

Research plan outline and strategy

2026-2030

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Introduction

Protecting and restoring ocean health, while addressing the need for scalable climate solutions, is a central motivation behind the development of marine carbon dioxide removal approaches. The Microalgae Carbon Fixation and Sinking (MCFS) methodology represents a controlled approach to marine carbon dioxide removal (mCDR) designed to facilitate the long-term sequestration of atmospheric carbon dioxide in the deep ocean. The approach uses engineered floating particles (“substrates”) that host local phytoplankton additional growth in surface waters. After a controlled growth phase, these substrates sink rapidly and efficiently, transporting the captured carbon through the water column to the deep ocean and seabed for long-term storage of hundreds to thousands of years.

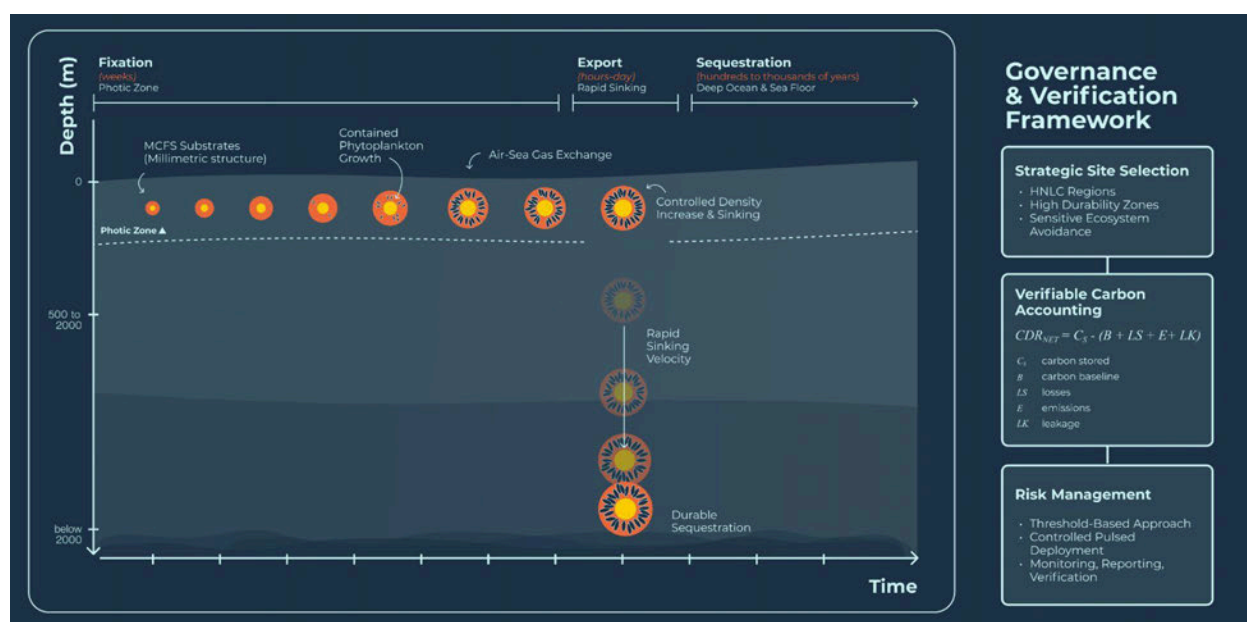


Figure 1. Schematic illustration of the phytoplankton growth on the Substrate (orange) and subsequent export into the deep ocean and sediment. During the fixation phase, microalgae (blue) start to accumulate within the Substrates. At the export phase, the Substrates and the accumulated microalgae and adjacent bacteria (fixed carbon) sink rapidly to the deep ocean, where the carbon is sequestered for at least 200 years (centuries to thousands). Note that this figure illustrates the capture and sinking process only, and does not account for the factors impacting the sequestration efficiency.

The purpose of this document is to present Gigablue’s Research Plan Outline and Strategy for 2026–2030 for MCFS activities offshore New Zealand. The document has been prepared for the New Zealand Ministry for the Environment (MfE) to provide a transparent overview of the scientific

research program that will guide the development, validation, and responsible scaling of MCFS operations. The research program integrates Gigablue's internal research objectives with scientific questions and areas of interest identified by the Ministry for the Environment.

The research strategy is designed to support the gradual development of MCFS activities from early experimental deployments toward larger operational scales. This progression is structured as a phased and evidence-based scale-up, in which each stage of deployment is supported by targeted research designed to address specific scientific uncertainties. Scaling decisions will be contingent upon the achievement of defined confidence benchmarks and validation points demonstrating adequate environmental understanding, monitoring capability, and technological performance.

To achieve these objectives, the research program is organized into three primary areas of investigation:

- *Environmental and Oceanographic Research*, focusing on understanding the baseline characteristics and evaluating potential environmental interactions across the surface ocean, water column, and seabed.
- *Measurement, Monitoring, Reporting, and Verification (MMRV) Methods*, focusing on the development and validation of techniques required to ensure ecosystem health, quantify carbon fixation, track substrate transport, and verify durable carbon storage.
- *MCFS Technology Research and Development*, focusing on the design, testing, and continuous improvement of substrate systems and associated operational technologies.

These research areas are closely integrated. *Environmental studies* inform operational thresholds and site suitability, *MMRV methods* provide the measurement framework required to ensure ocean health, quantify outcomes and verify performance, and *technology R&D* improves substrate design and deployment efficiency. Together, they support the responsible advancement of MCFS while maintaining compliance with environmental safeguards and methodological requirements.

The research program follows a chronological and iterative structure in which laboratory studies, modeling efforts, and field deployments progressively increase in scale. Early-stage activities focus on establishing environmental baselines, validating monitoring methods, and testing technological performance. Subsequent phases involve larger deployments designed to monitor environmental potential impact at scales sufficient to produce detectable signals. Throughout the program,

experimental design will ensure that research activities are capable of effectively answering the scientific questions under investigation.

