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Perchlorate-Reducing Biofilms Open a New Avenue for Martian Agriculture

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ABSTRACT

Mars stands out as a prime candidate for future human colonization. However, high concentrations of perchlorate in Martian soil pose a significant hazard to both animal and plant life. Microbe-mediated remediation has been reported as an effective method for Perchlorate reduction on Earth. The present study explored the perchlorate-reducing potential of microbial biofilms formed by bacteria and fungi isolated from soil samples collected at Ussangoda, Sri Lanka, a site with unique soil characteristics similar to the Martian regolith. Soil samples were collected from 11 random locations at Ussangoda, Sri Lanka. Perchlorate-reducing bacteria and fungi were isolated under both aerobic and anaerobic conditions, and their efficacy in perchlorate reduction was measured using Fourier Transform Infrared Spectroscopy. The results revealed that the bacteria and fungi tolerated perchlorate up to 0.5 M and reduced perchlorate up to 60% at 0.2 M concentration by forming fungal-bacterial biofilms. In comparison, bacteria-alone combination reduced perchlorate by up to 35.98% under the same conditions, while the control showed no significant reduction. These findings represent one of the highest rates of perchlorate reduction at elevated concentrations reported in a microbial setting to date. While the study demonstrates the potential of microbes isolated from Martian simulant (Ussangoda) soil as perchlorate bioremediating agents, further research is required to validate these results under conditions simulating the Martian environment, including atmospheric pressure, temperature, and soil properties. Additionally, future investigations should focus on elucidating the underlying mechanisms of perchlorate reduction and optimizing biofilm formulations for extraterrestrial applications.

KEYWORDS: Biofilms, Bioremediation, Mars, Martian soil simulants, Perchlorate.

INTRODUCTION

Perchlorate (ClO_4^-) is a monovalent inorganic chlorine oxyanion with high water solubility and stability. It is found in the environment from natural or anthropogenic sources.[1] Perchlorate is identified as an emerging contaminant as it pollutes water sources and causes environmental and human health impacts on Earth, in areas such as northern Chile and the southwestern region of the United States, where perchlorate is identified as a major water contaminant.[2]

Mars stands out as a leading candidate for future human expansion due to its Earth-like characteristics and recent discoveries confirming the presence of water.[3] However, the presence of perchlorate in Martian soil at concentrations ranging between 0.5% and 1%[4] indicates a barrier to human colonization due to its toxicity and negative effects on animal and plant life. Thus, in the context of colonizing Mars, removing perchlorate from the soil and water sources is essential. Physico-chemical methods such as ion exchange, adsorption, electrochemical reduction, membrane filtration, and use of catalytic converters, as well as phytoremediation, have limitations in such endeavors.[5] Thus, microbial bioremediation for perchlorate reduction is a viable option.[6] This is in line with the applicability of microorganisms for in-situ resource utilization (ISRU) in Martian research.[7]

Perchlorate-reducing bacteria (PRB) exhibit the ability to convert perchlorate into harmless chloride ions (Cl^-) through enzymatic processes.[8] The process is facilitated by the presence of perchlorate reductase (PcrAB), a specialized enzyme belonging to the dimethylsulfoxide reductase superfamily. Soil microorganisms such as *Pseudomonas,*[9] *Dechloromonas*, and *Azospira*[10] have demonstrated the capability to reduce perchlorate. These bacteria are typically classified as either facultative anaerobic or microaerobic, indicating their ability to thrive in environments with varying levels of oxygen availability. However, to date, there has been no evidence to support perchlorate reduction potential by fungal-bacterial biofilms.

Martian soil is known for its high iron content and scarcity of electron donors, factors that could influence microbial activity. While the potential of perchlorate-reducing bacteria (PRB) for remediating perchlorate contamination on Mars has not been widely explored, recent research by Levakov et al.[11] reveals that ferric and ferrous ions can inhibit perchlorate reduction by PRB. These findings highlight the importance of investigating the performance of PRB in simulated Martian soils to identify effective strategies for addressing perchlorate contamination on Mars.

The elemental composition of soils in Ussangoda National Park, Sri Lanka, reported by Vithanage et al.[12] reveals similarities with the elemental composition of Martian soils reported by Oravec et al.[13] Ussangoda, known for its limited biodiversity due to the high iron content in the soil, is a prime candidate for acquiring microbes that can adapt to Martian soil conditions. Here, we hypothesize that the microorganisms inhabiting the soils of the Ussangoda area may have adaptations to reduce perchlorate while overcoming iron interference. This study aimed to isolate microorganisms from soils in the Ussangoda area and to explore their perchlorate reduction ability for future applications, including bioremediation in the context of colonizing Mars.

MATERIALS AND METHODS

Study Area and Sampling Points

The study was conducted in Ussangoda National Park, located in the Hambanthota district of the Southern Province of Sri Lanka (6°05'55" N 80°59'12" E). The mean annual temperature of the Ussangoda area is 27.9 °C, and records less than 1250 mm of rainfall annually, including a long dry period of five months.[14] The soil is characterized as serpentine with high iron content. Georeferenced sampling points (n = 11) were randomly selected to represent the different areas of the field site.

