Evaluation and recommendation of sample preservation techniques for 16S rRNA sequencing in oilfield systems

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BACKGROUND

The implementation of metagenomics in oil and gas industry has drastically increased in the last several years. It is being used for a host of applications, including: detection of the origin of microbially influenced corrosion (MIC), biocide efficacy studies, and microbial content evaluations of injection water and water used for hydraulic fracturing. One major deficiency with the application of metagenomics, is the lack of standardized protocols. Due to the remote nature of oilfields, sample preservation and maintaining integrity of microbial population during transport is of particular concern. If not preserved properly, the microbial community composition can change between sample collection and DNA extraction.

OBJECTIVE

To evaluate several common preservation techniques that are suitable for remote oilfield locations and contribute towards the development of standard protocols for metagenomics analysis.

METHODOLOGY

Two samples were collected from an oilfield and shipped to the lab. Once received by the lab, the DNA was extracted from the control sample. Samples were then preserved using various methods and subjected to different scenarios including a simulated shipping delay and temperature cycling. Preserved samples were held for 7 and 14 days prior to DNA extraction and analysis. All samples were analyzed for total prokaryotes using qPCR and sequenced using Illumina 16s rRNA sequencing (515/806 primer).

Sample Collection

Well Water | Frac Pit Water

Sample Preservation

1. Control
2. LuminUltra GeneCount Chemical Preservation (7, 14 days)
3. Filtered and dried (7, 14 days)
4. Unpreserved (7, 14 days)

Temperature cycled (12 h at 20°C, 12h at 40°C)

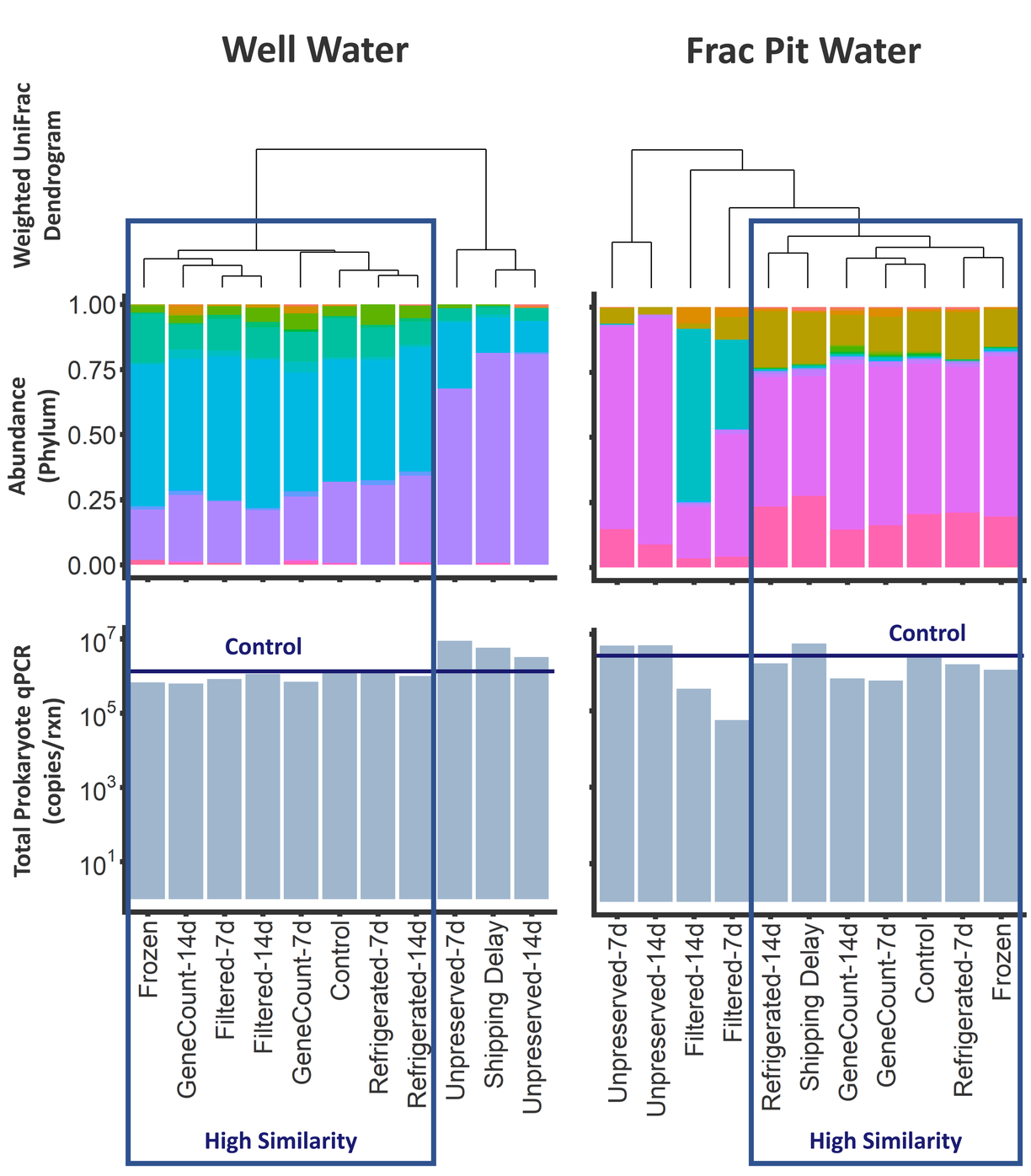
5. Refrigerated (7, 14 days)
6. Frozen (14 days)
7. Shipping delay (Refrigerated for 24 hours, room temperature for 3 days)