All research activities described in this plan will be conducted within a framework that prioritizes environmental protection, regulatory compliance, and scientific transparency. Deployment scales and operational parameters will remain within environmental thresholds defined through scientific analysis and validated by credible third-party review. Furthermore, all substrate compositions used in research deployments will comply with the MCFS methodology's substrate eligibility, regulatory compliance and safety criteria.

Funding for the research activities described in this plan may originate from multiple sources, including public research grants, academic collaborations, private investment, and carbon credit revenues associated with verified carbon removal activities.

This document provides an overview of the research topics, methods, and strategic framework that will guide MCFS scientific development between 2026 and 2030. The following sections describe the specific areas of research, the prioritization and methodological approach applied to each topic, and the phased research plan designed to build confidence and enable responsible scaling of MCFS activities.

Areas of research

The planned research program is organized into three primary scientific areas:

- *Environmental and Oceanographic Research*
- *Measurement, Monitoring, Reporting and Verification (MMRV) Methods*
- *MCFS Technology Research and Development.*

These three disciplines collectively address the key scientific and technical questions required to evaluate environmental safety, verify carbon sequestration, and support the continued development of mCDR in the New Zealand context, and specifically MCFS as a promising method for efficient and safe ocean restoration through carbon removal in the New Zealand waters.

Baseline Environmental Characterization of the AOI and thresholds setting

A foundational component of the research program is the characterization of environmental conditions within the Area of Interest (AOI). Establishing a robust environmental baseline is necessary to understand natural variability, identify sensitive ecosystem components, and define operational thresholds that ensure environmentally responsible deployment of MCFS substrates.

To ensure effective safety measures, each AOI will undergo a structured environmental characterization process. These parameters will either be newly defined or refined based on available data and additional research conducted as part of the MCFS research program:

1. Water current patterns characterisation: Ocean circulation patterns are characterized to understand surface and subsurface transport dynamics that influence substrate dispersion and transport.
2. Ventilation time of deep water: The residence time of deep-water masses confirms that ocean circulation patterns support long-term carbon storage of hundreds to thousands of years, and not less than 200 years.
3. Baseline characterisation of the water column: Includes surface water chemistry and biology.
4. Baseline characterisation of sediments: Includes sensitive species distribution, DO reduction sensitivity, organic carbon fate and remineralisation rates.
5. Setting operational thresholds for MCFS activities (substrate densities): Operational thresholds, based on environmental conditions and modeling results, ensure substrate densities remain well within environmentally safe thresholds.
 - a. Threshold setting for water column local nutrient depletion.
 - b. Threshold setting for sediment deposition - benthic smothering
 - c. Threshold setting for sediment deposition - dissolved oxygen depletion in deep water.

Key research topics

To support the safe and effective development of MCFS activities, a set of targeted research topics has been identified. These topics address the main scientific and technical questions required to

evaluate environmental interactions, develop reliable monitoring approaches, and improve MCFS technology performance. The blue shading indicates those topics that have been defined as being of scientific interest by the MfE.

1. Environmental & Oceanographic Research

- a. Surface and water column studies
 - i. Near-field and far-field nutrient concentration reduction under different MCFS deployment scenarios
 - ii. CO₂ air-sea fluxes during surface water DIC reduction activity
 - iii. Changes in species composition, biodiversity, and community structure of phytoplankton and bacteria due to MCFS activities relative to the baseline
- b. Benthic studies
 - i. Remineralization processes and the fate of the carbon in the deep ocean sediment-water interface
 - ii. Effects on benthic communities (DO, Nutrients, Organic matter load)
 - iii. Effects of DO concentration change over time until replenishment, and possible cumulative effect
 - iv. Chemical reaction with CaCO₃ (enhanced durability)
 - v. Effect of sunk substrate density on “smothering” of benthic communities
- c. Marine Macrofauna
 - i. Interest of marine birds and mammals in the floating substrates.
 - ii. Digestion impacts of substrates on macrofauna.

2. MMRV (Measurement, Monitoring, Reporting, Verification) approach

- a. Floating phase - Remote sensing techniques for tracking of floating substrate and phytoplankton growth dynamics.
- b. Sinking phase - Tracking of sinking substrates under diluted conditions (low densities of substrates in the water column).
- c. Post-sinking phase - Deep-sea sediments observation.
- d. Development of techniques and sensors to track and map the sunken substrates on the seafloor

3. Technology Research and Development (R&D)

- a. Substrate carbon accumulation efficiency and optimisation.
- b. Representative simulation of physical ocean conditions in the laboratory - Ocean Surface Energy (OSE) simulator.
 - i. Laboratory simulator development.
 - ii. Open ocean physical conditions' impacts on the performance and stability of the substrate.
- c. Sinking and shear force - Retention of phytoplankton on the sinking substrate

Research methods and strategy

To address the key research topics, the MCFS program applies multiple complementary approaches, including:

- A. Desktop studies: Assessment of current understanding from local and international literature, existing data, and prior research.
- B. Modelling studies: Predictive simulations of substrate dispersion, environmental impacts, and biogeochemical processes.
- C. Laboratory / On-Shore studies: In-Vitro microcosm experiments to test environmental impacts, biogeochemical processes and substrate performance.
- D. Field (In-Situ) observations and sampling: Deployment of substrates at various scales to measure real-world environmental responses and to validate models.

Research is structured over iterative stages:

- 1. Assessment of current knowledge: Compile and review all existing and available data to define the baseline understanding of each topic (Desktop Study).
- 2. Defining the level of confidence: Evaluate how well current knowledge supports safe and effective operations.
- 3. Threshold setting: Establish operational limits consistent with current understanding and confidence levels. Note that thresholds are established under third party supervision.
- 4. Formulating further research questions and methods: Identify knowledge gaps and plan

specific experiments to address them.

5. Integration into the wider experiment plan: Ensure studies across disciplines are coordinated and inform one another in a way that will increase the level of confidence in an effective manner.
6. Execution of the experimental plan: Conduct lab, modeling, and field experiments according to defined protocols.
7. Review and iteration: Update confidence levels and thresholds based on results, refine research questions, and repeat as necessary.