Collection of Soil Samples

At each sampling point, soil samples (20 g) were aseptically collected at 5 cm depth for isolating aerobic microbes and transferred into sterilized polythene bags. Samples were collected at 15 cm depth for isolating anaerobic microorganisms employing the technique described by Kanda.[15] Immediately after collection, each soil sample was transferred into a sterilized glass tube (15 ml) and secured with a rubber cap, and the headspace gas was removed using a sterile syringe and replaced with nitrogen gas (Purity 99.9%) to induce anaerobic conditions. Samples were transported to the Microbial Biotechnology unit of the National Institute of Fundamental Studies in Kandy, Sri Lanka, within 24 hrs of sample collection, and the samples were stored at 4°C.

Isolation of Anaerobic Microorganisms

Anaerobic microorganisms in the soil samples were isolated under anaerobic conditions using a previously described anoxic growth medium.[16] The growth medium was prepared in 1 L, the pH was adjusted to 7.2, and it was sterilized by autoclaving (20 min at 121°C, 15 psi). A Baker Ruskinn Anaerobic Workstation was utilized under a controlled atmospheric setting (5% H_2, 10% CO_2, and 85% N_2) for anaerobic culturing. The growth medium was transferred to airtight culture tubes (n=11) within the workstation. Each soil sample tube under anaerobic conditions was opened, and approximately one gram of soil was introduced to the anoxic growth medium. The culture tubes were incubated in the anaerobic workstation and monitored for cell growth. After cell growth in liquid culture tubes, solid culture plates were prepared by the spread plate method by transferring one milliliter of the cell suspension on anoxic growth medium (2% agar). Pure cultures were obtained by the serial streak plate technique.

Isolation of Aerobic Microorganisms

Aerobic microorganisms in the soil samples were isolated, and pure cultures were prepared using the same growth media[16] under aerobic conditions in a laminar flow cabinet.

Screening of the Isolates for Perchlorate Tolerance

The isolated anaerobic bacteria (n=2) were subjected to a perchlorate tolerance assay to evaluate their ability to grow in the presence of varying concentrations of sodium perchlorate. For the assay, the isolated strain was inoculated into an anoxic medium supplemented with sodium perchlorate at concentrations of 0.01M, 0.05M, 0.1M, 0.2M, 0.5M, and 1M. The medium was prepared by dissolving 28 g/L of agar in an anoxic environment to maintain strict anaerobic conditions. Inoculations were performed in triplicates to ensure the reliability of the results. The plates were incubated under controlled anoxic conditions at 30°C. Bacterial growth was monitored visually, and tolerance was determined based on the ability to form visible colonies at each perchlorate concentration. Colony formation was monitored at 24-hour intervals.

The isolated fungal strains (n=3) were subjected to a perchlorate tolerance assay to evaluate their ability to grow in the presence of varying concentrations of sodium perchlorate. For the assay, the fungal isolates were inoculated onto potato dextrose agar (PDA) supplemented with sodium perchlorate at concentrations of 0.01M, 0.05M, 0.1M, 0.2M, 0.5M, and 1M. The medium was prepared under anoxic conditions to ensure strict anaerobic growth. Inoculations were performed in triplicates to ensure the reliability of the results. The cultures were incubated under controlled anaerobic conditions, and colony formation was monitored at 24-hour intervals. The maximum perchlorate tolerance for each fungal strain was determined based on the ability to form visible colonies at the highest perchlorate concentration.

Determination of Perchlorate Reduction Efficiency of the Isolates by FTIR

The distinct bacterial (A, B) and fungal (W, Y, and P) isolates, which demonstrated perchlorate tolerance, were further studied through FTIR analysis. An experiment was designed with nine combinations to evaluate the bacterial isolates as pure cultures and in combination with fungal isolates. These combinations included individual bacterial isolates (A, B), a mixed bacterial culture (C), and combinations of bacterial isolates with fungal isolates: AW (A and W), AY (A and Y), AP (A and P), CW (C and W), CY (C and Y), and CP (C and P). A control (E), containing 0.2 M sodium perchlorate without any isolates, was also included. All combinations were prepared in triplicates, with 1.5 mL of the prepared isolates inoculated into anoxic growth media containing 0.2 M sodium perchlorate and incubated for 18 days.

After the incubation, 10µl of culture was treated with 100 mg of KBr. The formed pellets were analyzed using FTIR. Average absorption values were calculated using Origin Pro 2024 software. The perchlorate ion functional group of the spectra was identified by referring to the FTIR peak values for sodium perchlorate stated by Nyquist and Kagel.[17] The presence of perchlorate ion species is commonly related to the strong absorption bands around the 1050 - 1150 cm^{-1} range.

The perchlorate reduction efficiency *(PRE)* of each combination was calculated using the following equation.

$$PRE = \frac{(Pc - Ps)}{Pc} \times 100\%$$

Where, P_C = The FTIR peak value of the control sample at 1100 cm^{-1}, P_S = The FTIR peak value of the sample at 1100 cm^{-1}.

Confirmation of Biofilm Formation in Perchlorate-Reducing Bacteria and Fungi Using Congo Red Agar Method

Monocultures of Bacteria A and fungi (P, Y, W) were cultured in Perchlorate-Reducing Bacteria (PRB) selective media to evaluate biofilm formation. To prepare the cultures for inoculation, the monocultures were vortexed at 2,500 RPM for 10 seconds to ensure uniform cell suspensions. Aliquots of 100 µL were inoculated into the nutrient broth and PRB media-containing tubes (15 mL volume) in combinations of Bacteria A with each fungal species, specifically AY (Bacteria A with Fungi Y), AW (Bacteria A with Fungi W), and AP (Bacteria A with Fungi P).

