Australia's National
Science Agency

GISERA | Gas Industry Social and Environmental Research Alliance

Progress report

Microbial degradation of chemicals and fluids in aquifers of the Limestone Coast, South Australia

CSIRO | Australia Pacific LNG | QGC | origin | Santos | NSW Government | Australian Government Department of Industry, Science, Energy and Resources | Supported by Government of South Australia | Queensland Government | Northern Territory Government

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	Briefing document for sampling campaign	Aug-20	Nov-20	Completed
2	Sample collections- soil and water	Feb-21	Mar-21	Completed
3	Baseline microbial community profiling complete and raw data available	Nov-20	Apr-21	Completed
4	Chemical degradation and sole carbon growth assays complete and data prepared for final report	Dec-20	Aug-21	Completed
5	Impact and indicator taxa identified, and data prepared for final report	Dec-20	Aug-21	This milestone will be delivered early August 2022
6	Metagenomics	Feb-21	Sep-21	This milestone will be delivered early August 2022
7	Data analysis and reporting	Aug-20	Sep-21	This milestone will be delivered end of September 2022

Project schedule report

TASK 1: Logistics, planning, contacting stakeholders

BACKGROUND

During Task 1 we consult with colleagues in the South Australian Departments of Energy and Mining (DEM), and Environment and Water (DEW) to guide the sampling campaign to ensure that hydrogeological and physicochemical heterogeneity in the TLA is captured. In addition, we will contact relevant landholders who use water from the TLA. This will include replicated samples from viticulture, cattle and sheep farms, grain farms along with fruit, vegetable, and tree nut farms, and will ensure that the heterogeneity in the TLA is adequately covered.

TASK OBJECTIVES

1. Establish contacts with colleagues in the South Australian Departments of Energy and Mining (DEM), and Environment and Water (DEW) to guide the sampling campaign to ensure that hydrogeological and physicochemical heterogeneity in the TLA is captured.

2. Establish contacts with relevant landholders who use water from the TLA and identification of any permits, permission or consultation required for sampling.
3. Confirm the relevance of chemicals being tested in the project i.e., that they are still relevant for onshore gas production in the Limestone Coast region.
4. Identification of sites for aquifer collections. With a view to ensuring a good spread of sampling across the region.
5. Ordering and preparation of sampling equipment/reagents, vehicles, and OH&S considerations.

TASK OUTPUTS AND SPECIFIC DELIVERABLES:

This task will yield a series of documents describing the contacts, sampling sites, relevant permissions, sampling equipment and OH&S considerations.

PROGRESS REPORT

This milestone is complete.

The South Australian Departments of Energy and Mining (DEM), and Environment and Water (DEW) have been consulted regarding the hydrogeological and physicochemical heterogeneity in the TLA, and the regions for sampling have been determined to ensure that this heterogeneity will be met. Samples will be taken from across the TLA Hydrogeological Provinces 1 and 2, inclusive of the Zones 1A to 6A.

Consultation with the Department of Primary Industries and Regions, South Australia regional coordinator for the Limestone Coast, Department for Environment and Water and various Limestone Coast industry associations are complete and a list of landholders has been compiled for sampling. Sampling was confirmed with 10 landowners from 24.02-26.02 and 01.03-02.03. Only four land use types will be sampled (pasture, small seed, vegetable and orchard production). Preparations and ordering for the sampling campaign are complete. Staff are departed NSW on the 22/02/2021 and returned 06/03/2021.

TASK 2: Sampling campaign

BACKGROUND

Task 2 will involve two staff travelling to the Limestone Coast region of South Australia with the purpose of collecting aquifer samples across the region under a variety of land-use practices.

TASK OBJECTIVES

1. To collect triplicate preserved aquifer samples from the sites identified by Task 1.
2. To collect triplicate microbiological ('live') aquifer samples (under CO2 or other gas headspace) from each of the five land use types (viticulture, animal grazing, grain growing, fruit and tree nut farms, and vegetable farms).
3. To collect bulk aquifer samples (4x5L) to match the microbiological ('live') aquifer samples.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Collection of microbially preserved aquifer samples, anoxic aquifer samples and bulk samples to establish microcosms.

PROGRESS REPORT

This milestone is complete.

CSIRO successfully completed the sampling campaign collecting a total of 154 aquifer samples from 10 landowners across 21 sites. Microbially preserved aquifer samples were collected from all sites; live microbiological samples and bulk water samples were collected from 7 sites covering each of four land use types and six hydrogeological zones. The sampling campaign commenced 22nd February and finished 6th March 2021.

All landowners have been contacted to thank them for their assistance with the sampling campaign.

Sampling processing is underway

TASK 3: Baselining microbial communities

BACKGROUND

The microbially preserved aquifer samples will be subject to DNA extraction along with 16S rDNA sequencing.

TASK OBJECTIVES

The task will include the following objectives:

1. Filter microbially preserved samples onto 0.1µM PVDF filters.
2. Complete DNA extractions from all samples.
3. Process DNA for 16S NGS sequencing.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Raw sequencing data from microbial community profiling available.

PROGRESS REPORT

This milestone is complete

The filtration of microbially preserved samples from 21 sites, in triplicate for a total of 63 DNA extractions and the 16S NGS sequencing, has been completed. The raw sequencing data has been transferred, processed, and analysed by our external research sequencing contractor and is now available.

TASK 4: Microbial degradation and sole carbon growth trials

BACKGROUND

Replicated aquifer microcosms containing aquifer water will be established and used to determine the ability of aquifer microbes to degrade chemicals potentially used by industry. Chemical degradation will be determined either through direct measurement of the chemical where available at NATA accredited laboratories or measured indirectly through microbial growth on that compound.