Research topics are prioritized according to three main criteria:

1. *Level of possible impact*: Topics with higher potential influence on environmental impact, carbon sequestration efficacy or MRV efficiency are prioritized
2. *Level of confidence*: Topics with lower existing confidence require earlier or more intensive study to increase confidence.
3. *Experiment effectiveness*: Experiments are prioritized to provide the greatest knowledge gain

Research plan guiding principles

The MCFS research plan is organized into three integrated workstreams: environmental research, MRV methods, and Technology research and development. Research is phased according to increasing deployment scale, progressing from 100-200 tonnes/year in early pilot studies up to 250,000-500,000 tonnes/year by Q3 2031. Each phase is designed to build confidence, inform regulators, decision makers, operational decisions, and support safe scale-up.

Field Operations are guided by four key requirements :

1. **Legal and Regulatory Compliance**: All *in situ* activities will fully comply with all applicable local laws, regulations, and binding obligations.
2. **Substrate Compliance**: Every substrate version deployed will meet the eligibility criteria set defined in the MCFS methodology ([Appendix A](#)) for environmental safety and technical

suitability.

3. **Activity Thresholds:** Operations will remain within thresholds established and verified by accredited third parties.
4. **Experimental Design:** Experiments will be designed to effectively address the relevant scientific questions. Specifically, monitoring the impacts on the water column and benthos will require sufficient scales to generate measurable and meaningful results.

Research funding: Research activities outlined in this plan may be funded through a variety of sources, including grants, academic collaborations, investments, and carbon credit revenues.

Validation points:

Before each field deployment and subsequent scaling, new findings from lab, modeling, and prior in situ studies will be reviewed to determine if operational plans, thresholds, or experimental designs require adjustment. Any emerging knowledge gaps or research questions will be incorporated into the ongoing plan to ensure that scale-up decisions remain evidence-based, scientifically robust, and environmentally safe. The program is also committed to sharing new findings and learnings with the scientific community and relevant local stakeholders.

Proposed Scale-Up Management Scheme

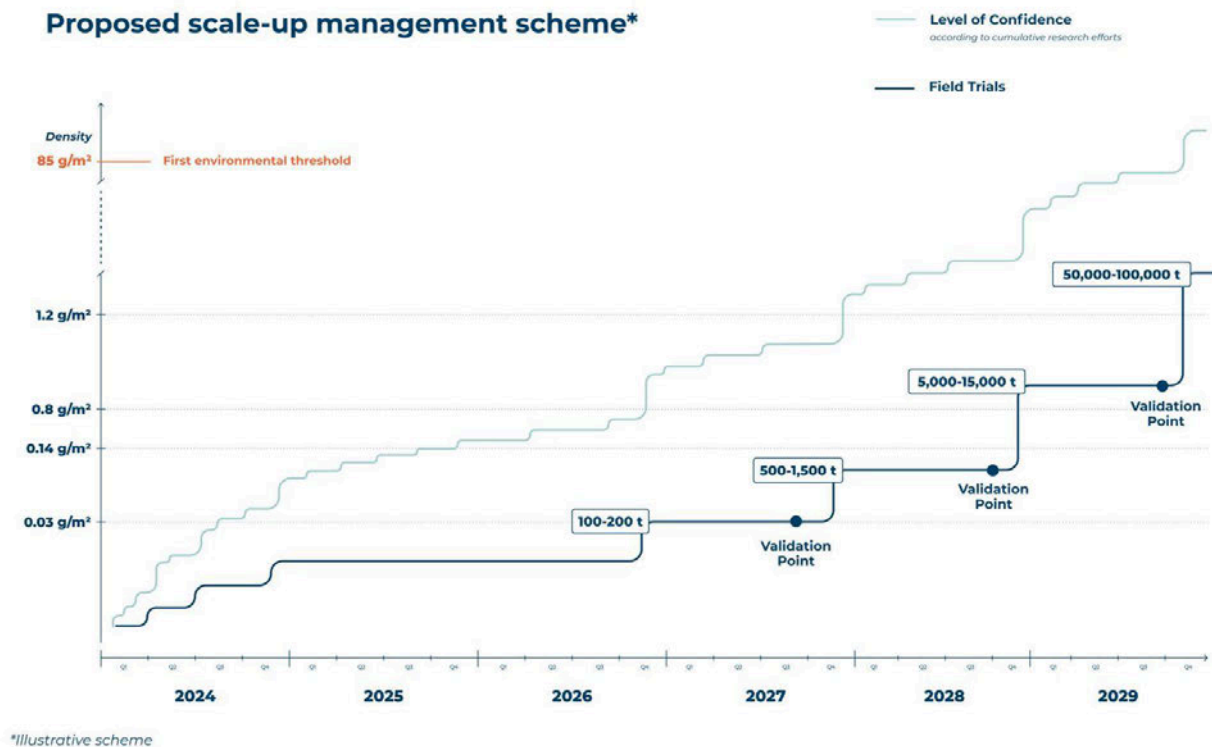


Figure 2. Proposed Scale-Up Management Scheme. Operational deployment scale (tonnes of substrate per year) is shown relative to substrate concentration density upon the seabed, alongside defined environmental threshold levels. Across all planned scales, operational concentrations remain well below these thresholds. The light blue line represents the progressive increase in confidence achieved through iterative research, driven primarily by evidence collected from in situ field experiments. The dark blue line illustrates field deployments completing a full MCFS cycle, from floating to subsequent sinking. Field experiments are season-dependent and are conducted during Q4–Q1 of each year. At its current stage, MCFS is focused on generating robust, evidence-based data to enable meaningful learning and inform responsible scale-up.

