness, process compatibility, and a cost structure that survives large-scale wet processing. Environmental burdens arise not simply from the molecular origin of the dye but from the complete colouration system, including feedstocks, reaction or fermentation energy, auxiliaries, salts, solvents, water, rinsing, wastewater treatment, and the lifetime of the coloured article. This systems perspective is particularly important for natural and microbial colourants. Natural provenance can reduce dependence on fossil-derived aromatic chemistry or enable renewable feedstocks, but it does not itself establish lower life-cycle impacts, nor does it guarantee low toxicity or adequate durability (Fried *et al*., 2022; Uddin *et al*., 2022).

Microbial pigments occupy a distinctive position within this landscape. In contrast with many plant-derived dyes, microbial production can be conducted independently of season and arable land, can potentially use side streams as carbon and nitrogen sources, and is amenable to metabolic and bioprocess engineering. At the same time, microbial systems introduce their own burdens: sterile or controlled cultivation, aeration or agitation, nutrient inputs, downstream recovery, solvent handling, biomass disposal, and the need to control strain safety and product purity. Reviews of bacterial pigments and biogenic textile colourants have therefore shifted from a simple natural-versus-synthetic dichotomy towards process-specific assessments of yield, robustness, recovery, application chemistry, functional performance, and environmental trade-offs (Rather *et al*., 2023; Venil *et al*., 2013).

Violacein is among the most intensively studied bacterial pigments. It is a deeply coloured bisindole metabolite produced by several Gram-negative bacteria, classically *Chromobacterium violaceum* and *Janthinobacterium lividum*, and by other taxa carrying homologous biosynthetic machinery. Its colour arises from an extended conjugated structure generated from L-tryptophan through a five-enzyme pathway, now conventionally described as VioA, VioB, VioE, VioD, and VioC. Early genetic descriptions of the pathway were refined by subsequent biochemical and structural work that clarified pathway intermediates, the catalytic role of VioE, and the oxygenation steps that distinguish violacein from deoxyviolacein (August *et al*., 2000; Balibar & Walsh, 2006; Sánchez *et al*., 2006; Shinoda *et al*., 2007). These advances make violacein attractive not only as a pigment but also as a model for connecting molecular pathway control with application-specific product properties.

Interest in violacein has historically been driven at least as much by its biological activities as by colouration. Reviews have documented antimicrobial, antiparasitic, antiviral, and cytotoxic activities and have motivated extensive work on natural-producer optimisation and heterologous biosynthesis (Ahmed *et al*., 2021; Choi *et al*., 2015; Durán *et al*., 2007; Park *et al*., 2021). For textiles, bioactivity creates an unusual opportunity: a pigment could, in principle, provide both colour and antimicrobial functionality. This prospect is not yet equivalent to a durable functional finish. Biological activity measured for purified or soluble pigment does not establish the dose retained on a fibre after repeated laundering, whether the active species remains accessible to microorganisms, whether it leaches into wash water or skin-contact conditions, or whether antimicrobial efficacy persists without unacceptable colour loss. The distinction between pigment bioactivity and validated functional-textile performance is therefore central to an industrial assessment. The broader literature on *C. violaceum* also documents the opportunistic pathogenicity of the native producer, separating organism-containment questions from the application safety of purified pigment (Durán *et al*., 2016).

Direct textile studies remain comparatively scarce. Foundational work demonstrated that bluish-purple pigments from *J. lividum* could colour silk, cotton, wool, nylon, acetate, and other fibres, while also identifying sunlight fading as a practical weakness (Shirata *et al*., 2000). A later multi-fibre study using *C. violaceum* pigment quantified colour strength and explored pH, temperature, and mordants across natural and synthetic substrates (Choi & Kim, 2009). Venil *et al*. (2016) expanded the fibre set and fastness testing, while Kanelli *et al*. (2018) integrated violacein production with polyamide 6.6 dyeing and demonstrated retained antibacterial and antifungal activity. More recently, Deeppal *et al*. (2024) characterised a *Chromobacterium piscinae* isolate and demonstrated dyeing of cotton and polyester fabrics. These studies establish feasibility, but they are heterogeneous in producer strain, pigment purity, extraction solvent, fabric construction, auxiliaries, dyeing route, colour metrics, and fastness protocols. Consequently, a darker shade or higher reported K/S in one experiment cannot be read as a general measure of violacein-fibre affinity across studies.

The production literature is substantially broader than the textile literature. Native producers have been optimised through medium composition and physical culture variables, including agitation and temperature, while heterologous expression has enabled pathway reconstruction in industrially familiar hosts and more controlled manipulation of flux (Jiang *et al*., 2010; Lu *et al*., 2009; Wang *et al*., 2009; Yang *et al*., 2007). Systems metabolic engineering has produced purified violacein from *Escherichia coli*, and *Corynebacterium glutamicum* has been engineered for high crude-pigment titres (Rodrigues *et al*., 2013; Sun *et al*., 2016). Agro-industrial substrates, including liquid pineapple waste and soybean meal, have been explored to reduce feedstock cost and couple pigment manufacture to residual-stream valorisation (Aruldass *et al*., 2015; Gohil *et al*., 2022). Yet comparisons are often misleading because the analytical object varies from total violet extract to crude violacein, mixed violacein/deoxyviolacein fractions, or purified violacein. This inconsistency matters for textile translation because minor congeners and residual matrix components can alter hue, hydrophobicity, adsorption, biological activity, and washing behaviour.

Recent violacein-specific studies continue to extend heterologous and process-engineering approaches and further indicate that production outcomes are sensitive to cultivation conditions and production-system design (Müller *et al*., 2024; Schick *et al*., 2024; Mehta *et al*., 2025).

Recent reviews have strengthened understanding of violacein biosynthesis, biological functions, and fermentation, including a 2025 synthesis focused specifically on microbial production (Zhang *et al*., 2025). Broader reviews have positioned microbial pigments within industrial biotechnology and sustainable textile colouration (Fried *et al*., 2022; Rather *et al*., 2023). What remains underdeveloped is a fibre-centred synthesis that connects the maturity of biosynthetic and production science to the much thinner evidence on adsorption, fixation, fastness, functional retention, and whole-process sustainability. In particular, the term dye-fibre interaction is often used as if molecular binding were experimentally established, whereas most violacein textile papers report colour outcomes rather than adsorption isotherms, binding energies, in situ spectroscopy, diffusion coefficients, or molecular simulations validated against experiment. A critical review must therefore separate direct evidence from mechanistic inference.

A recent review likewise identifies production, downstream processing, stability, and application evidence as distinct but interdependent areas that require continued integration when assessing violacein for industrial use (Scaramussa *et al*., 2025).

1.1 Scope, Research Gap and Objectives

This review evaluates microbial violacein as a textile colourant across three connected levels: biosynthetic formation and product composition, dyeing behaviour on major natural and synthetic fibres, and industrial translation under sustainability constraints. The central gap is the disconnect between relatively mature pathway and fermentation knowledge and the limited, methodologically heterogeneous evidence that defines how violacein actually interacts with fibres, survives use conditions, and performs within an industrially credible colouration system. The objectives are to clarify the biochemical basis of pigment formation, compare production strategies without conflating crude and purified outputs, critically synthesise direct textile studies, distinguish

demonstrated dye-fibre behaviour from chemically plausible but unverified mechanisms, and identify the environmental, safety, standardisation, and scale-up evidence required before sustainability claims can be defended. Biomedical applications unrelated to textile performance are outside the main scope except where they bear directly on antimicrobial functionality, toxicological interpretation, or product safety.

2. Methods for Literature Selection

2.1 Review Design and Rationale

A critical narrative review design was selected because the evidence base spans enzymology, microbial physiology, metabolic engineering, fermentation, extraction, polymer and fibre chemistry, textile colour measurement, functional finishing, toxicology, and sustainability assessment. These literatures use incompatible experimental units and outcomes, making a pooled quantitative synthesis inappropriate. The approach followed the principle that narrative reviews should make their search logic and selection criteria explicit while using critical integration to explain heterogeneity rather than merely catalogue studies (Snyder, 2019).

The review was therefore structured around mechanistic and translational questions: how violacein is formed and controlled, how production route affects the chemical object applied to textiles, what direct dyeing evidence exists for different fibres, which molecular interactions are supported rather than assumed, and which industrial claims remain unvalidated.

