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Isolation of Salt Tolerant Bacteria and Investigation of Perchlorate Biodegradation at High Salinity Conditions

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Isolation of Salt Tolerant Bacteria and Investigation of Perchlorate Biodegradation at High Salinity Conditions

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Background and Hypothesis

Perchlorate (ClO_4^-) is a by-product found in aerospace and defense programs as well as in fireworks and bleaching. But it is also known to be a harmful contaminant that negatively affects the endocrine system, being prevalent in major forms of water runoff.

Sources:

- Anthropogenic:** ammonium perchlorate used for military purposes and as rocket fuel: Aerojet facilities in CA, NV.
- Natural formation:** arid and semiarid environments.

Co-contaminants: Nitrate and chlorate

Electron Donor: Acetate

Electron Acceptor: Perchlorate

Degradation Pathway: $\text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{Cl}^-$

Treatment Technology: Physical (ion-exchange, membrane process), chemical (catalytic reduction), and biological/microbial reduction.

Biological Treatment: biodegradation of perchlorate to harmless chloride performed by naturally occurring bacteria. ClO_4^- serves as an electron acceptor for perchlorate reducing bacteria (PRBs) when an electron donor is provided.

High Salinity Issue: High levels of total dissolved solids (TDS), or salinity, have been shown to disrupt the activity of PRBs.

Hypothesis: Perchlorate biodegradation by halotolerant PRBs is possible within high salinity concentrations if they are acclimated in the correct conditions.

Problem Statement

The work done in the lab tested to see whether increasing salt conditions would still allow the soil bacteria (taken from a contaminated site) to degrade perchlorate. The percentages of salt (NaCl) ranged from 0% to 6.5%.

Methodology

- Concentrations of electron donor: acetate in 450 mg/L
- Concentrations of electron acceptor: perchlorate in 150 mg/L
- Concentration of DAP/urea: 25.4 mg-P L⁻¹, 115.3 mg-N L⁻¹



	Salt Concentration (%)	DI water (mL)	Nutrient (mL)	Buffer (mL)	Blended Composite Sample Soil (g)
0	89	1	10	5	
1	89	1	10	5	
2	89	1	10	5	
3	89	1	10	5	
4	89	1	10	5	
4.5	89	1	10	5	
5	89	1	10	5	
5.5	89	1	10	5	
6	89	1	10	5	
6.5	89	1	10	5	

Results & Discussion

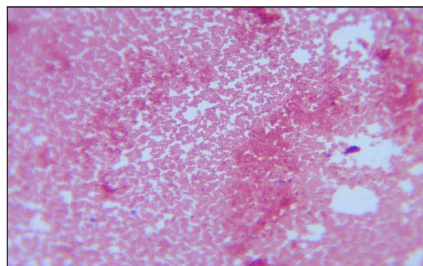


Fig 1: Microscopic bacteria at 2% buffer solution, with Gram-negative cocci present



Fig 2: Microscopic bacteria at 4.5% buffer solution, with Gram-positive bacilli and Gram-negative cocci present

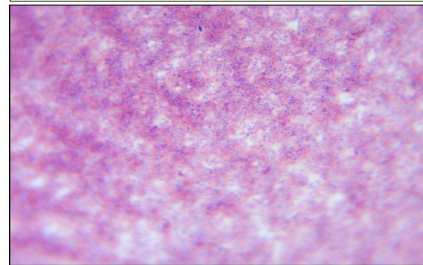


Fig 3: Microscopic bacteria at 5.5% buffer solution, with Gram-positive cocci present

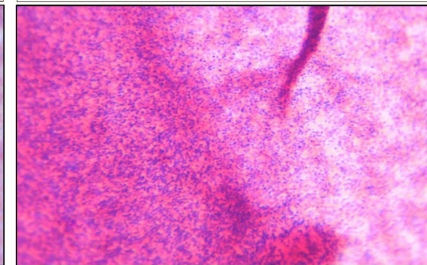
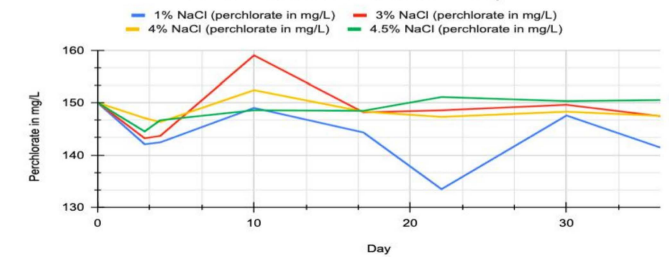


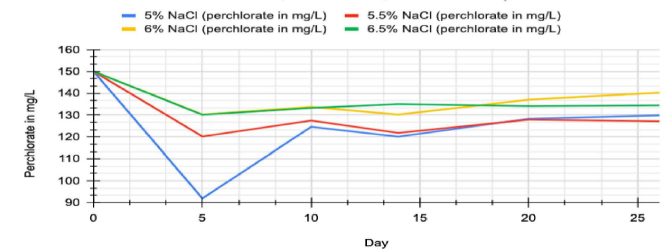
Fig 4: Microscopic bacteria at 6.5% buffer solution, with a mix of Gram-positive bacilli and Gram-negative cocci present

Results & Discussion

Perchlorate Degradation for Low Salt Concentration (1% NaCl, 3% NaCl, 4% NaCl, 4.5% NaCl)



Perchlorate Degradation for High Salt Concentration (5% NaCl, 5.5% NaCl, 6% NaCl, 6.5% NaCl)



- The perchlorate degradation heavily fluctuated through time, staying stagnant near the end
- Bacteria growth was prevalent in all salt concentrations, showing a range of Gram-positive bacilli and Gram-negative cocci

Conclusion

- At different salinity concentrations (up to 6.5% NaCl), the bacteria growth was observed in the cultures
- At high salinity level, 13% of perchlorate was degraded within 26 days
- Although the kinetic of perchlorate biodegradation is not high, there is still some PRBs that can support the biodegradation
- Acclimation time was not for too long, so if more time was given, they could adapt to the environment much more easily and degrade perchlorate
- Experiment is still ongoing