Research topics

1. Environmental & Oceanographic research

1.1. Environmental Planning and Modeling

This stream focuses on characterizing the optimal areas of MCFS deployment in New Zealand's EEZ. For example, the first Area of Interest (AOI) chosen for deployment is located in the Bounty Trough and Northern Campbell Plateau/Bounty Plateau, approximately 50–330 km east of Dunedin, New

Zealand. This location was chosen based on three key factors for effective carbon sequestration activity: seabed depth of ~1,200 meters (well below the permanent thermocline at ~100m deep), water chemistry characteristic of High Nutrient Low Chlorophyll (HNLC) conditions, and far from sensitive habitats.

- 1.1.1. Environmental Impact Assessment (EIA) and AOI Characterization: The first step is to characterize potential impacts of MCFS operations within the AOI and apply a [modeling framework](#) to support these assessments. The EIA will be updated iteratively as new data is being generated throughout the execution of the research plan.
- 1.1.2. Oceanographic Conditions Characterization: This involves describing the physical and biogeochemical baseline conditions of the AOI. A continually updated database will store background environmental and oceanographic data from all deployments, supporting both modeling and impact assessments. .
- 1.1.3. Lagrangian Models for Particle Dispersion: Regional connectivity and realistic release simulations will be tested and validated in the field repeatedly from Q4 2026 through Q4 2030. These simulations inform predictions of substrate transport, dispersion, and deposition patterns.
- 1.1.4. Ventilation Model: A high-resolution Lagrangian model will be applied to track deep-water trajectories, linking regional and lower resolution global models. The global model will evaluate the permanence of sequestered carbon per site, with a condition of a minimum 200-year storage period and up to thousands of years. .
- 1.1.5. Threshold Studies: Maximum concentration thresholds for substrates in the surface water and seabed will be established through desktop and model studies and verified via field studies. These studies are crucial for determining levels that must not be exceeded. The expected operational concentrations will then be tested against these established thresholds, and the operational plan will be adjusted based on the study findings.

- **Threshold for water column nutrient depletion**

This threshold is determined by modeling the impact of the operation's nutrient consumption on local phytoplankton growth rates.

The process involves:

- A. Running a dispersion model to calculate substrate concentrations throughout the fixation phase.
- B. Using these results to model nutrient consumption and resulting reductions in surface water nutrient concentrations.
- C. Calculate changes in phytoplankton growth rates.

The maximum allowable reduction in growth rates is set to the minimum intra-seasonal growth rate.

- **Benthic thresholds for dissolved oxygen reduction and smothering**

Initial thresholds are established by review and analysis of scientific literature on the natural variability of deep-water benthic dissolved oxygen (DO) at the AOI, determination of biomass-loaded substrates densities that can lead to DO reduction levels that preceding adverse benthic impacts, and / or could smother benthic communities.

Based on the established thresholds, the operational plan includes:

- A. Running a dispersion model to calculate substrate density settled on the sediments.
- B. Using these results to model the remineralisation related DO consumption.
- C. Comparing substrate densities on the seabed relative to the established smothering threshold.
- D. Comparing the modelled DO concentration reduction per expected substrates densities against the established threshold.

If any of these thresholds are exceeded, the deployment specifications will be adjusted. The DO reduction model results will be validated once operational scales allow reliable plume tracking .

1.2. Environmental & Oceanographic Studies

This stream focuses on the collection of *in situ* observations and samples across the water column and seafloor to characterize natural baseline conditions and evaluate the environmental responses to MCFS activities.

1.2.1. Surface and Water Column Studies

- **Background Studies:** Research will focus on characterizing natural conditions within the AOI. Activities include improving the understanding of ocean circulation patterns and pelagic ecosystems at different depths, collecting physicochemical background data, and studying the key food web components (e.g. abundance, production, and biodiversity). These studies are planned to run from Q1 2026 through Q4 2030.
- **Near Field Concentration Reduction of Nutrients:** Research will track macronutrient dynamics in surface waters during the fixation phase of a free-floating plume trial in order to quantify local nutrient uptake and evaluate potential short-term changes in nutrient availability. Field observations will be conducted between Q4 2026 and Q4 2030.
- **Far Field Concentration Reduction of Nutrients:** A global circulation model (either steady-state inverse or hindcast) will be used to simulate the transport of nutrient-depleted surface waters originating from the AOI through a 10-year tracer release simulation. The objective is to identify downstream regions where these waters may eventually outcrop and to demonstrate that each deployment will not cause nutrient concentrations (for nitrate, phosphate, or silica) to fall below established limitation thresholds in any downstream region. As scientific understanding of large-scale nutrient redistribution is still evolving, this framework is considered preliminary and is currently suitable for projects sequestering less than 1 Mtof carbon. As project scales increase, future refinements will include coupling biogeochemical and circulation models to better quantify wider-scale impacts.
- **CO₂ Air-Sea Gas Exchange (ASGE):** Research will use in-situ measurements to validate CO₂ exchange models. It will evaluate DIC dynamics in surface waters during the fixation phase. Measurements of carbonate system parameters will be

used to calculate expected ASGE. From Q3 2027 onward, eddy covariance measurements will be incorporated to investigate the relationship between vertical mixing rates, CO₂ gas-exchange rates, and short term DIC removal kinetics.

- Plankton Community Monitoring: Monitoring the natural planktonic community (bacteria and eukaryotes) during deployments will track potential changes in composition, abundance, and biodiversity relative to the baseline conditions.

1.2.2. Benthic Studies

This stream focuses on understanding the fate of exported substrates and associated carbon upon the seabed, and evaluating changes in biogeochemical dynamics and ecological responses at the deep ocean water-sediment interface.

- Effects on Benthic Communities: The research will assess potential impacts of substrate deposition on benthic communities within the AOI, including:
 - A. Review of scientific literature and publicly available benthic data within and proximal to the AOI and identify sensitive habitats, protected species, and vulnerable taxa, with findings incorporated into operational planning.
 - B. Habitat suitability modelling will be applied to predict the species distribution under limited data conditions.
 - C. An in situ benthic chamber study will be considered from Q3 2028 onward.
- Remineralization and Carbon Fate: Laboratory incubation experiments using sediment cores collected from the AOI will be conducted to study remineralization processes and the fate of the remineralized carbon at the sediment-water interface. This includes measurements of DO consumption, DIC and nutrient release, and remineralization rates.
- DO Concentration Change: An long-term in situ deep-sea monitoring system equipped with sensor arrays will be deployed in the sunken plume area to measure changes in DO over time. Deployment will begin once operational scales allow reliable plume tracking, with repeated retrieval and redeployment to assess cumulative impacts and recovery dynamics until replenishment.

