

Technical Annex

STEP – I&D&I Empresarial – Digital e Biotecnologia - Operações em Copromoção

**STEP – Business R&D&I – Digital and Biotechnology — Collaborative
Projects**

CALL MPr-2026-2

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Project Acronym and title: PRISMA - Platform for R&D in Industrial Scalable Microbial Pigments

Executive Summary

Textile dyeing remains one of the most environmentally intensive stages of textile manufacturing, largely due to the widespread use of petrochemical-derived dyes that require high energy and water inputs and generate hazardous effluents and are predominantly imported from Asian countries. Although microbial bio-based pigments have emerged as promising sustainable alternatives, their industrial adoption is still limited. The main bottleneck no longer lies in biological feasibility, but in manufacturing infrastructure, scale-up reliability, process robustness and the lack of experimental validation under industrially relevant conditions, particularly within the textile value chain. PRISMA was designed to address this gap by enabling the industrial-scale production and application of bio-based textile pigments through an integrated and validated development pathway. The project aims to bridge the transition from laboratory-scale bioprocess demonstrations to reproducible, cost-competitive and industrially deployable biomanufacturing solutions. To achieve this objective, PRISMA implements three strongly interdependent development lines. DL1 focuses on scalable microbial fermentation and feedstock valorisation, including continuous operation and the integration of low-cost agro-industrial waste streams. DL2 constitutes the enabling deep-tech core of the project, combining geometry-preserved 3D-printed bioreactors with AI-driven process intelligence to ensure predictable and robust scale-up from laboratory to pre-industrial scale. DL3 completes the value-chain loop through formulation, textile application and full-scale dyehouse validation under real industrial conditions.

Implemented by a complementary consortium comprising Chainreactor (lead), CEB-UM, INESC TEC, CITEVE and ATB, with the support of an external partner (Octarine Bio), PRISMA spans the entire value chain from bioprocess development to industrial demonstration. The project culminates in the operational qualification of a 2,000 L CMO-ready bioproduction facility, enabling the first European industrial-scale production of textile-grade bio-based pigments at cost parity with conventional dyes, while strengthening EU sustainability, strategic autonomy and manufacturing capacity in critical biotechnological and textile value chains.

Leading project beneficiary:	Golfinhos ilusionistas – Unipessoal LDA
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1 – Total number of project beneficiaries (2+3):	5
2 - Companies	2
3 – Research Performing Organisations (RPO's)	3
4 - Partners:	1 - Octarine Bio

Start Date:	01/01/2027
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End Date:	31/12/2029
Duration (months):	36

Eligible Investment:	13 196 428,98 €
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List of project beneficiaries

No.	Legal designation	Abbrev.	Entity Profile	RPO	Public/Private
1	Golfinhos ilusionistas – Unipessoal LDA	Chainreactor	Company	No	Private
2	Centre of Biological Engineering – University of Minho	CEB-UM	R&D Centre	Yes	Public
3	INESC TEC - Institute for Systems and Computer Engineering, Technology and Science	INESC TEC	R&D Centre	Yes	Private
4	CITEVE – Technological Centre for the Textile and Clothing Industry of Portugal	CITEVE	R&D Centre	Yes	Private
5	A.T.B.-Acabamentos Têxteis de Barcelos Lda	ATB	Company	No	Private

1. Project Description

1.1. Rationale and context

Describe the project rationale and needs addressed, clearly identifying each development line and their interdependencies.

Textile dyeing is one of the most environmentally intensive stages of textile manufacturing, largely due to the widespread use of synthetic dyes derived from petrochemical resources. The production and application of these dyes require high energy inputs and large volumes of water and chemicals, generating coloured effluents that are difficult to treat and pose risks to ecosystems and human health. In response to these challenges, biotechnological approaches based on microbial pigment production have emerged as promising alternatives. Such pigments are produced via fermentation from renewable feedstocks, reducing dependency on fossil resources while offering improved biodegradability, lower toxicity, and potential advantages in wastewater management and regulatory compliance¹. Within this context, Octarine Bio, a Danish synthetic biology company, developed the PurePalette™ technology, a fully bio-based pigment platform. Its technological approach combines cell-factory engineering, precision fermentation, and proprietary bioprocess know-how to enable the scalable production of pigments from renewable feedstocks. This technology significantly reduces water and energy consumption (61.5%), CO₂-equivalent emissions (69.5%), human toxicity potential (70%), and ecotoxic impact (52.3%) compared to conventional dye manufacturing routes. While initially targeting the textile sector, the developed pigments are also applicable to food, beverage, and cosmetics industries^{2,3}. Despite the substantial potential of bio-based pigments, key technical and industrial constraints persist. In particular, large-scale production remains challenging, as yields, downstream processing costs, and process variability limit economic competitiveness with established synthetic dyes. In addition, the performance of biopigments on textile substrates still lags behind conventional dyes with respect to colour fastness and shade reproducibility across different fibre types¹. PRISMA directly addresses this critical bottleneck faced by Octarine Bio and the broader bio-based colourant industry, which no longer lies in biological proof-of-concept, but rather in manufacturing infrastructure, process

development, and industrial-scale validation. Biomanufacturing scale-up is further constrained by the high cost of bioreactors, rigid and standardised reactor designs, and scale-dependent variability, which hinder the preservation of hydrodynamic and mass-transfer conditions and lead to unpredictable losses in yield and reproducibility. These limitations are particularly critical for microbial products, where competitiveness depends on rapid, reliable, and cost-efficient scale-up^{4,5}. Chainreactor responds to this gap through a patented 3D-printed bioreactor platform^{6,7} based on additively manufactured PPGF/silicone vessels with integrated sparging and impeller geometries, enabling geometry-locked scale-up up to 2,000 L⁷. By preserving key dimensionless parameters across all scales, the platform eliminates the primary source of scale-up variability observed in conventional reactors. This allows scale-up to proceed through controlled and documented steps, substantially reducing the need for costly pilot re-designs and accelerating process development. The PRISMA project provides an opportunity to further develop and experimentally validate the scale-up of Chainreactor's technology. In parallel, the growing complexity of modern bioprocesses has created a clear need for advanced process-intelligence and control frameworks. Long-duration fermentations, alternative feedstocks, and high-density cultures generate dynamic behaviours that cannot be adequately managed through conventional sensing and empirical control strategies. Without real-time interpretability and decision support, deviations are often detected only after yield or quality losses have already occurred, a limitation that becomes increasingly severe when transitioning from laboratory to industrial scale.

Consequently, PRISMA is designed to establish an end-to-end, experimentally validated development pathway—from upstream biomanufacturing and continuous operation, through downstream processing, to textile validation and industrial demonstration—capable of delivering reproducible pigment outputs and generating quantitative evidence under industrially relevant conditions. To address these needs, 3 complementary and interdependent development lines (DL) were defined:

- DL1** focuses on the scalable biomanufacturing of bio-based pigments, addressing the optimisation and validation of fermentation processes across successive scales, with emphasis on process stability, productivity retention, and reproducibility during scale-up. This line also includes the screening and integration of Portuguese agro-industrial waste streams as alternative carbon sources, contributing to cost reduction and sustainability.

- DL2** constitutes the deep-tech core of the project and addresses the need for advanced manufacturing infrastructure and process control through the adaptation, manufacturing, and validation of Chainreactor's bioreactor technology, together with the integration of an AI-driven process-intelligence layer to monitor and compensate for scale-dependent variability.

- DL3** focuses on industrial validation and application, ensuring that pigments produced and controlled under DL1 and DL2 are experimentally validated under representative industrial conditions, including formulation and application in textile processes within an Advanced Testing Bed (ATB).

The 3 development lines are strongly interdependent: DL1 generates the bioprocesses and material outputs, including those derived from alternative feedstocks, which are implemented and controlled using the infrastructure and digital tools developed in DL2, providing a scalable and predictable framework for process scale-up; DL3 completes the development loop by validating the integrated biomanufacturing system under industrial conditions and feeding performance

insights back into DL1 and DL2, ensuring progression toward industrial-level experimental demonstration. In this way, the PRISMA project responds to the persistent gap between laboratory-scale bioprocess demonstrations and their translation into robust, scalable, and industrially validated operations within the textile value chain.

References list: ¹Alves *et al.*, 2024, [DOI: 10.1016/j.tibtech.2024.02.010](https://doi.org/10.1016/j.tibtech.2024.02.010); ²<https://www.octarinebio.com/>; ³<https://portugaltextil.com/octarine-bio-acelera-nos-pigmentos-bio/>; ⁴Achinas *et al.*, 2020, <https://doi.org/10.3390/mi11090853>; ⁵Achleitner *et al.*, 2024, <https://doi.org/10.1016/j.jbiotec.2024.06.001>; ⁶Nathaniel Petre, A bioreactor vessel, a bioreactor assembly and a method of fabricating a bioreactor vessel, 2025 (national provisional patent: 20252007452783); ⁷Nathaniel Petre, A sparger for a bioreactor vessel and a bioreactor assembly with an integrated sparger, 2025 (European Patent Application nº 25227639.9); ⁸<https://www.chainreactor.bio/>;

Demonstrate alignment of the project's development lines with **STEP sectors, objectives, value chain intervention, and criticality conditions:**

- Describe and substantiate the proposed development line(s) in terms of **STEP Sector/Domain/Technology(ies)** (see Annex E of the Call Document)

-**DL1** directly contributes to the Biotechnologies sector through the development and scale-up of microbial fermentation processes leading to bio-based pigments as final products.

-**DL2** acts as the main enabling line, contributing to Biotechnologies, Digital technologies (AI) and Deep tech innovation by integrating advanced bioreactor systems and AI-driven process intelligence to ensure scalable and predictable industrial deployment.

-**DL3** completes the value-chain loop within Biotechnologies by validating the developed pigments under representative industrial textile conditions, reinforcing application readiness and resilience.

Sector: Biotechnologies | Domain: Bioprocess biotechnology techniques

PRISMA focuses the development, scale-up and industrial validation of microbial fermentation processes for the production of bio-based pigments for textile application. The project advances scalable fermentation strategies, including long-duration and continuous operation, integration of alternative and heterogeneous carbon sources, and the systematic management of scale-dependent process effects. These activities address key scientific and technological uncertainties related to process stability, productivity retention and reproducibility when transitioning from laboratory to industrially relevant scales. Essential downstream processing steps required for industrial applicability are integrated as part of the overall bioprocess workflow.

Sector: Digital technologies | Domain: AI technologies

The project develops and integrates AI-driven process-intelligence solutions, including sensor frameworks and data-driven decision-support models, to enable real-time monitoring, early deviation detection and process robustness under long-duration operation, variable feedstock composition and scale-dependent variability. These AI technologies are not developed as an end in themselves, but as critical enablers for predictable and resilient bioprocess scale-up.

Sector: Deep tech innovation

PRISMA incorporates enabling deep-tech manufacturing solutions that support industrial biotechnology. In particular, the project relies on geometry-preserved, additively manufactured 3D-printed bioreactors, which provide a flexible and cost-efficient infrastructure for experimental scale-up and industrial validation. This deep-tech component directly addresses structural

bottlenecks associated with conventional biomanufacturing infrastructure, reducing capital intensity and accelerating process development cycles.

- Describe development lines aligned with **STEP objectives**, **value chain position**, Annex D classification, and the inclusion of R&D and/or Productive Innovation actions. The intervention(s) should contribute to one or both of the following STEP objectives:
 - Strengthening the EU's strategic autonomy and competitiveness through the **development and manufacturing of critical technologies** (including industrialisation and scalability aspects such as pilot lines, first-of-a-kind facilities, quality assurance and standardisation); And/or
 - **Safeguarding and strengthening their respective value chains** (increasing the resilience of the intervened value-chain position(s))

DL1 Scalable biomanufacturing and feedstock valorisation targets the upstream value chain position, focused on the development and validation of microbial fermentation processes for bio-based pigment production. The intervention consists exclusively of R&D actions (industrial investigation), addressing critical technical barriers related to process stability, long duration continuous operation, scale up, and the integration of alternative agro-industrial feedstocks. The output of the DL1 is a final product: bio-based pigment for textile applications. By reducing reliance on refined fossil-based inputs and on synthetic pigments predominantly sourced from Asia, and validating robust bioprocesses under industrially relevant conditions, DL1 contributes to safeguarding and strengthening the value chain, while supporting the STEP objective of reinforcing EU technological autonomy in industrial biotechnology.

DL2I Advanced manufacturing infrastructure and digital process intelligence intervenes at the manufacturing enabling and process control layer of the value chain. It combines R&D actions (Experimental Development) related to the development and validation of geometry preserved 3D printed bioreactors, advanced sensing solutions, and AI driven process intelligence frameworks. These developments directly address scale up reliability, manufacturing predictability, and process robustness, which are recognised bottlenecks in non-pharmaceutical biomanufacturing, which will culminate in Productive Innovation actions corresponding to the establishment and operational qualification of a dedicated production facility (CMO Build). DL2 results in two types of outputs, in accordance with point (a) of Annex D: 1) Specific components and machinery 3D-printed, AI-enabled bioreactors (20 L to 2,000 L) for precision fermentation; and 2) Associated services through Chainreactor's CMO (contract manufacturing organisation) service. This will be a specialised, critical service for the development and manufacture of bio-based pigments (not only for Octarine Bio) that does not currently exist in the European market at the required scale and agility. These include AI-driven process intelligence, monitoring, optimisation and decision-support services, specifically tailored to continuous bioprocessing and pigment manufacturing. DL2 therefore contributes to the STEP objective of strengthening EU strategic autonomy and competitiveness through the development and industrialisation of critical enabling technologies, including advanced manufacturing systems, process monitoring, and digital optimisation tools, as well as by increasing domestic manufacturing capability for critical biotechnological processes and reducing dependence on external production infrastructures.

DL3I Industrial validation and application within the textile value chain intervenes at the downstream and application-oriented stage of the value chain, ensuring that bio-based pigments and associated processes are validated under representative industrial conditions. This line consists of R&D actions (Experimental Development), covering downstream processing, formulation, textile application, pilot scale validation, and industrial dyehouse demonstration. The output of the DL3 is a final product: bio-based pigment concentrates and powders across multiple colour classes, at commercial grade (OEKO-TEX®, REACH and EU Ecolabel compliant), for application in the European textile sector, with measurable reduction in water consumption, chemical use, and hazardous effluent.

Development Line	Value Chain intervention	Type of intervention (per Annex D (a) of the Call)	R&D actions	Innovative Productive Investment Assets
DL1	Upstream biomanufacturing and product development	Final products (<i>biotechnological colourants for textile applications</i>)	Development, optimisation and scale-up of microbial fermentation processes; validation of long-duration continuous operation; integration of alternative agro-industrial feedstocks; validation of robust bioprocesses under industrially relevant conditions	
DL 2	Manufacturing-enabling layer and process control	Specific components and machinery (<i>geometry-preserved 3D-printed bioreactors; pigment-specific DSP</i>) and Associated services (<i>AI-driven process intelligence and CMO service</i>)	Design, adaptation and validation of 3D-printed bioreactors (20 L–2,000 L); development and validation of advanced sensing and AI-driven monitoring, optimisation and decision-support tools; development and validation of a pigment-specific DSP configuration	Establishment and operational qualification of a dedicated production facility enabling Chainreactor's CMO service (Productive Innovation – CMO Build)
DL 3	Downstream industrial application and validation	Final products (<i>biotechnological colourants for textile applications</i>)	Experimental validation of pigments and processes in textile finishing, dyeing and industrial demonstration environments	

- Describe and substantiate the development line(s) in terms of the **STEP** criticality condition:
 - Introduce an innovative, emerging and cutting-edge element, into the EU internal market, with **significant economic potential** (see point (h) of Annex D of the Call for Proposals); and/or
 - Contribute to reducing or preventing strategic dependencies of the EU (see points (i), (j), (k), (l) and (m) of Annex D of the Call for Proposals).

Introduce an innovative, emerging and cutting-edge element, into the EU internal market, with significant economic potential: by combining continuous precision fermentation, AI enabled autonomous process control, and geometry preserved 3D printed bioreactor scale up into a single, industrially validated biomanufacturing platform. This combination does not currently exist within the European biomanufacturing landscape, particularly for the continuous production of secondary metabolites at pilot and pre-industrial scale with integrated AI driven control. Industrial biotechnology and biomanufacturing have been formally recognised by the European Commission as strategic enabling technologies for the EU's competitiveness and green transition, with a clear emphasis on accelerating scale up, industrial deployment and market uptake of bio-based solutions^{9,10}. PRISMA directly addresses these priorities by advancing a first of its kind European

pilot line that de risks continuous bioprocess scale-up from laboratory to 2,000 L using geometry locked bioreactor technology, significantly reducing capital intensity and development timelines compared to conventional infrastructure. The project further introduces AI driven process intelligence (including real time monitoring, optimisation and decision support) as an integral component of industrial biomanufacturing rather than as a standalone digital layer. This creates a replicable model for smart biomanufacturing that can be transferred to other bio-based production systems, reinforcing Europe's leadership in digitally enabled industrial biotechnology.

