

FLOW ASSURANCE

From Biological Activity to Flow Assurance: An Evidence-Qualification Framework for Biocatalytic and Bio-Derived Wax Control in Waxy Crude Oils

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Abstract

Wax accumulation and gel formation remain critical flow assurance obstacles in the extraction and conveyance of waxy crude oils, whereby precipitation, crystal development, aggregation, ageing, rheological behavior, and wall deposition are interrelated yet distinct processes. Traditional mitigation relies on temperature regulation, physical extraction, and chemical supplements. There is an increasing fascination with sustainable oilfield chemistry that has incorporated microorganisms, enzymes, biosurfactants, and renewable formulations as substitutes or enhancements. In many instances of this material, there is a paucity of demonstration about the intermediary causal processes, with signs of hydrocarbon biodegradation, paraffin utilization, or reductions in pour point and viscosity being regarded as proof of wax-deposition control. This comprehensive critique integrates literature from petroleum engineering, wax rheology, and microbiology with a systematic evidence-evaluation methodology, revealing four progressively robust assertions: biological activity, alteration of wax-related characteristics, diminished dynamic deposition, and engineering validation. The assessment delineates five realms of evidence, specifically biological functionality, compatibility with crude oil, change of the wax phase, response to flow assurance, and sustainable implementation. A six-tier qualifying framework is suggested, constraining the validity of a technological assertion to the evidence that has been substantiated. A novel seven-phase experimental approach is introduced, advancing from candidate identification and functional description, through crude oil compatibility, fundamental performance, and mechanistic validation, to dynamic deposition assessment and translation. A five-arm baseline control framework—untreated crude, formulation matrix, inactivated preparation, commercial standard, and active treatment—is suggested to differentiate catalytic, interfacial, carrier, and formulation influences. The synthesis indicates that biological systems present exciting research opportunities; nonetheless, the evidence for direct wax-control mechanisms is far less advanced compared to hydrocarbon degradation or biosurfactant activity. The essential research requirement is not merely further screening but rather reliable, crude-specific, dynamically evaluated data that connects biological activity to wax-phase behavior and pipeline-scale flow assurance.

Keywords: waxy crude oil; wax deposition; pour point depressant; biocatalyst; flow assurance; rheology; sustainable oilfield chemistry

1 Introduction

1.1 Waxy crude oil and the flow-assurance challenge

When the fluid reaches a temperature where its high molecular weight paraffins are not entirely dissolved, the extraction, transportation, and storage of waxy crude oil become challenging. The solubility of wax diminishes. Precipitation succeeded by nucleation, crystal formation, and aggregation. The characteristics of the oil and the temperature and hydrodynamic conditions it encounters can lead to the formation of interconnected crystals, which may significantly alter viscosity, yield stress, gelation propensity, and restart behavior. In pipelines, extra accumulation reduces the effective flow area, heightens pressure losses, and, in severe cases, jeopardizes safe operation (Aiyejina et al., 2011; Ruwoldt et al., 2017; Li et al., 2024). Consequently, wax deposition is more accurately seen as a multiscale, multiphysics issue rather than a result of any specific characteristic of crude oil. It encompasses the synergistic effects of phase equilibrium, precipitation kinetics, molecular diffusion, nucleation and growth, rheological alterations, heat and mass transfer, hydrodynamic shear, and deposit maturation, with the significance of each process varying according to crude composition, pipeline configuration, and operational parameters. Recent research has demonstrated that molecular diffusion or aging alone fails to account for the deposition observed in the field (Zhu et al., 2024; Li et al., 2024). This intricacy has clear ramifications for the evaluation of novel additives, encompassing biological options. The characteristics of low-temperature behavior include several factors such as pour point, wax appearance temperature (WAT), crystallization kinetics, crystal shape, gel-network strength, and dynamic deposition. A diminished pour point does not inherently indicate less wax accumulation or a slower rate of deposition during flow. Thus, this review is based on two assertions: (1) a lower pour point does not inherently indicate that wax deposition has been suppressed; (2) proven suppression of wax deposition does not inherently guarantee flow assurance at the field level, as factors such as dosage, thermal stability, crude compatibility, flow conditions, and long-term efficacy must be verified (Ruwoldt et al., 2019; Li et al., 2024).

1.2 Conventional approaches to wax control

The sector addresses its wax-related issues by thermal, mechanical, operational, and chemical methods. Thermal techniques (insulation, active heating) regulate the thermal history of the generated fluid, mechanical approaches, primarily pigging, eliminate the built-up wax, operational strategies modify production and cessation protocols, and chemical treatments alter precipitation, crystallisation, aggregation, gel-network formation, or deposit development (Aiyejina et al., 2011; Ala and Daraboina, 2024; Ogunwale et al., 2026). Chemical technologies encompass wax inhibitors, pour point depressants (PPDs), crystal modifiers, dispersants, solvents, and a growing array of hybrid, eco-friendly, and nanostructured formulations. A persistent issue is that no singular additive is universally effective. The efficacy of PPD and inhibitors depends on the molecular composition of the treatment agent, the carbon number distribution of the wax, the overall physicochemical characteristics of the material, the amount administered, and the existing temperature and flow conditions. Nonetheless, an identical total wax concentration does not inherently imply uniformity in composition, crystallization characteristics, and response to additives in crude oils; thus, total wax content alone is inadequate for forecasting treatment efficacy (Adams et al., 2018; Yang et al., 2018; Yao et al., 2022). Laboratory evaluations and empirical enhancements continue to be standard practices because of this crude-specific behavior, and recent analyses have advocated for a more profound mechanistic comprehension of the interactions among additive chemistry, crude composition, and

operational parameters (Cabanillas et al., 2016; Ala and Daraboina, 2024; Botett Cervantes et al., 2026).

1.3 The transition to sustainability and the necessity for a novel framework in biology

The impetus from environmental, economic, and regulatory factors has generated enthusiasm for green PPDs, renewable and bio-derived additives, biosurfactants, and various substitutes for conventional petroleum-based formulations (Li et al., 2024; Zhu et al., 2024; Dahwal et al., 2026). Nonetheless, a biological or renewable source does not inherently ensure superior environmental efficacy; comprehensive sustainability is contingent upon the entire life cycle and contextual application—including feedstock procurement, production efficiency, purification, formulation, storage, dosage, operational efficiency, and environmental impact (Joshikumar et al., 2026; Eastham and Leman, 2024). The evaluation thus regards bio-based origin, biological functionality, technological efficacy, and system-wide sustainability as four interconnected yet distinct characteristics, none of which can be deduced from the presence of another.

Biocatalyst-driven systems markedly diverge from conventional polymeric PPDs. The polymeric PPD is frequently assessed based on its molecular interactions with crude oil constituents. Conversely, a biological formulation may include diverse potential active components, including enzymes, proteins, metabolites, biosurfactants, extracellular products, and formulation residues, each of which could independently affect viscosity or pour point. Consequently, in the absence of adequate controls and mechanistic proof, any noted enhancement cannot be ascribed to enzyme function. The principal inquiry this review seeks to address is what quantity and category of evidence is necessary for a biological catalyst or biologically derived system to be legitimately classified as a wax-control or flow-assurance technology? The framework described in Sections 5–8 is meant to answer this question.

