Australia's National
Science Agency

GISERA | Gas Industry Social and Environmental Research Alliance

Progress report

Microbial communities and their ability to degrade prospective chemicals used in coal seam gas activities

Progress against project milestones

Progress against milestones/tasks are approved by the GISERA Director, acting with authority in accordance with the GISERA Alliance Agreement.

Progress against project milestones/tasks is indicated by two methods: Traffic light reports and descriptive Project schedule reports.

1. Traffic light reports in the Project Schedule Table below show progress using a simple colour code:

- **Green**:
  - Milestone fully met according to schedule.
  - Project is expected to continue to deliver according to plan.
  - Milestone payment is approved.
- **Amber**:
  - Milestone largely met according to schedule.
  - Project has experienced delays or difficulties that will be overcome by next milestone, enabling project to return to delivery according to plan by next milestone.
  - Milestone payment is withheld.
  - Milestone payment withheld for second of two successive amber lights; project review initiated and undertaken by GISERA Director.
- **Red**:
  - Milestone not met according to schedule.
  - Problems in meeting milestone are likely to impact subsequent project delivery, such that revisions to project timing, scope or budget must be considered.
  - Milestone payment is withheld.
  - Project review initiated by GISERA Director.

2. Progress Schedule Reports outline task objectives and outputs and describe, in the 'progress report' section, the means and extent to which progress towards tasks has been made.

Project schedule table

TASK NUMBER	TASK DESCRIPTION	SCHEDULED START	SCHEDULED FINISH	COMMENT
1	Logistics, planning, bore and soil selection	Jul 2022	Sept 2022	Complete
2	Literature review	Jul 2022	Aug 2022	Complete
3	Sampling campaign	Oct 2022	Mar 2023	Complete
4	Baselining microbial communities	Nov 2022	Aug 2023	Complete
5	Microbial degradation and sole carbon growth trials	Nov 2022	Dec 2023	Complete
6	Data analysis and project reporting	Nov 2022	March 2024	Complete
7	Communicate findings to stakeholders	Jul 2022	February 2024	This task will be completed May 2025

Project schedule report

TASK 1: Logistics, planning, bore and soil selection

BACKGROUND

During Task 1 staff will work to establish the sampling sites for soil and water samples required for downstream activities. Chemicals to be tested in subsequent tasks will be determined in Task 1 via consultation with the TRG. Up to 25 chemicals will be tested with priority given to chemicals with the greatest environmental and stakeholder concern. All chemicals will be verified as being planned for use in the Narrabri region by Santos. Task 1 will also include the safe and environmentally sensitive planning, provisioning, and logistics for the sampling campaign.

The study proposes to sample up to 48 soil samples and up to 48 surface and ground water samples, taking in agriculturally and ecologically important soils and waters of the region**.**

TASK OBJECTIVES

1) Establish water and soil sampling sites within the Narrabri region.
2) Conduct site selection and sampling campaign logistics.
3) Determination of chemicals of relevance for CSG production in the Narrabri region.
4) Preparation of sampling equipment/reagents;
5) Preparation for remote sampling fieldwork; and
6) Identification of any permits, permissions or consultation required for sampling.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

A briefing document will be prepared for the sampling campaign describing the outcomes of task objectives 1-6.

PROGRESS REPORT

A State Forrest Permit was obtained from the relevant authorities for permissions to sample within the Pilliga state forest just south of Narrabri prior to the campaign. Consultation with key local stakeholders and government officials connected the project to several landowners/stakeholders within the community of Narrabri for groundwater/surface water/soil sampling, in addition to council-run bores for groundwater sampling in the Narrabri region.

A Risk Assessment (ARA) was completed, covering the risk assessments and controls of field work including several Safe Work Instructions (SWIs) relevant to the nature of this sampling work, prior to the trip.

A significant quantity of laboratory reagents were prepared for the trip, these included ~50 bottles of preservation solution, ~50 water chemistry kits, and ~50 soil collection kits were prepared for field trip. Samples (and replicates) were identified from 11 groundwater, 12 Surface water, and 23 soil sites.