2.2 Information Sources

Live literature searching was undertaken in PubMed, Europe PMC, DOAJ, Semantic Scholar, OpenAlex, and Google Scholar.

Searches were supplemented by backward reference checking from high-quality reviews and key primary papers, forward and related-article searching where available, and verification against official journal or DOI landing pages. Retraction status was checked during reference verification, and retracted evidence was excluded from the substantive synthesis. Only sources whose bibliographic identity could be confirmed were retained. Publisher webpages were used for bibliographic and DOI verification but were not treated as literature databases.

2.3 Search Strategy and Search Period

The main search covered publications from 2000 to 13 June 2026, with older foundational studies retained when necessary to establish production or extraction history. Search concepts combined the pigment and its principal congener with textile, biosynthetic, production, and sustainability terms. Representative combinations included (violacein OR deoxyviolacein) AND (textile OR fabric OR dyeing OR colouration OR fibre OR fiber); violacein AND (biosynthesis OR *vioABCDE* OR metabolic engineering OR fermentation OR heterologous production); violacein AND (polyamide OR nylon OR cotton OR silk OR wool OR polyester OR rayon) AND (dye OR colour OR K/S OR fastness); and (microbial pigment OR biogenic colourant) AND (textile OR dyeing) AND (sustainability OR life cycle OR industrial OR scale-up). Searches were iterated with specific producer names and pathway genes when key mechanistic or process questions required greater precision.

2.4 Eligibility Criteria

Eligible publications were peer-reviewed original studies and high-quality reviews that directly informed violacein biosynthesis, regulation, production, extraction, characterisation, textile dyeing, fastness, antimicrobial textile performance, or industrial and environmental interpretation. English-language studies were prioritised because full bibliographic verification and claim assessment were required. Textile studies were included when the applied pigment could reasonably be identified as violacein-containing material and the fibre or fabric substrate and process were described. Broader natural-colourant and microbial-pigment reviews were used only for contextual sustainability and industrial comparisons. Studies concerned exclusively with biomedical effects were excluded unless they addressed safety or functionality relevant to textiles. Preprints were not used where peer-reviewed evidence was available, patents were not treated as effectiveness evidence, and retracted or bibliographically unverifiable records were excluded.

2.5 Screening, Duplicate Handling and Study Selection

Records were screened first for topical relevance from title and abstract information and then, where accessible, against the full article or detailed official record. Duplicate records were identified primarily by DOI and secondarily by matching title, authorship, and year. Selection emphasised direct primary evidence for the core claims of the review while retaining influential mechanistic studies and recent syntheses needed to interpret historical development. No numerical screening counts are reported because the process was designed for critical narrative synthesis rather than a prospectively registered systematic review. Sources with inaccessible or incomplete metadata were not used for claims requiring detailed study characteristics, and any record whose DOI or bibliographic identity remained uncertain was removed.

2.6 Critical Appraisal and Evidence Synthesis

Evidence was appraised according to design appropriateness, analytical definition of the pigment, culture and extraction transparency, use of controls, scale, repeatability, fibre specification, colour measurement, fastness methodology, relevance of functional assays, and the degree to which conclusions extended beyond measured outcomes. Production studies were interpreted cautiously when the reported output was crude violet pigment rather than chemically resolved violacein. Textile studies were given greater weight when they reported instrumental colour strength or CIELAB metrics, standardised or clearly defined fastness tests, and direct comparisons among process conditions. Mechanistic claims about dye-fibre interactions were classified as direct when supported by application data or molecular/analytical evidence and as inferential when derived mainly from pigment and polymer chemistry. Themes were developed iteratively around pathway control, bioprocessing, fibre-specific colouration, durability, functional effects, and industrial sustainability, with contradictions retained rather than harmonised artificially.

3. Violacein Chemistry and Biosynthetic Logic

3.1 Molecular Structure and Colour-Relevant Properties

Violacein is a conjugated bisindole metabolite produced from two L-tryptophan-derived units. Its central heterocyclic system and substituted indole rings generate a strongly absorbing blue-violet chromophore, while multiple nitrogen- and oxygen-containing functional groups create sites capable of hydrogen bonding and acid-base-sensitive interactions. The molecule is substantially less compatible with water than conventional ionic textile dyes, and practical extraction commonly relies on organic solvents. For textile processing this combination is consequential: the extended aromatic system favours dispersion and hydrophobic association with less polar substrates, whereas hydroxyl, carbonyl, and indolic N-H functions can plausibly participate in hydrogen bonding with amide- or hydroxyl-containing fibres. These are chemically reasonable interaction pathways, but they should not be mistaken for experimentally resolved binding mechanisms in dyed fibres. Direct studies of adsorption thermodynamics, solid-state pigment speciation, and bond-specific spectroscopy at the violacein-fibre interface remain sparse.

Violacein preparations are also chemically non-uniform unless purification is rigorous. Deoxyviolacein is formed when one hydroxylation branch of the pathway is bypassed or incomplete, and native or heterologous cultures can yield different ratios of congeners. Early work on production and extraction already distinguished crude purple material from more purified pigment, while later pathway reconstruction showed that host background can change the composition of pathway products (Jiang *et al*., 2010; Rettori & Durán, 1998). This compositional variability is more than an analytical nuisance. An altered violacein-to-deoxyviolacein ratio can shift polarity, hue, biological activity, and likely fibre partitioning. Accordingly, an industrial dye specification should define not only total absorbance or dry pigment mass but also chemical identity, purity, congener profile, residual solvent, and relevant fermentation-derived impurities.

3.2 The VioA-VioE Pathway: From L-Tryptophan to the Violet Chromophore

Genetic analysis of the *C. violaceum* pathway established the clustered organisation of genes required for violet-pigment biosynthesis, but the mechanistic model was subsequently refined substantially (August *et al*., 2000). The now accepted pathway begins with VioA, a flavin-dependent L-tryptophan oxidase that generates an oxidised tryptophan-derived imine. Structural and biochemical analysis of VioA clarified how substrate recognition and flavin chemistry initiate flux into the pathway (Füller *et al*., 2016). VioB, a haem-containing enzyme, promotes coupling of two activated tryptophan-derived intermediates. This dimerisation step is a major commitment point because the coupled intermediate is chemically reactive and can diverge into non-violacein products if subsequent handling is not correctly channelled.

VioE provides the decisive rearrangement that redirects the nascent coupled intermediate towards violacein biosynthesis rather than chromopyrrolic acid. Re-evaluation of the pathway demonstrated that VioE is not a dispensable accessory but a catalytic determinant of pathway identity (Sánchez *et al*., 2006). Structural work subsequently showed that VioE has a fold related to lipoprotein-transport proteins yet functions as a cofactor-independent catalytic chaperone, illustrating how pathway specificity can arise from control of a highly reactive intermediate rather than from classical redox chemistry (Ryan *et al*., 2008). The VioE product proceeds towards prodeoxyviolacein, after which the flavin-dependent oxygenases VioD and VioC establish the oxygenation pattern characteristic of violacein. In vitro reconstitution with VioA-E confirmed that the enzyme set is sufficient to transform L-tryptophan into violacein-related products (Balibar & Walsh, 2006).

Detailed intermediate analysis identified protoviolaceinic acid as a genuine pathway intermediate and clarified the contribution of VioC to the final oxidation sequence (Shinoda *et al*., 2007). The pathway is therefore better understood as a sequence of enzyme-controlled redox and rearrangement events followed by chemically assisted maturation, not as a single undifferentiated pigment-forming reaction. For process engineering, this matters because flux imbalance may change both total pigment titre and product composition. Increasing precursor supply without matching the catalytic capacity of downstream steps can accumulate intermediates or congeners, while altered oxygen availability can affect oxygenase-dependent stages. The textile consequence is direct: a fermentation process optimised only for apparent violet intensity may produce a different molecular mixture from one optimised for chemically quantified violacein.

3.3 Regulation, Producer Diversity and Product Variability

Violacein production is strongly coupled to producer physiology rather than being a constitutive chemical output. In *C. violaceum*, quorum-sensing circuitry contributes to population-density-dependent regulation, and later work identified negative regulatory control that adds further layers to the canonical activation model (Devescovi *et al*., 2017). In *J. lividum*, violacein production is associated with biofilm development and environmental adaptation, again emphasising that pigment formation can be integrated with stress and community behaviour rather than controlled solely by precursor availability (Pantanella *et al*., 2007). These regulatory differences help explain why apparently similar media and cultivation conditions can give markedly different yields across strains and why process parameters cannot be transferred between producers without validation.