2 Review methodology

2.1 Review design

This research was intended as a critical synthesis of evidence instead of a quantitative meta-analysis. A systematic literature review uncovered findings regarding wax precipitation, wax deposition, rheological properties, chemical inhibition, microbial and enzymatic hydrocarbon transformation, biosurfactants, and bio-derived flow enhancers. Quantitative pooling was not attempted because of the considerable variability in crude oil composition, wax content, carbon number distribution, additive characteristics and quantities, temperature and shear history, experimental design, and outcome descriptions. The objective was to evaluate the robustness and importance of evidence at various phases and to ascertain the minimal chain of evidence necessary for a biological system to be considered a wax-control technology.

The manuscript employs the PRISMA 2020 Statement as a standard for transparency in documenting identification, screening, eligibility, and synthesis choices (Page et al., 2021), but it does not qualify as a comprehensive systematic review: a pre-registered protocol, exhaustive database-level record counts, independent duplicate screening, and a formal risk-of-bias evaluation were absent from the foundational process. This distinction is deliberate—PRISMA serves as a reporting guideline rather than a substitute for a reproducible systematic review methodology. It can be accurately characterized as a rigorous analysis of the literature, guided by the tenets of systematic inquiry.

2.2 Information sources

The main sources utilized were Scopus and the Web of Science Core Collection, augmented by ScienceDirect for literature on petroleum and chemical engineering, ACS Publications for relevant chemistry articles, and Crossref for bibliographic validation. The investigation exhibited a bias favoring the Journal of Petroleum Science and Engineering (currently known as Geoenvironmental Science and Engineering), Fuel, Energy & Fuels, Chemical Engineering Journal, Applied Energy Environmental Protection, and Advances in Colloid and Interface Science. Fictitious record totals are excluded from this report, as the foundational workflow failed to retain complete database export files and search logs. The authors will preserve the precise search strings, search dates, export files, and screening log as extra material prior to submission, in case the publication necessitates systematic-review-level reproducibility.

2.3 Search strategy

The investigation encompassed three thematic categories: (A) waxy crude and flow assurance—'waxy crude,' 'paraffin wax,' 'wax precipitation,' 'wax deposition,' 'wax crystallization,' and 'wax rheology'; (B) control technologies—'flow assurance,' 'pour point depressant,' PPD, 'wax inhibitor,' 'wax dispersant,' 'flow improver,' 'crystal modifier,' pigging, and heating as applicable; and (C) biological or sustainable systems—biocatalyst*, enzyme*, microbial, biosurfactant*, biodegradation, 'bio-based additive,' 'bio-derived additive,' and 'renewable flow improver.' Boolean combinations were adjusted to conform to the syntax of each database. Citation searches in both directions, along with targeted author and title enquiries, revealed foundational studies and modern research that were overlooked by the keyword search. The primary investigation timeframe concentrated on 2015-2026, aiming to capture recent advancements in sustainable flow enhancers, sophisticated inhibitor chemistry, and biotechnology, while also preserving crucial earlier research necessary for understanding fundamental mechanisms.

2.4 Eligibility criteria

Research was incorporated if it presented evidence pertinent to at least one of the following: (i) biological or biochemical functionality; (ii) transformation related to hydrocarbons, paraffins, or wax; (iii) alteration of wax precipitation, crystallization, morphology, or rheology; (iv) dynamic deposition or pressure-loss characteristics; or (v) engineering, environmental, or economic assessment. Research was dismissed if it lacked relevance to petroleum wax behavior, failed to offer sufficient methodological specifics for critical analysis, replicated existing studies, or discussed general hydrocarbon biodegradation without a distinct association to wax-related behavior. Evidence about traditional chemical, thermal, and mechanical techniques was seen as comparative evidence rather than evidence of biological effectiveness.

2.5 Data extraction and evidence classification

Data was gathered based on a unified framework encompassing research aim, properties of crude oil or model fluid, wax composition and carbon-number details when available, biological system or additive, dosage and treatment parameters, temperature, pressure, shear history, analytical techniques, response metrics, controls, mechanistic insights, and operational significance. Each study was subsequently positioned on the six-step ladder (Section 8) at the apex level, substantiated by the methodologies and results presented. The synthesis distinctly differentiates between direct evidence, such as quantified wax-phase behavior or dynamic deposition resulting from a specific intervention,

and indirect evidence, including hydrocarbon biodegradation, biosurfactant generation, or general viscosity reduction where a wax-control mechanism remains unverified. No assertion was enhanced based on a singular favorable response characteristic.

2.6 Limitations

Significant constraints encompass inconsistent terminology within the literature, insufficient studies isolating a particular biocatalytic mechanism, irregular documentation of crude composition and wax characteristics, and restricted application of dynamic deposition experiments in biological research. The database encompasses whole-cell systems and multicomponent formulations that cannot be ascribed to a singular enzyme or molecule. Consequently, the proposed framework is to be seen as a provisional architecture for forthcoming research instead of an established regulatory or industry benchmark; its objective is to enhance cause differentiation, comparability, and the translation from laboratory findings to flow-assurance assessments.

3 Wax deposition as a multiscale petroleum-engineering problem

Wax deposition cannot be reduced to a solitary precipitation occurrence solely dependent on the overall wax concentration. It arises from mechanisms across various length and temporal scales, encompassing molecular interactions and phase behavior, nucleation, crystal growth, aggregation, gel-network development, rheological alterations, and deposit formation during flow, with the significance of each factor contingent upon the characteristics of the crude and the prevailing thermal, hydrodynamic, and transport conditions. Deposition cannot be ascribed to a singular mechanism across all operational settings (Li et al., 2024; Mehrotra et al., 2020; Yaghy et al., 2021). This critique examines wax accumulation across four interrelated domains: molecular/compositional, thermodynamic/kinetic, microstructural/rheological, and pipeline-scale deposition. These domains are interconnected—composition affects phase behavior, phase behavior impacts nucleation and growth, crystal characteristics shape network formation and rheology, and rheological/transport behavior subsequently determines the transport, deposition, aging, or removal of wax. An additive, which encompasses a biological candidate, might demonstrate efficacy at one level but lack a similar impact at another. Assertions need not to be indiscriminately extended from molecular or laboratory-scale information to pipeline-scale flow assurance.

3.1 Molecular and compositional domain

Crude oil constitutes a multifaceted chemical system with numerous components, wherein the wax-producing portion, primarily consisting of high-molecular-weight n-alkanes, crystallizes within a complicated chemical milieu rather than as a standalone system of pure alkanes. The overall wax content serves as a useful indicator, although it is insufficient for forecasting the reaction to additions. He et al. (2016) conducted a comparison of three waxy crude oils that had identical crystallizable wax content as determined by DSC, yet displayed significantly varied PPD responses. They ascribed this to variations in the long-chain n-paraffin composition, high-carbon-number segments, WAT, insoluble wax components, and crystal structure. The wax content assessed by DSC was inadequate to effectively forecast additive behavior in certain crude oils. Consequently, an equivalent total wax content does not inherently indicate analogous crystallization conduct, nor does comparable crystallization behavior guarantee a uniform reaction to inhibitors. This holds significant relevance for green, bio-derived, or biocatalyst-based treatments, as a positive outcome in a single crude oil should not be construed as indicative of widespread applicability.