From a market perspective, PRISMA targets the rapidly expanding market for bio-based textile dyes, which is being driven by EU regulatory and policy pressure to phase out hazardous and fossil derived substances. The EU Strategy for Sustainable and Circular Textiles and related regulatory instruments (including REACH and OEKO TEX® requirements) explicitly promote safer, low impact and industrially viable alternatives, creating strong and sustained demand for bio-based colourants¹¹. Sustainable dyes represent the fastest-growing segment of the global dyes market, with an estimated compound annual growth rate of approximately 8.2% over the 2023–2030 period¹². The commercial validation of bio-based pigments by Octarine Bio with leading fashion brands at major European fashion events further confirms market readiness and economic potential. By establishing a European based, AI enabled biomanufacturing platform and a specialised CMO service capable of delivering bio-based pigments at industrial scale, PRISMA strengthens EU strategic autonomy, reduces dependence on imported petrochemical dye intermediates, and positions Europe at the forefront of next generation sustainable materials manufacturing.

Contribute to reducing or preventing strategic dependencies of the EU: by addressing structural weaknesses in the textile dye and industrial biotechnology value chains that currently expose the EU to high levels of external dependence.

I-EU industrial and technological leadership: by integrating advanced bioprocessing (continuous fermentation) with enabling digital technologies and advanced manufacturing (geometry-preserved bioreactors), targeting a recognised European bottleneck: translating strong research capabilities into industrially deployable manufacturing systems at scale.

J-Contribution to critical infrastructures at EU level: by establishing and operationally validating a 2,000 L CMO-ready facility in Portugal. While not a pan-European infrastructure by itself, it is designed as a replicable reference asset (process + equipment + monitoring/control stack) that can be deployed at additional sites without fundamental re-engineering, supporting progressive reinforcement of EU mid-scale fermentation infrastructure. PRISMA approach is consistent with recent EU policy frameworks, which recognise industrial biotechnology and biomanufacturing as strategic enabling technologies and explicitly call for increased European production capacity to reduce reliance on non-EU manufacturing ecosystems⁹.

K-Increasing manufacturing capacity within the EU: PRISMA increases EU manufacturing capacity by shifting from laboratory demonstrations toward pilot and pre-industrial validation of bioprocesses and associated manufacturing systems (an EU identified gap in the transition from innovation to production in bio-based markets). This capacity increase is directly relevant for textile colourants value chain, which has experienced decades of relocation of dye and intermediate production outside Europe. Besides, PRISMA establishes re-usable digital and physical infrastructure, including validated AI process models, process intelligence frameworks and scalable

bioreactor configurations, that can be transferred beyond pigment production to other bio-based products and biomanufacturing value chains.

L-Strengthening security of supply of critical inputs: The European textile industry relies heavily on imported petrochemical dyes and dye intermediates, following the long-term relocation of dye manufacturing capacity away from Europe. Global production and consumption of synthetic dyes are now highly concentrated in Asia, particularly China and India, which together account for approximately 40–45% of global dye consumption and a dominant share of exports, including to European markets¹². Besides, the chemical sector has been identified as one of the EU's most exposed industrial domains in terms of import dependencies, increasing vulnerability to supply disruptions, regulatory divergence and price volatility¹³. PRISMA contributes to mitigating this dependency by enabling the development and scaling up of EU based production of bio-based textile pigments, substituting fossil derived dyes with biotechnological alternatives produced within the EU. By internalising pigment production through microbial fermentation using waste derived feedstocks, the project directly strengthens security of supply for a critical textile input.

M-Promoting positive cross border effects in the internal market: by structuring a transnational value chain that connects bioprocess development, digital technologies, manufacturing infrastructure, and industrial textile application across European countries. Biological know how and market demand anchored by a Danish biotech company are integrated with production, scale up, and industrial validation activities located in Portugal, facilitating cross border knowledge transfer, supply chain integration, and market access within the internal market. Moreover, the modular and replicable nature of the PRISMA platform supports future deployment by biotechnology and textile actors in other Member States, reinforcing internal market dynamics and reducing fragmentation in access to advanced biomanufacturing capabilities.

References list: ⁹European Commission (2024). *Building the future with nature: Boosting Biotechnology and Biomanufacturing in the EU* (COM(2024) 137 final); ¹⁰Joint Industry Statement (2024). *Boosting Biotechnology and Biomanufacturing in the EU*. IPA Europe / Europabio; ¹¹European Commission (2022). *EU Strategy for Sustainable and Circular Textiles* (COM(2022) 141 final); ¹²WorldMetrics. Dye Industry Statistics: Market Data Report 2026. 2026: <https://worldmetrics.org/dye-industry-statistics/>; ¹³Lefebvre, K. & Wibaux, P. (2024). Import Dependencies: Where Does the EU Stand? Policy Brief No. 47 https://www.international-economics.eu/PDF_PUB/pb/2024/pb2024-47.pdf

STEP technology domain / Technology	Value Chain intervention	Development and manufacturing critical technologies? (Y/N)	Contributing to the safeguarding and strengthening of their respective value chains (Y/N)	Innovative, emerging, cutting-edge with significant economic potential? (Y/N)	Reducing or preventing strategic dependencies? (Y/N)
Biotechnologies – Bioprocess biotechnology techniques	Final products (bio-based pigments)	Y	Y	Y	Y
Deep tech innovation – Advanced biomanufacturing systems (3D-printed bioreactors, DSP)	Specific components and machinery (bioreactors and pigment-specific DSP systems)	Y	Y	Y	Y
Digital technologies – AI technologies (process intelligence for biomanufacturing)	Associated services (AI-driven process intelligence and CMO service)	Y	Y	Y	Y

1.2. Objectives and methodology

Value Chain intervention	Innovative Characteristics	Unit of measurement / Technical specification	Current Market performance	Project Objective	Weight (%)
Upstream	I.C.1: Alternative carbon source usage	Yield to glucose corroboration (%)	-Existing but needs improvement	-Validate at least 3 Portuguese agro-industrial waste streams; -Obtain a yield-to-glucose corroboration rates >70% -Ensure that the transition to low-cost feedstocks maintains >85% of the baseline titre; -Reduce feedstock costs by 40-60%.	20
Manufacturing-enabling	I.C.2: Geometry-preserved bioreactor scale-up using additive manufacturing	-Working volume (L); -% scale-up error;	-Non-existent	-Validated scale-up from 20 L to 2,000 L with ≤20% deviation	30
Manufacturing-enabling	I.C.3: Predictive model of biology / AI Driven fermentation technology	Prediction Accuracy (%)	-Non-existent	-Achieve a MAPE (Mean Absolute Percentage Error) for key fermentation outcomes (titre, yield, productivity) < 15%	30
Downstream	I.C.4: Industrial bioproduction of textile-compatible bio-based dye:	-Cost production (€) - Annual production capacity (kg/year); -Dye performance and quality on textiles	-Existing but needs improvement	-Cost equal to synthetic dyes. -500–1,000 kg/year production -Validate pigments in full-scale industrial dyeing	20

Justification of **innovative characteristics** and **SMART characteristics** of the project objectives

I.C.1: Alternative carbon source usage: Current commercial precision fermentation relies almost exclusively on refined glucose or sucrose as carbon feedstock, typically sourced at EUR 300-500/ton. This creates both a cost floor and a supply chain dependency on commodity sugar markets. Academic literature has demonstrated feasibility of using agro-industrial by-products (molasses, fruit processing residues, whey permeate) as alternative carbon sources¹⁴, but yield-to-glucose corroboration rates remain inconsistent and poorly documented across organism classes. PRISMA targets systematic screening and validation of at least 3 Portuguese agro-industrial waste streams, achieving fermentable sugar yields competitive with refined glucose while reducing feedstock costs by 40-60%.

I.C.2: Geometry-preserved bioreactor scale-up using additive manufacturing: Commercially available bioreactors remain one of the largest barriers to scaling bio-based production. Current market leaders, including Sartorius (Germany), Eppendorf (Germany), Applikon Biotechnology/Getinge (Netherlands/Sweden), and IKA (Germany), supply high-quality stainless steel or glass bioreactors, but their equipment is optimized for pharmaceutical and academic

research rather than cost-sensitive industrial bioprocesses. However, conventional stainless-steel bioreactors change vessel geometry at every scale step. A 2L benchtop reactor has fundamentally different aspect ratios, impeller-to-vessel diameter ratios, and sparger configurations than a 200L pilot unit, which differs again from a 2000L production vessel. This forces complete process re-optimization at each scale, typically consuming 12-18 months and EUR 500K+ per transition. To date, no commercial bioreactor manufacturer currently offers geometry-preserved vessels across scales. Chainreactor's additive manufacturing approach enables identical geometry ratios from 1L to 2000L, eliminating re-optimization cycles and enabling direct process transfer. This is the project's primary technical moat and the foundation of its CMO graduation model.

I.C.3: Predictive model of biology / AI-Driven fermentation technology: Bioprocess AI remains fragmented in many non-pharmaceutical manufacturing contexts, often relying on retrospective batch data and limited integration across scales. PRISMA addresses this gap by developing an integrated process-intelligence stack (real-time smart sensing, data infrastructure, surrogate models, and digital twins) and validating its transfer to pilot and pre-industrial scales through the project's scale-up activities. The platform will be trained and refined using multi-scale process data generated across the PRISMA scale-up pathway, enabling improved prediction and decision support for titre, yield, and productivity under long-duration operation. The target of < 15% of MAPE for titre, yield, and productivity across scales is benchmarked against current academic best-in-class of approximately 60-70% for single-organism, single-scale models (limited to retrospective analysis).

I.C.4: Industrial bioproduction of textile-compatible bio-based dye: Bio-based pigments produced through microbial fermentation have demonstrated promising colour performance and favourable toxicological profiles at laboratory scale, including the absence of dermal irritation and low cytotoxicity, supporting their potential as safer alternatives to petrochemical dyes^{1,15}. Several companies have advanced microbial pigment technologies, including Octarine Bio (PUREPALETTE™)², Colorifix¹⁶, PILI (BioIndigo®)¹⁷, and HUUE¹⁸. However, current industrial deployment remains limited, with production largely confined to pilot or niche scales. The transition to industrial-scale manufacturing of microbial pigments continues to face significant challenges. In particular, limited fermentation yields, high downstream processing costs, and process variability constrain economic competitiveness with established synthetic dyes. In parallel, many existing bio-based pigments do not yet consistently meet industrial textile performance requirements, notably in terms of wash fastness and light fastness, nor do they achieve cost parity with commodity synthetic disperse dyes, typically priced at approximately EUR 5–15/kg. PRISMA directly addresses these barriers by developing and validating the industrial-scale production of PUREPALETTE™ pigments (upstream yield + downstream recovery efficiency) and targeting full certification-readiness and cost parity.

References list: ¹⁴Lopes & Ligabue-Braun, 2021. <https://doi.org/10.3389/fsufs.2021.589414>; ¹⁵Basto *et al.*, 2025. <https://doi.org/10.3389/fmicb.2025.1677799>; ¹⁶<https://www.colorifix.com/>; ¹⁷<https://www.pili.bio/en/homepage/>; ¹⁸<https://www.huue.bio/>;

SMART objectives

Specific: 1) Validate at least 3 Portuguese waste-derived carbon feedstocks for pigment fermentation with documented yield data; 2) develop, manufacture, and validate geometry-preserved 3D-printed bioreactors 200L, 500 and 2000L scales; 3) develop and deploy an AI-driven

process intelligence platform achieving < 15% of MAPE; 4) produce bio-pigment batches meeting ISO 105 textile testing standards for wash and light fastness and compatibility with industrial-scale dyeing process ; 5) commission a 2000L CMO-ready production facility.

Measurable: Feedstock yield (g fermentable sugar / g waste input); bioreactor scale transfer success (% titre retention between scales); AI prediction accuracy (% for titre, yield, productivity); textile performance (wash fastness grade, light fastness grade per ISO 105); pigment production cost (EUR/kg vs. synthetic benchmark).

Achievable: Bioreactor technology enters at TRL 5 with commercial units already deployed. Pigment fermentation enters at TRL 4 with Octarine Bio's validated strains and CEB-UM's fermentation expertise. AI/ML enters at TRL 4 and builds on INESC TEC's established bioprocess signal processing capabilities. All targets are grounded in demonstrated component-level performance.

Relevant: Directly aligned with EU Green Deal objectives, the EU Textile Strategy's push for sustainable textile chemicals, Portugal 2030 smart specialization priorities in biotechnology and advanced manufacturing, and the STEP programme's mandate for critical technology development and value chain resilience.

Time-bound: as each target is associated with specific activities, deliverables and milestones defined in the project Gantt chart, ensuring verification within the project's 36-month implementation period.

Explain key **technical challenges (TC)**, the **methodology** to address them, and the integration (or justified absence) of **multidisciplinary expertise**

TC1 | Usage of agro/food industry waste streams as carbon source: Waste-derived feedstocks vary in composition seasonally and across suppliers. Sugar content, inhibitor profiles (furfural, acetic acid, phenolic compounds), and solids loading all effect fermentation performance unpredictably. The methodological approach is systematic: CEB-UM will screen a minimum of 6 candidate streams from Portuguese agro-food producers, characterize each for fermentable sugar content, inhibitor concentration, and pre-treatment requirements, then down-select to 3 streams that meet threshold criteria for yield, cost, and supply reliability. Pre-treatment protocols (dilute acid hydrolysis, enzymatic saccharification) will be optimized per stream. This empirical screening approach avoids over-reliance on any single feedstock and builds a feedstock flexibility library for future use.

TC2 | Waste-derived feedstock type-dependent biology adjustments: Different carbon sources trigger different metabolic responses in pigment-producing organisms, affecting growth rate, pigment titre, and product profile. Rather than engineering the organism (which would create IP conflicts and regulatory complexity), PRISMA adjusts process parameters (temperature, pH, dissolved oxygen, feed rate) to accommodate each feedstock. CEB-UM will run comparative fermentation campaigns at 2L and 20L across the shortlisted feedstocks, mapping the parameter space for each organism-feedstock combination. This process-first (not strain-first) approach is a deliberate strategic choice that keeps IP clean and commercially defensible.

TC3 | Maintain yield and stability during scale-up from laboratory to industrial volumes: Scale-up is the defining challenge of industrial biotechnology. Conventional bioreactors introduce new mixing regimes, oxygen transfer limitations, and thermal gradients at each scale step. PRISMA's core

methodological innovation is geometry preservation: Chainreactor's 3D-printed vessels maintain identical aspect ratios, impeller-to-vessel diameter ratios, and sparger configurations from 1L to 2000L. This eliminates the largest source of scale-up variability. The remaining process variables (feed strategy, oxygen supplementation, foam control) are addressed through the AI-driven process intelligence layer (A3), which uses real-time soft sensor data to detect and compensate for deviations before they affect yield. Scale-up proceeds in defined steps (20L, 200L, 500L, 2000L), with each step requiring documented titre retention of at least 85% relative to the prior scale before advancing.

TC4 | Real-time process monitoring and model-based inference: PRISMA addresses this challenge through an integrated process-intelligence approach, combining physical and chemical sensing (e.g., pH, temperature, dissolved oxygen, pigment concentration), structured data acquisition, and model-based inference to estimate critical biological and process variables that are otherwise difficult to measure directly. INESC TEC will develop and validate predictive and surrogate models using multi-scale experimental data, enabling real-time interpretation and decision support during fermentation. The models are trained on datasets generated in Chainreactor's geometry-preserved bioreactor systems and are validated during scale-up activities in A6 under pilot and pre-industrial conditions, supporting early deviation detection and informed operational decision-making.

TC5 | Achieve textile performance standards and cost competence with synthetic dyes: Bio-based pigments must meet ISO 105 standards for wash fastness, light fastness, and rubbing fastness to be accepted by textile manufacturers. Current academic demonstrations fall short on these metrics, and pigment production costs remain 3-5x above synthetic equivalents. PRISMA addresses performance through systematic formulation development (CITEVE, A7) and industrial-scale application trials (ATB, A8), testing across multiple textile substrates and dyeing methods. Cost competitiveness is addressed upstream: waste-derived feedstocks reduce raw material costs, geometry-preserved scale-up eliminates re-optimization costs, and DSP optimization (A5) targets maximum pigment recovery with minimum energy input. The target is cost parity with commodity disperse dyes (EUR 5-15/kg) at the 2000L production scale.

Methodological & Multidisciplinary approach

PRISMA is inherently multidisciplinary. No single field of knowledge can deliver the project objectives in isolation. The consortium integrates six distinct disciplines, each contributing to specific activities while creating cross-disciplinary feedback loops: **Biotechnology** (CEB-UM)- fermentation science, microbial physiology, bioprocess engineering (core to A1, A2, A6). **Data science and AI** (INESC TEC)- machine learning, reinforcement learning, signal processing (core to A3). **Materials science and advanced manufacturing** (Chainreactor): additive manufacturing, polymer engineering, reactor design (core to A4, A6). **Chemical engineering** (Chainreactor + CEB-UM): downstream processing, separation science, process intensification (core to A5). **Textile engineering and chemistry** (CITEVE): dye formulation, application science, standardized testing (core to A7). **Industrial process engineering** (ATB): industrial dyeing systems, process integration, quality assurance (core to A8).