TASK OBJECTIVES

The task will include the following objectives:

1) Establish replicated anoxic microcosms with a CO2 headspace.
2) Spike microcosms with target compounds at realistic concentrations.
3) Incubate at realistic conditions i.e. aquifer temperature, dark, anoxic.
4) Harvest all water treatments after 120 days and prepare samples for chemical analyses.
5) Establish sole carbon source degradation trials.
6) 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 task has been completed with almost 1700 microcosms set up and spiked with chemicals of interest. The samples were established in anoxic conditions and incubated at ~25C in the dark to simulate aquifer conditions. After approximately 120 days samples were harvested for both DNA extraction and chemical analyses. The chemical analyses have been completed and data analysis is underway and will be reported in task 7. These microcosm experiments will be used for assessment of soil carbon degradation trials.

TASK 5: Bioinformatic analyses and identification of impact and indicator taxa

BACKGROUND

Aquifer microcosms will be subject to microbial community profiling after 120 days exposure to individual chemicals will be carried out to ascertain impacts on microbial communities and to potentially identify putatively useful indicator taxa for monitoring environmental impacts.

TASK OBJECTIVES

The task will include the following objectives:

1) Extract DNA from aquifer microcosms from Task 4 to determine changes in microbial community profiles.
2) Process DNA for 16S NGS sequencing.
3) Statistical and bioinformatics analyses of the resultant data from Tasks 3 and 5.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Experimental data on the microbial community changes in response to exposure to gas activity-related chemicals. Bioinformatic and statistical analyses complete and prepared for final report.

PROGRESS REPORT

In order to complete this milestone, DNA extractions from the ~1200 microcosm experimental samples, a single PCR amplification of 16S region, clean up and quantification, preparation of library, to followed by Illumina pair-end sequencing. On the move to Lindfield, some of our capacity to measure DNA concentrations in large batches was affected by equipment shortage. This has been rectified by accessing an instrument from a different BU. At present we anticipate that the DNA extractions, PCR, clean up, quantification and library preparation will be ready for submission for Illumina sequencing by June 30. After this data will be available in approximately 4 weeks and bioinformatic analysis will be complete approximately two weeks after receipt of DNA sequencing.

At present, we believe this milestone will be complete by early August 2022.

TASK 6: Metagenomics

BACKGROUND

For each chemical, one aquifer sample will be chosen based on the increase in cell number such that those aquifer samples that respond the most will be selected. DNA will be used from Task 4 and subject to whole genome, shot gun sequencing using 150bp PE reads. From the resultant metagenomic sequences contigs will be assembled and binned into genomes using a tri- or tetra-mer approach. The resultant bins (genomes) will then be subject to gene calling, gene annotation and pathway analyses with a view to identifying putative genes for chemical degradation. These data will be made available via a public data portal.

TASK OBJECTIVES

The task will include the following objectives:

1) Complete DNA sequencing
2) Complete metagenomic analyses (assembly, binning and annotation)
3) Putative genes involved in chemical degradation will be identified and data presented in the final report; and
4) Data will be available via a public portal.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

DNA sequence data, binned metagenomic contiguous sequences and annotations, and data available on a public portal linked to this project.

PROGRESS REPORT

This task is dependent on task 5 and has been delayed due to knock-on effects. For each chemical treatment, one aquifer sample will be chosen for metagenomic analyses, however, this is dependent upon the response elicited by the chemical treatment/aquifer sample. Task 5 will determine the response of each aquifer sample to the various chemical treatments. On completion of DNA extractions from Task 5, initial concentrations of DNA will be used to determine the aquifer samples

with the most elevated microbial response to chemical treatments, and these will undergo metagenomic analyses.

In order to deliver this milestone in a timely fashion, DNA quantification results from Task 5 will be used to determine the most suitable sample for metagenomic analysis. This will allow a time saving of approximately 4 weeks. We anticipate that this task will be complete by early August 2022.

TASK 7: Data analysis and reporting

BACKGROUND

The final report for this project will bring together microbial baseline data from aquifer samples across the region under differing land use practices. It will identify or describe microbial degradation, microbial community impact and useful indicator taxa for individual chemicals. These data will be combined with results from project W15 to inform requirements for future toxicological studies 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

The task will include the following objectives:

1) Reporting results and analyses from Tasks 2-6;
2) Integration of this studies results with those of project W15; and
3) Provide recommendations of chemicals with potential high residual risk requiring toxicological studies.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Final written report encompassing all the tasks outlined above and integration with the related project W15.

PROGRESS REPORT

This task is dependent on all previous tasks and has been delayed due to knock-on effects. Analysis of microbial community profiles and water chemistry from the baselining work (Task 3) have commenced. Detailed water chemistry of all samples are available; in broad terms all waters were neutral to alkaline and had low electrical conductivity with measured values between ~600-3000 µS cm^{-1}. This task will be completed by end of September 2022.

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
28/09/2020	Due to border closures, planning a field campaign has been difficult and therefore delayed.	All milestones extended by 2 months; the new project delivery date will be September 2021.	
27/10/21	COVID restrictions on entry to the laboratories, the decommissioning of the North Ryde site laboratories and relocation/refurbishment of the Lindfield site laboratories have all resulted in delays to this project.	Milestones 4, 5, 6 extended from June 2021 to January 2021 & milestone 7 extended from July 2021 to February 2022.	

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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.