Non-wax constituents, including aromatics, resins, and asphaltenes, influence the solubility milieu, viscosity, aggregation, and interactions of additives. Asphaltenes influence the perceived viscosity and the strength of gel structures (Yao et al., 2020; Liu et al., 2025), and the interactions between ethylene-vinyl acetate PPDs and asphaltenes align with the formation of composite particles (Yao et al., 2020), highlighting the importance of examining the behavior of inhibitors alongside non-wax crude components. Consequently, additive performance should be seen as a function of the additive structure, wax composition, non-wax components, and the interplay of operating conditions, rather than only the overall wax content. This principle is similarly relevant to biological entities, which face the same intricate, multifaceted, and multi-component surroundings and whose observable performance may be influenced by partitioning and stability factors that are independent of the inherent characteristics of the biological product.

3.2 Thermodynamic and kinetic domain

The reduction in temperature of waxy crude diminishes wax solubility, prompting nucleation, crystal development, aggregation, and the establishment of a network. Nonetheless, the initial instance of quantifiable precipitation does not signify the complete flow-assurance reaction: deposition persists to evolve through structural and transport mechanisms influenced by the changing thermal and hydrodynamic conditions (Mehrotra et al., 2020; Van der Geest et al., 2021). The initiation of wax precipitation, gelation, and deposit development are distinct phenomena that should be evaluated independently when assessing the efficacy of an addition. Traditional PPDs and wax inhibitors do not invariably avert precipitation itself, but rather generally alter crystal formation, morphology, liquid-wax mobility, or diminish certain hydrocarbon fractions upon cooling (Navaneetha Kannan et al., 2018). A pragmatic engineering objective is to modify the precipitated phase to achieve less crystal connectivity, a less robust structural framework, and enhanced flow. This must be substantiated through actual evidence rather than presupposed for any candidate, whether biological or otherwise.

3.3 Microstructural and rheological domain

The dynamics of flow depend not only on the quantity of solid wax produced but also on the spatial configuration and interconnectivity of the crystalline phase, which governs the apparent viscosity, shear sensitivity, yield stress, gelation, thixotropy, and viscoelastic properties. A solitary viscosity assessment at a specific temperature and shear rate yields restricted mechanistic understanding, as identical viscosity readings may arise from diverse microstructures, including diminished crystallized mass, modified crystal dimensions or morphology, weakened crystal-crystal interactions, or a more shear-responsive network, which remain indistinguishable without supplementary evidence. Ruwoldt et al. (2019) employed a blend of NMR, GC/MS, DSC, cross-polarized microscopy, and rheometry to examine inhibitor-wax interactions, whereas Makwashi et al. (2021) integrated waxy-crude characterization with viscometric and morphological evaluations of chemical inhibitors. Both investigations demonstrate that integrating macroscopic treatment response with microstructural data offers far more robust mechanistic validation than relying on a singular property assessment. This multi-faceted principle is also relevant to emerging biological technologies: a formulation that influences viscosity or pour point serves merely as preliminary evidence of efficacy, while more substantial assertions regarding wax modification, catalytic performance, or flow assurance at the pipeline level necessitate equally robust, multi-method evidence.

4 Conventional wax-control technologies and the limits of the current paradigm

The predominant method for tackling wax-related flow assurance challenges continues to be chemical treatment; nevertheless, recent analyses underscore that its efficacy is still largely contingent upon the characteristics of crude oil and operational parameters, with considerable mechanistic ambiguity persisting (Guin and Naiya, 2025; Ala and Daraboina, 2024). The performance is more accurately described as the result of an interplay between additive structure, crude composition, temperature, shear rate, contact time, and thermal and hydrodynamic history, rather than solely additive structure—which partially elucidates why expensive empirical trial-and-error selection persists (Fakroun and Benkreira, 2019; Ala and Daraboina, 2024). The identical principle is applicable, with heightened emphasis, to novel bio-based additives and biocatalyst-derived technologies: a candidate ought to be evaluated not on biological identity, enzyme efficacy, or isolated laboratory reactions, but on proven pertinence within the particular crude oil and operational context for which it is intended.

4.1 Polymeric pour point depressants

Polymeric PPDs represent the most extensively studied chemical approach to enhance low-temperature flow characteristics, functioning through their interaction with crystallising wax to influence crystal formation, morphology, aggregation, or network establishment rather than outright preventing precipitation. Deka et al. (2018) introduced tailored oleic-acid-derived polymeric additives for Indian waxy crude oils, evaluated based on pour point, apparent viscosity, gelation characteristics, viscoelastic attributes, crystal morphology, and cold-finger deposition – illustrating the comprehensive evidence standard this review endorses for any novel class of additives, whether biological or synthetic. The PPD reaction is influenced by polymer structure, molecular weight properties, functional group presence, wax carbon-number distribution, and the crude oil matrix. Variations in pour point, viscosity, yield stress, and deposit mass should not be regarded as equivalent indicators of inhibitor efficacy, as an additive may alter one parameter without a corresponding change in another. A primary issue is inadequate specificity. A PPD that proves effective in one type of waxy crude may be significantly less effective, or even harmful, in a chemically distinct crude, as the additive interacts not with a uniform 'wax' but with a variety of crystallisable hydrocarbons within a matrix of aromatics, resins, and asphaltenes. Consequently, an additive must be regarded as effective for a specific petroleum system and operational parameters rather than universally advantageous, a principle that similarly applies to biological candidates, where a positive reaction in one crude oil should initially be interpreted as merely specific evidence.

4.2 Surfactants, hybrids, and nanohybrid formulations

The influence of surfactants on interfacial characteristics and interactions among solid wax, liquid hydrocarbon, and other phases is not a uniform phenomenon but rather dependent on chemistry, concentration, chemical compatibility, and physical circumstances. Hybrid formulations utilizing polymers, surfactants, and/or nanoparticles aim for complementary effects; however, their amalgamation does not guarantee synergy. A hybrid should be deemed synergistic solely when its performance surpasses a definitive expectation derived from its constituent elements, rather than merely outperforming untreated crude. This criterion is applied directly to biological formulations that may include enzymes, metabolites, and surfactant-like substances, necessitating the separation of individual and collective contributions before any specific component can be linked to an observed effect. Numerous studies in the literature indicate that nanohybrid PPDs, which incorporate carbon,

silica, or metal-oxide nanoparticles inside polymeric matrices, have superior performance compared to conventional polymer-only PPDs regarding pour-point depression and shear resistance (Tan et al., 2024). Nanohybridization should not be regarded as a universal solution for crude specificity. The stability of nanoparticle dispersion, aggregation, and interaction with the petroleum matrix introduces additional variables, and a more intricate formulation does not inherently result in more predictable field performance.