Producer diversity also broadens the available bioprocess window. Psychrotrophic *J. lividum* isolates can require low temperature for strong pigment formation, whereas marine *P. luteoviolacea* responds markedly to agitation and aggregation state (Lu *et al*., 2009; Yang *et al*., 2007). *Duganella* species have shown comparatively rapid pigment accumulation under optimised culture conditions, while engineered hosts permit separation of pathway performance from native regulatory networks (Jiang *et al*., 2010; Wang *et al*., 2009). From an industrial perspective, the optimal producer is therefore not necessarily the strain with the darkest colonies or the highest shake-flask titre. Selection should account for temperature and oxygen demand, pathogenicity or biosafety, genetic stability, feedstock tolerance, pigment composition, broth rheology, downstream recovery, and compatibility with integrated dyeing.

Table 1 summarises the pathway elements that are most relevant to pigment specification and bioprocess control. The evidence is strongest for enzyme function and pathway order, whereas direct links between individual enzymatic control points and textile shade or fastness have rarely been tested. This distinction is important because metabolic engineering can tune a molecular output only if textile-relevant quality attributes are defined alongside titre.

Table 1. Biosynthetic and regulatory control points relevant to textile-grade violacein production

Control point	Established role	Textile/industrial relevance	Key uncertainty	Supporting references
VioA	FAD-dependent oxidation of L-tryptophan initiates pathway flux.	Precursor conversion and upstream flux influence total pathway productivity.	Effect of VioA flux on congener ratio and textile shade has not been mapped systematically.	Füller *et al*., 2016; Balibar & Walsh, 2006
VioB	Haem-dependent coupling of two activated tryptophan-derived units.	Controls formation of the bisindole scaffold that underpins the chromophore.	Reactive intermediates and side-product formation under industrial oxygen/redox conditions remain poorly characterised.	Balibar & Walsh, 2006; Sánchez *et al*., 2006
VioE	Catalytic rearrangement channels the coupled intermediate towards prodeoxyviolacein rather than shunt products.	Critical for product identity and therefore pigment composition.	Quantitative relationships between VioE expression, purity and textile performance are not established.	Ryan *et al*., 2008; Sánchez *et al*., 2006
VioD	FAD-dependent oxygenation on one indole branch.	Influences hydroxylation state and violacein/deoxyviolacein distribution.	Industrial cultures rarely report oxygenase flux together with fibre-specific colour metrics.	Balibar & Walsh, 2006; Shinoda *et al*., 2007
VioC	Late oxygenation contributes to the characteristic violacein oxidation pattern.	Late-stage conversion affects chemical identity and potential polarity.	Incomplete conversion may alter colour and adsorption, but this remains largely untested on textiles.	Balibar & Walsh, 2006; Shinoda *et al*., 2007
Quorum-sensing and negative regulators	Population-dependent and repressive controls modulate pathway expression in native producers.	Regulatory architecture affects batch-to-batch productivity and timing of pigment formation.	Transferability across strains and large-scale reactors is uncertain.	Devescovi *et al*., 2017; Pantanella *et al*., 2007

Note. FAD = flavin adenine dinucleotide. The table distinguishes established biochemical roles from application-level uncertainties; it does not imply that each control point has been independently validated for textile outcomes

4. From Native Producers to Fermentation Platforms

4.1 Native Producers and Culture Control

The practical history of violacein production illustrates a recurring difficulty in comparing bioprocess claims. Rettori and Durán (1998) established an early workflow for production, extraction, and purification, but later studies expanded the range of strains, media, analytical methods, and definitions of yield. In *Duganella* sp. B2, medium and process optimisation produced a reported 1.62 g/L crude violacein after 32 h in shake-flask culture (Wang *et al*., 2009). A psychrotrophic *J. lividum* isolate from a glacier produced violet pigment strongly at temperatures below 20 °C, with yield reported relative to dry biomass rather than reactor volume (Lu *et al*., 2009). These examples are often presented side by side as evidence of rising productivity, yet the units and chemical definitions are not equivalent. Biomass-normalised pigment, spectrophotometrically estimated crude extract, and chromatographically quantified purified violacein answer different questions.

Physical culture variables can be as influential as medium composition. In *P. luteoviolacea*, agitation altered growth form and violacein production, with lower agitation or more static conditions favouring pigment accumulation in the studied strain (Yang *et al*., 2007). The result cautions against assuming that greater oxygen transfer will always improve a pathway containing oxidative enzymes. Agitation also alters aggregation, shear, gas-liquid transfer, and potentially quorum-sensing dynamics; the net phenotype is strain- and reactor-dependent. Such findings have industrial consequences because a condition that works in a flask may not reproduce in a stirred tank where mixing, oxygen gradients, and morphology differ.

Recent isolation studies continue to show this strain specificity. A new *Janthinobacterium* sp. isolate reached about 114 mg/L under an optimised yeast-extract/fructose medium after 120 h, while glycerol was not favourable under the tested conditions (Ishimoto *et al*., 2024). By contrast, glycerol supported a fed-batch strategy in the *J. lividum* process developed for polyamide dyeing (Kanelli *et al*., 2018). The apparent disagreement is informative rather than contradictory: carbon-source effects depend on transport, central metabolism, regulation, and strain background. A commercial programme should therefore avoid treating a single preferred carbon source as a species-wide rule.

4.2 Heterologous Production and Metabolic Engineering

Heterologous expression changes the engineering problem from persuading a native organism to overproduce a secondary metabolite to balancing a defined pathway within a chosen chassis. Reconstruction of the *Duganella* sp. B2 pathway in different hosts demonstrated that host context affects both amount and composition of the recovered pigments. *Citrobacter freundii* produced a high crude violacein titre in that study, whereas other hosts generated different distributions of violacein-related products (Jiang *et al*., 2010). The result reinforced a key quality principle for textile production: a conserved gene cluster does not guarantee an invariant dye preparation. Earlier heterologous-expression work also established that environmental-DNA-derived violacein biosynthetic genes can be functionally expressed outside the native producer, providing a foundational demonstration of pathway portability (Brady *et al*., 2001).

Systems metabolic engineering in *E. coli* subsequently combined precursor supply, pathway expression, and process control to produce violacein and deoxyviolacein at preparative scale. Rodrigues *et al*. (2013) reported 710 mg/L violacein in fed-batch culture and crystallised product at 99.8% purity. Although the mass concentration was lower than some later crude-pigment reports, the work is especially informative because chemical purity was explicitly resolved. For application science, a lower but well-defined titre may be more valuable than a higher number derived from unresolved extract, since it allows reproducible mass dosing and a direct link between molecular identity and textile response.
C. glutamicum has also been engineered as a production host. Sun *et al*. (2016) reported an optimised crude-violacein concentration of 5.436 g/L in a 3 L fermentation, with a volumetric productivity of 47 mg/L/h. The high titre demonstrated substantial pathway capacity in a tractable industrial microorganism, but the qualifier 'crude' remains important. It is also inappropriate to infer pigment safety from the established use of a production host in industrial biotechnology. Host biosafety, product toxicology, residual host-cell components, and downstream purity are separate regulatory questions.

4.3 Agro-Industrial Feedstocks and Downstream Recovery

Using residual biomass or food-processing side streams is one of the strongest conceptual arguments for a bio-based violacein process, but it is also an area where sustainability rhetoric can exceed measured evidence. Aruldass *et al*. (2015) used liquid pineapple waste as substrate for *C. violaceum* UTM5 and scaled cultivation to a 50 L bioreactor. The reported output exceeded 16 g/L for the recovered violet pigment, and the process simultaneously improved several conventional measures of the pineapple effluent. Purification showed that the pigment contained violacein and deoxyviolacein. The study is therefore valuable as a demonstration of waste-stream valorisation, but its reported pigment yield should not be interpreted as 16 g/L of analytically pure violacein.