The critical integration points are: 1) the data pipeline from bioreactor sensors (A4) through AI models (A3) back to fermentation control (A1, A2, A6) this requires biotechnology, data science, and materials science to co-develop sensor specifications and data schemas; 2) textile performance

results feed (A7, A8) back into upstream processing and downstream formulation choices (A5, A6) - this requires textile engineers and bioprocess engineers to define shared quality metrics and feedback protocols; 3) DSP development (A5) which sits at the interface of bioprocess engineering and industrial manufacturing, requiring both CEB-UM and Chainreactor to co-optimize for biological yield and industrial scalability simultaneously.

1.3. Technical and Financial Maturity

Indicate the **initial TRL**, demonstrate the **consortium's technological expertise**, justify the route to **productization of the results**, and prioritize **proven solutions**, exceptionally justifying the need for non-proven solutions.

The proposed development lines of PRISMA start from **TRL 4–5** and are designed to reach **TRL 7–8** by the end of the project, through progressive validation under pilot and pre-industrial operating conditions.

DL1I Scalable biomanufacturing and feedstock valorisation: Alternative carbon feedstocks have been extensively studied at the academic level for decades, and industrial fermentation using agro-industrial feedstocks is already implemented in several sectors, particularly for biofuels, enzymes and bulk bio-based ingredients. In particular, scientific literature and prior work carried out by CEB-UM have demonstrated the feasibility of fermenting agro-industrial by-products at laboratory scale. PRISMA builds on this established foundation by validating and optimising this proven approach for specific pigment-producing microorganisms supplied by Octarine Bio and Portuguese agro-industrial waste streams, thereby applying well-understood principles in a new biological and industrial context. In parallel, Octarine Bio's technological approach, which combines cell-factory engineering, precision fermentation and proprietary bioprocess know-how, enables the scalable production of pigments from renewable feedstocks. As a result, the biological production of microbial pigments and associated formulations is validated at laboratory and bench scale (TRL 4), with demonstrated proof of concept and reproducibility under controlled conditions. Under the LOI with Octarine Bio, CEB-UM will conduct preliminary fermentation trials with pigment-producing microorganisms in Chainreactor's 20L bioreactors prior to programme start, establishing baseline growth and pigment accumulation data. Octarine Bio's broader biological platform, which includes commercially demonstrated PurePalette strains at pilot scale, provides the biological knowledge base from which PRISMA's novel manufacturing process development departs. Within the project, productization of DL1 is achieved through the definition and validation of standardised fermentation protocols, feedstock specifications and operating windows that can be directly transferred to pilot and pre-industrial operation (TRL 7).

DL2I Advanced manufacturing infrastructure and digital process intelligence: Chainreactor has designed, manufactured, validated, and commercially deployed 3D-printed PPGF/silicone bioreactors from 250mL to 20L with external customers (TRL5). The patented integrated sparging system⁷ delivers controlled gas distribution validated across multiple fermentation organisms. Cloud telemetry is live and streaming from deployed units. INESC TEC has validated AI/ML frameworks for bioprocess data in laboratory environments in prior projects (TRL4), while Chainreactor's cloud platform has demonstrated real-time fermentation telemetry capture, storage, and processing.

Productization is achieved by transforming individual technological elements into an operational and transferable manufacturing-enabling platform, comprising validated bioreactor designs, integrated sensing and data-acquisition architectures, and AI-based process-intelligence tools. These elements are progressively deployed and tested during scale-up activities, ensuring that the resulting solutions can be directly integrated into industrial production environments without fundamental redesign (TRL7).

DL3I Industrial validation and application within the textile value chain: Octarine Bio's pigments were designed from the outset for integration into industrial dyeing processes and are currently validated at laboratory and bench scale (TRL 4). DL3 focuses on systematically validating pigment performance, robustness and reproducibility along the textile value chain under increasing scale conditions. CITEVE has conducted preliminary application trials with bio-based pigment compounds, establishing baseline compatibility with industrial textile processes and providing a validated pilot environment for standardised performance testing. Final industrial validation is carried out by ATB, enabling evaluation under real production conditions and confirming compatibility with existing industrial dyeing infrastructure (TRL8). Productization of DL3 results in the definition of pigment formulations, application protocols and performance benchmarks aligned with industrial standards, enabling direct transfer of results to manufacturing environment.

Productization pathway: The R&D outputs from DL1, DL2, and DL3 converge on a single productizable asset: a 2000L CMO-ready bioproduction facility capable of producing textile-grade bio-pigments at cost parity with synthetic dyes (TRL8). Productization does not depend on any single breakthrough but on systematic integration of individually proven components. The CMO Build (A11) is classified as Productive Innovation and represents the direct commercialization vehicle.

Describe the **innovative productive investments**, identifying the companies responsible for their implementation, and justify: Describe **innovative productive investments**, the responsible companies, and their alignment with **R&D activities** and **market objectives**.

Chainreactor is the sole company responsible for the implementation of the innovative productive investment component of the project, corresponding to A11 - CMO Build. Productive investment is intrinsically and directly complementary to the R&D activities carried out within the project, forming the essential bridge between technology development, validation, and industrial deployment. The R&D activities developed under A1–A6 are focused on the development and validation of upstream bio-based pigment production processes, including precision fermentation, scale-up, and process optimization. A7–A8 address downstream validation, namely the application of the produced pigments under industrial textile conditions. A11 translates these validated processes into a permanent and operational manufacturing infrastructure, enabling industrial production under real market conditions. The productive innovation investment to be implemented by Chainreactor includes the following main components:

- Site preparation and industrial facility fit-out;
- Integrated production infrastructure, comprising a 2,000L industrial bioreactor, an industrial downstream processing (DSP) line (filtration and spray drying), and associated utilities, clean-in-place (CIP) systems, and biosafety-compliant installations, enabling continuous and reliable industrial operation.

- **Industrial process monitoring, control, and automation systems**, including a **production sensor suite** and **PLC/SCADA control**, ensuring real-time supervision, reproducibility, and scalable manufacturing performance.
- **Digital and IT infrastructure**, encompassing **IT systems**, **SCADA/MES software**, and **LIMS solutions**, supporting manufacturing execution, quality control, batch traceability, and integration between physical production assets and digital platforms.
- **Operational resilience and continuity systems**, namely **backup power** and **UPS infrastructure**, safeguarding critical production and control processes.
- **Intangible operational enablers**, including **DSP technical know-how licensing**, as well as **production management and scheduling software**, enabling efficient planning, coordination, and optimization of CMO activities.

Therefore, the productive investment establishes Chainreactor's first Contract Manufacturing Organization (CMO) production unit, designed with an initial capacity to operate 2–4 industrial production batches per month, generating bio-pigment volumes sufficient for industrial-scale customer trials. This scale is fully adequate and proportionate to the project's target market objectives, namely supplying Octarine Bio's initial European manufacturing requirements and serving as a reference facility to support subsequent CMO customer acquisition. In this context, the productive investment is fully aligned with the ambition of the project, ensuring technological continuity from R&D to market, enabling industrial exploitation of the results, and positioning Chainreactor as a strategic manufacturing partner within European bio-based value chains.

2. Implementation Capacity

2.1. Consortium description

The project will be carried out by a consortium comprising Chainreactor, acting as the lead beneficiary, together with CEB-UM, INESC TEC, CITEVE and ATB as co-beneficiaries, leveraging their complementary competencies and expertise for the successful development and implementation of the project activities. Together, they cover all critical stages of the value chain of the project's main output.

>**Chainreactor**, founded in 2024, is a deep tech biotech company combining 3D printing (additive manufacturing) and machine learning to make bioreactors scalable and accessible. Its mission is to democratize access to biotechnology, enabling researchers, entrepreneurs, and communities to use these solutions to tackle complex global challenges. The company aims to reach 1 million liters of bioreactor capacity by 2030, promoting a distributed and sustainable approach to biotechnological innovation.

Team project comprising 19 members, including both existing staff and 16 new hires, with 12 members classified as critical HR. This reflects the fact that the company is relatively young and that part of its workforce is currently allocated to other ongoing projects. The team project includes members with expertise in bioreactor and process engineering, additive manufacturing and 3D-printing technologies, AI/ML for bioprocess, fluid dynamics and reaction engineering, materials science, fermentation scale-up, and downstream processing engineering.

-**Nathaniel Petre**: CEO of Chainreactor, with a background in design engineering and additive manufacturing focused on sustainability and circular solutions. His professional trajectory combines hands-on experience in scalable 3D-printing systems with leadership in

innovation-driven, environmentally responsible industrial initiatives. Within PRISMA, his role is primarily dedicated to overall project governance, strategic coordination of the consortium, dissemination of results and IP management, ensuring that the project outcomes are aligned with industrial scalability, market relevance and long-term impact.

-Tânia Chilima: Chief Scientific Officer (CSO) at Chainreactor, with an internationally recognised research and industry track record at the intersection of bioprocess engineering, advanced bioreactor platforms and AI-driven process optimisation. She holds an EngD in Biochemical Engineering from University College London and brings over a decade of experience leading the development, scale-up and commercialisation of next-generation bioreactor systems from benchtop to 2,000 L industrial scale, including 3D-printed and automated bioprocess platforms. Within PRISMA, her expertise is central to the scientific and technological leadership of the project, guiding bioreactor design, scale-up strategies, process intelligence integration and the translation of advanced R&D outcomes into robust, industrially deployable biomanufacturing solutions.

-David Birgé-Cotte: is the CTO at Chainreactor, with an interdisciplinary background combining hardware and software design, biotechnology and sustainable engineering. His professional experience includes the development of modular bioreactor and automation systems, as well as innovation projects at the interface of digital technologies and bio-based systems. Within PRISMA, his participation is focused on to providing technical inputs related to system design, dissemination and innovation support activities.

>CEB-UM is a long-standing R&D research centre from University of Minho combining fundamental life sciences with engineering to deliver value added products and processes for Food, Chemical, Biotech, and Environmental Industries. CEB brings together more than 280 researchers and holds a portfolio comprising about 1500 peer-reviewed publications in last 5 years, dozens of patents, more than 15 spin-off companies, and hundreds of funded R&D projects, demonstrating its strong contribution to the generation of new knowledge and the training of highly specialised HR. CEB-UM includes research groups specialising in bioprocess engineering; biomass, bioreactors and bioproducts for industrial applications; and molecular biotechnology and precision fermentation, supported by well-equipped laboratories and extensive analytical and characterisation infrastructure. Team project comprising 18 members, of whom 8 are critical HR, and 3 are new hires. This includes members with expertise in fermentation science, microbial physiology, bioprocess engineering, analytical chemistry, biomass and feedstock characterisation. A summary of the CV of critical HR (and their respective profiles in the project) is presented.

-Lígia Rodrigues: is an Associate Professor with Habilitation at the UMinho, with an internationally recognised research career in industrial biotechnology and bioprocess engineering, focused on microbial cell factories and bioprocesses for the production of high-value bioactive molecules. She has authored over 220 scientific publications and coordinated multiple national and European R&D projects. Within PRISMA, her expertise supports advanced bioprocess strategies and scale-up-oriented process development under industrially relevant conditions.

-Joana Rodrigues: is a tenure-track Assistant Professor at the UMinho and a senior researcher at CEB-UM, with an internationally recognised research career in synthetic biology and fermentation engineering. She holds a PhD in Bioengineering Systems developed in collaboration with MIT and has extensive experience in the design and optimisation of engineered microbial cell factories for

high-value compound production. Within PRISMA, her expertise is central to continuous fermentation strategies and scale-up-oriented bioprocess optimisation.

-José Teixeira: is a Full Professor of Biological Engineering at the UMinho, with an internationally recognised research career in industrial biotechnology and bioprocess engineering, focused on the valorisation of agro-industrial residues and bio-based processes. He has authored over 800 peer-reviewed scientific publications and supervised extensive PhD and post-doctoral research. Within PRISMA, his expertise is key to advanced bioprocess development, scale-up strategies and the translation of laboratory-scale innovations into robust industrial processes.

-António Vicente: is a Full Professor of Biological Engineering at the UMinho, with an internationally recognised research career in bioprocess engineering and advanced bio-based materials, particularly focused on advanced bioreactor systems and the valorisation of bioresources. He has authored over 380 scientific publications, holds several patents, and has supervised numerous PhD and post-doctoral researchers. Within PRISMA, his expertise supports the development of advanced processing technologies and scalable bioprocess solutions for sustainable industrial applications.

-Cristina Rocha: is an Assistant Researcher at the CEB-UM, and has an established research career in sustainable bioprocessing and bioresource valorisation. Her work focuses on green extraction and downstream technologies, cascading approaches for agro-food and algae by-products, and the development of sustainable bio-based materials. She has co-authored over 70 scientific publications, and supervised PhD, post-doctoral and MSc researchers. Within PRISMA, her expertise contributes to sustainable processing strategies and DPS process strategies.

>INESC TEC is an internationally oriented Associate Laboratory with more than 30 years of experience in research and technology transfer, with recognised expertise in artificial intelligence, data science and bioengineering. Its mission emphasises end-to-end knowledge generation and science-based innovation, ensuring the translation of data infrastructures and sensing frameworks into validated digital tools capable of operating under pilot and pre-industrial conditions. The institute benefits from extensive, well-equipped laboratories and a large multidisciplinary research environment, supporting the development, integration and validation of sensing pipelines, data analytics, modelling and optimisation methods. In addition, INESC TEC has an extensive and well-established track record in the participation and coordination of collaborative R&D projects. Team project comprising 22 members, of whom 2 are critical HR, and 2 are new hires. This includes members with expertise in data science and advanced analytics, AI/ML, signal processing, sensor integration and soft-sensing, machine vision, mathematical modelling and optimisation, process systems engineering, digital twins and decision-support systems. A summary of the CV of critical HR (and their respective profiles in the project) is presented.

-Davide Carneiro: is an Associate Professor at the Polytechnic Institute of Porto and a senior researcher at INESC TEC with internationally recognised research career in artificial intelligence, focused on machine learning, meta-learning and explainable AI. He has authored over 190 scientific publications and coordinated and/or participated in multiple R&D projects. Within PRISMA, his expertise is central to the development of AI-driven models, data-driven optimisation strategies and the integration of advanced analytics across the bioprocess workflow.

-Luís Coelho: is a senior researcher at INESC TEC and Coordinator of the Centre for Applied Photonics, with an internationally recognised research career in optical sensing technologies. His

expertise focuses on optical fibre sensors, thin-film and nanostructured coatings, and spectroscopy-based sensing for real-time monitoring of environmental and industrial systems. Within PRISMA, his role supports the development and integration of advanced optical sensing solutions for *in-situ* process monitoring and validation.

>**CITEVE** is a private, non-profit technological centre active since 1989, acting as a reference organisation for research and development, innovation and technology transfer within the Portuguese textile and clothing sector. Since 2017, CITEVE has served as the managing authority of the Portuguese Textile Cluster, supporting sector-wide engagement and the diffusion of innovative technologies. CITEVE is supported by extensive physical and technological infrastructure, including pilot plants equipped with advanced textile technologies covering dyeing, finishing and functionalisation, coating and lamination, and printing. In addition, its laboratories are internationally accredited for a wide range of tests and standards, providing robust performance assessment, including colour and fastness testing, and ensuring certification readiness. As a recognised technological centre providing testing, certification, consultancy and training services to industry, particularly SMEs, CITEVE has an extensive experience in the participation and coordination of collaborative R&D projects. Team project comprising 10 members, of whom 3 are critical HR, and 2 are new hires. This includes members with expertise in textile engineering and chemistry, pigment formulation and stabilisation, analytical and performance testing & compliance. A summary of the CV of critical HR (and their respective profiles in the project) is presented.

-**Rosa Maria Silva**: is a senior specialist at CITEVE with more than three decades of experience in textile chemistry, dyeing, finishing and colour management. Her expertise spans industrial dyeing and finishing processes, colourimetry, technology transfer and technical consultancy, with extensive involvement in national and European R&D and innovation projects. Within PRISMA, she supports advanced dyeing, finishing and colour validation activities, contributing to the industrial applicability and robustness of the developed solutions.

-**Margarida Fernandes**: is a senior technician at CITEVE with a background in applied biology and biotechnology. Her work focuses on laboratory and pilot-scale activities related to textile dyeing, including experimental support, pilot testing, and technical management of national and European R&D projects. Within PRISMA, she contributes to laboratory and pilot-scale validation activities, supporting dyeing trials, and formulation testing.

-**Ana Gonçalves** is a researcher at CITEVE with an established research career in bioprocess engineering and environmental biotechnology. Her expertise focuses on biomass valorisation, wastewater treatment and process optimisation, with experience spanning both academic and applied industrial contexts. Within PRISMA, her role supports pigment characterisation across different feedstocks, dye formulation development and testing, and the integration of sustainable bio-based solutions in textile and industrial applications.