The Congo Red Agar (CRA) media was prepared by dissolving 37 g/L of Brain Heart Infusion Agar (BHIA), 5% sucrose, and 0.08% Congo Red in distilled water, followed by sterilization via autoclaving at 121°C for 15 minutes. The prepared cultures were inoculated aseptically onto the CRA plates and incubated at 30°C for 72 hours to allow for biofilm formation. Biofilm formation was confirmed by visually observing the appearance of dark red-coloured spots on the CRA plates, indicating the presence of biofilms, as Congo Red binds to extracellular polymeric substances (EPS) produced by the biofilm-forming microorganisms.

The CRA method utilizes Congo red dye to differentiate between biofilm-forming and non-biofilm-producing bacteria based on their ability to bind the dye.[18] Biofilm producers typically exhibit distinct colouration, forming dark red to black colonies due to strong dye binding, while non-biofilm producers may appear light pink or white.[19] Biofilm-forming bacteria produce extracellular polysaccharides that interact with Congo red, leading to differential staining and visual identification on the agar. Congo red is a hydrophilic, symmetrical sulfonated azo dye that can form complexes with polysaccharides in a triple helix conformation.[20] The Congo red staining binds with biofilm matrix polysaccharides, marking the biofilm producers distinctly.[20] Microorganisms producing an EPS matrix during biofilm formation interact with Congo red dye, resulting in strong coloration.[21]

Confirmation of Biofilm Formation by Microscopic Method

Combined cultures of Bacteria A with each fungal species, specifically AY (Bacteria A with Fungi Y), AW (Bacteria A with Fungi W), and AP (Bacteria A with Fungi P), established on PRB selective media and nutrient broth media, were incubated for 72 hours at 30 °C. After incubation, the cultures were stained with lactophenol cotton blue to confirm biofilm formation. Following staining, the cultures were carefully examined under a light microscope to identify and observe the biofilm structures.

Assessing the Perchlorate Reduction in Martian Soil Simulants

Based on the determined perchlorate reduction efficiencies of various isolate combinations, the best-performing combination was selected for further experimentation. Monocultures of the selected isolates, A and P, were cultivated in nutrient broth and incubated at 30°C for 96 hours to allow biofilm formation. The presence of biofilms was confirmed using the Congo red agar method, supplemented with microscopic observations. Once biofilm formation was established, the biofilms were introduced into sealed tubes containing Martian soil simulants. These simulants were supplemented with sodium perchlorate at initial concentrations of 1% (W/W) and 0.5% (W/W), simulating the perchlorate content found in Martian soils.

The experiment was conducted under Martian atmospheric conditions with 100% CO_2 and incubated at 20°C to replicate the typical surface temperatures on Mars. After 10 days of incubation,

the perchlorate reduction activity was analysed using Fourier Transform Infrared (FTIR) spectroscopy, with particular focus on the perchlorate absorption peak at 1076 cm^{-1}, to assess the extent of perchlorate reduction.

The perchlorate reduction efficiency (PRE) of each combination was calculated using the following equation.

$$PRE = \frac{(Pc - Ps)}{Pc} \times 100\%$$

Where, P_C = The FTIR peak value of the control sample at 1076 cm^{-1}, P_S = The FTIR peak value of the sample at 1076 cm^{-1}.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics software (Version 29.0.2.0). To assess the differences between combinations, a one-way analysis of variance was performed. This statistical test was selected to evaluate whether there were significant differences in the means across the different combinations. Following the ANOVA, Tukey's Honestly Significant Difference test was applied for pairwise comparisons between the groups, allowing for the identification of specific differences between combinations when the overall ANOVA results indicated statistical significance. All statistical tests were conducted at a significance level of $*p < 0.05$.

RESULTS

Characterization of Soil Microorganisms

Two distinct bacterial isolates (A & B) and three distinct fungal isolates (W, Y & P) were isolated from the Ussangoda soil samples. The colony characteristics of fungal isolates are given in Table 1 and Figure 1, and bacterial isolates are given in Table 2.

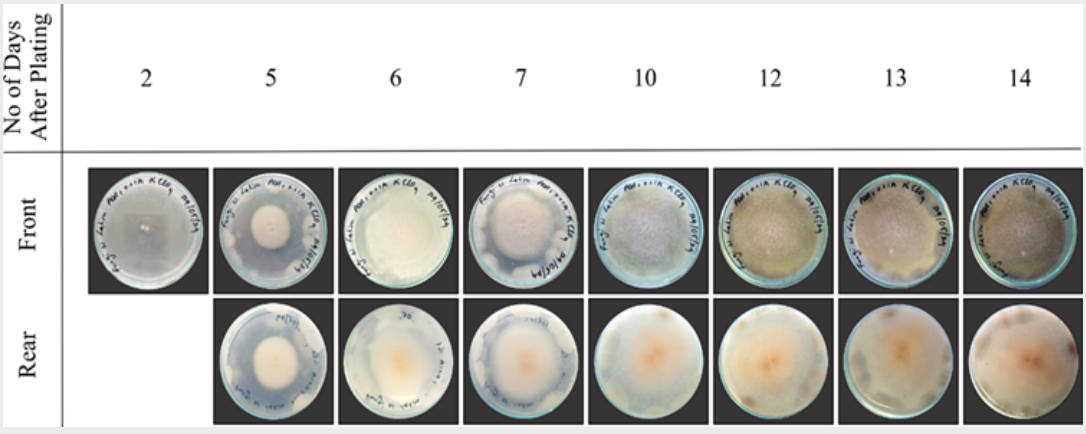
Table 1. Characteristics of fungal strains isolated from Ussangoda soil samples.