4.3 Green and emerging PPDs

The rising interest in sustainable petroleum technology has prompted research into additives that are based, in whole or in part, on renewable feedstocks. The term "green" must not serve as a replacement for technical qualifications: a material's biological or renewable origin does not inherently ensure wax-deposition inhibition, operational compatibility, environmental advantages, low toxicity, biodegradability under suitable conditions, production scalability, or life-cycle sustainability. A bio-based origin does not signify verified flow-assurance performance or confirmed ecological benefits—this distinction is the rationale for the certification structure presented in Sections 5–8.

4.4 Why empirical screening remains insufficient

Laboratory assessment has conventionally served as the initial phase in managing chemical wax, succeeded by pilot or field trials as required. This methodology has produced beneficial treatments; however, it continues to be expensive and ineffective due to a lack of a clearly defined relationship between laboratory assessments and operational efficacy, with recent evaluations highlighting that the reliance on expensive and predominantly empirical trial-and-error techniques endures because of an insufficient predictive comprehension of the behavior of wax-control agents across different crude types and operational scenarios (Ala and Daraboina, 2024; Gao et al., 2026). The challenge lies not in the act of screening itself, which is essential, but in conducting screening without an adequately predictive, multiscale mechanistic framework and failing to consider more than one or two readily observable parameters like pour point or viscosity. Field systems encompass fluctuating temperature profiles, axial thermal gradients, diverse shear and flow conditions, shutdown and restart cycles, deposit aging, and compositional variability that may not be replicated by simplified laboratory experiments. Consequently, the experimental hierarchy must be organized in such a way that each phase presents a more operationally pertinent inquiry, advancing from empirical evaluation to the systematic technology-qualification route delineated in Section 9. Consequently, the evaluation of conventional wax-management systems is encapsulated in Table 1.

Table 1. Comparative summary of conventional wax-control technologies.

Technology class	Principal intended function	Evidence commonly reported	Principal limitation for mechanistic interpretation
Polymeric PPDs	Modify wax crystallisation and low-temperature flow behaviour	Pour point and viscosity	Performance can be strongly crude-specific
Wax inhibitors	Reduce adverse effects of crystallisation/deposition	Static inhibition metrics	Mechanism may not be directly demonstrated
Surfactants	Modify physicochemical/interfacial interactions	Pour point, viscosity, dispersion behaviour	Multiple possible pathways complicate causal attribution

Technology class	Principal intended function	Evidence commonly reported	Principal limitation for mechanistic interpretation
Polymer–surfactant hybrids	Combine complementary effects	Comparative laboratory performance	Apparent improvement does not automatically demonstrate synergy
Nanohybrid PPDs	Enhance crystallisation/structural modification	Thermal and rheological measurements	Dispersion stability and crude-specific interactions limit transferability
Green/bio-derived additives	Renewable material platforms	Static fluid-property improvement	Renewable origin does not establish sustainability or field performance
Biological/biocatalyst candidates	Proposed modification of wax-related behaviour	Biological activity and preliminary fluid testing	Biological activity alone does not establish a petroleum flow-assurance mechanism

5 Biological systems relevant to petroleum flow assurance

Microorganisms and their byproducts can interact with hydrocarbons, interfaces, and complex multiphase systems through various fundamentally distinct mechanisms, including with hydrocarbons, interfaces, and intricate multiphase systems through various fundamentally distinct mechanisms, such as enzymatic substrate transformation, biosurfactant-facilitated interfacial alteration, microbial hydrocarbon degradation, and the synthesis of extracellular polymers and other metabolites. Consequently, the phrase 'biological wax-control agent' is rather broad unless the active ingredient and its intended mechanism of action are detailed. A biological formulation that improves pour point, viscosity, or wax-appearance characteristics may contain various active ingredients, and it is scientifically inappropriate to solely ascribe the observed effect to enzymatic catalysis until the specific component is analyzed.

5.1 Biocatalysts and biosurfactants: a necessary distinction

A fundamental conceptual differentiation is crucial prior to the systematic application of biological methodologies in flow-assurance investigations. The present IUPAC definition of a biocatalyst refers to an enzyme or enzyme complex, whether cell-free or within intact cells, that facilitates metabolic processes or substrate transformations. A biocatalyst is characterized by its ability to facilitate a chemical reaction, wherein an enzyme and substrate create an enzyme-substrate complex that results in the formation of the enzyme and product. In the realm of petroleum, asserting that an enzyme functions as a wax-control biocatalyst necessitates proof of the involvement of a chemically identifiable substrate or substrate category, that a quantifiable conversion occurs, that this conversion is associated with catalytic activity, that suitable controls eliminate non-catalytic interpretations, and that the chemical transformation can be credibly connected to the observed petroleum-fluid response. The discovery of an enzyme, or its activity on a different laboratory substrate, does not constitute adequate evidence of catalytic modification of wax-forming hydrocarbons. In contrast, biosurfactants are amphiphilic substances derived from biological sources, exhibit structural diversity, and primarily function through adsorption at interfaces, self-assembly, and the reduction of surface or interfacial tension, rather than through direct chemical transformation of substrates (Ali et al., 2024; Hernández et al., 2023). Consequently, biocatalysts and biosurfactants are not interchangeable; yet, a single whole-cell microbial system can produce both catalytic enzymes and biosurfactants. This issue of attribution is exemplified in the historical literature on petroleum.

Wang et al. (2022) documented the elimination of wax and the decrease in viscosity of waxy crude oil subjected to treatment with *Ochrobactrum intermedium*, a complete bacterial culture where hydrocarbon breakdown, metabolite generation, and putative biosurfactant activity were all possibly significant. The results endorse the general significance of biological systems in relation to waxy-crude behavior, although they do not conclusively demonstrate a particular enzyme-driven wax-regulation mechanism. Consequently, this review advocates for the precise characterization of biological materials based on their actual attributes—whether it be a complete cellular biological treatment, a biologically sourced multicomponent formulation, an enzyme-inclusive preparation, a biocatalyst (only after demonstrating activity and substrate conversion), a biologically sourced surfactant, or a biological formulation with an indeterminate mechanism—with the necessary evidence proportionately adjusted. A formulation should not be classified as a biocatalytic wax inhibitor unless it has been proven to be catalytic and that this is pertinent to the proposed mechanism for petroleum fluid.

5.2 Biological functionality under petroleum-relevant conditions

The value of a biological substance is not solely dictated by its intrinsic activity but also by the capacity of that activity to endure the conditions vital for petroleum production and transportation. The self-organization and interfacial properties of biosurfactants are influenced by their molecular structure, temperature, pH, and ionic strength (Jin et al., 2025), akin to how enzymatic activity is contingent upon the enzyme type, substrate availability, and environmental conditions. The performance exhibited in a conventional water assay does not inherently signify efficacy in waxy crude oil. A candidate must be assessed for thermal stability, salinity resilience, pH consistency, resistance to crude oil constituents, suitability for water-in-oil or oil-in-water systems, durability throughout the treatment duration, distribution between aqueous and hydrocarbon phases, substrate accessibility, and maintenance of quantifiable functionality after exposure to crude oil. Consequently, standard-assay activity needs to be regarded as an indicator of biological discovery rather than as direct proof of petroleum flow-assurance efficacy.