- Chemical Reaction with CaCO_3 (Enhanced Durability): A desktop study will evaluate the potential for chemical reaction between carbon and CaCO_3 in the sediment, where present, during remineralization, and their implications for enhanced carbon storage durability.
- Substrate Density and "Smothering": A seabed observation study utilising systems such as DTIS - Deep Towed Imaging System of the sunken plume will be used to assess substrate deposition densities, and associated carbon loading on sediments relative to model and laboratory estimations. This approach will be implemented once deployment scales allow effective plume tracking until it reaches the seabed..

1.2.3. Marine Macrofauna and Digestion Impacts

- Interest of Large Marine Fauna: On-deck (on the vessel) and Drone-based observations of marine mammals in the plume area will be conducted to observe, document, and study the behavior of large marine fauna with the substrates. Passive Acoustic Monitoring (PAM) with a hydrophone array will be used during deployment to confirm the absence of marine mammals at the deployment site.
- Digestion Impacts: Laboratory simulations of representative marine macrofauna digestion systems will be conducted to test substrate breakdown under ingestion scenarios, with results incorporated into the Impact Assessment (IA) will be updated.
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2. MRV: Plume Tracking and Monitoring

This stream focuses on the development, improvement and validation of methods to track and monitor the MCFS activity across all operational phases .

- ### 2.1. Floating Phase : Techniques for tracking floating substrate plume, model validation and substrate performance monitoring will be continuously improved or developed if needed. This will include:

- #### 2.1.1. Combined SAR and optical satellite imagery to assess plume dispersion over time.

- 2.1.2. Drifters will be deployed to validate modeled plume trajectories (up to ~20 days), and measurements will be used to refine models by quantifying the effects of currents and wind on particle transport.
- 2.1.3. Particle plume sampling will be conducted during the floating, sinking and post-sinking phases, collecting both water and substrate samples for relevant parameters.
- 2.2. Sinking Phase (Tracking Diluted Substrates): As the plume disperses and transitions to the sinking phase, tracking will utilize echosounder and ROV-based observations. Results from in-situ trials will be used to develop and improve methods for detecting and tracking low-density, sinking substrate plumes.
- 2.3. Post-Sinking Phase (Seafloor Mapping): A dedicated sensor will be developed to track and map substrates on the seafloor, including the application of fluorescent-based detection methods for chlorophyll- on deep water seabed .

3. R&D: Substrate Performance

This stream focuses on the continuous development, testing, and validation of substrate performance and stability. Gigablue conducts ongoing optimization of the substrate evaluated for carbon accumulation efficiency (performance). Our primary goal is to develop the substrate formulation with the highest carbon accumulation efficiency while strictly utilizing environmentally safe materials only and minimizing environmental impact. Once an optimal formulation is selected, manufacturing begins and each batch is tested against the strict eligibility criteria and QA requirements detailed in Appendix 1 ([Substrate Testing and Requirements for Eligibility](#)).

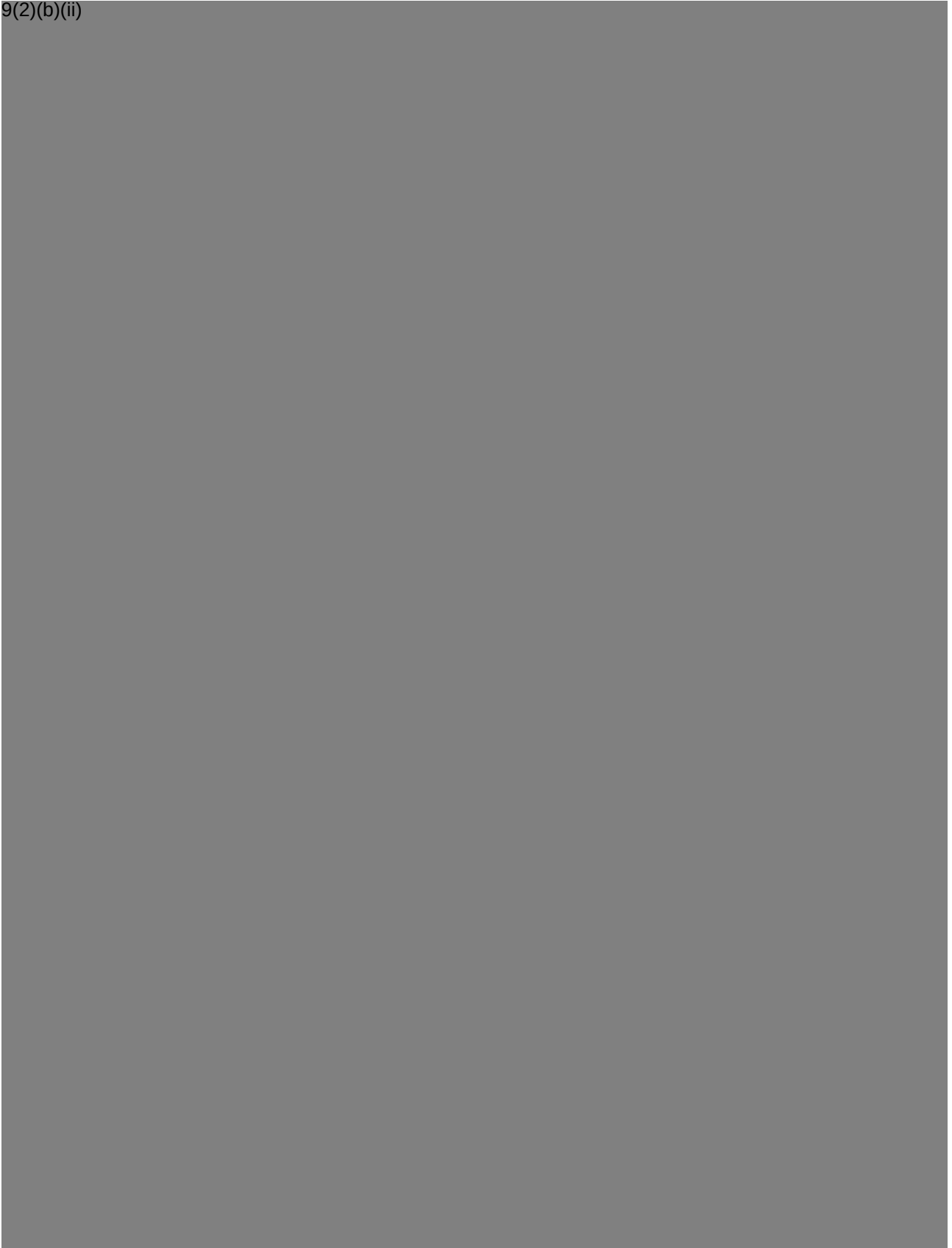
- 3.1. Substrate Performance: Laboratory studies and in situ ocean pens experiments (tens of kilograms scale) will be conducted to evaluate phytoplankton growth and carbon accumulation within the substrate, running from **Q1 2026 through Q4 2030** alongside ongoing R&D optimization. This includes:

- Quantifying the effect of sinking velocity and shear forces on biomass retention during descent.
 - Quantifying biomass loss due to remineralization during the sinking phase.
 - Assessing the effect of different macronutrient regimes on substrate performance.
- 3.2. Ocean Surface Energy (OSE) simulator development: To simulate energy applied on the substrates while it is floating at the surface water glider-based measurements of turbulence in the upper mixed layer and drone-based observations of the surface dynamics together with image processing techniques will be conducted. The data gathered will be used to develop a laboratory-based simulator capable of reproducing representative ocean conditions for continuous substrate testing and optimization.
- 3.3. Substrate Eligibility Testing: Eligibility testing for each manufactured substrate batch prior to deployment is a continuous activity spanning from Q1 2026 through Q4 2030 ensuring compliance with the technical and safety requirements defined in [Appendix A](#).

Research Plan Gantt

The research plan is structured through a series of annual Gantt charts, each representing one operational cycle from Q4 of a given year through Q3 of the following year. This structure aligns with the seasonal nature of operations, with in situ field activities conducted during Q4–Q1 (Austral spring and summer), and the remaining period dedicated to laboratory work, data analysis, modeling, and desktop studies. Following each yearly Gantt, a set of defined confidence benchmarks is provided, which must be met to enable progression to the subsequent phase and support responsible scale-up of operations.

9(2)(b)(ii)



Confidence Benchmarks prior to 500-1,500 Tonne Scaling

- **Plume monitoring and tracking methodology**

Demonstrated ability to effectively track both floating and sinking plumes and evaluation of tracking accuracy and identification of any required technical improvements.

- Field-based model validation

- **Environmental baseline and biochemical impact**

Validation of AOI baseline characterization through comparison with *in-situ* field operational data, specifically confirming that observed biochemical changes remain within the defined thresholds.

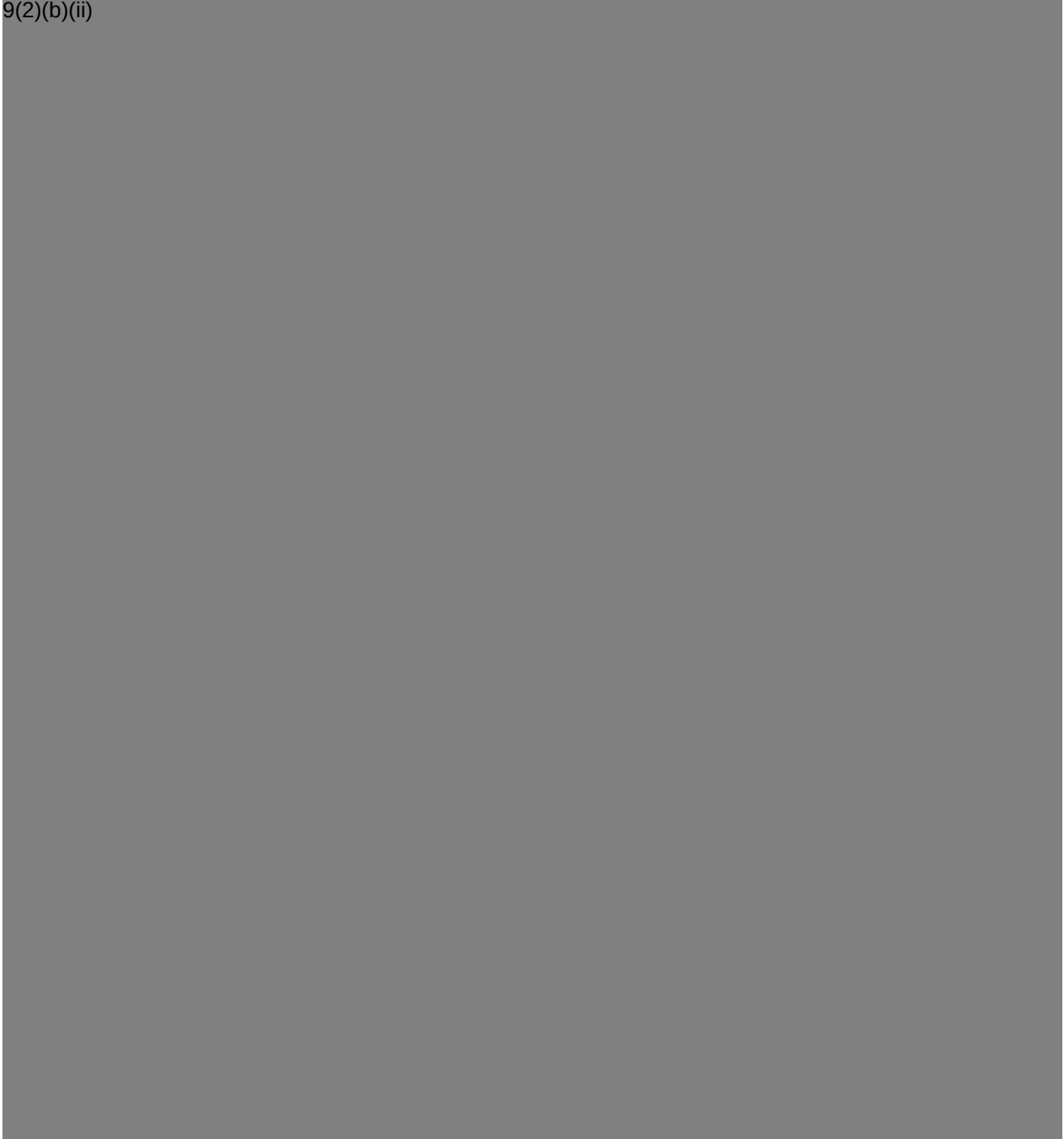
- **Benthic impact and operational optimization**