Isolate	Colony		
	Colour	Texture	Morphology / Other
Y	Yellow	Velvety	Center colour changes to dark green upon maturation. Parallel grooves and exudate formation noted. Dark red soluble pigment on the reverse side.
W	White	Powdery	Center and margin colours remains white throughout lifecycle. No grooves, exudate, or pigment formation.
P	Pink	Velvety	Microscopic observation shows hyaline hyphae with a globose head.

Table 2. Characteristics of Bacterial Strains Isolated from Ussangoda Soil Samples

Isolate	Colony Characteristics		Cell Morphology
	Colour	Shape	
A	Golden yellow	Circular	Cluster forming coccus bacteria
B	White	Circular	Cluster forming coccus bacteria

Fungal isolate – W



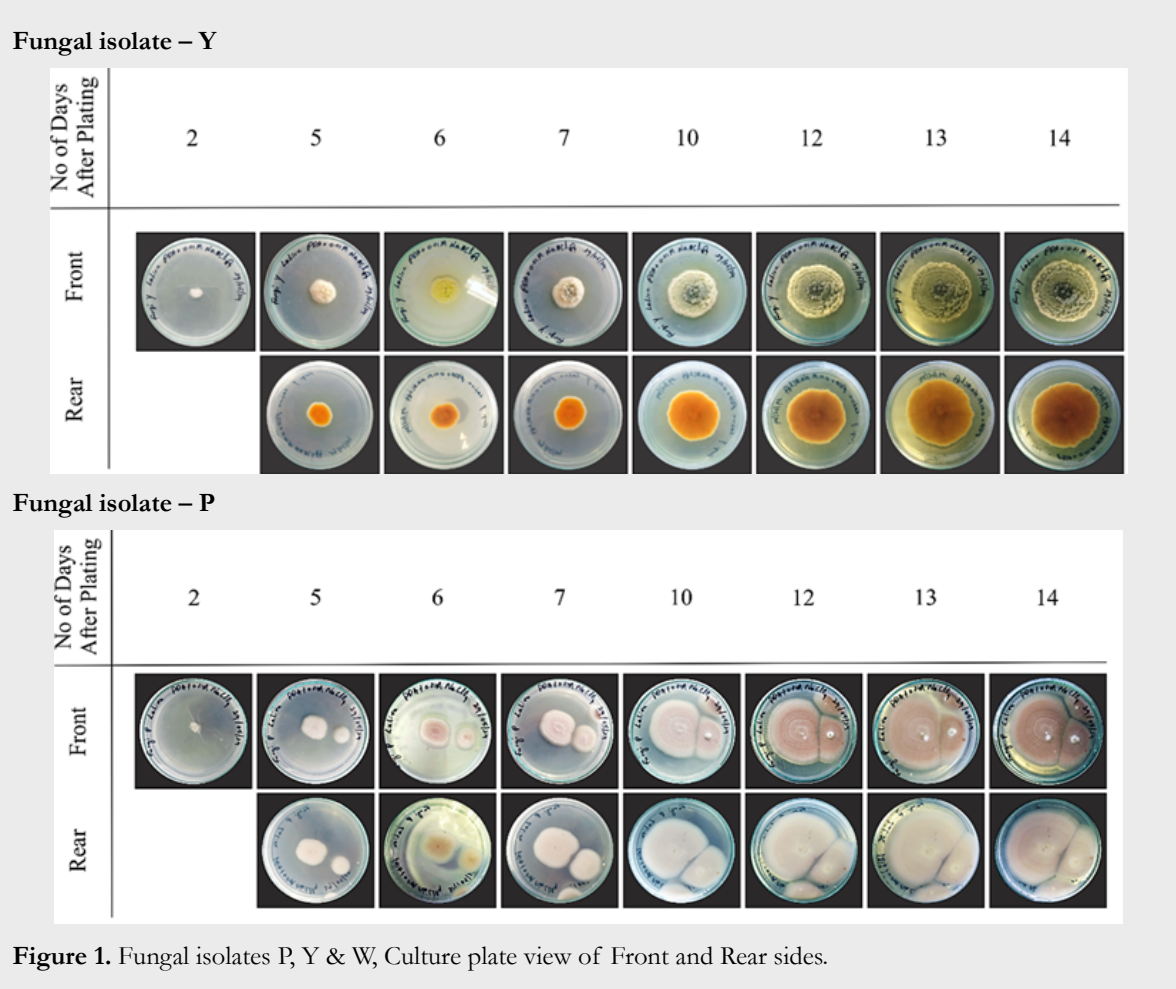


Figure 1. Fungal isolates P, Y & W, Culture plate view of Front and Rear sides.

Perchlorate Tolerance of the Isolates

Based on the duration for colony initiation, the perchlorate tolerance of fungal isolates Y and P was found to be up to 0.2 M $NaClO_4$, while bacterial isolate B and fungal isolate W tolerated up to 0.5 M $NaClO_4$ concentrations (Table 3). None of the isolates grew in 1 M $NaClO_4$ medium. At $NaClO_4$ concentrations higher than 0.2 M, a different colony morphology was observed in Bacteria A, with the colony colour changing from white to golden yellow. This new bacterial setting was subsequently considered as a new combination (Bacteria setting A) in the study.