>**ATB** is an industrial textile company specialising in technical dyeing, digital printing and advanced textile finishing, with decades of experience operating under full-scale industrial conditions. The company operates at industrial scale using state-of-the-art equipment for textile dyeing processes, with a strong focus on quality control and process consistency. Within the PRISMA project, ATB acts as an industrial demonstrator, and its physical and technological infrastructure as a dyeing, printing and finishing operator enables the execution of full-scale dyehouse compatibility

assessments and industrial production runs, which are critical for the final validation and market readiness of the project results. Team project comprising 14 members, of whom 2 are critical HR. This includes members with expertise in industrial textile dyeing processes, textile finishing operations, quality control and assurance, process optimisation, quality and compliance. A summary of the CV of critical HR (and their respective profiles in the project) is presented.

-Carla Silva: is a Chemical Engineer at ATB, with almost 3 decades of experience in the textile dyeing and finishing industry. Her professional background spans quality management, laboratory operations and customer liaison, including active involvement in production interfaces, pricing, sustainability initiatives and R&D activities. Within PRISMA, her role supports the industrial demonstrator activities at ATB, ensuring the development and optimisation of more environmentally friendly dyeing and finishing processes, conducting laboratory trials and quality assessment.

-Joana Faria: is a Textile Engineer, with experience in industrial and laboratory environments within the textile dyeing and finishing sector. Her professional activity focuses on the optimisation of dyeing and finishing processes, colour formulation and quality control, as well as the development and implementation of innovative and sustainable textile solutions at industrial scale. Within PRISMA, her role supports industrial and laboratory trials, quality control activities and the integration of novel dyeing technologies and sustainable processes under real production conditions.

It should be noted that that detailed information on each critical team member can be found in the CVs submitted along with the application for this project.

Based on the above, it can be concluded that the PRISMA consortium brings together complementary partners that collectively cover all key stages of the project's value chain, forming a fully integrated consortium. **Chainreactor** leads the technological and industrial development, providing scalable bioreactor solutions and coordinating the transition from laboratory research to pre-industrial and industrial implementation. **CEB-UM** ensures the scientific robustness of the upstream bioprocesses through its expertise in fermentation science and bioprocess engineering, while **INESC TEC** contributes advanced capabilities in data science, artificial intelligence and digital process intelligence. **CITEVE** adds critical textile-sector know-how, pilot-scale infrastructure and accredited testing and certification capacity, and **ATB** completes the consortium by acting as an industrial demonstrator, enabling full-scale dyehouse validation under real manufacturing conditions. Finally, it should be noted that, although **Octarine Bio** is not a formal beneficiary of the consortium, it is a strategic project partner that anchors PRISMA to a concrete industrial use case. As the developer of the PurePalette™ bio-based pigment platform, Octarine Bio provides validated pigment-producing microorganisms, advanced know-how in synthetic biology and precision fermentation, and target performance and cost benchmarks for textile applications, ensuring that the project developments are aligned with real market and industrial needs. A Letter of Intent (LOI) from Octarine Bio is submitted as an annex to this application.

2.2. Identification of Activities

Activity No.	Activity Title	Classification	Responsible beneficiary	
			Nº	Abbrev

1	Continuous fermentation development	Industrial Research	2	CEB-UM
2	Feedstock characterisation, screening and validation	Industrial Research	2	CEB-UM
3	AI-Driven process intelligence development	Experimental Development	3	INESC TEC
4	Bioreactor design development	Experimental Development	1	Chainreactor
5	DSP process development	Experimental Development	1	Chainreactor
6	Bioreactor scale-up	Experimental Development	1	Chainreactor
7	Textile application & validation	Experimental Development	4	CITEVE
8	Industrial textile dyeing process demonstration	Experimental Development	5	ATB
9	Promotion, dissemination & IP	Dissemination and Promotion	1	Chainreactor
10	Project Management	Technical Management	1	Chainreactor
11	CMO Build	Productive Innovation	1	Chainreactor

Provide a concise description of the **structure and logic of the work plan**. Identify the **main activities foreseen** and their **interdependencies**.

PRISMA is planned to occur over 36 months, starting in January 2027, following a clear path from feedstock characterization and preparation to fermentation to process optimization and digitization to scaling up production to industrial level validation. The project's work plan is structured into 11 major activities, which are interdependent, covering Industrial Research, Experimental Development, Dissemination and Promotion, Technical Management, and Productive Innovation. Each activity includes a set of tasks with associated milestones and deliverables. The work plan starts with upstream bioprocess development and feedstock validation activities (**A1–A2**), where baseline fermentation performance is established and alternative agro-industrial feedstocks are screened and optimised. These activities generate the foundational biological, process and compositional data required for all downstream developments. In parallel, process intelligence, bioreactor engineering and downstream processing activities (**A3–A5**) are developed in close interaction with upstream tasks. Data generated during fermentation campaigns feeds the process-intelligence activities (**A3**), enabling model development and decision-support tools. Advanced manufacturing and bioreactor design (**A4**) provide the physical infrastructure required for predictable scale-up, while downstream processing optimisation (**A5**) ensures recovery efficiency, stability and cost control. These activities are mutually dependent and are iterated based on upstream performance and application requirements. All technological components converge in the scale-up and integration activities (**A6**), which form the central validation hub of the project. During this phase, upstream processes, bioreactor systems, monitoring frameworks and downstream routes are jointly validated under pilot and pre-industrial operating conditions, confirming robustness, reproducibility and transferability. Following technical scale-up, the work plan transitions to application and industrial-context validation activities (**A7–A8**). Pilot textile application trials and standardised testing (**A7**) establish performance benchmarks, which are subsequently validated under real industrial dyeing conditions (**A8**). Results from these activities provide feedback to upstream processing and formulation choices, ensuring alignment with industrial requirements. The work plan culminates in the CMO Build activity (**A11**), which integrates the validated outputs of all previous activities into a 2,000 L CMO-ready bioproduction facility, serving as the project's principal productization and commercialisation vehicle.

2.3. Description of Activities

Activity No.	Activity Title	
A1	Continuous fermentation development	
	Start date: 02.01.2027	End date: 31.12.2027

Objective	
A1 aims to establish the performance benchmark for pigment production under standard 72h fed-batch conditions to serve as the project's technical control group, as well as, to validate a long-term (21-day) continuous fermentation system using cell-retention technology, targeting at least 80% of the baseline titre productivity.	
Description of the activity	
<u>T1.1 Baseline fermentation characterization M1-M6 Lead: CEB-UM; Participants: Octarine Bio, Chainreactor & INESC TEC</u> This task focuses on establishing the standard performance baseline using Octarine Bio proprietary yeast strains. The task will be carried out in close collaboration with INESC TEC within the scope of Activity A3, ensuring interdisciplinary integration between fermentation development, process analysis and data-driven process intelligence. Fermentations will be conducted in lab-scale stirred-tank bioreactors (5-20 L) using analytical-grade minimal media and high-concentration dextrose (>500 g/L) as the primary carbon source under 72 h fed-batch operation mode. Carbon evolution rate, oxygen uptake rate, respiratory quotient, specific yields, titres and productivities will be monitored. Analytical monitoring will be performed via HPLC-DAD/RID for routine quantification of titres and residual sugars. Additionally, LC-MS/MS will be employed for the structural validation and peak purity analysis of the pigments, ensuring the baseline profile matches the reference standards provided by Octarine Bio. Additionally, spectrophotometric assays will be used to assess colour consistency, and biomass evolution will be monitored via optical density and dry cell weight measurements, enabling correlation with metabolic exchange data and data-driven modelling inputs developed within A3. As output, a comprehensive baseline performance map will be obtained. This data will serve as the control group for all subsequent tasks and activities, providing the target benchmarks that continuous processes must aim to match or exceed. The achievement of less than 10% variance in pigment yields relative to these reference benchmarks will constitute a key technical milestone, authorising the transition from fed-batch operation to continuous fermentation mode in subsequent tasks. <u>T1.2 Continuous fermentation development M5-M12 Lead: CEB-UM; Participants: Chainreactor, INESC TEC</u> This task focuses on the conversion of the baseline 72h fed-batch process into a long-term continuous operation. Using the baseline data from T1.1, the team will implement cell-retention strategies (perfusion, settler) to decouple cell growth from pigment production and maximize volumetric productivity. Initial trials will use 20 L bioreactors provided by Chainreactor to optimize key operational parameters, specifically dilution rates and bleed rates, to maintain a high-density steady-state culture. The core of this task is the 21-day continuous operation validation. The objective is to demonstrate process robustness and stability over extended periods, ensuring that the system can maintain a constant output without loss of genetic stability, metabolic drift or loss of viability. The team will monitor the steady-state performance to ensure that the transition from fed-batch to continuous mode does not compromise the biochemical efficiency of the yeast strains. The primary goal is to achieve a titre retention of >80% in continuous mode compared to the 72h fed-batch benchmark established in T1.1 (this is the "go/no-go" point for scaling up and introducing alternative feedstocks). To monitor long-term steady-state, flow cytometry will be employed to assess cell viability and population health. Pigment quality and profile stability will be verified periodically through UPLC-MS to detect any potential metabolic drift or degradation products during the 21-day run.	
Effort in FTEs and investments (per beneficiary)	
Chainreactor: 17,3 FTEs CEB-UM: 25,62 FTEs INESC TEC: 12 FTEs	
Description of deliverables and milestones	
D1.1: Baseline fermentation performance map; D1.2: Continuous process optimization report; M1.1: Fed-batch Baseline Validated; M1.2: Successful Steady-State Achievement; M1.3: Successful completion of a 21-day continuous campaign.	

Activity No.	Activity Title	
A2	Feedstock characterisation, screening and validation	
	Start date: 02.01.2027	End date: 31.12.2027
Objective		
A2 focuses on the systematic identification, characterization and selection of alternative, low-cost carbon sources (e.g., sugar cane molasses, fruit pomace, oat bran/flour) to replace the analytical-grade dextrose baseline. It addresses the economic bottleneck of precision fermentation by utilizing local side-streams as sustainable feedstocks. The		

overarching goal is to achieve a media reformulation targeting a 40% to 60% reduction in raw material costs, while ensuring full metabolic compatibility with engineered yeast cell factories and maintaining high-productivity standards.

Description of the activity

T2.1 Analysis of alternative carbon sources | M1-M4 | Lead: CEB-UM; Participant: Chainreactor

The team will conduct a comprehensive screening of various low-cost alternative carbon sources (at least 3-5 priority side-streams for lab-scale analysis), performing quantitative analysis of total and individual sugar profiles. The analysis also includes measuring total solids, ash content, nitrogen content, phosphorus, and essential minerals to determine the nutritional adequacy of each source for the target yeast strains. As an output, a comprehensive characterization database and a ranking matrix of alternative feedstocks based on fermentable sugar content and regional availability will be obtained.

For solid or heterogeneous biomass, the material will undergo mechanical milling followed by aqueous extraction or thermochemical pretreatment and subsequent quantitative acid hydrolysis to release both free sugars and matrix-bound carbohydrates (e.g., starch, cellulose, hemicellulose) into the liquid phase. For liquid or syrup-based side-streams, samples will be diluted, filtered, or centrifuged to remove suspended solids. Quantitative analysis of the resulting total sugar profiles will be performed using HPLC-RID. Total crude protein and nitrogen content will be determined by Kjeldahl digestion. Phosphorus and essential minerals such as magnesium, potassium and calcium will be quantified using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) following nitric acid digestion of the raw material. Total Solids and Ash Content will be determined gravimetrically according to standard National Renewable Energy Laboratory (NREL) analytical procedures to standardize the feedstock concentrations.

T2.2 Inhibitor profiling | M2-M5 | Lead: CEB-UM; Participant: Chainreactor

The team will screen selected feedstocks for common fermentation inhibitors, specifically focusing on furan derivatives (furfural, hydroxymethylfurfural), organic acids (acetic, formic, levulinic acids), and phenolic compounds. These inhibitors will be quantified by HPLC with a DAD. The resulting detailed chemical map for each feedstock will inform the refining or detoxification requirements necessary for the subsequent biological phases.

T2.3 Media preparation and strain robustness for alternative feedstocks | M2-M9 | Lead: CEB-UM; Participant: Chainreactor & Octarine Bio

This task focuses on converting raw side-streams into standardized fermentable media and, where required, on aligning the yeast's metabolic capabilities with the complex chemical profile of the selected side-streams. Raw side-streams will be processed into standardized fermentable hydrolysates through appropriate pre-treatment, hydrolysis, clarification and, where necessary, detoxification steps, aiming to obtain consistent sugar profiles and nutrient compositions suitable for fermentation. In parallel, the compatibility between the selected hydrolysates and the yeast strains will be systematically assessed, addressing tolerance to potential inhibitory compounds and the efficient co-utilization of mixed sugar streams. Adaptive laboratory evolution and/or rational metabolic engineering will be applied to improve strain robustness under the chemical conditions imposed by real feedstock-derived media. The combined optimization of media formulation and strain performance will result in a validated, low-cost fermentation medium and a biologically robust production system, ensuring readiness for long-term continuous fermentation and subsequent scale-up activities. The strategy specifically targets the replacement of expensive synthetic nitrogen sources and salts with side-stream-derived nutrients, aiming for a 40% to 60% reduction in total raw material costs compared to the synthetic dextrose baseline. Only the feedstocks that meet both the stability criteria and the targeted cost-reduction thresholds will be selected as primary or fallback candidates for the subsequent validation phases.

T2.4 Continuous fermentation with alternative feedstocks | M6-M12 | Lead: CEB-UM; Participant: Chainreactor, INESC TEC & CITEVE

This task integrates the optimized hydrolysates from T2.3 and, if necessary, the adapted strains into the continuous fermentation setup established in T1.2. The focus is on evaluating the long-term impact of side-stream impurities on steady-state stability, cell-retention efficiency (membrane fouling/settler performance), and pigment quality. A 21-day continuous campaign will be performed to ensure that the transition to low-cost feedstocks maintains >85% of the baseline titre and fulfils the specific pigment yield and colour reproducibility standards, ensuring that the use of heterogeneous feedstocks does not compromise the chromatographic purity and colour consistency of the target compounds. This task will be supported by complementary lab-scale textile dyeing and substrate characterisation assays performed by CITEVE, providing iterative feedback on tintorial affinity and colour performance of pigments produced from different feedstocks. This input will also guide feedstock selection and process optimisation prior to downstream processing and scale-up. Moreover, A2 will be carried out in close collaboration with INESC TEC within the scope of A3.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 22,45 FTEs | **CEB-UM:** 26,12 FTEs | **INESC TEC:** 3 FTEs | **CITEVE:** 18,45 FTEs

Description of deliverables and milestones

D2.1: Comprehensive chemical characterization dataset of agri-food and industrial side-streams; **D2.2:** Inhibitor mapping and threshold definition; **D2.3:** Standardized hydrolysate preparation and media reformulation protocol; **M2.1:** candidate streams mapped and screened; **M2.2:** Feedstock selection (at least 3 screened) for continuous fermentation trials; **M2.3:** Strain/Media compatibility and robustness confirmed.