5.3 Plausible mechanisms as testable hypotheses

Four mechanistic classifications may feasibly elucidate the influence of biological systems on waxy-crude behavior and should be regarded as testable hypotheses rather than definitive truths, with the requisite evidence escalating significantly from a broad observation of enhanced bulk flow to a precise assertion of catalytic hydrocarbon transformation. ***Mechanism A - Alteration of interface.*** Biologically synthesised surfactants can adhere to critical surfaces, modifying local physicochemical interactions and influencing dispersion and aggregation dynamics. Supporting evidence must encompass the chemical characterization of the surfactant fraction, surface/interfacial activity under pertinent temperature and salinity conditions, crude compatibility, and a clearly established, empirically validated connection between interfacial modification and wax-related efficacy. The activity of biosurfactants by itself does not lead to the prevention of wax deposition. ***Mechanism B - Modification of wax crystalline structure.*** A biological formulation can physically interact with crystallising wax and affect nucleation, growth, morphology, or aggregation similarly to a conventional PPD mechanism. Evidence must encompass DSC (thermal transitions), cross-polarized microscopy (crystal morphology and aggregation), XRD (crystalline structure), and GC-MS (compositional variation), with bulk performance substantiated by thermal, morphological, and compositional data being significantly more robust than bulk response alone; furthermore, the biological origin should not diminish this standard. ***Mechanism C—Alteration in the gel matrix.*** The biological component

might alter the connectivity or mechanical integrity of the crystalline structure, although it does not inherently affect the quantity of wax precipitate. This theory necessitates regulated rheological data—temperature-sensitive viscosity, shear-rate analyses, yield stress, thixotropic behavior, storage and loss moduli, and frequency analyses—ideally combined with microscopy and DSC instead of deduced from a solitary viscosity assessment. **Mechanism D - Catalytic chemical alteration.** This represents the most challenging hypothesis: that a biological catalyst alters wax-forming hydrocarbons chemically to enhance their flow characteristics. Considering the recognized variety of microbial alkane-activation and oxidation pathways, this is generally feasible; nonetheless, the documented microbial hydrocarbon metabolism does not constitute verified wax-control catalysis in a specific crude. For a claim to be deemed credible, it must entail an active biocatalyst interacting with a specific substrate to achieve a quantifiable chemical transformation, resulting in identifiable products, maintaining mass-balance integrity, and exhibiting an impact on wax-phase or flow characteristics, validated through pre- and post-GC-FID/GC-MS compositional evaluations, while incorporating suitable controls (untreated crude, active preparation, heat-inactivated preparation, enzyme-depleted fraction, medium blank, and, if applicable, a positive control) to differentiate between catalytic and non-catalytic influences, ensuring that the loss of a single chromatographic peak is not interpreted as evidence of biological conversion, and establishing a temporal relationship that connects sustained biological activity to chemical transformation. A decrease in a substantial hydrocarbon proportion, absent any recorded alteration in wax-related characteristics, does not inherently provide proof of an effective flow-assurance method.

5.4 Implications

The most compelling inference from the literature is not that biocatalysts have successfully supplanted traditional PPDs, but rather that biological systems offer several scientifically credible pathways—interfacial, crystal, network, or catalytic—through which the behavior of petroleum fluids could be modified, necessitating demonstration of the relevant pathway instead of mere assumption. The framework rejects the notion that isolating a microorganism and identifying an enzyme validates biocatalytic wax inhibition. It requires progress that isolating a microorganism and identifying an enzyme confirms biocatalytic wax inhibition, necessitating advancement through biological identity, functional characterization, stability and crude compatibility, regulated petroleum-fluid testing, direct mechanistic evidence, dynamic deposition testing, and deployment evaluation. The fundamental principle is that the validity of any technological assertion must not exceed the robustness of the corroborating evidence.

6 Transitioning from observed response to mechanism to technology validation

A significant methodological hazard in evaluating biological and multicomponent wax-control formulations is to misconstrue an observed enhancement in petroleum-fluid quality as evidence of a particular process. Viscosity, pour point, or perceived gel strength may be diminished due to dilution, solvent or carrier influence, surfactant efficacy, physical dispersion, modified thermal history, or genuine chemical alteration of wax. The rheology of waxy crude is governed by interconnected compositional, thermal, crystallization, and structural mechanisms (Li et al., 2024). Consequently, a noted alteration in performance should be regarded as an initial basis for a series of rival hypotheses to be experimentally tested rather than as a definitive conclusion.

6.1 Various interpretations for an identical reaction

Possible mechanisms encompass catalytic transformation by the designated enzyme or enzyme system; interfacial alteration by biosurfactants or alternative surface-active metabolites; influences of carriers, solvents, or formulation matrices; mere dilution or variations in thermal history; and non-specific interactions of formulation elements with wax, asphaltenes, or other crude constituents. Generally, a biological substance is provided within a carrier or formulation matrix (such as water, buffer, residual growth medium, or extraction solvent), which means that the observed response may partially represent the matrix rather than the intended active ingredient, and without a matrix control, these influences cannot be accurately distinguished. Nonetheless, waxy-crude oils exhibit significant sensitivity to cooling rates, shear history, and aging duration, implying that unregulated variations in thermal or mechanical history between treated and control samples could produce a deceptive treatment effect. Consequently, the experimental framework must segregate the active element and include appropriate negative and positive controls.

6.2 A baseline five-arm control architecture

In this evaluation, we propose a minimum five-condition experimental framework (Table 2) for any biological wax-control candidate to differentiate these opposing ideas.

Table 2. Five-arm baseline control architecture for biological wax-control candidates.

Condition	Composition	Primary purpose
C1 — Untreated crude	Crude oil only	Establishes baseline petroleum-fluid behaviour
C2 — Formulation matrix	Crude + carrier/matrix, no active biological component	Isolates solvent, dilution and carrier effects
C3 — Inactivated preparation	Crude + validated heat- or chemically inactivated biological preparation	Tests whether the response depends on retained biological activity
C4 — Commercial PPD benchmark	Crude + a representative commercial PPD	Establishes relative technical competitiveness
T — Active formulation	Crude + active biological formulation	Measures the full candidate response

The fundamental comparison lies between the active treatment and the formulation-matrix control (C2), rather than solely between the active treatment and the crude untreated (C1); if the active formulation and the matrix control exhibit comparable behavior, there is scant evidence for a distinct biological effect. If biological activity is explicitly eliminated (C3) and the response remains unchanged, a catalytic rationale becomes less credible. Should the reaction be markedly diminished, a catalytic rationale is suggested (but not substantiated only by this finding), as inactivation methods may modify protein or surfactant composition irrespective of the decline in activity. The commercial-PPD benchmark (C4) differs from the mechanism controls (C1-3); C4 evaluates the candidate's performance against current technology, whereas C1-3 investigate the underlying causes of the observed response. All evaluations must be conducted under identical, clearly specified thermal and shear conditions (initial temperature, cooling rate, equilibration duration, applied shear, measurement temperature and duration, and ageing period), with sufficient independent replicates and suitable statistical analysis, as a solitary measurement does not provide proof of consistent performance.