TASK 2: Literature review

BACKGROUND

Task 2 will involve a detailed review of existing work being done on the geology, surface water and groundwater chemistry, and microbiology of the Narrabri region. Furthermore, microbial biodegradation of CSG-related chemicals will be reviewed in light of the relevance to the Narrabri region. A wide range of literature including publicly accessible reports, scientific journals and web-based resources will be used. Task 2 will also help inform Task 1. CSIRO Myall Vale will also be contacted to determine what, if any, unpublished data are available on the microbiology of these soils.

TASK OBJECTIVES

1) Review previous work as published in reports, journals, and web-based resources; and
2) Use this information to inform Task 1.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

A written document that comprehensively reviews previous work will be prepared.

PROGRESS REPORT

This work is complete and will be included in the final report. An extensive review of existing work on the geology, surface water and groundwater chemistry, and microbiology of the Narrabri area has been conducted. A wide range of literature including publicly accessible reports, scientific journals and web-based resources were used. The report encompasses a basic introduction to the Narrabri region, land use and geology, a review of the Gunnedah and Surat basins, Cenozoic alluvium, hydrogeology, soil cover and composition, and previous microbiology work.

There is significant work that has been undertaken in vertosolic soils of the cotton growing areas around Narrabri. It is noteworthy, however, that almost no microbial data occurs within the NGP area. The project literature review has primarily focused on the areas of and directly surrounding the NGP, however, a summary of relevant microbial work undertaken for and by researchers working on cotton has also been included.

We are awaiting final confirmation about work done to date in the cotton-growing regions of the Narrabri area from staff at CSIRO Myall Vale, discussions have been completed with non-CSIRO staff with expertise in Microbiology at Narrabri regarding work completed by CSIRO and allied researchers from Myall Vale.

TASK 3: Sampling campaign

BACKGROUND

Task 3 will involve two staff travelling to the Narrabri region for the purpose of collecting water and soil samples.

TASK OBJECTIVES

1) Microbially preserved water and soil samples will be collected from sites identified in Task 1 for microbial baselining;
2) Four “live”, anoxic water samples will be collected from sites identified in Task 1 for biodegradation experiments;
3) Four large-volume water samples will be collected from sites identified in Task 1 for preparation of media for biodegradation experiments and chemical baselining;
4) Collection of microbially preserved soil samples from each of the two major soil types for microbial baselining; and
5) Large volume soil samples will be collected from sites identified in Task 1 for biodegradation experiments and chemical baselining.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Collection of preserved, oxic and anoxic samples to establish chemical biodegradation microcosms

PROGRESS REPORT

This task is complete and required two CSIRO staff to travel to Narrabri from Sydney and Brisbane in early June 2023. Across the course of ~1 week, sample sites from the first round of collections were revisited and samples collected. In total, 25 soil samples were collected from in, and around, the prospective site of the Narrabri Gas Project. In addition, we recollected nine of the eleven groundwater samples from the first round of field work. The remaining two groundwater samples will be collected on GISERAs behalf by landholders. Most surface water samples were collected; however, some creeks were dry at this time of year and samples were unavailable. All samples were shipped from Narrabri and have safety arrived in appropriate laboratories in Sydney and Brisbane.

TASK 4: Baselining chemistry and microbial communities

BACKGROUND

The microbially preserved water samples and the soil samples will be subject to DNA extraction along with 16S rDNA sequencing, and, for the soil samples ITS DNA sequencing.

TASK OBJECTIVES

1) Complete DNA extractions from all samples.
2) DNA samples prepared and sent to external sequencing provider; and
3) Bioinformatics completed for microbial baselining of all samples.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Raw sequencing data from microbial community profiling available.

PROGRESS REPORT

This milestone is complete.