Soybean meal provides a second example. Gohil *et al*. (2022) reported 496 mg/L crude violacein with 2% soybean meal, a 1.62-fold increase relative to Luria-Bertani medium under the tested conditions. Such results suggest that complex agro-industrial substrates can supply both carbon and nitrogen while reducing reliance on refined medium components. Yet industrial benefits depend on local availability, compositional variability, sterilisation burden, solids handling, extract colour, and downstream impurity removal.

A nominally low-cost waste feedstock can raise purification costs if proteins, lipids, phenolics, or suspended solids co-extract with the pigment.

Downstream recovery remains a central sustainability bottleneck because violacein is poorly water-compatible and is frequently extracted with ethanol, methanol, acetone, or related organic solvents. Solvent recovery can reduce net consumption, but energy for evaporation and solvent losses still contribute to process impacts. This is one reason the simultaneous fermentation and dyeing route of Kanelli *et al*. (2018) is conceptually important: it attempts to transfer pigment from a producing culture directly to polyamide fabric and thereby bypass a conventional solvent-extraction step. The approach does not remove downstream questions; instead, it shifts them towards broth impurities, biomass separation, sterilisation, residual nutrients, shade control, and wastewater treatment. Whether this trade is favourable at factory scale is unknown because comparative life-cycle or techno-economic analyses have not yet been reported for violacein textile dyeing.

Table 2 compares representative production platforms while retaining the analytical labels used by the original studies. The wide numerical range is not a league table of performance: differences in host, scale, cultivation time, chemical purity, extraction, and reporting basis prevent direct ranking. The most important industrial lesson is that future studies should report a chemically resolved pigment specification together with volumetric productivity and recovery yield.

Table 2. Representative violacein production platforms and comparability limitations

Producer/chassis	Process or feedstock	Scale/context	Reported output	Why direct comparison is limited	Supporting references
Duganella sp. B2	Optimised native-strain culture	Shake flask	1.62 g/L crude violacein after 32 h	Crude pigment estimate; strain- and assay-specific.	Wang *et al*., 2009
Citrobacter freundii carrying *Duganella* pathway	Heterologous pathway reconstruction	Laboratory culture	1.68 g/L crude violacein	Host-dependent product mixture and crude measurement.	Jiang *et al*., 2010
Escherichia coli	Systems metabolic engineering and fed-batch	Bioreactor/fed-batch	0.71 g/L violacein; crystallised product 99.8% purity	Lower apparent titre than some crude reports but chemically defined final product.	Rodrigues *et al*., 2013
Chromobacterium violaceum UTM5	Liquid pineapple waste	50 L bioreactor	16.256 ± 0.440 g/L violet pigment	Violet pigment contained violacein and deoxyviolacein; not pure-violacein titre.	Aruldass *et al*., 2015
Corynebacterium glutamicum	Engineered heterologous production	3 L fermentation	5.436 g/L crude violacein; 47 mg/L/h	Crude-pigment basis; purity and congener distribution limit comparison.	Sun *et al*., 2016
Janthinobacterium lividum	Glycerol-fed batch linked to polyamide dyeing	Laboratory fed-batch	1.828 g/L crude violacein	Application-oriented process; crude pigment and direct textile integration differ from purified-product studies.	Kanelli *et al*., 2018
Chromobacterium violaceum	Soybean meal medium	Shake flask	0.496 g/L crude violacein at 48 h	Complex substrate and crude estimate; local feedstock quality may affect recovery.	Gohil *et al*., 2022
Janthinobacterium sp. isolate	Yeast extract/fructose medium optimisation	Shake flask	0.114 ± 0.017 g/L at 120 h	Different strain, assay and optimisation objective; illustrates strain-specific carbon response.	Ishimoto *et al*., 2024

Note. Reported outputs are reproduced in the analytical categories used by the cited studies. They should not be normalised mentally as equivalent pure-violacein titres. ± indicates reported standard deviation where available

5. Direct Evidence for Textile Dyeing

5.1 Fibre Scope and Colour Yield

The most important fact about violacein textile application is the small size of the directly relevant evidence base relative to the fermentation literature. Shirata *et al*. (2000) isolated bluish-purple pigment-producing bacteria identified as *J. lividum* and tested pigment-containing material on a broad fibre set. Silk, cotton, wool, nylon, vinylon, acetate, and related substrates could be coloured, demonstrating that violacein-containing pigment was not restricted to a single polymer class. The study also showed that

hue depended on substrate and process conditions. Its breadth remains useful, but colour yield and molecular uptake were not characterised using the full range of instrumental methods now expected in textile dyeing research.

Choi and Kim (2009) provided a more explicitly comparative multi-fibre assessment using pigment from *C. violaceum* CV107. The pigment was extracted with 80% acetone and characterised by liquid chromatography/mass spectrometry before application to cotton, silk, wool, diacetate, triacetate, acrylic, and nylon 66. Reflectance-based K/S values showed maxima in the approximately 540-580 nm region, consistent with violet colouration. Dyeing response varied strongly with substrate, pH, and temperature, confirming that a single recipe cannot represent all fibres. The study is particularly valuable because it treated violacein as a textile dyeing problem rather than merely showing that a fabric could become purple.

Venil *et al*. (2016) broadened application testing with violacein from *C. violaceum* UTM5 on cotton, silk, rayon, jacquard rayon, silk satin, and cotton/polyester fabric. The work demonstrated visible colouration and assessed several mordants as well as fastness properties. Comparison with a conventional reactive-dye control showed that the synthetic control could produce deeper colour under the tested conditions, which is an important corrective to claims that microbial pigments are already performance-equivalent.

A bio-based dye does not need to outperform every synthetic colourant to be useful, but claims of substitution require fibre-specific benchmarks rather than aesthetic photographs alone.

The strongest quantitative case is presently polyamide 6.6. Kanelli *et al*. (2018) compared three routes: simultaneous fermentation and dyeing, dyeing with sonicated culture, and dyeing with cell-free extract. Simultaneous fermentation and dyeing produced the highest reported total colour difference, $\Delta E = 74.81$, and $K/S = 22.01$. These values indicate substantial colour development within that experimental system and show that a producing culture can act as both pigment generator and dye bath. The process is scientifically significant because it integrates production and application, but it remains a laboratory-scale demonstration. Industrial translation would require control of contamination, fabric-to-liquor ratio, broth solids, oxygen transfer, shade reproducibility, and post-dyeing removal of microbial residues.

Deeppal *et al*. (2024) added a newly characterised *C. piscinae* isolate to the production landscape and used ethanol-extracted pigment for cotton and polyester dyeing. The pigment identity was supported by high-performance liquid chromatography, Fourier-transform infrared spectroscopy, and proton nuclear magnetic resonance. Textile application was demonstrated with sodium chloride added during dyeing. The paper describes sodium chloride as a mordant, but from a textile-chemistry perspective it is more appropriately interpreted as an inorganic electrolyte or exhaustion aid than as a classical coordination-forming mordant such as a metal salt. This terminology matters because mechanistic claims about fixation should follow the chemistry of the auxiliary rather than its label.

5.2 Effects of pH, Temperature, Auxiliaries and Process Route

Process-condition evidence is heterogeneous but yields several useful principles. Choi and Kim (2009) found high colour strength around alkaline pH and elevated temperature, with an optimum near pH 10 and 70 °C under their multi-fibre conditions. The pH effect may reflect a combination of pigment speciation, fibre surface chemistry, and changes in aggregation or solubility, rather than a single ionisation event. Because different fibres have different tolerance to alkaline conditions, the practical optimum must be constrained by substrate damage and finishing requirements. Wool and silk, for example, cannot simply inherit a recipe optimised for a robust synthetic polymer without assessing handle and mechanical integrity.

Mordant effects are similarly non-universal. Choi and Kim (2009) reported that tested mordants did not improve colour density or overall quality in their system. Venil *et al*. (2016) tested alum, ferric sulfate, copper sulfate, and calcium hydroxide; some conditions, particularly slaked lime, altered or deepened colour on selected fabrics. These results do not establish a general 'best mordant'. Metal salts can change colour through complexation, fibre modification, pH shifts, or pigment precipitation, but they can also introduce ecotoxicity and wastewater-treatment burdens that undermine a sustainability claim. A credible sustainable process should therefore prefer auxiliaries only when their gains in fixation or fastness are quantified and outweigh their additional chemical and environmental load.