Within the limitations of this study, the perchlorate tolerance of fungal isolates was assessed only in solid PDA media containing sodium perchlorate. To obtain more accurate results on actual tolerance, it is recommended to use a range of growth media (both solid and liquid). For example, DMSZ growth medium (3% malt extract, 0.3% soya peptone) as described by Heinz et al.[22] and other media optimized for facultative anaerobic fungi should be considered.

When conducting the perchlorate susceptibility assay, it was noticed that sodium perchlorate concentrations higher than 1 M interfered with agar solidification. Therefore, it is suggested to use liquid media for future studies using sodium perchlorate concentrations higher than 1M.

Table 3. Duration of Colony Initiation in Perchlorate Tolerance Assay at Sodium Perchlorate Concentrations Ranging from 0.01M to 1M.

Isolate	Duration for Colony Initiation (Days)					
	0.01 M	0.05 M	0.1 M	0.2 M	0.5 M	1 M
B	2	2	5	7	25	X
W	2	2	3	5	9	X
Y	2	3	5	7	X	X
P	2	5	7	7	X	X

Bacteria (B), Fungi (W, Y & P), X - No growth was observed.

Perchlorate Reduction Efficiency

The FTIR analysis confirmed the reduction of perchlorate by the different fungal-bacterial biofilms. Reduction of perchlorate was interpreted by the peak in the absorbance spectrum at 1100 cm^{-1} (Figure 2a). According to the calculated perchlorate removal efficiencies, bacteria setting A alone reduced perchlorate by 32.37%, while bacteria setting B alone achieved a 35.98% reduction (Figure 2b). As noted in the perchlorate susceptibility assay, at an

initial perchlorate concentration of 0.2 M, bacteria setting B may have transformed into bacteria setting A under the experimental conditions, leading to the higher reduction rate observed in the bacteria setting B-only compared to the bacteria setting A-only.

Biofilm Formation in Perchlorate-Reduction

Dark-coloured colonies or spots, ranging from brown to black, were observed on inoculated plates, indicating EPS secretion by biofilms in combinations AP and AW, whereas no such secretion was noted in combination AY. In the AY combination, weak EPS formation was initially detected (Figure 3); however, increased biofilm formation was observed after 14 days.

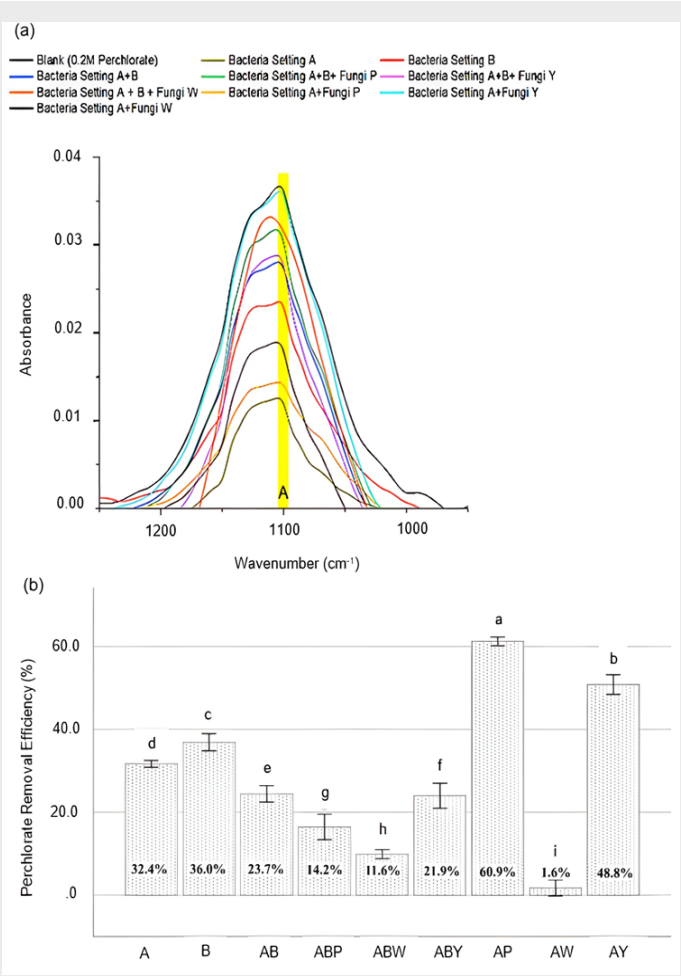


Figure 2. FTIR absorbance peaks of Sodium Perchlorate under tested combinations. Perchlorate reduction efficiency (%) of different microbial combinations. A- Bacteria setting A, B- Bacteria setting B, a mixed bacterial culture (AB), and combinations of bacterial isolates with fungal isolates: ABP (Bacteria A, B and fungi P), ABW (Bacteria A, B and fungi W), AP (Bacteria A and fungi P), AW (Bacteria A and fungi W), AY (Bacteria A and fungi Y). Different letters indicate statistically significant differences between groups ($^{*}p < 0.05$).

Confirmation of Biofilm Formation by Microscopic Method

Bacterial cells were observed adhering to fungal structures, supporting the findings from the Congo Red Agar method (Figure 4).

The staining method facilitated the identification of distinct biofilm formations, where bacterial cells were visibly clustered around fungal hyphae, further confirming the presence of biofilms in the combined cultures.