6.3 Separating performance, mechanism, and operational qualification

The framework identifies four inquiries that are frequently merged in scholarly discourse: (1) did the intervention provide a quantifiable petroleum response (performance evidence)? Was the response replicable and more effective than suitable controls (comparative evidence)? What physicochemical or biological mechanism generated the response (mechanistic evidence derived from DSC, microscopy, compositional analysis, rheology, and substrate/product analysis)? Does the reaction enhance pipeline-related performance in representative dynamic settings (operational evidence)? These four tiers are not interchangeable, and a treatment may fulfill one without necessarily fulfilling the subsequent one: mechanistic proof that a formulation alters crystal morphology does not inherently suggest diminished pipeline deposition, just as a quantified decrease in laboratory viscosity does not inherently indicate enhanced hydraulic restart efficacy. The qualifying procedure should consequently refrain from transitioning immediately from a manageable laboratory characteristic to a field-scale technological assertion. Figure 1 encapsulates the manner in which the evidence produced by the control architecture in Section 6.2 integrates into the five-domain framework and the six-level ladder established in Sections 7 and 8.

7 A five-domain conceptual framework

This review, expanding on Sections 3–6, suggests that biologically sourced wax-control agents should be assessed across five interrelated evidence domains, advancing from biological identity to sustainable application: (I) biological functionality, (II) compatibility with crude oil, (III) modification of the wax phase, (IV) response for flow assurance, and (V) sustainable deployment (Figure 1). Any failure in a domain should prompt reevaluation or restructuring instead of unthinking advancement. Intense microbial activity coupled with inadequate crude compatibility signifies an inappropriate candidate; favorable crude compatibility without detectable wax alteration indicates a feeble mechanism; wax alteration without rheological enhancement denotes restricted engineering significance; and robust technical performance alongside a deficient environmental or economic profile suggests constrained sustainability.

7.1 Domain I — biological functionality

This domain defines the biological system and its active functions. Isolating a microorganism that can diminish wax deposition does not, in isolation, constitute adequate proof of mechanism; the system must be sufficiently characterized to ascertain whether the observed effect is due to microbial proliferation, enzymatic breakdown, extracellular metabolites, biosurfactant synthesis, direct crystal interaction, modified wettability, or a combination thereof. Minimum characterization must encompass microbial identity (molecular identification), phenotype (hydrocarbon-utilization profile), enzyme identity and kinetics when applicable, metabolite identity (GC-MS/LC-MS/NMR), biosurfactant activity (surface/interfacial tension, emulsification index, critical micelle concentration), stability under varying temperature/pH/salinity, hydrocarbon tolerance, storage stability, and reproducibility across distinct biological batches. A candidate may advance to Domain II only after biological identity, active function, and reproducible activity have been collectively confirmed; a favorable screening outcome lacking the identification of the underlying active function constitutes biological discovery evidence rather than validated wax-control technology.

7.2 Domain II — crude-oil compatibility

The behavior of wax is distinctly dependent on the crude oil type, so formulation efficacy should be assessed against a defined array of petroleum conditions rather than a solitary laboratory setting. Significant variables are crude type, wax content, carbon-number distribution, WAT, API gravity, viscosity, water cut, salinity, pH, pressure, temperature, cooling rate, shear history, dose, contact time, additive ageing, and asphaltene/resin concentration. This assessment suggests prioritizing the development of crude-response maps—systematic associations between crude-oil attributes and treatment performance—to advance the field from the overarching question of whether a formulation is effective to the engineering inquiry of the specific crude-oil and operational conditions that influence its efficacy, dosage, and mechanism. An advisable minimum compatibility matrix includes multiple representative crude oils and wax concentrations; temperatures above and below WAT; salinity of formation water and seawater; a range of water cuts; both sub-optimal and optimal dosages; static to high-shear conditions; varying asphaltene/resin content; and both fresh and aged formulations, with explicit records of any precipitation, emulsion instability, or negative interactions observed.

7.3 Domain III — wax-phase modification

This area connects the mechanistic disparity between biological function and petroleum efficacy. The objective needs to be to demonstrate variation across different scales, encompassing molecular composition, crystallization behavior, crystal shape, aggregation, gel structure, and deposition behavior. Two additives might produce comparable pour-point reductions yet possess inherently dissimilar crystalline architectures, gel strengths, and deposition characteristics. Any mechanistic assertion must be substantiated by a minimum of two complementary analytical methodologies, preferably three or more: DSC for crystallization/melting transitions and enthalpy; cross-polarized microscopy for assessing crystal morphology, size, and aggregation; XRD for elucidating crystal structure; GC-FID/GC-MS for analyzing n-alkane and hydrocarbon distribution; FTIR for examining functional-group interactions; NMR for investigating molecular mobility and phase behavior when applicable; and rheometry for evaluating yield stress, gel strength, viscoelasticity, and thixotropy, as illustrated for traditional crystal modifiers and dispersants by Sun et al. (2022). Interpretation must differentiate among biodegradation, co-crystallization or crystal-growth interference, interfacial adsorption, modifications in crystal morphology, crystal dispersion, diminished crystal-crystal interactions, changes in wettability, biosurfactant-facilitated interfacial alterations, and disruption of the three-dimensional gel matrix. These procedures are analogous to those set for traditional chemical PPDs, and biological candidates ought to meet a similar evidentiary criterion.

7.4 Domain IV — flow-assurance response

This field signifies the essential shift from laboratory chemistry to petroleum engineering: microbial proliferation, hydrocarbon breakdown, biosurfactant synthesis, or crystal alteration only attain technological significance when they are converted into improved flow characteristics and reduced deposition risk. A five-tiered evidence hierarchy is suggested in this domain: (A) pour-point response, which serves as a preliminary screening tool but is inadequate on its own; (B) viscosity-temperature behavior across the production and transport spectrum; (C) yield stress and gel strength, encompassing storage/loss moduli and thixotropy, due to the intricate viscoelastic and time-dependent nature of waxy-crude gels; (D) dynamic wax-deposition assessments—including deposition rate, deposit thickness, mass, composition, hardness, aging, adhesion, and the influence of shear and

temperature gradients—emphasizing that a formulation that lowers pour point may not necessarily mitigate wall deposition; and (E) flow-loop or validated pipeline-scale simulations under representative conditions, detailing pressure-drop reduction, critical deposition temperature, deposit thickness and rate, flow capacity, restart pressure, and pumping-energy requirements. The primary engineering issue in this field is whether the therapy improves the transport mechanism, rather than being solely a laboratory characteristic.