The route by which pigment reaches the fibre may be as important as the formulation. Solvent-extracted violacein offers a more defined dosing material and separates fermentation from dyeing, which can simplify quality control but adds extraction and solvent-recovery stages. Cell-free broth extracts can reduce biomass carry-over but still require separation. Simultaneous fermentation and dyeing eliminates conventional pigment isolation and creates a continuous transfer environment from cells or broth to fibre (Kanelli *et al*., 2018). These routes should be considered distinct process archetypes rather than minor variants. Their environmental and economic profiles will differ in solvent use, residence time, sterility requirements, energy demand, effluent composition, and compatibility with existing dyeing machinery.

5.3 Fastness and Retained Antimicrobial Function

Fastness determines whether initial colouration survives use. Shirata *et al*. (2000) described fastness as broadly comparable with several vegetable dyes under the tested conditions but identified sunlight fading as a major weakness. Venil *et al*. (2016) likewise found that some washing, rubbing, and related fastness properties were fair to excellent depending on fabric and condition, while light performance was less robust. These findings converge on photostability as a probable limitation, although methods, exposure

conditions, and rating scales differ. The lack of a harmonised, cross-fibre fastness dataset prevents a single durability grade from being assigned to violacein.

Light fading is chemically plausible because a large conjugated chromophore can undergo photo-oxidative transformation, especially in the presence of oxygen, fibre-bound metal ions, or reactive finishing residues. Yet the exact photoproducts and degradation kinetics of fibre-bound violacein are poorly resolved. Stabilisation strategies therefore remain empirical. Ultraviolet absorbers, antioxidants, encapsulation, pigment derivatisation, or fibre pretreatments may improve durability, but each intervention changes cost, chemistry, and potentially biodegradability. Before such strategies are promoted, the failure mechanism should be established using controlled irradiation coupled to chromatographic and spectroscopic analysis of both colour loss and chemical transformation.

Functional performance is most convincingly demonstrated in the polyamide study of Kanelli *et al*. (2018), where dyed fabrics showed antibacterial activity against *Staphylococcus aureus* and antifungal activity against *Candida albicans* and *Candida parapsilosis* under the authors' test conditions. The result demonstrates that bioactivity can be retained after dyeing, but it should not be generalised to every violacein-dyed textile. Functional durability after repeated laundering, the amount of pigment released into wash liquor, the relationship between K/S and antimicrobial dose, and the balance between surface availability and fixation have not been established adequately. A pigment that leaches may show strong short-term antimicrobial activity while performing poorly as a durable finish and raising exposure or environmental concerns.

Table 3 collates the direct textile studies retained in this review. Their collective strength is proof of cross-fibre colouration and the demonstration of an integrated polyamide process; their collective weakness is methodological fragmentation. No single study simultaneously resolves pigment purity, adsorption/fixation, standardised fastness across multiple fibres, wash-cycle durability, leaching, toxicology, and process-level environmental performance.

Table 3. Direct evidence for violacein-containing pigments in textile dyeing

Study	Pigment source/form	Fibre(s)	Key process/performance finding	Critical limitation	Supporting references
Shirata *et al*. (2000)	*J. lividum* bluish-purple pigment; cell/pigment preparations	Silk, cotton, wool, nylon, vinylon, acetate and others	Demonstrated broad fibre dyeability; shade depended on substrate and conditions; sunlight fading identified.	Limited modern instrumental colour and interfacial analysis.	Shirata *et al*., 2000
Choi & Kim (2009)	*C. violaceum* CV107 pigment; 80% acetone extract, LC/MS characterised	Cotton, silk, wool, diacetate, triacetate, acrylic, nylon 66	K/S maxima near 540-580 nm; process response depended on pH and temperature; tested mordants did not improve colour density.	Cross-fibre design strong, but molecular fixation mechanism not measured.	Choi & Kim, 2009
Venil *et al*. (2016)	*C. violaceum* UTM5 violacein-containing extract	Cotton, silk, rayon, jacquard rayon, silk satin, cotton/polyester	Mordants altered shade in selected cases; fastness ranged from fair to excellent; conventional control yielded deeper colour in some comparisons.	Light fastness remained weaker; pigment composition and fixation not fully resolved.	Venil *et al*., 2016
Kanelli *et al*. (2018)	*J. lividum* crude violacein, sonicated culture or cell-free extract	Polyamide 6.6	Simultaneous fermentation/dyeing gave ΔE 74.81 and K/S 22.01; antimicrobial/antifungal activity retained.	Laboratory-scale process; long-term laundering, leaching and industrial broth control require validation.	Kanelli *et al*., 2018
Deeppal *et al*. (2024)	*C. piscinae* AMA-5 ethanol-extracted, HPLC/FTIR/1H-NMR characterised pigment	Cotton and polyester	Demonstrated colouration with sodium chloride used as an auxiliary.	Textile outcomes less comprehensively quantified; NaCl is better regarded as an electrolyte/exhaustion aid than a classical mordant.	Deeppal *et al*., 2024

Note. K/S = Kubelka-Munk colour-strength factor; ΔE = CIELAB total colour difference; LC/MS = liquid chromatography/mass spectrometry; HPLC = high-performance liquid chromatography; FTIR = Fourier-transform infrared spectroscopy; 1H-NMR = proton nuclear magnetic resonance

6. Dye-Fibre Interactions: Demonstrated Evidence and Mechanistic Inference

6.1 Polyamide and Protein Fibres

Polyamide provides the clearest starting point for analysing dye-fibre interactions because it combines strong direct colouration evidence with chemically plausible binding modes. Polyamide 6.6 contains regularly spaced amide groups embedded in a partly crystalline hydrocarbon backbone. Violacein contains hydrogen-bond donor and acceptor sites as well as extended aromatic surface area. It is therefore reasonable to propose a combination of hydrogen bonding, dispersion forces, and hydrophobic partitioning, followed by diffusion into accessible amorphous regions. Kanelli *et al*. (2018) demonstrated high colour strength in polyamide under simultaneous fermentation/dyeing, but the experiment did not isolate the contribution of each molecular force. Thus, the macroscopic result supports substantial affinity while the microscopic mechanism remains inferential.

Temperature dependence in textile dyeing is consistent with a diffusion-controlled component. Heating can increase polymer-segment mobility, reduce aggregation of hydrophobic pigment species, and accelerate transport from bath to fibre. Yet stronger colour at higher temperature does not prove a particular chemical bond. To move beyond inference, future work should couple adsorption isotherms and kinetic modelling with solid-state or attenuated-total-reflectance infrared spectroscopy, Raman mapping, surface-sensitive mass spectrometry, or molecular simulations benchmarked against measured uptake. The goal is not to identify one exclusive bond but to quantify the relative contributions of partitioning, hydrogen bonding, ionic effects, and physical entrapment.

Silk and wool also contain polypeptide chains and therefore offer amide-rich environments, but their supramolecular structures and side-chain chemistries differ markedly from synthetic polyamide. Silk fibroin has extensive ordered beta-sheet domains and less ordered regions that govern accessibility, whereas wool keratin contains charged, polar, hydrophobic, and disulfide-containing side chains. Direct studies show that violacein-containing preparations can colour both fibres (Choi & Kim, 2009; Shirata *et al*., 2000; Venil *et al*., 2016). It is plausible that hydrogen bonding and hydrophobic association contribute, with pH altering both pigment state and the net charge of accessible amino-acid residues.

However, there is insufficient evidence to assign a dominant binding mechanism or to predict uptake solely from protein content.

6.2 Cellulosic Fibres

Cotton and regenerated cellulose present a different chemical problem. Cellulose is rich in hydroxyl groups and has a highly hydrogen-bonded, hydrophilic network, whereas violacein is comparatively hydrophobic and lacks the strongly water-solubilising ionic groups characteristic of many reactive or direct dyes. Hydrogen bonding between pigment and cellulose is chemically possible, but aqueous compatibility and access to the fibre interior are likely to limit exhaustion. Physical adsorption to accessible surfaces, dispersion interactions with less hydrated regions, and entrapment during drying may all contribute. Demonstrated cotton and rayon colouration confirms that these mechanisms are sufficient for visible uptake, but it does not establish covalent fixation (Choi & Kim, 2009; Venil *et al*., 2016).