Assessing the Perchlorate Reduction in Martian Soil Simulants

FTIR analysis was conducted, focusing on the perchlorate absorption peak at 1076 cm^{-1} (Figure 5a, b). Furthermore, Perchlorate reduction efficiencies were calculated based on the FTIR absorbance peaks of Perchlorate. At 0.5% perchlorate concentration, undiluted AP biofilms (initial cell count: 1.21×10^{7} cells/mL) achieved a perchlorate reduction of 39.5% ($^{**}p < 0.001$), compared to the control. Diluted AP biofilms (cell count: 1.21×10^{5} cells/mL) demonstrated a reduction of 16.2% ($^{**}p < 0.001$), compared to the control. At 1% perchlorate concentration, undiluted biofilms demonstrated a reduction of 33.29% ($^{**}p < 0.001$), while diluted biofilms showed a reduction of 21.34% ($^{**}p < 0.001$), compared to the control, which showed no reduction (Figure 5c, d).

DISCUSSION

Biofilms formed by bacteria A and fungi P and W, i.e., AP and AW combinations, demonstrated significant EPS secretion, which facilitates microbial attachment and protection, contributing to higher perchlorate reduction efficiencies. In contrast, the combination of bacteria A with fungi Y, i.e., AY combination, exhibited delayed biofilm formation, which may explain its weaker initial reduction performance. The microscopic observations confirmed bacterial-fungal biofilm formation, with bacterial cells clustering around fungal hyphae. This mutualistic interaction likely enhanced the reduction of perchlorate, as evidenced by the FTIR results.

The ability of the isolates to reduce perchlorate under anaerobic conditions in iron-rich Ussangoda soils provides promising insights for extraterrestrial applications. The undiluted AP biofilm exhibited the highest perchlorate reduction efficiency (39.5% at 0.5% perchlorate concentration), highlighting its potential for bioremediation in extreme environments. In contrast, diluted biofilms displayed reduced efficiency, underscoring the critical role of cell density in bioremediation processes. A recent study investigating perchlorate reduction in methane-based biofilm reported a maximum perchlorate removal percentage of 48.3% at an initial perchlorate concentration of 10 mg/L.[23] In contrast, the findings of the current study revealed a significantly higher percentage of perchlorate reduction, even at a concentration 2000 times higher, with an initial concentration of 19,890 mg/L.

These findings highlight the importance of further research focused on optimizing biofilm formation and enhancing perchlorate reduction efficiency under Martian-like conditions.

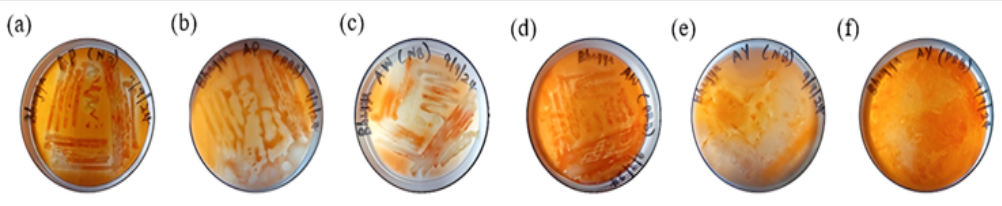


Figure 3. Biofilm formation observed in (a) AP combination cultured in NB media (b) AP combination cultured in PRB media (c) AW combination cultured in NB media (d) AW combination cultured in PRB media (e) AY combination cultured in NB media (f) AY combination cultured in PRB media.

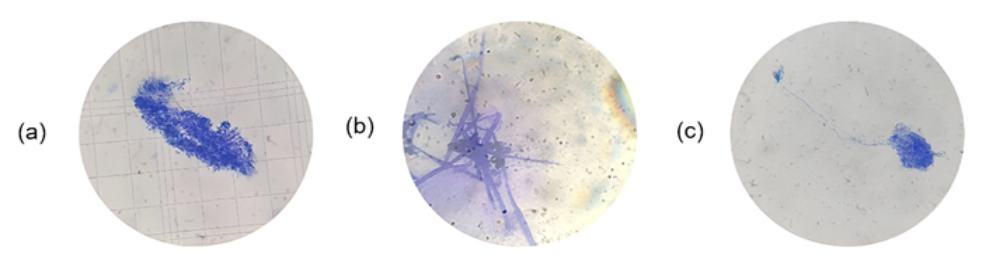


Figure 4. Microscopic observation of biofilm formation of microbial combinations grown on PRB media and incubated for 7 days (a) AY combination (b) AW Combination (c) AP Combination, Microscopic view at (400x).