7.5 Domain V — sustainable deployment

This domain protects against the idea that biological origin always indicates sustainability, since a biodegradable formulation may still require energy-intensive purification, expensive cold-chain storage, or problematic field-injection infrastructure. Sustainability should be evaluated across five dimensions: technical effectiveness (deposition decrease, dosage, persistence, thermal/salinity stability, compatibility with production chemicals, effectiveness under dynamic flow); environmental effectiveness (biodegradability, marine toxicological effects, persistence, bioaccumulation potential, production waste, energy as well as water consumption, greenhouse-gas emissions), ideally evaluated through life-cycle assessment with a distinctly specified functional unit and system limits rather than assumed from bio-based sources (Fred et al., 2022; Blanco-Cejas et al., 2023; Arunkumar et al., 2026); economic viability, which involves comparing total treatment expenses — feedstock, manufacturing/purification, formulation/storage, application and monitoring costs — against avoided pigging, heating, chemical treatment, downtime and production reductions, rather than cost per kilogram of additive independently; safety (microbial pathogenicity, biological containment, metabolite toxicity, corrosion and formation-damage implications, regulatory specifications); and capacity for expansion (reproducibility of biological production, feedstock access, fermentation productivity, downstream recovery, formulation stability, batch-to-batch uniformity and field-injection logistics), which the broader biosurfactant as well as microbial-EOR literature identifies as a pervasive barrier to industrial implementation (Janamatti et al., 2019; Chafale and Kapley, 2022; Varjani et al., 2022).

7.6 Derivation of the framework and its boundaries

The five-domain framework was founded on the shared need for evidence throughout the literature, rather than assuming that biological identity solely determines a category of technology. Research was classified into categories based on biological functionality, compatibility with petroleum fluids, alteration of the wax phase, a quantifiable flow-assurance response, or evidence of sustainability/translation, with the domains reflecting interrelated evidence enquiries rather than distinct experimental categories. The six-tier ladder established in Section 8 was subsequently constructed by organizing the most robust defensible assertion from discovery to deployment. This is a theoretical structure for qualification that has not been validated through field development decisions; subsequent research should assess its reproducibility through anticipated application to autonomous candidate systems.

8 A six-level evidence-qualification ladder

The five-domain framework is implemented via a six-tier evidence ladder that delineates the most robust scientific assertion justifiably supportable at each phase of inquiry. The fundamental tenet of the ladder posits that the robustness of a technological assertion must not surpass the robustness of

the corroborating evidence and that advancement from one tier to another must be substantiated rather than presumed (Table 3).

Table 3. Six-level evidence-qualification ladder.

Level	Evidence stage	Minimum evidence	Scientific claim justified
1	Biological discovery	Organism/enzyme/metabolite identified; preliminary activity demonstrated	Candidate biological system has potential functional activity
2	Preliminary petroleum response	Pour point and/or viscosity response against appropriate controls	Preliminary low-temperature flow improvement
3	Mechanistic and comparative validation	Replicated experiments, controls, dose-response, statistical analysis, DSC/microscopy/compositional evidence	Candidate modifies wax crystallisation, morphology or rheology through a demonstrable mechanism
4	Crude-specific flow-assurance validation	Multiple crude systems; rheology and deposition testing across relevant temperature/shear conditions	Reproducible crude-specific wax-control performance
5	Dynamic engineering validation	Flow-loop testing and/or validated pipeline simulation under representative conditions	Engineering-level flow-assurance potential demonstrated
6	Sustainable deployment and scale-up	TEA, LCA, safety, scalability, formulation stability and field/pilot evidence	Technically, economically and environmentally credible deployable technology

Appropriate phrasing at Level 1 is limited to the applicant's commitment to wax-control implementation. It would be hasty to define the chemical as a confirmed inhibitor. At Level 2, a pour point or viscosity response must be documented as a first enhancement in low-temperature flow, rather than as proof of deposition prevention. Level 3 necessitates an untreated control (ideally a commercial-PPD positive control), various treatment concentrations, repeated experiments, statistical significance assessment, a dose-response correlation, and at least DSC, microscopy, and rheological data; a study meeting this criterion can justifiably assert that the formulation alters wax crystallization or crystal morphology and induces a quantifiable rheological change—significantly more substantial than merely stating a percentage pour-point reduction. At level 4, the mechanism must be applicable to two or more representative crude oils that vary in wax content, salinity, water cut, temperature, dosage, ageing, and shear, and the phrase “crude-specific” should characterize any performance assertion at this level, as a formulation validated solely in one crude oil should not be deemed universally effective. At Level 5, it is necessary to describe the deposition rate and pressure-drop response from a flow-loop or validated pipeline simulation, which constitutes the minimum evidence for a formulation to be regarded as possessing engineering-level potential. Level 6 amalgamates technical, environmental, economic, safety, and scale-up evidence, signifying the stage where pilot or field-scale assessment is scientifically warranted; nonetheless, it must be differentiated from complete commercial validation unless actual field data has been produced. This framework tackles the prevalent issue in innovative bio-based flow-assurance studies, the tendency to directly extend laboratory-scale biological performance to real-world applications. The ability of a microbe to decompose paraffin does not inherently imply that it will inhibit deposition inside a pipeline, nor does a decrease in pour point indicate a diminishment in dynamic deposition. Biological degradation, the formation of biosurfactants, crystal alteration, and enhancements in flow are interconnected yet different processes that must be assessed and documented individually (Wang et al., 2024). Table 4

elucidates this notion by delineating the minimum evidence necessary to substantiate each of the four claim categories identified in the literature; papers discussing potential biological wax-control agents must explicitly indicate the claim type their research corroborates and refrain from asserting claims that surpass it.

Table 4. Minimum evidence required to support each claim type.

Claim type	Minimum evidence	Evidence that is insufficient alone	Permitted conclusion
Biological activity	Defined organism/enzyme/metabolite and reproducible activity assay	Hydrocarbon degradation alone without wax relevance	Candidate has biological functionality relevant to further testing
Wax-related modification	Wax content/composition plus DSC/WAT/microscopy and/or compositional evidence	Pour-point or viscosity change alone	Treatment modifies at least one wax-related property
Dynamic deposition control	Controlled deposition experiment with untreated and formulation controls; deposition mass/rate and uncertainty reported	Static pour-point or viscosity improvement alone	Treatment reduces deposition under the tested dynamic conditions
Engineering qualification	Crude-specific dynamic validation, stability, safety, economic and scale-up evidence	Laboratory screening or biodegradability alone	Candidate is suitable for defined pilot/field qualification

9 Proposed experimental qualification framework

The theoretical framework is converted into a pragmatic, seven-phase petroleum-engineering research initiative in this segment, which includes biological screening, biochemical characterization, crude-oil compatibility, wax-phase examination, rheology, dynamic deposition evaluation, and sustainability analysis. The procedures are clearly associated with the five domains and the evidence hierarchy to establish a route from environmental sampling to pilot-scale validation, with definitive go/no-go decision criteria at every phase (Table 5).

Table 5. Seven-stage experimental qualification framework.