The cellulosic evidence also explains why mordant and electrolyte language requires care. Classical metal mordants can form coordination complexes with some natural dyes and/or change fibre surface chemistry, but Choi and Kim (2009) found no general improvement from their tested mordants. Slaked lime altered colour in selected Venil *et al*. (2016) conditions, which may reflect pH-driven pigment or substrate changes rather than stable metal coordination. Sodium chloride in the *C. piscinae* study may promote exhaustion by changing ionic strength and activity coefficients, but it is not a classical metal mordant (Deeppal *et al*., 2024). Mechanistic studies should therefore measure bath depletion, desorption, and chemical state rather than infer fixation from colour deepening alone.

If cellulosic fibres are to become a major violacein application, pretreatment may be necessary. Cationisation, biopolymer coatings, plasma treatment, enzyme-assisted surface modification, or bio-based binders could increase affinity, but they create a new sustainability balance. A process that requires an energy-intensive or toxic pretreatment to fix a natural pigment may offer little environmental advantage. The correct benchmark is therefore not maximum K/S at any chemical cost, but acceptable colour and fastness with a transparent auxiliary burden per kilogram of textile.

6.3 Polyester and Other Synthetic Fibres

Polyester is relatively hydrophobic and crystalline, with limited polar functionality compared with polyamide. For small non-ionic disperse dyes, industrial colouration relies on hydrophobic partitioning and diffusion into polymer free volume at elevated temperature or with carriers. Violacein's aromatic character suggests that disperse-like partitioning could occur, and direct cotton/polyester and polyester demonstrations show that visible colouration is possible (Deeppal *et al*., 2024; Venil *et al*., 2016).

However, the larger and more multifunctional structure of violacein should not be assumed to behave identically to conventional disperse dyes. Quantitative diffusion coefficients, equilibrium partition constants, and high-temperature stability on polyester have not been established.

Acetate, triacetate, acrylic, vinylon, and mixed fabrics appear in early or comparative dyeing studies, but the evidence is too limited for confident mechanistic ranking (Choi & Kim, 2009; Shirata *et al*., 2000). Blends add another level of complexity because the two fibre components may take up different amounts of pigment, creating cross-dyeing effects and shade non-uniformity. A cotton/polyester fabric that looks uniformly violet at macroscopic scale may still contain very different pigment concentrations in the two phases. Fibre-resolved microscopy or selective extraction is needed before blend behaviour can be predicted reliably.

6.4 Photostability, Aggregation and Pigment Microstructure

The dye-fibre interface is also shaped by pigment aggregation and microstructure. Hydrophobic pigments can aggregate in aqueous baths, reducing effective molecular concentration while generating particulate deposition that may raise apparent surface colour but lower rubbing or washing durability. Extraction solvent, residual broth components, pH, temperature, and congener composition can all influence aggregation. This provides a plausible explanation for some study-to-study variability, but direct particle-size and aggregation measurements are rare in violacein dyeing studies. Reporting absorbance spectra alone is insufficient because spectral broadening or shifts can reflect aggregation as well as molecular environment.

Photostability represents the clearest intersection between molecular chemistry and textile performance. Light-driven oxidation can disrupt conjugation, while fibre chemistry and residual metal ions may sensitise or catalyse degradation. The observed sunlight and light-fastness weaknesses in early and later textile studies support a genuine application concern (Shirata *et al*., 2000; Venil *et al*., 2016). Yet a mechanistic photochemistry of fibre-bound violacein has not been established. Future stabilisation should therefore be evaluated through simultaneous colour, chemical, and physical measurements: reflectance spectra and CIELAB coordinates, chromatographic profiling of extractable pigment and photoproducts, mass-loss or leaching measurements, and microscopy of the fibre surface.

Table 4 separates observed dyeing outcomes from mechanistic interpretations. This distinction prevents chemical plausibility from being presented as proof. Across fibres, the evidence is strongest for macroscopic uptake and weakest for molecularly resolved bonding, diffusion, and fixation.

Table 4. Fibre-specific interpretation of violacein colouration and evidence confidence

Fibre class	Direct observations	Plausible interaction modes	Evidence confidence	Priority experiment	Supporting references
Polyamide 6.6 / nylon	High colour strength and large ΔE demonstrated; integrated fermentation/dyeing feasible.	Hydrogen bonding to amide groups, hydrophobic/dispersion interactions, diffusion into amorphous regions.	Moderate for affinity; low for molecular mechanism.	Adsorption kinetics/isotherms plus ATR-FTIR or Raman mapping and wash-desorption mass balance.	Kanelli *et al*., 2018; Choi & Kim, 2009
Silk and wool	Visible colouration across multiple studies; process response varies with pH/temperature.	Hydrogen bonding, hydrophobic association, possible pH-dependent ionic contributions from protein side chains.	Moderate for dyeability; low for dominant interaction.	Fibre-specific uptake and spectroscopy across controlled pH with mechanical-damage assessment.	Choi & Kim, 2009; Shirata *et al*., 2000; Venil *et al*., 2016
Cotton and rayon	Visible colouration demonstrated; mordant effects inconsistent.	Surface adsorption, hydrogen bonding, dispersion forces and physical entrapment; limited intrinsic aqueous affinity.	Moderate for dyeability; low for fixation mechanism.	Bath-depletion mass balance, desorption and comparison of untreated versus low-impact cationised/biopolymer-treated cellulose.	Choi & Kim, 2009; Deeppal *et al*., 2024; Venil *et al*., 2016
Polyester / blends	Polyester and cotton/polyester colouration demonstrated.	Hydrophobic partitioning and diffusion are plausible; blend components may take up pigment unequally.	Low to moderate; little quantitative mechanistic evidence.	High-temperature diffusion/partition study with fibre-resolved microscopy or selective extraction.	Deeppal *et al*., 2024; Venil *et al*., 2016
Cross-cutting light exposure	Sunlight/light fading repeatedly identified as weaker performance dimension.	Photo-oxidation of conjugated chromophore; aggregation and fibre/metal environment may modify rate.	Moderate for performance problem; low for degradation mechanism.	Controlled irradiance with colourimetry plus LC-MS identification of photoproducts and leachables.	Shirata *et al*., 2000; Venil *et al*., 2016

Note. ATR-FTIR = attenuated-total-reflectance Fourier-transform infrared spectroscopy; LC-MS = liquid chromatography-mass spectrometry. Interaction modes are explicitly labelled plausible where not directly demonstrated

7. Sustainability and Industrial Translation

7.1 Feedstocks, Energy, Solvents, Water and Effluent

The proposition that violacein is sustainable rests on several credible but incomplete premises. Microbial production can replace petrochemical synthesis with renewable biological conversion, can potentially use low-value residual feedstocks, and may occur under milder temperatures than some synthetic dye chemistries. The pineapple-waste and soybean-meal studies support the feasibility of using agro-industrial substrates, while broader microbial-pigment reviews identify resource flexibility as a core industrial advantage (Aruldass *et al*., 2015; Gohil *et al*., 2022; Rather *et al*., 2023). Yet environmental performance depends on the whole process. A low-cost carbon source does not offset inefficient aeration, refrigeration for psychrotrophic strains, long batch time, solvent-intensive extraction, repeated washing, or poor light fastness that shortens garment life.

Temperature deserves special attention. Some native producers favour relatively cool culture, which can reduce heating but may require active cooling in warm climates or large fermenters. Likewise, agitation can increase electricity demand, yet low agitation may decrease mass transfer or process homogeneity even when it favours pigment formation in a particular strain (Lu *et al*., 2009; Yang *et al*., 2007). These trade-offs cannot be represented by pigment titre alone. A meaningful environmental inventory should quantify electricity, steam, cooling, water, medium components, solvent make-up, cleaning chemicals, and wastewater treatment per kilogram of textile at a defined shade depth.

Solvent use is the most obvious downstream burden. Conventional violacein recovery frequently depends on alcohols or ketones, and a textile process may then require the pigment to be re-dispersed in another bath. Solvent recycling can improve the balance but needs distillation and containment. Direct or simultaneous dyeing offers a route to avoid this double handling, yet the broth itself becomes part of the colouration chemistry. Nutrients, salts, cellular fragments, proteins, and metabolites may affect fibre uptake and effluent oxygen demand. Thus, process integration should be evaluated by complete mass and energy balance rather than by counting the number of unit operations.