Activity No.	Activity Title	
A3	AI-Driven process intelligence development	
	Start date: 01.03.2027	End date: 01.06.2029
Objective		
A3 aims to develop an integrated AI-driven process intelligence framework enabling real-time and continuous monitoring, optimisation, and control of fermentation processes along with productivity and quality control of the final-coloured products. The activity combines data infrastructure, model predictive control (MPC) combined with reinforcement learning, digital twins, and transfer learning to create adaptive and scalable bioprocess systems. It supports a Manufacturing-as-a-Service model and ensures optimisation not only of bioreactor performance but also of downstream pigment application in textile processes, contributing to efficient and industrially relevant bio-based production.		
Description of the activity		
<u>T3.1 Bioprocess data lake development and experimental data architecture M3 – M9 Lead: Chainreactor; Participants: INESC TEC, CEB-UM</u>		
This task establishes the data infrastructure backbone to support AI-driven optimisation of fermentation processes across the PRISMA platform. A scalable bioprocess data lake will be designed and deployed to enable real-time ingestion, storage, and management of heterogeneous data streams from Chainreactor bioreactors, laboratory systems, and downstream validation environments. This includes control and monitoring of process parameters (e.g., pH, temperature, dissolved oxygen), <i>in situ</i> sensor data, experimental metadata, and analytical results. The task also includes the definition of a standardised data architecture, ensuring consistency, interoperability, and traceability across all experimental activities. Integration with CEB-UM's LIMS system will enable full linkage between experimental design, execution, and results. Data governance mechanisms will be implemented following FAIR principles, ensuring data quality, accessibility, and reusability for AI model development.		
<u>T3.2 Sensing solutions and bioprocess data acquisition frameworks M3 – M24 Lead: INESC TEC; Participants: Chainreactor, CEB-UM</u>		
This task develops the sensor and data acquisition framework required to enable reliable, real-time monitoring of fermentation processes in Chainreactor bioreactors. The definition of sensing requirements will be carried out in close collaboration with other activities, particularly A1, A2, A4 and A6, to ensure alignment between process needs, reactor configurations, and AI modelling requirements. This includes identifying the critical variables to be monitored and specifying the corresponding sensing technologies and data acquisition strategies. Based on these requirements, a set of relevant sensing technologies will be selected, developed and integrated in industrial settings.		
<i>T3.2.1. Sensors for precision fermentation: pH, Temperature and Dissolved oxygen:</i> This sub-task focuses on the deployment and integration of sensing technologies available in the INESC TEC portfolio for real-time, continuous, <i>in situ</i> monitoring of precision fermentation processes for microbial pigment production. Accurate monitoring of key cultivation parameter (pH, temperature and dissolved O ₂) is essential to ensure stable microbial growth, metabolic performance and pigment synthesis, particularly under long-duration operation and variable process conditions. Culture pH is a critical variable influencing microbial metabolism and pigment production. Given the use of saline, low-cost mineral media, conventional glass pH electrodes are unsuitable due to limited accuracy and stability. Instead, a custom fluidic electrochemical pH sensor will be employed, based on a poly(thionine) film fabricated by electrochemical polymerisation. This platform allows rapid, automated and stable potentiometric pH measurements under high-salinity conditions and is expected to maintain sensitivity throughout continuous fermentation campaigns of at least 21 days. Oxygen transfer is a well-known limitation in aerobic bioprocess scale-up. Continuous dissolved O ₂ monitoring will therefore be implemented using an optical sensor based on oxygen-sensitive fluorescent dyes immobilised in gas-permeable membranes. Optical excitation and signal transduction will enable reliable, non-invasive monitoring of oxygen availability during fermentation. Temperature sensors will be integrated to ensure precise thermal control and to compensate for temperature-dependent drift in pH and dissolved O ₂ measurements.		

Additional temperature sensing points may be deployed within Chainreactor bioreactors to account for geometry-dependent thermal gradients.

T3.2.2. On-site sensor for monitoring microbial biomass: After optimization of nutrient feed rates and considering the bioreactor dimensions (space), optical density (OD) measurements will allow the quantitative assessment of the cultured microbial population. Advanced optical schemes will be designed and implemented through the selection of highly efficient light sources, including high-performance LEDs and lasers, to precisely match microbial spectral bands. Arrays of emitter-detector configurations will be tested to maximize penetration depth, spatial resolution, and signal integrity. The microbial biomass concentration will be determined by measuring the OD and converting it to a dry weight concentration via a calibration curve (OD for a serially cultured sample with a known dry weight concentration). The microbial growth will be continuously measured over the cultivation period by measuring the turbidity inside the bioreactor (under agitation). The system can be implemented directly on low-volume bioreactors by integrating dedicated optical windows. If the microbial population saturates the OD signals, an auto sampling strategy will be implemented to reduce the optical path on external sensing units placed side-by-side to the bioreactors.

T3.2.3. Sensors for bio-pigments colour profiling and concentration: This sub-task focuses on the development and deployment of an online optical sensing tool for real-time monitoring of bio-pigment colour profile and concentration during fermentation and downstream processing. Pigment production is the final output of the upstream microbial fermentation, and its quality must be assessed continuously to ensure consistency, purity and process robustness. The approach relies on spectrophotometric analysis of the characteristic absorption spectra of each pigment class, enabling simultaneous estimation of pigment concentration, based on absorbance intensity, and spectral purity, through detection of single versus multiple absorption bands across the visible range. These sensors will be deployed at strategic locations, including in the upstream bioreactor for continuous monitoring of pigment accumulation and downstream, after pigment extraction and separation, to support quality control.

The sensing system will integrate a multi-wavelength light source covering the visible spectral region, with independently modulated wavelengths to improve signal discrimination and robustness. Transmitted or scattered light will be collected by photodetectors to reconstruct wavelength-resolved absorbance spectra. Sensor performance and accuracy will be validated against standard offline analytical methods, such as HPLC, available at CEB-UM, ensuring reliable operation under the process conditions defined in A1 and A2.

T3.2.4. Sensors validation, device integration and data communication: This task focuses on the *in-situ* validation of the developed sensor systems at lab-scale relevant conditions before industrial scale-up and demonstration (A6). This will be carried out by initially assessing the performance, stability, and reliability of each sensor in comparison with standard reference measurements. Based on these results, the sensing solutions will be calibrated and refined to ensure robustness, scalability, and suitability for long-term continuous operation. Subsequently, the individual sensors will be jointly integrated and validated as a multi-parametric monitoring system, enabling continuous, real-time acquisition of fermentation and product quality data. The task includes the integration of the sensors with the bioreactor hardware and with the data platform developed in T3.1, ensuring synchronized data acquisition, basic signal processing, and reliable data transmission to the digital twin environment. This integrated setup will support process monitoring, optimisation, and early detection of deviations during continuous microbial fermentation, enabling timely intervention/adjustments.

T3.3 Bioprocess surrogate models for process optimisation | M6 – M18 | Lead: INESC TEC Participants: Chainreactor, CEB-UM & CITEVE

This task focuses on the development of surrogate models to support the understanding and optimisation of the bioprocess by capturing the relationships between operating conditions and key performance indicators. The models will be built using experimental data generated in A1, A2, A4 and A6, together with sensor data, process variables and analytical results stored in the data lake (T3.1). Machine learning and optimisation techniques will be applied to generate simplified, data-driven representations of the process that enable sensitivity analysis and efficient exploration of the operating space.

The surrogate models will be used iteratively in close interaction with experimental activities, providing insights that guide the definition of targeted experiments and sampling strategies. This model-guided approach will progressively refine process knowledge, reduce experimental effort, and support subsequent digital-twin development and control activities in T3.4. This will reduce the experimental burden (costs, time) associated with trial-and-error optimisation in the sense that this model-guided experimentation approach will ensure that new campaigns are focused on the most relevant regions of the design space, accelerating process learning and increasing the value extracted from each experimental run.

T3.4 Digital Twin and Control Agent | M6 – M30 | Lead: INESC TEC; Participants: Chainreactor & CEB-UM

This task develops a digital twin-based control framework enabling real-time, closed-loop optimisation of fermentation processes across different bioreactor configurations. Each bioreactor will be instantiated as a dedicated digital twin, capturing its specific geometry, scale, operating conditions, and dynamic biological behaviour. These digital twins will be developed by combining mechanistic process knowledge with data-driven models, calibrated using experimental data from A1, A2, A4 and scale-down models from A6, ensuring accurate representation of process dynamics across systems. These digital twins will include data driven process intelligence tools that integrate multi parametric data streams to characterize the biological and operational state of the fermentation process. Building on the physical sensing infrastructure developed in T3.2 and the data platform established in T3.1, this task aims to transform raw sensor data into meaningful process indicators that support monitoring, optimization, and decision making across different operating conditions. Advanced data analytics and machine learning techniques will be applied to combine online measurements with analytics methods, enabling the assessment of key performance indicators such as process stability, productivity trends, pigment formation dynamics, and colour profile evolution. Developed models will provide an integrated interpretation of the process behaviour, capturing complex interactions between biological responses, operational parameters, and product quality attributes. The resulting process intelligence layer will deliver consistent, real-time insights into fermentation performance and system health, supplying reliable inputs to downstream optimization, digital twin calibration, and control activities. On top of the digital twin framework, advanced control strategies will be implemented, including model predictive control (MPC) and reinforcement learning (RL) agents. These agents will interact with the digital twins and real systems to learn optimal control policies, dynamically adjusting process parameters (e.g., feed rates, aeration, agitation) according to the evolving biological state and process objectives. A key innovation of this task is the implementation of a transfer learning approach across digital twins. The information generated by each individual twin will be contextually structured and exploited to enable knowledge reuse across different bioreactors. This allows the identification of shared patterns and scale-invariant behaviours, supporting faster adaptation to new reactor configurations and promoting continuous improvement of control strategies over time. The framework operates as a continuous learning loop, where data from experiments and operations are fed back into models, enabling progressive improvement of optimisation and control strategies. The framework will be progressively validated from simulation to real fermentation environments, demonstrating robust and adaptive control across different scales and operating conditions.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 33,65 FTEs | **CEB-UM:** 5,05 FTEs | **INESC TEC:** 189,5 FTEs

Description of deliverables and milestones

D3.1: Data platform operational; **D3.2:** Technical Report on Sensor Suite Validation; **M3.1:** Bioprocess datalake deployed; **M3.2:** Integration and validation of sensors functionality; **M3.3:** Digital twins and transfer learning model.

Activity No.	Activity Title	
A4	Bioreactor design development	
	Start date: 02.01.2027	End date: 31.12.2027
Objective		
A4 aims to adapt, refine, and validate a bioreactor design specifically adapted to continuous microbial pigment production, ensuring alignment with the process requirements defined in upstream fermentation activities. By translating biological and operational specifications into engineering solutions and validating alternative configurations at small scale, this activity aims to deliver a consolidated and scalable reactor design, formally frozen and ready for pilot-scale implementation in subsequent scale-up activities.		
Description of the activity		
<u>T4.1 Design specification M1 – M3 Lead: Chainreactor; Participants: CEB-UM & INESC TEC</u>		
This task focuses on the definition of the technical and functional requirements for the bioreactor systems to be developed within the project, considering the operational needs of continuous fermentation processes for microbial pigment production. This includes operating modes, target productivities, oxygen transfer requirements, mixing performance, shear constraints, and compatibility with saline, low-cost fermentation media. Engineering requirements will be formalised covering mechanical design, materials of construction, sensor and actuator integration, sterilizability, clean-in-place (CIP) procedures, and modularity for scale-up. The task will involve the preparation of detailed design briefs, preliminary engineering drawings, and component specifications, which will define the basis for prototype development.		

T4.2 Pigment-specific geometry and configuration development | M3 – M12 | Lead: Chainreactor; Participants: INESC TEC

This task addresses the development and refinement of bioreactor geometries and internal configurations tailored to the specific requirements of pigment-producing microbial systems. Based on the design specifications defined in T4.1, this task includes optimising internal geometry for homogeneous mixing under varying viscosities, integrating optical access points for inline colour monitoring, and evaluating light-exposure requirements for photosensitive compounds. Iterative design adjustments will be performed and informed by experimental feedback from fermentation trials, enabling the progressive optimisation of reactor configuration for pigment production performance and operational robustness.

T4.3 Small-scale comparative testing and design validation | M6 – M12 | Lead: Chainreactor; Participants: CEB-UM

This task focuses on the experimental evaluation and benchmarking of prototype bioreactor designs against standard stirred-tank reactor configurations at the 5 L scale (for controlled benchmarking and rapid iteration of specific design features). Comparative testing will assess mixing efficiency, mass-transfer performance, sensor reliability, and pigment stability under representative process conditions. The task will involve repeated experimental runs using alternative configurations and operating regimes. In addition, down-scale testing in preparation for increased working volumes will be conducted to identify potential scale-related constraints prior to pilot-scale implementation.

T4.4 Design freeze for scale-up | M10 – M12 | Lead: Chainreactor

This task concludes the design development phase by consolidating the results of the comparative testing into a finalised bioreactor design ready for scale-up and fabrication of a 200 L pilot unit. The selected design will be formalised through detailed engineering documentation, including final drawings, technical specifications, and assembly guidelines.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 65 FTEs | **CEB-UM:** 2,5 FTEs | **INESC TEC:** 10 FTEs

Description of deliverables and milestones

D4.1: Reactor design dossier; **D4.2:** Sensor integration and PCB/PCB interfaces report; **D4.3:** Comparative test report; **M4.1:** Bioreactor design requirements defined; **M4.2:** Pigment-Specific Bioreactor Configuration Validated at Small Scale; **M4.3:** Final bioreactor design frozen for scale-up & Sensors validated in lab;

Activity No.	Activity Title	
A5	DSP process development	
	Start date: 01.09.2027	End date: 31.12.2028
Objective		
A5 aims to design, develop and validate an integrated downstream processing (DSP) strategy for the efficient recovery, purification and stabilisation of microbial pigments produced under continuous fermentation conditions. The outcome of A5 will be a validated, application-ready pigment format and a consolidated DSP framework technically prepared for subsequent scale-up and industrial validation activities.		
Description of the activity		
<u>T5.1 DSP route definition and process requirements M9–M12 Lead: Chainreactor; Participants: Octarine Bio, CEB-UM & CITEVE</u>		
This task focuses on the definition of downstream processing (DSP) routes suitable for the recovery and stabilization of microbial pigments produced under continuous fermentation conditions. Based on the characteristics of the fermentation broth obtained in A1 and A2, alternative DSP strategies will be defined, considering operational compatibility with continuous production, pigment physicochemical properties, and target application requirements foreseen by CITEVE, ATB and Octarine Bio. This task provides an integrated DSP process framework, ensuring technical coherence across all downstream operations.		
<u>T5.2 Study of filtration and separation processes M12–M18 Lead: Chainreactor; Participants: CEB-UM & CITEVE</u>		
This task addresses the development and optimisation of solid-liquid separation steps required to efficiently remove biomass and suspended solids from the fermentation broth. Separations approaches, including tangential flow filtration (TFF), centrifugation, and pre-treatment strategies, will be tested and compared under representative process conditions. The performance of each approach will be evaluated in terms of separation efficiency, robustness under continuous operation, fouling propensity, and impact on pigment quality.		

T5.3 Pigment extraction and purification development | M12–M24 | Lead: Chainreactor; Participants: CITEVE

This task focuses on the development of extraction and purification strategies to recover microbial pigments. Several extraction methods will be evaluated, including solvent-based extraction, adsorption/desorption systems, and aqueous phase manipulation, with the objective of maximizing pigment recovery while preserving colour properties and chemical stability. Experimental optimisation will address compatibility with scalability, regulatory constraints and cost targets aiming to reduce energy use by >15%.

T5.4 Pigment stabilisation and drying process development | M15–M24 | Lead: Chainreactor; Participants: CITEVE

This task addresses the stabilisation and final conditioning of purified pigments to ensure shelf life, ease of handling, and suitability for downstream application and testing activities. Different stabilisation and drying approaches, including spray-drying and alternative concentration or drying techniques, will be evaluated at small scale. The activity will assess the impact of process conditions on pigment stability, colour retention, and physical form, ensuring compatibility with pilot-scale implementation and downstream industrial validation.

To highlight that A5 will be supported by preliminary formulations developed and application at lab-scale textile dyeing assays, including the textile substrate characterisation (colorimetric characterization) performed by CITEVE, providing feedback on how DSP choices influence colour performance and application suitability.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 45,1 FTEs | **CEB-UM:** 11,7 FTEs | **CITEVE:** 25,1 FTEs

Description of deliverables and milestones

D5.1: DSP route definition and requirements report; **D5.2:** Solid–liquid separation and clarification optimisation report; **D5.3:** Pigment extraction and purification protocol; **D5.4:** Stabilised pigment formulation and drying report; **M5.1:** DSP route defined and validated at lab scale; **M5.2:** Robust clarification strategy validated; **M5.3:** Extraction and purification targets achieved; **M5.4:** Application-ready pigment format validated.

Activity No.	Activity Title	
A6	Bioreactor scale-up	
	Start date: 01.01.2028	End date: 31.12.2029
Objective		
A6 aims to experimentally validate the scalability of the continuous microbial pigment fermentation process from pilot to pre-industrial scale. This activity aims to demonstrate reliable transfer of the biological system and operating regimes to bioreactor volumes from 200 L to 2000 L, while maintaining process stability, productivity, and pigment quality, supported by robust monitoring and control frameworks to enable subsequent industrial demonstration.		
Description of the activity		
A6 will be supported by complementary lab-scale textile dyeing and substrate characterisation assays performed by CITEVE, using representative pigment samples obtained during scale-up campaigns to verify colour reproducibility and application-relevant properties across scales.		
<u>T6.1 Scale-up to 200 L and 500 L M13-M24 Lead: CEB-UM; Participant: Chainreactor, CITEVE & INESC TEC</u>		
This task focuses on the sequential scale-up of the continuous fermentation process, transitioning from the optimized lab-scale environment to pilot-scale bioreactors provided by Chainreactor. The objective is to validate the robustness of the engineered yeast strains and the consistency of the alternative hydrolysates at industrially relevant volumes. Initial scale-up will be performed in a 200 L stirred-tank bioreactor. This phase focuses on fine-tuning the oxygen transfer rates (kLa) and heat dissipation, which are critical for maintaining pigment metabolic pathways in larger volumes. The continuous feeding strategy, utilizing the standardized side-stream hydrolysates (from A2), will be monitored for 14-day steady-state periods to evaluate cell-retention efficiency and potential foaming issues common with complex feedstocks. Upon successful validation at 200 L, the process will be scaled to a 500 L bioreactor for an extended 21- to 30-day demonstration run. This stage serves as a pre-industrial demonstration, where the focus shifts to process stability and downstream integration. Key performance indicators (KPIs) including volumetric productivity, pigment titre, and biomass yield will be benchmarked against the lab-scale baseline. During both stages, Process Analytical Technology (PAT), including online monitoring of dissolved oxygen, pH, temperature, and off-gas analysis (carbon evolution rate, oxygen uptake rate), will be utilized to ensure real-time process control. Samples will be		

collected at regular intervals for HPLC analysis to confirm that the 40-60% feedstock cost-reduction does not negatively impact the pigment's chemical and optical profile at scale.