Stage	Objective	Representative activities	Framework alignment
1. Discovery	Identify biological candidates with measurable wax-control potential	Environmental sampling from hydrocarbon-associated sites; isolation and culture; screening for hydrocarbon utilisation, paraffin degradation, extracellular enzyme and biosurfactant production; candidate ranking	Domain I; Evidence Level 1
2. Functional characterisation	Determine the biological function responsible for the observed activity	Enzyme activity, metabolite and biosurfactant characterisation; substrate specificity; temperature/pH/salinity dependence; fractionation to separate active component from matrix	Domain I; Evidence Levels 1–2
3. Crude-oil compatibility	Determine whether the formulation remains functional in representative petroleum systems	Testing across crude type, wax content, salinity, water cut, temperature, dosage, contact time, shear and ageing; phase-separation, precipitation and activity-retention checks	Domain II; Evidence Levels 2–3

Stage	Objective	Representative activities	Framework alignment
4. Basic performance	Determine whether treatment measurably improves pour point and viscosity–temperature behaviour	Pour-point testing against untreated, matrix and commercial-PPD controls; viscosity across the WAT-spanning temperature range	Domain IV; Evidence Level 2
5. Mechanistic testing	Establish the mechanism of wax modification	Integrated DSC, cross-polarised microscopy, GC/GC-MS and rheology linking biological activity to chemical, crystal and rheological change	Domain III; Evidence Level 3
6. Dynamic performance	Determine whether structural/rheological improvement reduces deposition under flow	Cold-finger screening; controlled deposition-cell testing; flow-loop validation reporting deposition rate and pressure drop	Domain IV; Evidence Levels 4–5
7. Translation	Determine whether the candidate can progress to pilot/field evaluation	Formulation and storage stability; techno-economic assessment; life-cycle assessment; safety assessment; scale-up planning	Domain V; Evidence Level 6

Advancement through stages should rely on established qualification criteria instead of being automatic: a candidate advances from Stage 1 only after identification and screening collectively reveal pertinent activity; from Stage 2 only after the active component (enzyme, metabolite, or biosurfactant) has been characterized; from Stage 3 only after stability and dose–response are confirmed under petroleum-relevant conditions; from Stage 4 only after pour-point and viscosity–temperature data demonstrate a genuine, reproducible enhancement; from Stage 5 only after DSC, microscopy, compositional, and rheological evidence collectively establish a credible mechanism; and from Stage 6 only after cold-finger, deposition-cell, and ideally, flow-loop testing indicate diminished deposition under representative dynamic conditions. An essential experimental framework at every phase must encompass an untreated crude control, a solvent/formulation-matrix blank, the biological treatment, a commercial-PPD standard, and, when feasible, a validated positive biological control, alongside multiple dosages, sufficient replication, and suitable statistical evaluation (including descriptive statistics, confidence intervals, analysis of variance, dose-response modelling, and, when multiple crude properties and operational variables are examined concurrently, response-surface or alternative design-of-experiments approaches).

10 Comparative assessment of wax-control technologies

A significant evaluation of thermal, mechanical, chemical, nanomaterial-based, and biological wax-control technologies must take into account their effectiveness in reducing wax deposition as well as their mechanisms, energy demands, crude-oil specificity, intervention needs, environmental consequences, scalability, technological maturity, and supporting evidence. No technology is uniformly superior, and effectiveness is largely contingent on context. Thermal management and pigging exhibit advanced technology readiness but demand significant energy or intervention; polymeric PPDs and surfactants possess established chemical-control mechanisms yet are defined by notable crude specificity; nanohybrids demonstrate potentially robust performance but entail formulation, environmental, and scale-up uncertainties; biological systems offer prospects for renewable feedstocks and innovative wax modification pathways but presently necessitate substantially stronger evidence regarding mechanism, crude compatibility, reproducibility, dynamic deposition, safety, economics,

and scale-up to be considered viable alternatives (Pandian et al., 2021; Mahir et al., 2021; Li et al., 2024). Consequently, the research deficiency identified in this review is not the lack of an additional wax-control additive, but rather the absence of a cohesive evidentiary framework linking biological function to molecular wax alteration and engineering-scale flow assurance. This is the void that the five-domain framework and evidence ladder seek to address.

11 Research gaps and future priorities

Four research priorities emerge directly from the just-concluded study. Initially, it is imperative to perform a systematic and reproducible evaluation of biological wax-control candidates, clearly distinguishing catalytic, interfacial, and formulation-matrix contributions through the five-arm control architecture described in Section 6.2. The current literature seldom differentiates these mechanisms with sufficient controls. Secondly, the substitution of singular crude, singular condition demonstrations with justifiable assertions regarding the operational parameters within which a candidate operates, as determined by the crude-response mapping, entails a methodical examination of how treatment efficacy fluctuates with wax content, carbon-number distribution, WAT, salinity, water cut, and asphaltene/resin composition. Third, biological candidates necessitate dynamic, flow-representative assessments (cold-finger, deposition-cell, and flow-loop examinations). The majority of the existing database relies on static pour-point or bottle-test results that fail to demonstrate applicability at the pipeline size. Fourthly, a comprehensive techno-economic and life-cycle evaluation for the production and implementation of biological wax control should occur concurrently with laboratory development, rather than subsequently, to guarantee that sustainability assertions are substantiated by evidence rather than merely presumed from biological origin.

12 Conclusion

Wax accumulation and gel formation in waxy crude oils are complex processes that encompass thermodynamics, kinetics, crystal development, aggregation, ageing, rheology, and transport; thus, pour point or viscosity alone cannot determine wax-deposition regulation. Traditional thermal, mechanical, and chemical methods continue to serve as significant benchmarks; however, their efficacy is frequently rudimentary and dependent on specific conditions. Biological systems, including whole-cell cultures, biosurfactants, bio-derived formulations, and enzyme systems, have viable research prospects; nonetheless, the existing evidence is inconsistent and fails to support equating biological activity with proven pipeline-scale wax management. The primary contribution of this review is a framework for evidence qualification that connects biological functionality, compatibility with crude oil, alteration of the wax phase, flow assurance reaction, and sustainable implementation. The six-tier evidence hierarchy and seven-phase experimental framework delineate increasingly rigorous evidence standards, while the five-arm control structure establishes a foundational design for differentiating active treatment effects from formulation, carrier, interfacial, and non-catalytic factors; supplementary controls should be incorporated as necessitated by a candidate's chemistry. The literature offers concrete evidence that certain biological systems are capable of degrading paraffinic hydrocarbons, generating biosurfactants, modifying fluid characteristics, and, in certain investigations, influencing wax-related behavior. The data linking a specific biocatalytic mechanism to less dynamic deposition is significantly less advanced than the evidence for biodegradation or alterations in static fluid properties. The primary research deficiency is thus causal and translational: determining if a specified biological function can consistently alter wax behavior in typical crude oils

and diminish deposition under regulated temperature and hydrodynamic circumstances. Subsequent research must encompass crude-fluid characterization, biochemical evaluations, differential scanning calorimetry or comparable thermal assessments, compositional analyses, microscopy, rheological studies, regulated deposition trials, and flow-loop verification, while documenting suitable controls, replication, uncertainty, and crude-specific operational parameters consistently. Sustainability assertions ought to be substantiated by safety, biodegradation, life-cycle, and techno-economic data rather than only deduced from biological provenance. Collectively, the framework provides a shared evidentiary lexicon for evaluating biological and traditional wax-control technologies without presuming similarity in mechanisms or levels of development; its efficacy should ultimately be assessed prospectively, using independent candidate systems and pertinent field performance metrics.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Data Availability

No new experimental dataset was generated. The evidence base consists of published literature and the analytical framework developed in this review. A supplementary literature matrix (Supplementary Material) documents the study-by-study evidence underlying Sections 3–10.

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