No comparative cradle-to-gate life-cycle assessment of violacein textile colouration against a functionally equivalent synthetic dye process was identified in the verified literature used for this review. Similarly, no robust textile-specific techno-economic analysis was found that integrates fermentation yield, downstream recovery, dye-house liquor ratio, fastness-related rework, solvent recovery, wastewater treatment, and pigment selling price. This absence is decisive: sustainability should be treated as a hypothesis supported by promising process features, not as an established property of the pigment.

7.2 Process Integration, Shade Consistency and Quality Standards

Industrial colouration is built around reproducibility. A microbial pigment process must deliver a defined shade across lots despite variation in inoculum state, raw materials, fermentation history, congener composition, and downstream recovery. This is more demanding than producing a visually violet extract. Current literature often reports absorbance or total crude pigment without a release specification for violacein/deoxyviolacein ratio, unidentified congeners, moisture, residual solvent, endotoxin or biomass residues, and colour strength on a standard substrate. Reviews of violacein production have repeatedly noted the diversity of measurement approaches, and recent fermentation syntheses continue to call for improved process standardisation (Choi *et al*., 2015; Park *et al*., 2021; Zhang *et al*., 2025).

A textile-grade specification should combine analytical chemistry and application testing. High-performance liquid chromatography or ultra-high-performance liquid chromatography with authentic standards should quantify violacein and major congeners. UV-visible spectra can serve as a rapid secondary quality check but should not be the sole mass assay. A standard reference fabric and recipe can then translate chemical purity into K/S and CIELAB coordinates. Process-control limits should include batch-to-batch ΔE, residual solvent, microbial contamination, and defined wash, rubbing, perspiration, and light fastness. Such standardisation would also make fermentation comparisons more useful, because a productivity gain could be judged against a constant product-quality target.

Simultaneous fermentation and dyeing is especially sensitive to quality control. The highest colour strength in the Kanelli *et al.* (2018) polyamide work arose in a biologically active system, where pigment formation and fibre uptake occurred together. Scaling this concept may require enclosed equipment with validated cleaning and sterilisation, control of bioburden after dyeing, and procedures to ensure that viable production organisms do not remain on the final textile. The method may also conflict with dye-house expectations for rapid bath turnaround. Its value will depend on whether eliminated extraction steps compensate for longer biological residence times and more demanding hygienic control.

7.3 Safety, Leaching and Regulatory Considerations

The term natural can obscure safety questions. Violacein is biologically active, and some of the same properties that motivate antimicrobial textiles also justify toxicological scrutiny. Aruldass *et al*. (2015) reported cytotoxicity of their purified pigment preparation in V79-4 Chinese hamster lung cells and a low selectivity index under the tested bioactivity framework. This finding does not demonstrate that a violacein-dyed textile is unsafe, because exposure route, dose, binding to fibre, metabolism, and formulation differ. It does show that microbial origin cannot be used as a surrogate for dermal safety.

Textile safety assessment should therefore begin with exposure. Total pigment content is insufficient; the relevant quantities are extractable and leachable pigment under sweat, sebum, laundering, abrasion, and foreseeable ageing conditions. Chemical identity

of leachates should be determined because degradation products may differ from the parent pigment. Skin-contact applications would require appropriate cytotoxicity, irritation, sensitisation, and, where indicated, genotoxicity assessment using validated frameworks. Environmental evaluation should include biodegradation, aquatic toxicity, and fate of pigment-containing wash water. For antimicrobial textiles, the potential for chronic low-level release and the relationship between antimicrobial efficacy and exposure should be considered together rather than in separate development programmes.

Production-organism safety is a second, independent issue. *C. violaceum* is known as an opportunistic pathogen in humans, so native-strain manufacture requires stringent containment and downstream removal even if the pigment itself meets safety criteria. Heterologous expression in better-characterised industrial hosts can reduce some manufacturing risks, but it does not eliminate the need for product purity and toxin testing. A regulatory dossier should therefore distinguish organism risk, process impurity risk, pigment toxicology, textile exposure, and environmental release. The pathogenic potential of the native producer is documented in the broader *C. violaceum* literature (Durán *et al*., 2016).

7.4 Economics, Scale-Up and the Meaning of Industrial Readiness

Scale-up evidence remains uneven. The 50 L pineapple-waste fermentation demonstrates that violacein-containing pigment can be produced beyond flask scale, and 3 L engineered-host work shows that high volumetric titres are possible (Aruldass *et al*., 2015; Sun *et al*., 2016). These studies are valuable, but industrial textile supply requires more than a successful batch. Long campaigns must demonstrate genetic and phenotypic stability, contamination control, consistent oxygen and heat transfer, reproducible downstream recovery, solvent recycling, and reliable pigment specification. Seasonal and supplier variation in waste-derived feedstocks must also be absorbed without unacceptable colour drift.

Economic comparison must be functional rather than mass-based. The relevant denominator is not cost per kilogram of crude purple extract but cost to achieve a specified colour strength and durability on a defined textile. A more expensive high-purity pigment could be competitive if it exhausts efficiently, requires little auxiliary chemistry, and gives durable colour at low dose. Conversely, a low-cost crude pigment can become expensive if bath uptake is poor, solvent losses are high, or light fading increases re-dyeing and product rejection. The same principle applies to antimicrobial value: a premium is defensible only if functional performance persists through a realistic service life.

Current evidence therefore supports an intermediate technology-readiness interpretation. Biosynthesis is well established; fermentation is engineerable; and textile colouration is demonstrable across several fibre classes. What is missing is an integrated industrial evidence package. The principal bottlenecks are not the existence of a violet chromophore or the possibility of making it microbially, but standardised pigment quality, reproducible fibre-specific dyeing, light durability, long-term functional retention, exposure-based safety, and comparative process sustainability. Progress on these fronts would be more consequential than another isolated report of a darker flask culture.

Table 5 translates common industrial claims into the evidence actually available and the validation still required. The central pattern is that most favourable claims are plausible and partly supported, but few have been tested at the system boundary needed for industrial decision-making. This evidence gap is particularly pronounced for comparative environmental impact, cost, long-term durability, and safety after ageing.

Table 5. Industrial and sustainability claims for violacein textile colouration: evidence and validation needs

Claim	Current evidence	Main qualification	Required validation	Supporting references
Renewable, potentially lower-impact colourant	Microbial production and use of pineapple waste or soybean meal are demonstrated.	Renewable feedstock does not account for energy, solvent, water, wastewater or product lifetime.	Comparative cradle-to-gate and, where possible, cradle-to-grave LCA at matched shade/fastness.	Aruldass *et al*., 2015; Gohil *et al*., 2022; Fried *et al*., 2022
Scalable production	50 L native-producer and litre-scale engineered-host fermentations have been reported.	Scale-up does not yet establish long-run stability, downstream recovery or textile-grade consistency.	Pilot campaigns with mass balance, productivity, purity, solvent recovery and lot-to-lot shade control.	Aruldass *et al*., 2015; Sun *et al*., 2016; Zhang *et al*., 2025
Broad fibre applicability	Colouration demonstrated on protein, cellulosic and synthetic fibres.	Methods and pigment preparations differ; molecular fixation is poorly resolved.	Common protocol across fibre classes with uptake, fixation, K/S, ΔE and standard fastness.	Choi & Kim, 2009; Shirata *et al*., 2000; Venil *et al*., 2016
Solvent reduction through process integration	Simultaneous fermentation and polyamide dyeing achieved strong colouration.	Broth residues, hygiene, residence time and effluent burden may replace extraction burdens.	Pilot dye-house comparison with solvent, water, energy, cleaning, bioburden and effluent metrics.	Kanelli *et al*., 2018
Antimicrobial	Polyamide fabrics	Durability, dose,	20-50 wash-cycle efficacy,	Kanelli *et al*.,

Claim	Current evidence	Main qualification	Required validation	Supporting references
functional textile	retained antibacterial and antifungal activity in vitro.	leaching and safety after repeated washing are unresolved.	leachate analytics, exposure assessment and standardised microbiological testing.	2018; Durán *et al*., 2007
Safe natural pigment	Microbial origin and bioactivity are established.	Natural origin is not a safety endpoint; cytotoxicity has been reported in cell-based assays.	Dermal and environmental safety assessment of defined pigment and aged-textile leachates.	Aruldass *et al*., 2015; Park *et al*., 2021
Economically competitive dye	High crude titres and low-cost feedstocks indicate technical potential.	No textile-specific TEA at matched colour depth and durability was identified.	TEA linked to chemical purity, dye uptake, recovery, auxiliaries, energy, effluent treatment and rejection rates.	Rather *et al*., 2023; Zhang *et al*., 2025

Note. LCA = life-cycle assessment; TEA = techno-economic analysis. Validation needs are proposed from the gaps identified in the evidence synthesis rather than claimed as completed studies

8. Future Directions

The immediate research priority is to define the product before optimising the process. Violacein studies should report the concentrations of violacein, deoxyviolacein, and major related products using validated chromatographic methods with authentic standards, together with extraction recovery and a mass balance. Crude pigment can remain a legitimate industrial product if it is intentionally specified, but it should not be reported interchangeably with pure violacein. This standardisation would allow genuine comparison of producer strains, metabolic-engineering strategies, and textile recipes and would make it possible to identify whether congener composition affects hue, adsorption, fastness, or antimicrobial activity (Park *et al*., 2021; Zhang *et al*., 2025).