Verification of laboratory-based assessments of benthic disturbance and identification of any adjustments to the defined thresholds.

- **Macrofauna interaction and digestion verification**

Assessment of marine life attraction to the floating plume and validation of substrate digestibility (based on OSE simulator tests).

9(2)(b)(ii)



Confidence Benchmarks for 5,000–15,000 Tonne Scaling

- **Marine macrofauna interaction**

Assessment of potential attraction of higher trophic levels to the plume.

- **Plume dynamics and tracking methodology efficiency**

Evaluation of tracking accuracy for both floating and sinking plume, including identification and implementation of necessary technical sensor or modeling adjustments .

- **Biochemical impact and baseline deviation analysis**

Quantification of changes in water column biogeochemistry (specifically DO and macronutrients) relative to baseline conditions ensuring results remain within defined thresholds .

9(2)(b)(ii)



Confidence Benchmarks for 80,000–120,000 Tonne Scaling

- **Environmental safety and threshold compliance**

Validation of dissolved oxygen, nutrient, and sediment deposition data to confirm

compliance with defined environmental thresholds and refine future deployment protocols if needed.

- **Benthic ecosystem integrity**

Assessment of seafloor impacts, specifically substrate accumulation and potential smothering, to optimize deployment density.

- **Trophic interaction and ingestion risk**

Verification that macrofauna do not exhibit significant attraction to floating plumes and that ingestion risks remain minimal.

- **Water column biochemical stability**

Quantification of deviations in biogeochemical parameters from baseline conditions ensuring they remain within established safety thresholds.

9(2)(b)(ii)



Pre-Scaling Readiness: 250k–500k Tonne Operational Topics

- **Cumulative environmental impact and sustainability**

Assessment of long-term ecological risks, including cumulative benthic impacts from repeated deployments.

- **Substrate reliability and performance efficiency**

Evaluation of the operational reliability and substrate performance to ensure commercial viability at scale.

- **Biochemical variance and threshold buffer analysis**

Quantification of water column deviations relative to baseline conditions and remaining safety margins relative to regulatory thresholds.

- **Benthic oxygen dynamics and recovery rates**

Verification of how substrate deposition affects seafloor Dissolved Oxygen (DO) and whether natural recovery rates can stay within the defined thresholds with continuous, large-scale operational cycles.

9(2)(b)(ii)



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Appendix A. Substrate Testing and Requirements for Eligibility

The Microalgae Carbon Fixation and Sinking (MCFS) substrate must undergo extensive testing and analysis to confirm its eligibility for deployment, focusing on its physical properties, chemical composition, toxicity, and performance kinetics. These tests and analyses, as were defined by the Puro.earth MCFS methodology, ensure the substrate promotes carbon fixation efficiently while minimizing potential environmental harm.

I. Composition and Physical Property Analysis

Before deployment, the substrate must be analyzed to characterize its chemical and physical makeup:

Analysis/Requirement	Details
Detailed Characterization	The Substrate composition must be detailed. The material components must be non-toxic and non-hazardous (organic material and inorganic material/minerals).
Micronutrient Content	Trace elements (micronutrients) must be up to 2% of the total mass of the Substrate. Iron must be included as one of these micronutrients.
Micronutrient Form/Stability	All added micronutrients must be in an oxide form and possess a zero to low tendency to dissolve (determined as insoluble under ocean conditions—salinity, pH, temperature). This minimizes nutrient leaching into the surrounding waters.
Particle Dimensions	The range of diameters or geometrical faces of each Substrate unit must be between 0.5 mm and 100 mm. For a single deployment, the size variance must fall within a relative standard deviation of 50%.
Density (Pristine)	The density of the solids comprising the pristine substrate must be at least 0.01 g/cm ³ higher than the density of the water at the deployment site.
Total Mass Measurement	The dry weight of each batch must be measured prior to deployment, as close in time to the deployment as possible, using reliable, calibrated weighing equipment.

Containment Mechanism	The Substrate must demonstrate a mechanism for containment of phytoplankton, such as physical entanglement within a porous structure, to minimize the loss of accumulated biomass due to physical forces.
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II. Toxicity and Contaminant Analysis

The Substrate must be tested for potentially toxic elements (PTEs) and other contaminants to ensure it poses a low risk to marine fauna.