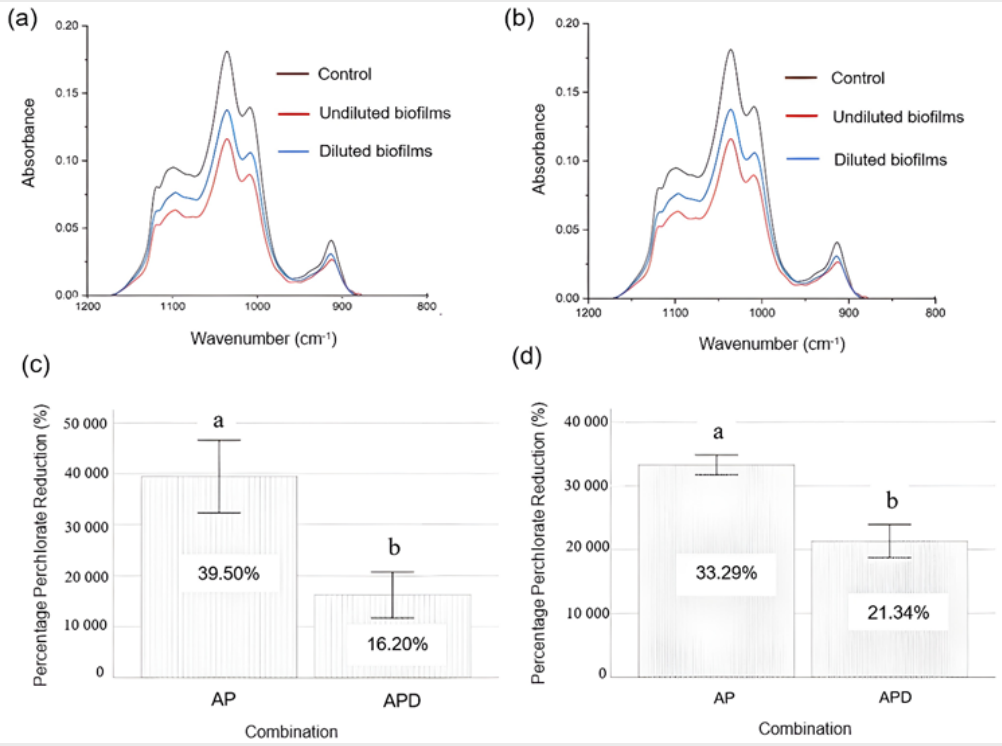


Figure 5. (a) Perchlorate absorption analysis at 1076 cm^{-1} from FTIR showing perchlorate reduction in Martian simulant soils (0.5% w/w initial perchlorate concentration) under different biofilm combinations. (b) Perchlorate absorption analysis from FTIR at 1076 cm^{-1} showing perchlorate reduction in Martian simulant soils (1% w/w initial perchlorate concentration) under different biofilm cobinations (c) Perchlorate reduction percentages relative to control by the biofilm combination AP at 0.5% (w/w) and (d) Perchlorate reduction percentages relative to control by the biofilm combination AP at 1% (w/w) sodium perchlorate. AP: biofilm of bacterium A and fungus P; APD: diluted biofilm of bacterium A and fungus P. Different letters indicate statistically significant differences between groups.

Perchlorate Tolerance of Microorganisms

The study demonstrated varying perchlorate tolerance among the isolates. Fungal isolate W exhibited the highest tolerance, up to 0.5 M $NaClO_4$. This finding suggests that these isolates possess mechanisms, potentially involving enzymatic detoxification or osmotic regulation, to withstand high perchlorate levels. Fungal isolates Y and P showed limited tolerance (up to 0.2 M $NaClO_4$), with slower colony initiation rates at higher concentrations. This indicates a potential connection between the isolates growth rate and perchlorate resistance, which could be explored further in future studies. The interference of high sodium perchlorate concentrations with agar solidification also points to the need for liquid media in future studies. A recent study[24] reported up to 25% discovered perchlorate reduction and by a bacteria with the maximum perchlorate tolerance of 10,000 mg/L $KClO_4$ (equivalent to a perchlorate ion concentration of 0.072 M). These bacteria show promise for the bioremediation of perchlorate contamination, particularly in high-salinity conditions. Another perchlorate-tolerant species, halotolerant yeast *D. hansenii*, was found, exhibiting a tolerance of 2.4 M $NaClO_4$,[22] which has the highest microbial perchlorate tolerance reported to date, surpassing the previous record of the bacterium *Planococcus halocryophilus*.

Future studies should focus on confirming the perchlorate reduction efficiency of the monocultures and mixed cultures by employing advanced analytical methods such as ion chromatography and liquid chromatography-mass spectrometry. Additionally, the exact perchlorate tolerance of the isolates can be assessed using liquid broth media, providing more precise insights into their capabilities. The selected isolates should also be inoculated into perchlorate-containing Martian simulant soils and incubated under Mars-like environmental conditions to evaluate their perchlorate reduction efficiency in simulated extraterrestrial settings. Furthermore, a UV tolerance assay can be conducted to quantify the isolates' resistance to radiation, which poses a significant challenge when establishing such cultures on Mars. To quantify the biofilm formation rates, methods like tissue culture tube assay can be employed, further advancing our understanding of their potential applications in extreme environments.

CONCLUSION

This study aimed to isolate microorganisms from soils in the Ussangoda area and explore their perchlorate reduction ability for future applications, including bioremediation in the context of colonizing Mars. Three fungal isolates with perchlorate tolerance ranging from 0.2 M to 0.5 M and a bacterial isolate with perchlorate tolerance of 0.5 M were identified. Mixed cultures of these isolates were tested in a 0.2 M perchlorate-containing medium, showing a reduction ranging from 1.63% to 60.9%. With high perchlorate tolerance, the ability to survive under extreme environments, and high efficiency in perchlorate reduction, these mixed cultures have potential applications in the bioremediation of Martian soil and water. Future studies are necessary to identify the isolated bacteria and fungi at the species level. This will help to understand the potential of these microbes beyond perchlorate tolerance.

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AUTHOR CONTRIBUTIONS

Mahesh Premarathna and Lahiru Kavinda: Conceptualization, Lahiru Kavinda, Bhagya Prasadini, Ravindu Pathirana, and Hashan Premarathna: Conducting laboratory analyses, Mahesh Premarathna: Supervision of laboratory analyses, Lahiru Kavinda, Bhagya Prasadini, Ravindu Pathirana, and Dumindu Ariyarathne: Writing - original draft, and Mahesh Premarathna, Gamini Seneviratne, and Ishara Manawasinghe: Writing - review and editing.