A second priority is a controlled fibre-mechanism programme. The most informative design would use one chemically defined pigment batch across standardised cotton, regenerated cellulose, wool, silk, polyamide 6.6, polyester, and selected blends.

Experiments should quantify bath depletion, fixation after rinsing, equilibrium uptake, kinetic parameters, K/S, CIELAB values, and desorption. These measurements should be paired with spectroscopy and microscopy capable of resolving whether pigment resides mainly on the surface or penetrates the fibre. Molecular simulation can be useful, but only when its predictions are tested against uptake and spectroscopic evidence. Such a programme would convert the present qualitative language of hydrogen bonding and hydrophobic interaction into a predictive dyeing model.

Photostability is the most urgent performance problem. Future studies should expose dyed textiles to controlled irradiance while recording colour change as a function of dose and identifying chemical degradation products. The design should vary oxygen availability, humidity, fibre type, pigment purity, congener ratio, and relevant metal ions. Stabilisation strategies should then be compared on the basis of retained colour after exposure, not simply initial K/S. A promising additive should also be evaluated for washing durability, toxicology, and its own environmental burden, because a stabiliser that compromises the sustainability case would merely transfer the problem.

Process integration deserves pilot-scale testing, particularly for polyamide where the evidence is strongest. A comparison among purified-pigment dyeing, cell-free crude extract, and simultaneous fermentation/dyeing should hold shade depth and fabric mass constant. Outcomes should include pigment productivity, solvent use, water use, electricity, heating/cooling, process time, cleaning demand, viable-cell removal, effluent chemical oxygen demand, total organic carbon, dye-house throughput, and lot-to-lot ΔE. This would reveal whether bypassing extraction produces a net gain or simply moves burdens to hygiene, residence time, and wastewater treatment.

Waste-derived feedstocks should be advanced from proof-of-concept to supply-chain studies. Pineapple waste and soybean meal show that complex residual streams can support pigment formation (Aruldass *et al*., 2015; Gohil *et al*., 2022), but future work should quantify composition variability, pretreatment requirements, storage stability, microbial contamination, transportation distance, and the effect of feedstock impurities on pigment purification. Continuous or fed-batch strategies should be compared with batch culture at a fixed chemical specification. The objective should be resource efficiency per unit of textile colouration, not merely maximum crude pigment concentration.

Safety and function must be studied in the same ageing framework. Antimicrobial textiles should be challenged through repeated laundering, artificial sweat, abrasion, ultraviolet exposure, and storage, with simultaneous measurement of antimicrobial activity, colour, pigment content, and leachate composition. This would determine whether functionality persists because pigment is strongly retained or whether efficacy depends on release. Exposure-relevant toxicology should focus on defined pigment and the actual leachable fraction from finished textiles. Such evidence is necessary for skin-contact products and would prevent antimicrobial claims from being separated from realistic user and environmental exposure.

Longer-term research should integrate life-cycle assessment and techno-economic analysis with process development rather than adding them after a high-yield fermentation has been optimised. Prospective models can identify whether yield, productivity, solvent recovery, fermentation temperature, dye-bath liquor ratio, fastness, or pigment dose is the dominant environmental and cost

driver. Sensitivity analysis can then direct biological and textile engineering towards the variables that materially change system performance. Comparative assessment should use a functionally equivalent benchmark, such as a synthetic violet colourant delivering the same shade depth and fastness on the same fibre, rather than comparing kilograms of chemically dissimilar dye.

Finally, the field would benefit from shared minimum reporting standards. Production studies should state strain identity, analytical pigment definition, culture conditions, scale, titre, productivity, recovery and purity. Textile studies should report fabric composition and construction, liquor ratio, pigment dose, pH, temperature, auxiliaries, bath exhaustion, fixation after rinsing, K/S or equivalent reflectance data, CIELAB coordinates, and clearly identified fastness standards. Functional-textile studies should add wash-cycle durability and leaching. Adoption of a common reporting core would reduce false comparisons and make future reviews and industrial decisions substantially more reliable.

9. Conclusions

Microbial violacein has a credible scientific basis as a textile colourant, but the maturity of the underlying science is uneven. The enzymatic pathway from L-tryptophan to violacein is well defined, and both native and engineered microorganisms can produce substantial quantities of violet pigment. Bioprocess engineering has demonstrated control through strain selection, medium design, heterologous expression, fed-batch operation, and the use of agro-industrial substrates. These advances establish that microbial manufacture is technically feasible and potentially compatible with renewable or residual feedstocks. They do not, by themselves, establish textile sustainability.

Direct dyeing evidence demonstrates that violacein-containing preparations can colour polyamide, silk, wool, cotton, regenerated cellulose, polyester-containing fabrics, and several additional synthetic fibres. Polyamide currently has the strongest integrated evidence, including high instrumental colour strength and direct dyeing during fermentation with retained antimicrobial activity. Across the wider fibre set, proposed hydrogen bonding, hydrophobic association, dispersion forces, diffusion, and physical entrapment are chemically plausible, but molecularly resolved dye-fibre interactions remain largely unproven. The field should therefore distinguish measured colouration from inferred binding mechanism.

The principal barriers to industrial translation are inconsistent pigment definition, weak comparability among production titres, incomplete fibre-specific fixation data, limited light fastness, uncertain long-term antimicrobial durability, solvent-intensive recovery in many workflows, and insufficient exposure-based safety evidence. Most importantly, no direct comparative life-cycle or textile-specific techno-economic evidence currently demonstrates that violacein colouration is categorically more sustainable than functionally equivalent synthetic dyeing. The defensible position is that violacein is a promising, biologically manufactured colourant with routes to lower-impact processing, especially through waste-derived feedstocks and process integration, but its sustainability is conditional on how it is produced, recovered, applied, stabilised, used, and treated at end of life.

Industrial progress will depend less on reporting another visually intense culture and more on integrating analytical chemistry, fibre science, durability, toxicology, and systems engineering. Standardised pigment specifications, common textile testing, mechanistic adsorption studies, photostability research, pilot-scale integrated dyeing, leachate-based safety testing, and functionally matched environmental and economic comparisons are the evidence needed to convert violacein from an attractive laboratory pigment into a defensible commercial colouration technology.

10. Limitations

As a critical narrative review, this synthesis is intrinsically susceptible to selection and interpretive bias despite the use of a transparent search strategy, explicit eligibility criteria, live bibliographic verification, and critical appraisal. The evidence base is also highly heterogeneous: studies differ in microbial strain, fermentation scale, extraction solvent, pigment purity, analytical definition, textile substrate, dyeing recipe, colour metric, and fastness method, which prevents formal quantitative pooling and limits direct ranking. Some relevant literature may be absent because the review relied on accessible scholarly sources and English-language evidence that could be verified reliably rather than subscription-only coverage or unverifiable records.

The direct textile literature is small relative to the broader violacein production literature, and industrial-scale dye-house trials, long-term wash-cycle studies, photodegradation chemistry, leachate toxicology, life-cycle assessment, and textile-specific techno-economic analysis are especially sparse. Consequently, several molecular dye-fibre interactions discussed in this review are explicitly mechanistic inferences grounded in known pigment and polymer chemistry rather than directly measured interfacial phenomena. Rapid development of microbial production and downstream technologies may also change the relative importance of current bottlenecks after the stated search date.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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