T6.2 2000 L scale-up and pre-industrial demonstration | M25–M36 | Lead: Chainreactor; Participants: CEB-UM, INESC TEC & CITEVE

This task focuses on the experimental scale-up of the validated continuous microbial pigment fermentation process to pre-industrial scale, using bioreactor volumes of up to 2000 L. Building on the operating regimes and performance benchmarks established in T6.1, its objective is to verify process robustness, reproducibility, and feasibility under conditions approaching industrial deployment. Scale-dependent constraints related to mixing, oxygen transfer, heat removal, feeding strategies, and cell-retention performance will be assessed. Operating regimes validated at pilot scale will be transferred to the 2000 L system and tested in representative long-duration continuous campaigns. The primary technical objective is to demonstrate stable steady-state operation while maintaining at least 80% of the volumetric productivity observed at pilot scale, confirming process transferability across scales. During these campaigns, fermentation will be supported by monitoring and control tools developed in A3, applied as operational instruments for process supervision. Experimental data will be used to characterise process stability, productivity retention, and pigment quality consistency using combined online monitoring and offline analytical assays. The task addresses process-level scalability, while the dedicated evaluation of sensing, monitoring, and control frameworks at large scale is undertaken separately in T6.3.

T6.3 Control and optimization of large-scale fermentation process | M15 – M30 | Lead: INESC TEC; Participants: Chain Reactor

This task focuses on the experimental validation of sensing, monitoring, and data interpretation frameworks when transferred from laboratory and pilot scales to large-scale continuous fermentation systems. Building on the sensor technologies, data acquisition pipelines, and modelling frameworks developed in Activity A3, its objective is to assess their robustness, signal quality, and reliability under large-volume bioreactor conditions.

Sensing solutions for pH, T, and dissolved O₂ will be adapted and tested at a large scale to evaluate measurement stability, redundancy strategies, and resistance to long-term drift during the extended operation period. Given the increased optical path lengths and geometric constraints of large-scale bioreactors, the feasibility and limitations of optical biomass monitoring will be assessed. If necessary, alternative sensing configurations, including bypass-based optical measurement systems with controlled optical paths, will be evaluated to ensure reliable biomass quantification. Optical sensing approaches for pigment concentration and colour profile monitoring will also be assessed under scale-up conditions and benchmarked against reference analytical methods to verify accuracy and long-term stability. The outcomes of this task will ensure that large-scale fermentation data are sufficiently reliable and informative to support process supervision, modelling, and decision-making during scale-up and pre-industrial operation. The validation of the large-scale sensing and monitoring framework will be based on quantitative criteria defined prior to each scale-up campaign, including data availability above 95% during continuous operation, acceptable long-term signal drift over campaigns of at least 21 days, predefined maximum deviation from reference offline measurements, and near-real-time data transmission compatible with process supervision and decision-making.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 234,40 FTEs | **CEB-UM:** 90 FTEs | **INESC TEC:** 58,5 FTEs | **CITEVE:** 18 FTEs

Description of deliverables and milestones

D6.1: Pilot-scale scale-up validation report (200 L and 500 L); **D6.2:** Pre-industrial scale-up and process robustness report (2000 L); **D6.3:** Large-scale monitoring and sensing tools validation report; **M6.1:** Pilot-scale continuous operation validated (M18, M24); **M6.2:** Pre-industrial continuous fermentation demonstrated . **M6.3:** Sensors for monitoring and control validated at large scale.

Activity No.	Activity Title	
A7	Textile application & validation	
	Start date: 01.12.2027	End date: 30.06.2029
Objective		
A7 aims to develop, optimise and validate textile dyeing, coating, and printing processes based on PRISMA pigments, assessing their applicability and performance across different fibre types. This activity aims to establish robust, fibre-specific finishing formulations and processing protocols at laboratory and pilot scale, ensuring adequate colour		

strength, fixation, and fastness properties, and providing the technical basis for subsequent large-scale textile validation and industrial demonstration.

Description of the activity

T7.1 Development of formulations for textile finishing application | M12 – M21 | Lead: CITEVE; Participants: Chain Reactor, CEB-UM & ATB

This task focuses on the development and preliminary validation of finishing formulations based on PRISMA pigments for textile dyeing, coating, and printing applications. An initial screening of the produced pigments will be carried out to identify the most promising candidates for application on different textile substrates (e.g. cotton, polyester, wool, lyocell, and their blends). Laboratory-scale textile finishing trials will be performed using representative dyeing (exhaust and impregnation), coating, and printing techniques. Dyeing protocols and formulation parameters will be adapted to the specific physicochemical characteristics of each fibre type. For coating and printing applications, water-based formulations will be developed using bio-based polymer matrices, including appropriate cross-linkers and rheological modifiers. Different pigment concentrations will be assessed to evaluate colour development and process compatibility. Selection of the most suitable pigments and formulations will be based on colour strength (K/S values), preliminary fastness performance (washing and light), and formulation behaviour, including dispersibility, stability, and absence of sedimentation or phase separation. Where relevant, both formulated pigment preparations and clarified fermentation broths (after cell removal and appropriate conditioning) will be explored to assess their suitability for direct textile application. The developed formulations will be further characterised in terms of physicochemical stability and homogeneity using standard analytical techniques for particle size, dispersion stability, and sedimentation behaviour.

T7.2 Laboratorial and pilot scale textile finishing trials | M13– M30 | Lead: CITEVE; Participants: Chain Reactor & ATB

This task focuses on the validation and scale-up of the dyeing, coating, and printing formulations developed in T7.1 for different textile fibres. Dyeing processes will be validated under both exhaust and continuous dyeing, with systematic optimisation of key operational parameters such as liquor ratio, temperature profiles, and processing time. Selected formulations will be further validated at pilot scale using increased textile loads to assess process robustness and reproducibility. Innovative low-liquor-ratio dyeing approaches for cellulosic fibres will also be evaluated to reduce water, energy, and chemical consumption while ensuring adequate colour diffusion and fixation. For continuous dyeing, process parameters related to fabric impregnation and fixation will be adjusted to ensure uniform colour uptake during scale-up. Coating and digital printing processes will be validated through the optimisation of application parameters, including layer thickness, drying and curing conditions, and printing resolution, adapted to each textile substrate and formulation. Post-application treatments, including curing and pre- and post-treatments, will be assessed to improve pigment fixation and fastness properties.

T7.3 Evaluation of fastness performance of the finished textiles | M14 – M30 | Lead: CITEVE; Participants: ATB

The finished textile materials will be characterized for their colour coordinates (CIEL*a*b* colour space) and quality control assessment of colour fastness to wash (ISO 105-C), light (ISO 105-B02), rubbing (ISO 105-X12), perspiration (ISO 105-E04). Taking in consideration the obtained fastness results, the formulations composition and finishing processes parameters may need to be optimized to accomplish the fastness standards.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 5,31 FTEs | CEB-UM: 5,72 FTEs | CITEVE: 106,45 FTEs | ATB: 47,7 FTEs

Description of deliverables and milestones

D7.1: Selected PRISMA pigments and validated application routes; **D7.2:** Validated textile finishing formulations; **D7.3:** Textile performance and fastness validation report; **M7.1:** Textile formulations technically validated; **M7.2:** Application performance validated across target fibres; **M7.3:** Technology readiness for industrial uptake confirmed.

Activity No.	Activity Title	
A8	Industrial textile dyeing process demonstration	
	Start date: 01.12.2028	End date: 31.12.2029
Objective		
A8 aims to validate the industrial applicability of PRISMA bio-based pigments within real textile manufacturing environments. This activity aims to demonstrate dyehouse compatibility, production-scale performance, and process reproducibility under representative industrial conditions, while generating robust technical, techno-economic, and		

environmental evidence to support informed decision-making for downstream industrial adoption and sustainability assessment.

Description of the activity

T8.1 Dyehouse compatibility assessment | M24 – M30 | Lead: ATB; Participants: CITEVE

This task assesses the compatibility of PRISMA bio-based pigments with industrial dyehouse operations under representative conditions used by ATB. Building on the formulations and performance ranges defined in A7, the task evaluates pigment behaviour in real dyeing workflows, identifying operational constraints, adaptation needs, and applicable processing windows at industrial scale. The interaction of PRISMA pigments with standard dyehouse equipment, auxiliaries, and operating conditions (e.g. temperature, pH, liquor ratio, and process duration) will be investigated, with particular focus on pigment dispersibility, process stability, and colour development. Initial industrial-scale dyeing trials will be carried out by ATB on selected textile substrates (e.g. cotton, polyester blends, cellulosic fibres, next-generation bio-based and circular-economy fibres, and functional textiles). Colour performance, shade consistency, and process robustness will be evaluated using standard colorimetric methods, with technical support from CITEVE. Where limitations are identified, experimental adjustments to processing parameters will be explored to define compatible dyeing conditions for PRISMA pigments. Lastly, ATB will use its industrial dyeing lines (jet, overflow, continuous processes like CPB) to simulate real production environments. The outcome of this task will be the definition of robust industrial operating windows for PRISMA pigments, ensuring compatibility with existing dyehouse infrastructure and enabling predictable scale-up towards continuous and production-scale operations.

T8.2 Production-scale dyeing trial | M30 – M36 | Lead: ATB

This task focuses on the experimental validation of PRISMA bio-based pigments under production-scale with fermentation process 2000L and dyeing conditions at ATB, following the compatibility boundaries and operating windows defined in T8.1. The objective is to demonstrate the technical feasibility, robustness, and reproducibility of PRISMA pigments when applied in full-scale industrial dyeing runs representative of real manufacturing operations. The task will evaluate process operability, colour uniformity, shade reproducibility, and batch-to-batch consistency under continuous production-relevant conditions, accounting for normal industrial variability in raw materials and operating parameters. Colour performance will be assessed using standard industrial colourimetric methodologies, while process robustness will be evaluated through monitoring of key operational indicators such as process stability, handling behaviour, and repeatability across successive dyeing runs. Particular emphasis will be placed on demonstrating process reproducibility and robustness under industrial conditions, targeting colour variation across batches, while maintaining stable process performance over extended production runs. This task is critical to validate PRISMA pigments as a viable drop-in alternative to conventional synthetic dyes in industrial environments.

T8.3 Techno-economic and environmental assessment | M30 – M36 | Lead: ATB; Participants: Chainreactor & CITEVE

This task addresses the techno-economic and environmental assessment of PRISMA bio-based pigments applied to textile production, using experimental and industrial data generated in previous tasks, in particular the production-scale dyeing trials performed in T8.2. The objective is to analyse process performance, resource efficiency, and environmental impacts without duplicating experimental or industrial activities. ATB will consolidate and structure primary industrial data from production-scale dyeing trials, including process duration, resource consumption (water, energy, chemicals and auxiliaries), yields, reprocessing rates, and operational efficiency. Based on these validated datasets, CITEVE will carry out a Life Cycle Assessment (LCA) of textile products dyed with PRISMA pigments in accordance with NP EN ISO 14040:2006 and NP EN ISO 14044:2006, using GaBi software, complemented where necessary with recognised secondary databases. In parallel, techno-economic indicators will be derived to contextualise production-scale performance and sustainability potential relative to conventional dyeing pathways. These analyses will rely exclusively on existing data and will not involve additional production trials. ATB will play a central role in the generation and validation of high-quality primary industrial data, including real-time measurement of water consumption, energy use, chemical inputs, process duration, yields, and operational efficiency. These datasets will ensure the accuracy and industrial relevance of the LCA models developed by CITEVE, significantly reducing reliance on secondary data sources. The integration of real industrial data across the value chain will support full traceability and alignment with emerging regulatory frameworks, including the Ecodesign for Sustainable Products Regulation (ESPR) and Digital Product Passport requirements. Through this activity, ATB ensures that PRISMA outcomes are validated under real industrial conditions, bridging the gap between biotechnology development and textile manufacturing. This significantly reduces adoption risk and accelerates the commercial deployment of bio-based pigments within the European textile industry.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 2,85 FTEs | **CITEVE:** 38,05 FTEs | **ATB:** 70,32 FTEs | **CEB-UM:** 1,40 FTEs

Description of deliverables and milestones

D8.1: Dyehouse compatibility and operating windows report; **D8.2:** Production-scale dyeing performance report; **D8.3:** Techno-economic and environmental assessment report; **M8.1:** Dyehouse compatibility validated under industrial conditions; **M8.2:** Reproducible production-scale dyeing demonstrated; **M8.3:** Sustainability and techno-economic performance assessed.

Activity No.	Activity Title	
A9	Promotion, dissemination & IP	
	Start date: 01.06.2027	End date: 31.12.2029
Objective		
A9 aims to ensure the protection and dissemination of the knowledge and results generated within the PRISMA project. This activity supports future industrial valorisation by aligning intellectual property management with scientific dissemination and controlled knowledge transfer across the project's value chain.		
Description of the activity		
<u>T9.1 Industrial dissemination M6-M36 Lead: Chainreactor; Participants: CITEVE, ATB</u> This task addresses the dissemination and promotion of project results to industry stakeholders through: project updates and results published on Chainreactor's website and social media channels; preparation and distribution of press releases to biotech and textile industry media; production of at least 2 white papers on waste-to-pigment bioprocessing and geometry-preserved bioreactor scale-up; blog content targeting potential CMO customers, bio-based ingredient buyers, and textile brands exploring sustainable supply chains. Participation in industrial conferences and trade fairs including:ACHEMA (Frankfurt, 2027), BioFach (Nuremberg, 2028), Future Fabrics Expo (London, 2028/2029), European Forum for Industrial Biotechnology (EFIB, 2028), Performance Days (Munich, 2029), TechTextil Frankfurt (2028), Premier Vision (Paris, 2029) and Milano Unica (Milan, 2029). Organization of at least 2 stakeholder workshops (M18, M30) demonstrating PRISMA bio-pigment samples and production capabilities to textile industry adopters. <u>T9.2 Scientific dissemination M6-M36 Lead: Chainreactor; Participants: CEB-UM, INESC TEC, CITEVE</u> This task addresses the dissemination and promotion of scientific results through: -Peer-reviewed publications • CEB-UM: at least 2 papers regarding development and optimization of bioprocess for high value pigment production using alternative carbon sources. • INESC TEC: at least 3 papers on AI-based bioprocess control for efficient pigment production; at least 1 paper on real-time monitoring of microbial fermentation; at least 1 paper on sensing solutions for bio-pigments quality assessment. • Chainreactor: at least 1 paper on geometry-preserved bioreactor scale-up methodology (together with CEB-UM). • CITEVE: at least 1 paper regarding textile finishing applications of novel PRISMA pigments. -Thesis supervision • CEB-UM: at least 2 master theses: "Development and optimization of bioprocesses for high-value pigment production" and "Valorization of agri-food side-streams for sustainable biotechnology: Nutritional characterization and circular economy potential in pigment bioprocesses." • INESC TEC: at least 2 master theses: "Development of sensing tools for in situ monitoring of critical parameters in microbial fermentation"; "Sensing solutions for evaluation of bio-pigments production for textile applications". • CITEVE: at least 2 master thesis: "Dyeing process optimization for the biotechnological pigments on different textile materials" and "Bio-Based Coating and Printing approaches for the application of biotechnological pigments on textile materials" -Conference participation: • CEB-UM: at least 2 participations in conferences (e.g., European Congress on Biotechnology). • INESC TEC: at least four participations in specialized conferences (Application of Optics and Photonics - AOP, TBD, PT, 2028; EUROPT(R)ODE, Porto, PT, 2028; Photonics Europe, TBD, Europe, 2029; Photonics West, TBD, USA, 2028). • CITEVE: at least four participations in specialized conferences, - two national (e.g. IJUP, Porto, PT, 2028, 2029; Application of Optics and Photonics (AOP), PT, 2028) and two international (EUROPT(R)ODE, Porto, PT, 2028; Photonics Europe, Strasbourg, France, 2029; EUROPT(R)ODE, Porto, PT, 2028).		

- Chainreactor: ACHEMA (Frankfurt, 2027), BioFach (Nuremberg, 2028), Future Fabrics Expo (London, 2028/2029), European Forum for Industrial Biotechnology (EFIB, 2028), Performance Days (Munich, 2029).

T9.3 IP protection | M12-M36 | Lead: Chainreactor; Participants: CEB-UM & INESC TEC

Chainreactor's IP strategy uses provisional patent filings to enable safe disclosure at conferences while preserving priority dates. Accordingly, the following sub-tasks will be carried out under this task:

- technology watch update on existing patent landscape for 3D-printed bioreactors, sensor systems, and bio-based textile dyes (M12);
- filing of at least 1 provisional patent application with the USPTO covering geometry-preserved bioreactor design and/or integrated soft sensor architecture (M18);
- evaluation of European patent filing routes via EPO based on project results (M30);
- drafting of final patent application(s) for post-project submission.