All samples were subject to filtering through a 0.1uM filter to recover cells, the cells were then subject to DNA extraction using a commercial kit. The DNA was then sent to Molecular Research Labs (Texas USA). The DNA samples were then subject to barcoding (joining a short, unique piece of DNA to the sample), and DNA sequencing. The DNA sequences and unique barcodes were then returned to Australia and subject to analysis using an in house (GHAP) pipeline. This allowed the digital fragments of DNA to be grouped and compared to databases to be able to classified. Tables of species, their relative abundance at given sites are available by request, and will form part of the final report with appropriate discussion of taxonomic and ecological implications.

TASK 5: Microbial degradation and sole carbon growth trials

BACKGROUND

This task is designed to provide biodegradation data for chemicals used in onshore gas activities to alleviate community concern in the event of a spill. Replicated microcosms containing either the soils or aquifer water will be established and used to determine the ability of microbes in these environments to degrade chemicals potentially used by the CSG industry. Chemicals will be grouped and tested in up to four mock industrial applications (e.g. drilling, workovers, surface facility treatments). These applications, and the chemicals used in them, will be determined through consultation with the TRG. Chemical degradation will be determined via microbial growth assays and community profiling. A small subset of five chemicals will be directly measured using analytical chemistry techniques. These chemicals will be determined through consultation with the TRG to choose those of most concern to the community. In addition, sole carbon growth trials (using all chemicals individually) will be conducted in soil mimicking liquid media and directly in aquifer water samples. Only samples collected in the first sampling collection (October 2022) will be used for setting up biodegradation trials.

TASK OBJECTIVES

1) Establish replicated microcosms.
2) Spike microcosms with target chemicals at concentrations as consulted with TRG;
3) Incubate at *in situ* conditions i.e. for soil microcosms, incubate at field relevant conditions (local temperatures and day/night cycle will be reproduced in the laboratory) for aquifer water microcosms relevant subsurface temperature will be used in the absence of light;
4) Harvest all soil treatments after four weeks and prepare samples for DNA sequencing and chemical analyses;
5) Harvest all water treatments after twelve weeks and prepare samples for DNA sequencing and chemical analyses;
6) Establish sole carbon source experiments;
7) Incubate at relevant field conditions;
8) Inspect cultures for visual signs of growth and prepare samples for DNA sequencing; and
9) Statistical analyses of the resultant data.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Replicated experimental data on the degradation of target compounds. Data prepared for analysis and final reporting.

PROGRESS REPORT

This milestone is completed.

Two different microcosm experiments were conducted for this study: 1) a community impact assessment; and 2) a sole carbon experiment. All groundwater samples were treated under strict anaerobic conditions in a dedicated oxygen-free chamber, while the remaining soil and surface water samples were treated, as in their natural environment, under oxic conditions. All chemicals and their concentrations used were derived via consultation with the stakeholders involved.

For the community impact assessment, two different groundwaters, two different soils, and one surface water sample from the study area were placed into 70 sterile containers each and subjected to chemicals used by the onshore gas industry proposed for the Narrabri area. Each microcosm contained 25 mL of sample. Of these 70 samples from each location, ten replicate samples were subjected to a cocktail of chemicals used in: 1) "workover processes": hydrocarbons, C12-C15, n-alkanes, isoalkanes, cyclics, <2% aromatics; alkanes, C11-15-iso; and isotridecanol, ethoxylated; and 2) "drilling processes": monoethanolamine; ethoxylated alcohol. A further thirty samples were subjected to the following biocides: dazomet (ten replicate samples), glutaraldehyde (ten replicates), and oxazolidine (ten replicates). The remaining twenty samples were controls. The samples were incubated for approximately three months at room temperature. Upon completion of the incubation period, one-third of these samples were sent for chemical assessments to commercial laboratories to determine the extent of degradation of the compounds used in the offshore gas industry. The remaining samples had their DNA extracted and sent off for sequencing by a commercial company in the USA.