DATA AVAILABILITY

Data are available from the corresponding author upon reasonable request.

DECLARATIONS

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Conflict of Interests

The authors declare no competing interests including financial or non-financial interests.

Ethical Approval

Not applicable

REFERENCES

1. Gallardo-Carreño I, Moreno-Paz M, Aguirre J, et al. A Multiplex Immunosensor for Detecting Perchlorate-Reducing Bacteria for Environmental Monitoring and Planetary Exploration. *Front Microbiol.* 2020;11:590736.
2. Steinmaus CM. Perchlorate in Water Supplies: Sources, Exposures, and Health Effects. *Curr Environ Health Rep.* 2016;3(2):136-143.
3. Nazari-Sharabian M, Aghababaei M, Karakouzian M, et al. Water on Mars-A literature review. *Galaxies.* 2020;8:40.
4. Catling DC, Claire MW, Zahnle KJ, et al. Atmospheric origins of perchlorate on Mars and in the Atacama. *J Geophys Res Planets.* 2010;115.
5. Srinivasan A, Viraraghavan T. Perchlorate: Health Effects and Technologies for Its Removal from Water Resources. *Int J Environ Res Public Health.* 2009;6:1418-1442.
6. Keaney D, Lucey B, Finn K. A Review of Environmental Challenges Facing Martian Colonisation and the Potential for Terrestrial Microbes to Transform a Toxic Extraterrestrial Environment. *Challenges.* 2024;15:5.
7. Averesch NJH. Choice of Microbial System for In-Situ Resource Utilization on Mars. *Front Astron Space Sci.* 2021;8.
8. Wang O, Coates JD. Biotechnological Applications of Microbial (Per) chlorate Reduction. *Microorganisms.* 2017;5(4):76.
9. Steinberg LM, Trimble JJ, Logan BE. Enzymes responsible for chlorate reduction by *Pseudomonas* sp. are different from those used

for perchlorate reduction by *Azospira* sp. *FEMS Microbiol Lett*. 2005;247:153-159.

10. Coates JD, Achenbach LA. Microbial perchlorate reduction: rocket-fuelled metabolism. *Nat Rev Microbiol*. 2004;2:569-580.
11. Levakov I, Han J, Ronen Z, et al. Inhibition of perchlorate biodegradation by ferric and ferrous iron. *J Hazard Mater*. 2021;410:124555.
12. Vithanage M, Rajapaksha AU, Oze C, Rajakaruna N, et al. Metal release from serpentine soils in Sri Lanka. *Environ Monit Assess*. 2014;186(6):3415-3429.
13. Oravec HA, Asnani VM, Creager CM, Moreland SJ. Geotechnical Review of Existing Mars Soil Simulants for Surface Mobility. In: 17th Biennial International Conference on Engineering, Science, Construction, and Operations in Challenging Environments, 2021;157-170.
14. Weerasinghe HAS, Iqbal MCM. Plant diversity and soil characteristics of the Ussangoda serpentine site. *J Natn Sci Foundation Sri Lanka*. 2011;39:355-363.
15. Kanda K. Rapid Anaerobic Incubation Method for Measuring N Mineralization Potential in Soil. *JIRCAS Journal*. 2000;8:33-40.
16. Bruce RA, Achenbach LA, Coates JD. Reduction of (per)chlorate by a novel organism isolated from paper mill waste. *Environ Microbiol*. 1999;1:319-329.
17. Nyquist RA, Kagel RO. Infrared Spectra of Inorganic Compounds. In: Handbook of Infrared and Raman Spectra of Inorganic Compounds and Organic Salts, Academic Press, Michigan, 1971;1-18.
18. Lee JS, Bae YM, Han A, et al. Development of Congo red broth method for the detection of biofilm-forming or slime-producing Staphylococcus sp. *LWT*. 2016;73:707-714.
19. Prabhu K, Prasathkumar M, Sivaraman J, et al. Phytochemical characterization, antibacterial, and anti-biofilm efficacy of Mangifera indica seed kernel: A preliminary study using in vitro and in silico approaches. *J King Saud Univ Sci*. 2023;35:102688.
20. Xiong Z, Tian X, Wang G, et al. Development of a high-throughput screening method for exopolysaccharide-producing Streptococcus thermophilus based on Congo red. *Food Res Int*. 2022;162(Pt B):112094.
21. Mahto KU, Vandana, Priyadarshanee M, et al. Bacterial biofilm and extracellular polymeric substances in the treatment of environmental pollutants: Beyond the protective role in survivability. *J Clean Prod*. 2022;379: 134759.
22. Heinz J, Krahn T, Schulze-Makuch D. A New Record for Microbial Perchlorate Tolerance: Fungal Growth in NaClO4 Brines and its Implications for Putative Life on Mars. *Life (Basel)*. 2020;10:53.
23. Lv PL, Jia C, Wei CH, et al. Efficient perchlorate reduction in microaerobic environment facilitated by partner methane oxidizers. *J Hazard Mater*. 2024;466:133683.
24. Acevedo-Barrios R, Bertel-Sevilla A, Alonso-Molina J, et al. Perchlorate-Reducing Bacteria from Hypersaline Soils of the Colombian Caribbean. *Int J Microbiol*. 2019:6981865.