IP generated by CEB-UM and INESC TEC within their respective activities is governed by the Consortium Agreement (Clause 8), with Chainreactor holding first-right licensing for commercial application of jointly generated IP.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 12,6 FTEs | **CEB-UM:** 5,43 FTEs | **INESC TEC:** 17 FTEs | **ATB:** 9,45 FTEs

Description of deliverables and milestones

D9.1: Material for communication and dissemination actions; **D9.2:** Mission reports related to participation in conferences and exhibitions; **D9.3:** Scientific publications; **D9.4:** Provisional and final patents. **M9.1:** Dissemination of project results for the industrial sector and stakeholders; **M9.2:** Dissemination of project results to the scientific community; **M9.3:** Completion of the patent submission.

Activity No.	Activity Title	
A10	Project Management	
	Start date: 02.01.2027	End date: 31.12.2029
Objective		
A10 aims to ensure effective project governance, compliance, risk control, and quality assurance, enabling the coordinated execution of PRISMA activities and the timely delivery of high-quality results.		
Description of the activity		
<u>T10.1 Consortium coordination M1-M36 Lead: Chain Reactor; Participants: CEB-UM, INESC TEC, CITEVE, ATB</u>		
This task ensures the effective coordination and governance of the PRISMA consortium throughout the project duration. A structured governance framework will be established, comprising a Steering Committee with quarterly meetings involving all beneficiaries, a Technical Committee meeting monthly with activity leaders, and ad-hoc bilateral coordination meetings as required. The Steering Committee will be chaired by Chainreactor's CEO, while the Technical Committee will be chaired by Chainreactor's CTO. Decision-making procedures will follow the rules defined in the Consortium Agreement (Clause 5). Project coordination activities include the formal onboarding of all partners in Month 1, covering data management protocols, reporting templates, and communication channels. All meetings will be documented through formal minutes, and action items will be monitored using a shared project workspace to ensure transparency, accountability, and timely execution of project activities.		
<u>T10.2 Reporting & compliance M1-M36 Lead: Chain Reactor; Participants: CEB-UM, INESC TEC, CITEVE, ATB</u>		
This task ensures the timely preparation and submission of all technical-scientific and financial reports required by ANI, in full compliance with applicable guidelines and contractual obligations. Each beneficiary will submit quarterly activity-level progress reports to Chainreactor, which will consolidate them into programme-level reports for submission. Financial reporting will be conducted in accordance with ANI rules, with each beneficiary responsible for maintaining supporting expenditure documentation. Chainreactor will verify the consistency and eligibility of reported costs prior to submission. An external grant-management consultancy will provide administrative support for compliance verification and preparation of payment requests.		
<u>T10.3 Risk management M1-M36 Lead: Chain Reactor; Participants: CEB-UM, INESC TEC, CITEVE, ATB</u>		
This task ensures the systematic identification, monitoring, and mitigation of project risks throughout the PRISMA implementation. A structured risk-management process will be applied, based on a risk register maintained by Chainreactor and reviewed quarterly at Steering Committee level. Identified risks will be classified into technical, operational, financial, and external categories, with each risk assessed in terms of probability and potential impact.		

Mitigation measures, responsible owners, and escalation thresholds will be defined for each risk. Critical risks identified in Section 2.6 will be monitored through dedicated indicators to support timely corrective actions and ensure project continuity.

T10.4 Quality assurance | M1-M36 | Lead: Chain Reactor; Participants: CEB-UM, INESC TEC, CITEVE, ATB

This task ensures the quality, consistency, and technical robustness of all project deliverables through a structured quality-assurance framework. Each deliverable will undergo an internal review by the responsible beneficiary, followed by a cross-review by at least one additional consortium partner, prior to final acceptance by Chainreactor. In addition, gate reviews will be conducted at Months 12 and 24 to assess overall programme progress, deliverable quality, and achievement of key milestones. These reviews will support informed go/no-go decisions for subsequent project phases, namely the transition to pilot-scale activities at M12 and to 2000 L scale-up activities at M24.

Effort in FTEs and investments (per beneficiary)

Chainreactor: 40,7 FTEs | **CEB-UM:** 7,78 FTEs | **INESC TEC:** 18,50 FTEs | **ATB:** 9,65 FTEs

Description of deliverables and milestones

D10.1: Interim technical-scientific reports and corresponding payment requests; **D10.2:** Final technical-scientific report and final payment request (M36); **M10.1:** Successful completion of the project (M37)

Activity No.	Activity Title	
A11	CMO Build	
	Start date: 01.12.2027	End date: 31.12.2029
Objective		
A11 aims to establish and operationally qualify an innovative production facility enabling the industrial deployment of PRISMA technologies, translating validated R&D results into production-ready capacity and ensuring seamless integration with downstream industrial dyeing activities.		
Description of the activity		
<u>T11.1 Site preparation and facility design M6-M12 Lead: Chain Reactor; Support: ATB</u>		
This task addresses the preparation of the selected site and the design of a dedicated production facility to support the industrial deployment of the PRISMA technology. Building on the technical requirements defined in previous activities and on the collaboration with ATB, the task includes securing the lease agreement. A facility layout will be developed comprising 5 functional zones: 1) a fermentation hall hosting the 2000 L bioreactor and associated utilities; 2) a downstream processing area for filtration and spray drying; 3) laboratory and quality-control spaces; 4) storage areas for feedstocks and finished products; and 5) office/control room facilities. The layout will be optimised for safe operation, efficient process flow, and future scalability, with a total usable area of approximately 150–185 m ² . The task also includes the preparation of construction permits and BSL-1 biosafety documentation, the definition of electrical, water, and drainage specifications, and the engagement of local contractors for required civil works.		
<u>T11.2 Equipment procurement and installation M12-M24 Lead: Chain Reactor; Support: ATB</u>		
This task covers the procurement, installation of the productive equipment required for the industrial deployment of the PRISMA technology. Key assets include a 2000 L 3D-printed bioreactor system with integrated sensor suite manufactured by Chainreactor, downstream processing equipment for filtration and spray drying, a clean-in-place (CIP) system, and associated utility connections (e.g. compressed air, chilled water, and steam where required). Laboratory analytical equipment will also be acquired to support in-process quality control. The task includes equipment installation, system integration, and commissioning through successive validation runs, initially using media-only tests followed by inoculated test batches, in order to verify operational readiness prior to full production-scale use.		
<u>T11.3 Commissioning and operational qualification M24-M36 Lead: Chain Reactor; Support: CEB-UM & ATB</u>		
This task focuses on the commissioning and operational qualification of the production facility and installed equipment. Activities include verification of utilities, performance qualification of key assets, and validation of the production process using fermentation protocols developed in Activity A2 and scaled in Activity A6. The task also includes the documentation of standard operating procedures (SOPs) for production, cleaning, and quality-control activities, and a final operational readiness assessment to confirm the facility's capacity to support regular production campaigns.		

Effort in FTEs and investments (per beneficiary)
Innovative productive investments were described in section 1.3, with a total investment of 2 730 500,00€
Description of deliverables and milestones
D11.1: Industrial production facility operational and qualified; M11.1: Facility operational qualification completed.

2.4. summary table of deliverables

Deliverable No.	Activity No.	Deliverable title	Delivery date	Responsible beneficiary		Deliverable type	Dissemination level
				No.	Abbrev.		
D1.1	A1	Baseline fermentation performance map	M6	1	CEB-UM	Report	Confidential
D1.2	A1	Continuous process optimization report	M12	1	CEB-UM	Report	Confidential
D2.1	A2	Comprehensive chemical characterization dataset of agri-food and industrial side-streams	M4	1	CEB-UM	Report	Confidential
D2.2	A2	Inhibitor mapping and threshold definition	M5	1	CEB-UM	Report	Confidential
D2.3	A2	Standardized hydrolysate preparation and media reformulation protocol	M6	1	CEB-UM	Report	Confidential
D3.1	A3	Data platform operational	M9	3	INESC TEC	Prototype	Confidential
D3.2	A3	Technical report on sensor suite validation	M24	3	INESC TEC	Report	Confidential
D4.1	A4	Reactor design dossier	M12	1	Chainreactor	Document	Confidential
D4.2	A4	Sensor integration and PCB/PCB interfaces report	M12	1	Chainreactor	Report	Confidential
D4.3	A4	Comparative test report	M12	1	Chainreactor	Report	Confidential
D5.1	A5	DSP route definition and requirements report	M12	1	Chainreactor	Report	Confidential
D5.2	A5	Solid-liquid separation and clarification optimisation report	M18	1	Chainreactor	Report	Confidential
D5.3	A5	Pigment extraction and purification protocol	M24	1	Chainreactor	Document	Confidential
D5.4	A5	Stabilised pigment formulation and drying report	M24	1	Chainreactor	Report	Confidential
D6.1	A6	Pilot-scale scale-up validation report (200 L and 500 L)	M24	2	CEB-UM	Report	Confidential
D6.2	A6	Pre-industrial scale-up and process robustness report (2000 L)	M36	1	Chainreactor	Report	Confidential
D6.3	A6	Large-scale monitoring and sensing tools validation report	M30	3	INESC TEC	Report	Confidential
D7.1	A7	Selected PRISMA pigments and validated application routes	M21	4	CITEVE	Report	Confidential
D7.2	A7	Validated textile finishing formulations	M30	4	CITEVE	Demonstrator	Public
D7.3	A7	Textile performance and fastness validation report	M30	4	CITEVE	Report	Confidential
D8.1	A8	Dyehouse compatibility and operating windows report	M24	5	ATB	Report	Confidential
D8.2	A8	Production-scale dyeing performance report	M30	5	ATB	Report	Confidential
D8.3	A8	Techno-economic and environmental assessment report	M36	5	ATB	Report	Confidential
D9.1	A9	Material for communication and dissemination actions;	M36	All		Document	Public
D9.2	A9	Mission reports related to participation in conferences and exhibitions;	M36	All		Report	Confidential

D9.3	A9	Scientific publications	M36	1 Chainreactor; 2 CEB UM; 3 INESC TEC; 4 CITEVE	Document	Public
D9.4	A9	Provisional and final patents	M36	1 Chainreactor	Document	Confidential
D10.1	A10	Interim technical-scientific reports and corresponding payment requests	M12 M24	All	Report	Confidential
D10.2	A10	Final technical-scientific report and final payment request	M36	All	Report	Confidential
D11.1	A11	Industrial production facility operational and qualified	M30	1 Chainreactor	Report	Confidential

2.5. summary table of milestones

Milestone No.	Activity No.	Delivery date (Month 0+N)	Milestone title	Means of verification (<i>must be quantifiable</i>)
M1.1	A1	M5	Fed-batch baseline validated	D1.1
M1.2	A1	M9	Successful steady-state achievement	D1.2
M1.3	A1	M12	21-day continuous campaign completed	D1.2
M2.1	A2	M2	Candidate feedstock streams mapped and screened	D2.1
M2.2	A2	M6	Feedstock selection for continuous fermentation trials	D2.2; D2.3
M2.3	A2	M9	Strain/media compatibility and robustness confirmed	D2.3
M3.1	A3	M24	Bioprocess datalake deployed	D3.1
M3.2	A3	M22	Sensor integration and functionality validated	D3.2
M3.3	A3	M30	Digital twins and transfer learning validated	D3.3
M4.1	A4	M3	Bioreactor design requirements defined	D4.1
M4.2	A4	M9	Pigment-specific bioreactor configuration validated	D4.3
M4.3	A4	M12	Final bioreactor design frozen for scale-up	D4.1; D4.2
M5.1	A5	M12	DSP route defined and validated at lab scale	D5.1
M5.2	A5	M18	Robust clarification strategy validated	D5.2
M5.3	A5	M24	Extraction and purification targets achieved	D5.3
M5.4	A5	M24	Application-ready pigment format validated	D5.4
M6.1	A6	M24	Pilot-scale continuous operation validated	D6.1
M6.2	A6	M36	Pre-industrial continuous fermentation demonstrated	D6.2
M6.3	A6	M28	Large-scale sensing and monitoring validated	D6.3
M7.1	A7	M21	Textile formulations technically validated	D7.1
M7.2	A7	M30	Application performance validated across fibres	D7.2; D7.3
M7.3	A7	M30	Technology readiness for industrial uptake confirmed	D7.3
M8.1	A8	M24	Dyehouse compatibility validated under industrial conditions	D8.1
M8.2	A8	M30	Reproducible production-scale dyeing demonstrated	D8.2
M8.3	A8	M36	Sustainability and techno-economic performance assessed	D8.3
M9.1	A9	M36	Industrial dissemination completed	D9.1
M9.2	A9	M36	Scientific dissemination completed	D9.2; D9.3
M9.3	A9	M36	Patent submission completed	D9.4
M10.1	A10	M37	Project successfully completed	D10.2
M11.1	A1	M30	Facility operational qualification completed	D11.1

2.6. Description of critical risks for Project Management

Project management risk event	Event description	Probability of occurrence (1 = Low to 5 = High)	Impact on project delivery	Planned mitigation measures
Delays in provision of deliverables by beneficiaries	Given the technical complexity and interdependence of activities, delays in the timely delivery of technical deliverables by one beneficiary may propagate to downstream activities, affecting integration and validation steps.	4	Delays in milestones achievement, disruption of the critical path (notably scale-up and industrial validation), and risk of misalignment with the Gantt schedule.	Clear allocation of responsibilities, quarterly monitoring through the Project Steering Committee, early warning mechanisms via monthly technical meetings, and buffer periods embedded in the Gantt for integration-intensive activities. Introduce early warning escalation protocols and allow temporary task redistribution if delays occur.
Changes in tasks	Given the R&D nature of PRISMA (fermentation, DSP, AI integration), technical findings may require redefinition of tasks, especially in Activity 1 (bioprocess optimization) and Activity 5 (DSP route). For example, a pigment pathway may require alternative extraction or different reactor conditions.	4	If not managed, this can cause delays in downstream activities (scale-up, validation) but typically does not compromise overall project feasibility.	Establish a flexible work plan governance through the Steering Committee, allowing controlled task reallocation. Implement quarterly technical reviews to validate task relevance. Maintain buffer tasks and contingency pathways (e.g., alternative DSP routes, strain engineering for waste carbon resource digestion by the organism).
The effort required to deliver is significantly different from what was planned in the application	Scale-up, DSP optimization, or continuous fermentation may require significantly more time and experimentation than estimated, especially at 200L–2000L transition.	4	Underestimation of effort in scale-up or DSP may result in resource shortages, extended timelines, and budget pressure. Critical activities (e.g. 200L–2000L scaling or continuous operation) may be delayed, affecting industrial demonstration readiness.	Include effort buffers in critical activities (A5, A6, A9). Use iterative validation loops rather than fixed pipelines. Prioritize high-impact experiments and maintain scope prioritization mechanisms via Steering Committee.
Delays in installation and qualification of the CMO Build facility (A11)	Procurement, installation or commissioning delays of production equipment or utilities may affect the timely availability of the CMO-ready facility.	3	Postponement of pre-industrial validation and reduced time window for industrial operation within the project duration.	Early site preparation and permitting (with the support of ATB), phased equipment procurement, close coordination with suppliers, and alignment of A11 timeline with upstream validation milestones to minimise idle dependencies.

Communication and coordination issues among beneficiaries	The multidisciplinary nature of the consortium and the number of interlinked activities increase the risk of miscommunication or misalignment between partners.	2	May lead to misalignment in technical specifications, duplicated work, or integration failures between upstream, DSP, and AI components. This reduces efficiency and can cause rework, delays, and inconsistencies in deliverables.	Establish a clear communication structure: weekly operational calls, monthly WP meetings, shared documentation platform. Assign single points of contact per partner. Use standardized reporting templates and dashboards.
Deviations from the defined budget	Unexpected costs may arise from equipment, raw materials, pilot runs, or process failures during scale-up and DSP development.	3	Budget overruns may force reprioritization or reduction of project scope, delay procurement or pilot activities, and potentially affect co-financing capacity, putting pressure on project continuity and compliance.	Implement budget monitoring per WP on a quarterly basis. Allocate a contingency budget margin. Reprioritize non-critical activities if needed. Ensure early communication with FI Group for budget adjustments.
Lack of qualified human resources	Specialized skills (bioprocess scale-up, DSP, AI for bioprocessing) are scarce and may delay execution, especially at critical phases.	3	Can delay execution of specialized tasks (bioprocess optimization, DSP, AI integration), reducing productivity and increasing reliance on external support. This may lead to timeline slippage and reduced technical performance.	Plan early recruitment strategy. Combine internal hiring with partner expertise. Use external consultants where needed. Cross train team members to reduce dependency on single profiles. Establishment of partnerships with academic institutions aimed at recruiting qualified human resources.
Deliverables do not reach the expected quality	Technical outputs (e.g. pigment stability, yield, reactor performance, AI models) may not meet industrial or validation standards.	4	Affects technical validation, industrial applicability, and KPIs, particularly for pigment performance, process stability, and AI control systems. This may require rework, additional testing cycles, and could impact final project evaluation and funding outcomes.	Define clear KPIs and acceptance criteria per deliverable. Implement internal validation checkpoints before submission. Use iterative testing cycles and involve validation partners (CITEVE) early.