Sample Analysis

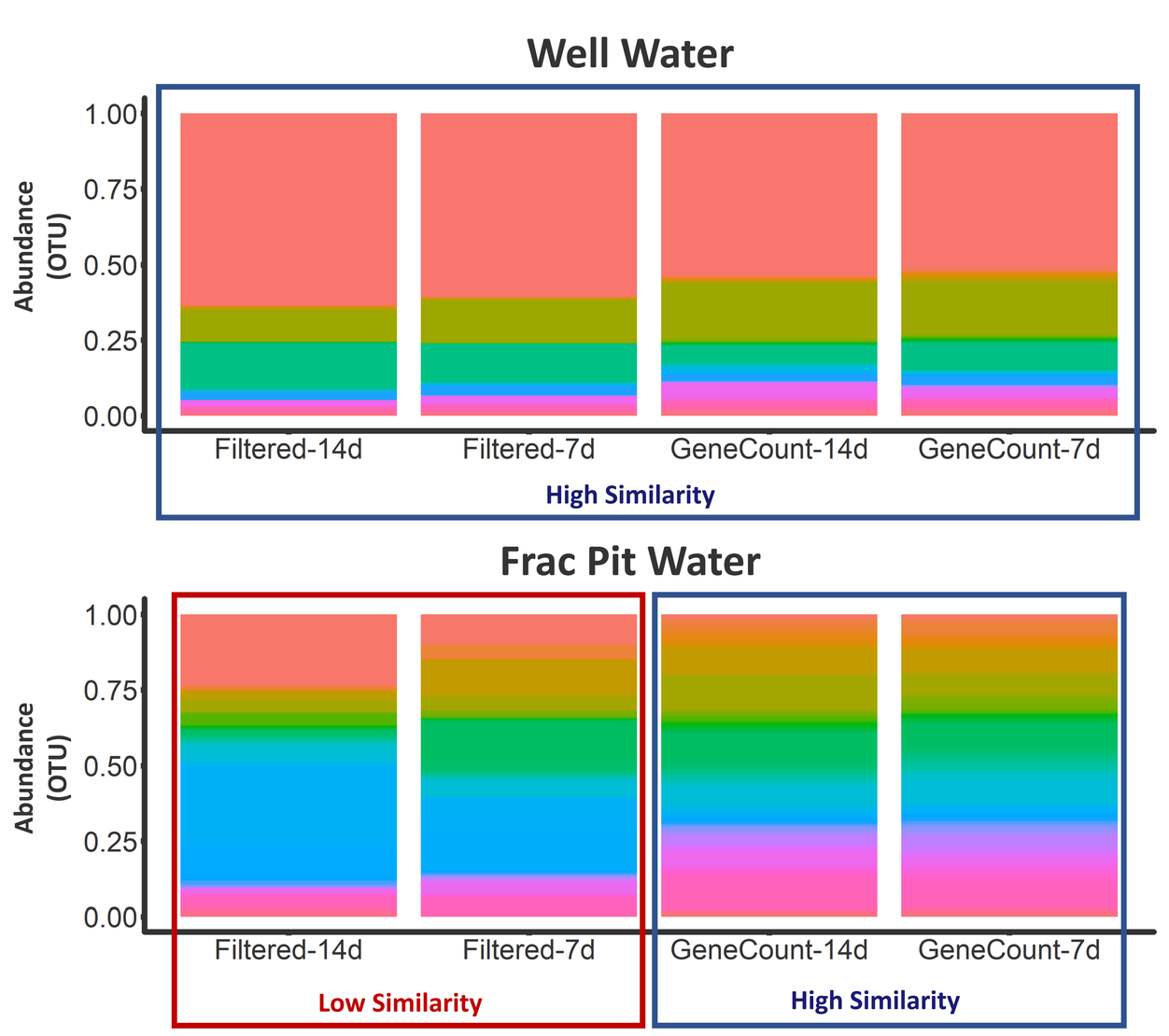
qPCR – Total Prokaryotes | 16S rRNA Sequencing

Two samples were also preserved in the field using LuminUltra's GeneCount chemical preservation and by filtering and air drying. Preserved samples were shipped to the lab where they were further temperature cycled and analyzed after 7 and 14 days.

RESULTS – IN LAB



RESULTS– IN FIELD



OUTCOMES – IN LAB

1. There were three preservation techniques that gave similar results to the control in both samples: freezing, refrigeration and GeneCount chemical preservation.
2. Filtering and drying had similar results to the control in well water (lower alpha diversity) but not in frac pit water (higher alpha diversity), which shows that it does not universally preserve all samples. There was a considerable increase in Bacilli-associated genera in the filtered frac pit water.
3. The GeneCount chemical preservation kit did result in some additional low abundance OTUs, especially in the highly turbid well water. It is hypothesized that this is due to the extended extraction period offered by chemical preservation which resulted in the lysing of additional and more robust cells.

OUTCOMES – IN FIELD

1. Well Water – Similar to the laboratory results, filtration and GeneCount chemical preservation gave similar results for both hold times. Compared to each other the preservation methods were also quite similar.
2. Frac Pit Water – The GeneCount chemical preservation kit gave similar results after 7 and 14 days of temperature cycling. Like the laboratory results, the filtered samples had low similarity when compared to each other and compared to the chemically preserved samples. The filtered samples were characterized by relative increases in Pseudomonas (day 7), Bacillus (day 7 and 14) and Streptomyces (day 7 and 14) compared to the chemically preserved samples.

CONCLUSIONS

Various preservation methods were tested on two oilfield sample matrices. Three preservation methods performed well for both samples: freezing, refrigeration and GeneCount chemical preservation. Filtration and air drying worked well for one sample, but not the other. This shows that testing multiple sample types is important when evaluating preservation methods.

The results show that chemical sample preservation is recommended, particularly in cases where cold-chain transport is not possible.