The sole carbon experiment aimed to determine the typical microorganisms involved in the degradation of the above chemicals by culturing them on the compounds as their only carbon source. For this project, eight vials from each location (two soils, two groundwaters, and one surface water) were subjected to the same chemicals as mentioned in the community impact assessment project except that the chemicals were introduced separately and not as a cocktail. Each of these chemicals were placed as an aqueous solution of 24 mL into a sterile vial and subsequently inoculated with either 1 mL of sample water or soil. After one week of incubation at room temperature, 1 mL of solution from that vial was placed into a new sterile vial that contained a fresh solution of chemicals. After eight rounds of these serial dilutions, each vial is now believed to contain only microorganisms able to degrade the compounds of interest. These samples have now been filtered and their DNA extracted for sequencing by a commercial company in the USA.

TASK 6: Data analysis and project reporting

BACKGROUND

The final report for this project will collate baseline data with microbial degradation, microbial community impact and useful indicator taxa for individual chemicals. These data will inform requirements for future research and will provide information for a range of stakeholders.

Critical evaluation of the results is needed to understand the experimental outcomes of this study.

TASK OBJECTIVES

1) Reporting results and analyses from Tasks 3-5;
2) Provide research options for chemicals with high residual risk of environmental impact.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Final written report encompassing all the tasks outlined above.

PROGRESS REPORT

All data have been analysed and the report is complete. The report 'Chemical and microbial baseline studies and biodegradation experiments of chemical compounds used in coal seam gas

activities in the Narrabri region, NSW' which includes a discussion of research options emerging from the research project is available for public viewing.

TASK 7: Communicate project objectives, progress and findings to stakeholders

BACKGROUND

Communications of GISERA research are an important component of outreach and dissemination of findings to diverse audiences.

TASK OBJECTIVES

Communicate project objectives, progress and findings to stakeholders through meetings, knowledge transfer sessions, factsheets and journal articles, in collaboration with GISERA Communications officers.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Communicate project objectives, progress and results to GISERA stakeholders according to standard GISERA project procedures which may include, but are not limited to:

1) Knowledge Transfer session with Government/Gas Industry
2) Presentation of findings to Community members/groups
3) Preparation of article for GISERA newsletter and other media outlets e.g. The Conversation
4) Revision of project factsheet to include final results (a factsheet is developed at project commencement, and another will be prepared at completion)
5) Peer reviewed scientific manuscript ready for submission to a relevant journal

PROGRESS REPORT

This task is partially completed.

The Knowledge Transfer session for this project has been conducted. A draft manuscript has been completed and will be submitted after the final report for this project is publicly released.

Options for communicating the results are being drawn up.

This task will be completed in May 2025.

Variations to Project Order

Changes to research Project Orders are approved by the GISERA Director, acting with authority, in accordance with the GISERA Alliance Agreement. Any variations above the GISERA Director's delegation require the approval of the relevant GISERA Research Advisory Committee.

The table below details variations to research Project Order.

Register of changes to Research Project Order

DATE	ISSUE	ACTION	AUTHORISATION
05/04/23	Due to the second campaign of field work being slightly delayed, milestone 3 will now be complete in the first week of June when the field trip is being planned	Milestone 3 delivery date extended from March 2023 to June 2023	
03/07/23	Due to delays in the field work and with chemical supplies, delivery of Milestone 4 & 5 will be delayed.	Milestone 4 and milestone 5 delivery date extended from June 2023 to August 2023	
20/10/23	Risk assessments (task 5) for some new chemicals required additional risk mitigation and safety documentation to be prepared and approved.	Milestone 5 delivery date extended from August 2023 to December 2023 Milestone 6 pushed back from December 2023 to March 2024 Milestone 7 pushed back from December 2023 to February 2024.	

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GISERA is a collaboration between CSIRO, Commonwealth and state governments and industry established to undertake publicly-reported independent research. The purpose of GISERA is to provide quality assured scientific research and information to communities living in gas development regions focusing on social and environmental topics including: groundwater and surface water, greenhouse gas emissions, biodiversity, land management, the marine environment, and socio-economic impacts. The governance structure for GISERA is designed to provide for and protect research independence and transparency of research.