2.7. Project Management

In order to ensure effective management of the entire project, an activity focused on the technical and financial management of the project was defined (A10 – Project Management). PRISMA employs a centralized coordination model led by Chainreactor as lead beneficiary, with structured mechanisms to ensure timely inputs from all consortium partners on whom project execution depends.

Governance structure: The project operates through a two-tier governance model. The Steering Committee meets quarterly and includes senior representatives from all five beneficiaries. It is chaired by Chainreactor's CEO and holds authority over strategic decisions, budget reallocations above 10% of any activity budget, milestone acceptance, and dispute resolution. Decisions follow the procedures established in the Consortium Agreement (Clause 5). The Technical Committee meets monthly and comprises the lead researcher or engineer for each active activity. It is chaired by Chainreactor's CTO and manages day-to-day technical coordination, inter-activity dependencies, and early identification of risks or delays.

Coordination and cooperation mechanisms: Planning, task tracking, and partner communication are managed by Chainreactor through a shared digital project workspace accessible to all beneficiaries. Each activity lead submits a brief monthly progress update (technical status, upcoming milestones, resource needs, risks) to Chainreactor, who consolidates these into the project-level view. Bilateral coordination meetings between activity leads are scheduled as needed, particularly at integration points where outputs from one activity feed into another (e.g., A1 feedstock data to A2 fermentation; A4 sensor specs to A3 AI development).

Reporting: All beneficiaries maintain their own financial and technical records. Chainreactor compiles and submits consolidated technical-scientific reports and payment requests to ANI at M12, M24, and M36. An external grant management consultancy, engaged by Chainreactor under subcontract, supports administrative compliance, expenditure verification, and reporting format alignment with ANI requirements.

Adequacy for project size and complexity: The quarterly Steering Committee prevents strategic drift without over-burdening partners. The monthly Technical Committee ensures that interdependencies are actively managed. The formal gate reviews at M12 and M24 (defined in A10) provide structured go/no-go decision points that prevent resource commitment to downstream activities before upstream validation is confirmed. Chainreactor's dependence on technical inputs from CEB-UM (biology), INESC TEC (AI/ML), CITEVE (textile testing), and ATB (industrial application) is explicitly recognized and managed through the monthly reporting cycle and bilateral coordination protocols.

3. Impact

3.1. Economic and/or business impact

1) Chainreactor

Chainreactor's economic valorization strategy is focused on the industrial exploitation of high-technology-intensive goods and knowledge-intensive services resulting directly from the PRISMA project's development lines. Rather than operating as a downstream product company, Chainreactor positions itself as an enabling mid-stream infrastructure provider, capturing value through manufacturing platforms, process intelligence and specialised industrial services that connect innovation to large-scale application. In this context, Chainreactor positions itself as the "factory of the future" for biotechnology developers, acting as the essential infrastructure and CMO partner that de-risks the transition from laboratory validation to industrial production.

The project targets the bio-based textile pigment segment, a niche within the global textile dye market, which exceeds EUR 7 billion annually, while bio-based pigments currently represent less than 1% of total supply. This imbalance reflects a strong substitution opportunity driven by regulatory pressure, brand-level sustainability commitments and increasing restrictions on fossil-derived colorants. PRISMA-developed pigment production processes are specifically designed to integrate into existing industrial dyeing and

finishing lines, minimising switching costs for textile manufacturers. More broadly, PRISMA will position Chainreactor within the fast-growing precision fermentation market, projected to expand significantly by 2030, and within a global bioreactor market valued at approximately USD 20 billion. The company's addressable market is a specific segment comprising precision fermentation scale-up infrastructure and CMO capacity for small and mid-size biotechnology companies lacking in-house manufacturing, including approximately 400+ precision fermentation startups across Europe. This segment is characterised by fragmented demand, limited access to affordable scale-up infrastructure and a growing need for flexible, geometry-preserved bioprocessing solutions. Chainreactor's value proposition directly addresses one of the most critical pain points in biotechnology: scale-up failure, by offering geometrically identical bioreactors across volumes, from 250 mL benchtop systems to 2,000 L industrial units, ensuring that processes behave identically from development to production.

The anchor commercial relationship for market entry is Octarine Bio (Denmark), developer of the PurePalette bio-pigment platform, which has raised EUR 12.8 million in Series A financing, achieved commercial launch at London and Copenhagen Fashion Weeks, and secured signed off-take agreements with global fashion brands. Under a signed letter of intent, Octarine Bio has committed to evaluating Chainreactor as its primary CMO partner for commercial-scale pigment production. This partnership provides early industrial validation of the PRISMA platform, demand-side visibility and a customer reference to support subsequent export-driven market entry.

The competitive landscape for bio-based textile pigments remains nascent. The most visible competitor, Colorifix (UK), operates a vertically integrated model spanning organism engineering, fermentation and dyeing services, which limits scalability and ties production to proprietary biology. In contrast, Chainreactor's differentiation is structural: it does not commercialize pigments but rather provides the manufacturing infrastructure and AI-enabled process intelligence required by any bio-based ingredient producer to scale. This horizontal model enables technology neutrality, reuse across multiple organisms and customers, and significantly higher scalability. Conventional CDMOs (e.g. Lonza, Samsung Biologics) serve pharmaceutical markets at cost structures unsuitable for early-stage bio-based ingredients, and no European provider currently offers geometry-preserved scale-up combined with AI-driven fermentation at volumes and prices appropriate for the textile chemicals market. Chainreactor further strengthens its competitive moat through vertical integration, offering an end-to-end CMO value proposition that takes customers from bioreactor to market-ready powder, integrating downstream steps such as cell separation, extraction and spray-drying. PRISMA therefore occupies a defensible white space between laboratory-scale innovation and commodity chemical manufacturing.

Market entry and scale-up follow a phased strategy. During the project implementation period (2027–2029), Chainreactor validates its manufacturing platform through bio-pigment production at the 2,000 L Barcelos facility, while simultaneously expanding its bench-scale bioreactor fleet to generate cross-organism process data. These deployments feed a central AI-driven process-intelligence platform, strengthening predictive scale-up capabilities and reducing time-to-market for new customers. The AI platform is also leveraged as a recurring SaaS layer, providing clients with biological process intelligence, yield prediction and automated control functions. From the Consolidation Year onwards (2031+), the company expands CMO services to additional bio-based ingredient producers and adjacent sectors such as food ingredients, cosmetic actives and industrial enzymes, initially across Europe and subsequently into North America.

Export activity is based on direct B2B commercialization. Bench-scale bioreactors are marketed through digital channels and targeted direct outreach, while CMO services are developed through bilateral commercial agreements. CITEVE's management of the Portuguese Textile Cluster (over 6,000 companies) provides a complementary channel for bioreactor adoption among domestic textile producers transitioning toward bio-based dyes. This cluster also functions as a high-density industrial testbed for validating and refining the bioreactor-as-a-service and CMO model before replication across other European biotechnology

hubs. No distributor network is required given the technical sophistication and concentration of the customer base. International export markets initially prioritized include the USA, United Kingdom, Denmark, Switzerland, Sweden, Germany, Canada and Spain, reflecting regions with advanced bio-based chemicals adoption, strong textile and biotechnology ecosystems and favourable regulatory environments.

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By the Consolidation Year, Chainreactor will be positioned as a mid-stream manufacturing infrastructure provider within the bio-based value chain, connecting upstream biological innovation with downstream industrial users. Competitive advantages include geometry-locked bioreactors that eliminate repeated scale-up optimisation, an AI-driven process-intelligence platform trained on cross-scale fermentation data, an operational 2,000 L CMO facility with validated production procedures, and a growing portfolio of protected intellectual property. Long-term value creation is driven by platform extensibility, recurring revenues from hardware access, AI-enabled services and CMO production, and cumulative proprietary process knowledge. Forecast market objectives indicate progressive revenue growth through hardware subscriptions and CMO production, with turnover expected to reach 3 656 500,00 € and international turnover of 1 828 250,00 € by the consolidation year (2031).

2) ATB

ATB's strategy for the economic valorization of the project results is aligned with the integration of medium-high technology-intensive processes into its existing industrial activity. ATB is positioned mid-stream within the textile value chain, operating as a finishing and processing partner for downstream manufacturers and brands rather than as a final product exporter.

Through PRISMA, ATB will adopt bio-based pigments validated under industrial conditions, positioning itself as an early mover in sustainable textile finishing. The project enables the company to operate with medium-high technology intensity, combining bio-based inputs, validated industrial dyeing protocols, and accredited sustainability certification.

The target market for the valorization of the project results is primarily the Portuguese textile sector, serving national industrial clients, with application in textile segments. Given ATB's mid-chain position, international market exposure occurs predominantly through indirect export channels. Therefore, the geographic focus remains predominantly domestic, with residual activity in international markets such as Belgium, Germany, Spain, France, United Kingdom and Tunisia. Therefore, the project will allow ATB to operate in higher value-added segments requiring enhanced environmental compliance and technical performance.

Potential competitors include conventional textile finishing companies that have not integrated bio-based or sustainability-driven processes, namely Carvema, Barceltinge and ABM and therefore face increasing regulatory and market pressure. ATB differentiates itself through demonstrated industrial capability, technical know-how, and the early adoption of sustainable finishing solutions validated under project conditions.

Market entry is not limited to new geographic or sectoral expansion but is primarily driven by the strengthening of ATB's position within its existing client base, while simultaneously enabling access to new domestic and international clients with higher sustainability, traceability, and regulatory requirements. Through PRISMA, ATB will be able to address emerging market segments linked to sustainable and bio-based textile solutions, positioning itself to capture new business opportunities both within Portugal and in selected European markets where demand for compliant and low-impact textile processing is rapidly increasing.

In the Consolidation Year, ATB will be positioned as an advanced industrial partner within the Portuguese textile value chain, capable of supporting downstream manufacturers and brands in responding to regulatory requirements such as sustainability reporting and traceability. Competitive advantages include enhanced process sustainability, regulatory readiness, and reinforced client loyalty. ATB's contribution to export performance is therefore structural, enabling the competitiveness of Portuguese textile exports rather than generating direct export flows.

Market objectives for ATB are therefore defined not only by the consolidation of existing turnover and client relationships, but also by the progressive acquisition of new clients and entry into higher value-added market segments enabled by the adoption of PRISMA technologies. The project is expected to support incremental revenue generation associated with sustainable textile finishing services, strengthening ATB's competitive positioning and expanding its presence in both domestic and international markets. Total turnover is estimated at 18 492 485,86 €, with international turnover of 924 906,44 € in the consolidation year (2031), reflecting the company's enhanced capability to respond to global sustainability-driven demand.

3.2. If applicable, justify (quantifying) the project's contribution to reducing or preventing strategic dependencies of the EU in one or more dimensions

Contribution to strengthening security of supply of critical inputs, components and technologies: PRISMA reduces EU dependency on 3 critical supply chain nodes: 1) petrochemical dye intermediates (replaced by bio-based pigments from local waste feedstocks); 2) conventional stainless steel bioreactors imported primarily from German and US manufacturers (replaced by domestically 3D-printed bioreactors at 60-70% lower cost); 3) external CMO services for precision fermentation (replaced by an EU-based CMO capability accessible to European biotech companies at price points 5-10x below pharma-grade CMOs). By building all 3 capabilities within Portugal, PRISMA eliminates single-source dependencies at the infrastructure, process, and product levels simultaneously.

Contribution to critical infrastructures at EU level: by creating a 2000L CMO-ready bioproduction facility in Portugal's Norte region, directly contributing to the EU's critical biomanufacturing infrastructure base. The facility is designed as a replicable template: the same geometry-preserved bioreactor system, sensor architecture, and AI control platform can be deployed at additional sites without re-engineering. This modularity addresses the EU's recognized deficit in mid-scale fermentation capacity. The alternative protein sector alone requires three orders of magnitude more fermentation capacity than is currently available in Europe by 2030. PRISMA's facility model provides a scalable, cost-efficient path to closing that gap.

Contribution to increasing manufacturing capacity of critical value chains within the EU: A central contribution of PRISMA lies in increasing EU manufacturing capacity along the textile colourants value chain, which has experienced decades of relocation of dye and intermediate production outside Europe. PRISMA establishes domestic EU manufacturing capacity for bio-based textile pigments using locally sourced waste feedstocks, directly reducing this import dependency. At full operation, the CMO platform targets a production capacity of 2–4 batches per month, corresponding to approximately 500–1,000 kg per year of textile-grade bio-pigments. This represents the first European-manufactured bio-pigment supply at industrial scale. The waste-to-pigment value chain is entirely localized within Portugal: feedstocks sourced from Portuguese agro-food producers, fermentation and DSP at the Barcelos facility, textile validation at CITEVE and ATB.

Contribution to the EU industrial and technological leadership: PRISMA establishes European-first capability in three technology areas where no EU-based commercial solution currently exists: 1) geometry-preserved bioreactors manufactured by additive manufacturing across production-relevant scales (1L to 2000L); 2) an integrated AI-driven process intelligence framework enabling real-time and continuous monitoring, optimisation, and control of fermentation processes along with productivity and quality control of the final products; and 3) a validated manufacturing process for bio-based textile pigments at cost parity with

petrochemical dyes. These capabilities position the EU ahead of US and Asian competitors in precision fermentation infrastructure. In addition, the PRISMA project creates at least 2 patent families that secure European control over critical bioprocessing technologies. These patents reduce reliance on non-EU technologies, support EU-based industrialisation of bio-based products and enable controlled dissemination and cross-border adoption within the internal market, while consolidating bioreactor manufacturing know-how and production capacity in Europe.

Contribution to promoting positive cross-border effects in the internal market: by structuring a transnational value chain that connects bioprocess development, digital technologies, manufacturing infrastructure, and industrial textile application across European regions: i) the Octarine Bio LOI (Denmark) creates a Portugal-Denmark commercial manufacturing relationship, with bio-pigments produced in Barcelos for a Copenhagen-based biotech company serving global fashion brands; ii) PRISMA's bench-scale bioreactor deployments serve customers across the EU (UK, Netherlands, Germany), building an installed base that feeds data back into the AI platform; 3) the modular and replicable nature of the PRISMA platform supports future deployment by biotechnology and textile actors in other Member States, reinforcing internal market dynamics and reducing fragmentation in access to advanced biomanufacturing capabilities.

- Complete the table below by selecting market risk events

Market risk event	Event description	Probability of occurrence (1 = Low to 5 = High)	Planned mitigation measures
Low adoption of bio-based pigments by the textile industry	Textile manufacturers may be reluctant to replace established synthetic dye supply chains due to cost uncertainty, certification requirements and conservative procurement practices, slowing market uptake of bio-based pigments.	3	Validation of demand through an anchor customer (Octarine Bio); industrial-scale trials generating robust cost and performance data; third-party certification and testing by CITEVE; direct engagement with textile manufacturers via CITEVE's industry network to accelerate early adoption.
High production costs compared to synthetic dyes	Bio-pigment production costs may fail to reach parity with synthetic dyes (EUR 5–15/kg) due to lower-than-expected fermentation yields, high downstream processing costs or feedstock price variability.	4	Qualification of multiple waste-derived feedstocks to reduce cost volatility; geometry-preserved scale-up to avoid costly re-optimisation; DSP optimisation to reduce energy and operating costs; temporary focus on higher-margin applications if cost parity is delayed.
Competing bio-based dye solutions reaching the market earlier	Other bio-dye developers (e.g. Colorfix) may achieve industrial-scale production earlier, potentially capturing initial market share and first-mover advantages.	2	Positioning Chainreactor as a technology-agnostic manufacturing and CMO platform rather than a dye producer; leveraging competitor success to validate demand for CMO capacity; serving multiple bio-pigment developers, including competitors of vertically integrated players.
Rapid technological evolution in biomanufacturing	Advances in alternative biomanufacturing approaches (e.g. new synthetic biology)	2	Use of organism- and process-agnostic bioreactor and AI platform; applicability of the PRISMA infrastructure to multiple

	strategies or non-fermentation-based systems) could reduce demand for fermentation-based production in the textile sector.		bioprocesses beyond pigments; diversification into adjacent markets such as food ingredients, cosmetics and industrial biotechnology.
Challenges in protecting intellectual property	Key innovations such as geometry-preserved bioreactor design and process-intelligence architectures may be reverse-engineered or independently developed by competitors before full patent protection is secured.	2	Early provisional patent filings to secure priority dates; protection through a combination of hardware IP, proprietary process data and trained AI models; governance of IP ownership and access through the Consortium Agreement to prevent information leakage.