Analysis/Requirement	Details
PTE Concentrations	Evidence that concentrations of PTEs in the Substrate do not exceed the limits defined in applicable local, regional, national, or international legislation.
Heavy Metals	Analysis must cover heavy metals that could leach and bioaccumulate, including, but not limited to, mercury (Hg), lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn). (For example, prior toxicity tests indicated no adverse effects from the comprising materials—cellulose with manganese and iron—on algae or mussels, and ICP-MS analysis showed metal/metalloid contaminants were well below water quality guidelines).
Organic Contaminants	Levels of polycyclic aromatic hydrocarbons (PAHs) and polyfluoroalkyl substances (PFAS) must be analyzed.
Pesticides	Levels of pesticides, including organophosphates, neonicotinoids, and herbicides, must be analyzed when applicable.
Toxicity Thresholds	All contamination levels obtained must be reported and compared against established safety thresholds. If any threshold is exceeded, the Substrate is considered ineligible.
Micronutrient Leaching	Laboratory-based tests must be conducted on pristine substrates to quantify the leaching of micronutrients and trace elements using elemental analysis methods (such as ICP-OES or ICP-MS).

III. Performance and Kinetic Testing (Laboratory-Based)

Laboratory experiments must be performed on the substrate before deployment to model its behavior in the ocean, specifically regarding sinking and decomposition:

Test/Analysis	Purpose and Methodology Details
Structural Stability	Incubation experiments conducted over a minimum of 30 days in artificial seawater under simulated oceanic mixing conditions (e.g., using an orbital shaker or Turbulence Induced Nano-Cosm, TINS) to assess the % mass unit remaining.
Floatation Time	Laboratory experiments must determine the floatation time (in days), which cannot exceed 30 days. This is defined by assessing the percentage of units that remain floating versus those that sink to the bottom of the testing vessel.
Sinking Rate	Column tests using measuring cylinders must be conducted to determine the sinking rate once the substrates reach a sinking condition (density greater than water density). The required minimum sinking velocity is 20 m/hr.
Maximum Phytoplankton Growth Rate (μ_{max})	Incubations in HNLC seawater must be performed to evaluate the maximum growth rates of local phytoplankton. The rate is calculated from the phytoplankton biomass accumulated over the experiment duration.
Remineralization Rate (d_0)	Laboratory measurements, onboard the monitoring vessel, are required to evaluate the non-sinking remineralization rate of the biomass attached to the unsunk substrate, which is a key input for calculating the sinking efficiency ($SE_{project}$).

Appendix B. Modeling Framework

Several distinct modeling approaches are utilised for planning, quantification, risk assessment, and monitoring throughout the project lifecycle. These models range from local, high-resolution physical simulations to global-scale circulation models used for determining carbon durability and efficiency. The models and modeling frameworks include:

I. Planning and Deployment Models

These models support operational decisions, site selection, and the strategic distribution of substrates.

- **Physical Oceanographic Model:** This local-scale model for the Area of Interest (AOI) characterizes circulation patterns, stratification, and water mass interaction, resolving three-dimensional ocean circulation. It has a horizontal spatial resolution of at least $\sim 1/12$ validated against observational data such as satellite-derived sea surface temperature.
- **Lagrangian Particle Tracking Model:** This model is utilized before deployment to simulate Substrate dispersion and trajectory driven by 3D current fields, wind, and surface wave parameters. It generates 3D particle trajectories over time, surface dispersion maps, and seabed deposition maps. It is essential for calculating the Substrate deployment density.
- **Life Cycle Assessment (LCA) Model:** A model that calculates the whole life cycle **Project Emissions** associated with the MCFS activity, following ISO-14040/44 and ISO-14064-2 principles. The model must be a digital tool (spreadsheet or non-spreadsheet) enabling transparent verification and must account for operational emissions and embodied emissions.

II. Environmental Risk and Baseline Models

These models are used to anticipate, assess, and mitigate environmental risks associated with MCFS operations, particularly concerning nutrient cycling and oxygen levels.

- **Global Circulation Model (Nutrient Robbing):** Used to identify the downstream regions that may be impacted by nutrient robbing (macronutrient depletion) resulting from the MCFS activity. It performs a water mass connectivity analysis to determine where surface waters of the AOI outcrop after subducting, and then assesses whether nutrient limitation thresholds are crossed in those connected downstream regions.
- **Sediment-Water Interface Model (Multi-Box Model):** Used to assess the risk of dissolved oxygen (DO) depletion in the deep water overlying the sediments. This model simulates the impact of Substrate addition on DO consumption at the sediment-water interface, taking into account bottom current velocity and baseline DO concentration.
- **Phytoplankton Growth Rate Model/Calculation:** Used to quantify any change in natural phytoplankton growth rate due to the MCFS activity. This includes the determination of the natural intra-seasonal minimum specific growth rate, which depends on variables like maximum growth rate and the Monod constant. The model also calculates nutrient uptake based on the generic Redfield ratio (C:N:P = 106:16:1).
- **Truscott-Brindley (TB) Model:** Used during the optimal environmental conditions for deployment to simulate time-dependent population sizes of phytoplankton and zooplankton grazers to maximize net carbon fixation efficiency.

III. Quantification and Durability Models

These models are essential for determining the total net CO₂ removal by calculating the components of the overall quantification equation (MCFS Puro.earth methodology).

- **Air-Sea Gas Exchange Model (AS) (Direct Ocean Removal (DOR) Model/Zhou et al. (2025) Framework):** This model is used to quantify the air-sea gas exchange term (AS), which accounts for the fraction of the surface ocean CO₂ deficit caused by MCFS that equilibrates with the atmosphere. The DOR model results, based on the same modeling framework as Zhou et al. (2025), are used to assess the CO₂ uptake efficiency. The model is a general circulation model coupled with an ocean biogeochemistry model, resolving 690 global regions.
- **Durability and Ventilation Loss Model (Nowicki et al., 2024):** This global ocean circulation model is used to quantify Carbon Losses and ensure site eligibility. It estimates the fraction of remineralized Dissolved Inorganic Carbon (DIC) that is re-emitted to the atmosphere over the minimum 200-year storage period. The model employs a steady-state global ocean circulation model coupled with a carbon biogeochemical model, which is validated using modern tracers.

Whenever it is possible, the models will be validated by the data obtained from in situ trials monitoring.