Extracting Active Enzymes from soils as a Measure of Bioremediation Potential.

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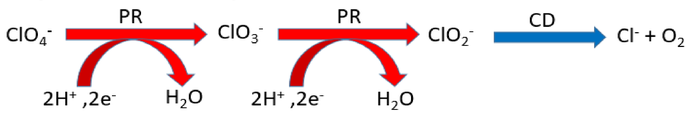
INTRODUCTION

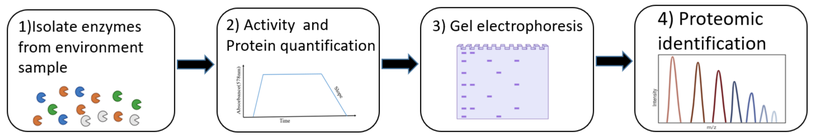
- Identifying pollutant-degrading enzymes from the environmental samples such as soil and/or groundwater can inform the bioremediation potential.
- Extracting active enzymes from complex soil matrices is challenging.
- Two methods for extracting active enzymes from soil were analyzed.
- The direct method involves lysing the cells within the soil matrix, while indirect involves separating first the cells from the soil then followed by lysis.
- Perchlorate reductase from *Azospira oryzae (A.o.)* was used as a model enzyme system to test soil-enzyme extraction.

Enzymatic reduction of perchlorate



- Perchlorate reductase (PR)
- Chlorite dismutase (CD)

Proteomic analysis and identification of enzymes extracted from environmental samples.

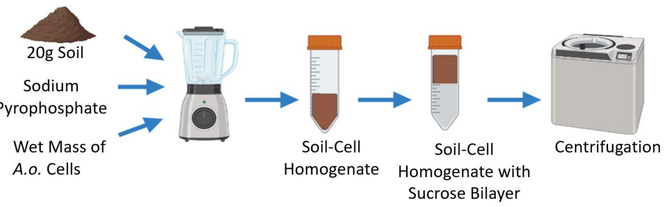


METHODS

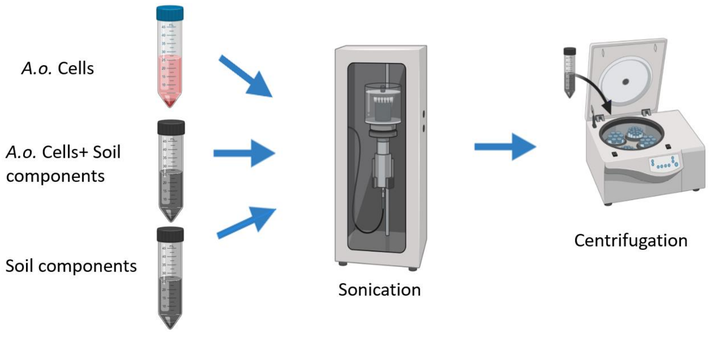
- *A.o.* was grown anaerobically and loaded in soil to test extraction methods;

I) Direct Method - Modified Novipure Soil Protein Extraction Kit
II) Indirect method – Three steps labeled below as (A), (B), and (C)

(A) Spike and extract the cells from soil using the sucrose density gradient centrifugation (SDGC)



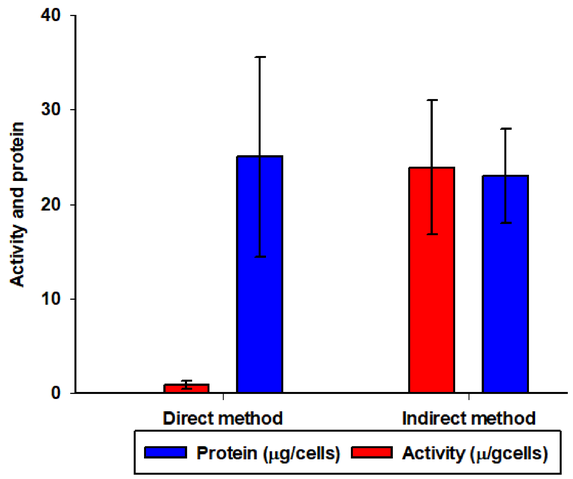
(B) Lyse cells and (C) centrifuge lysate to obtain the free enzymes



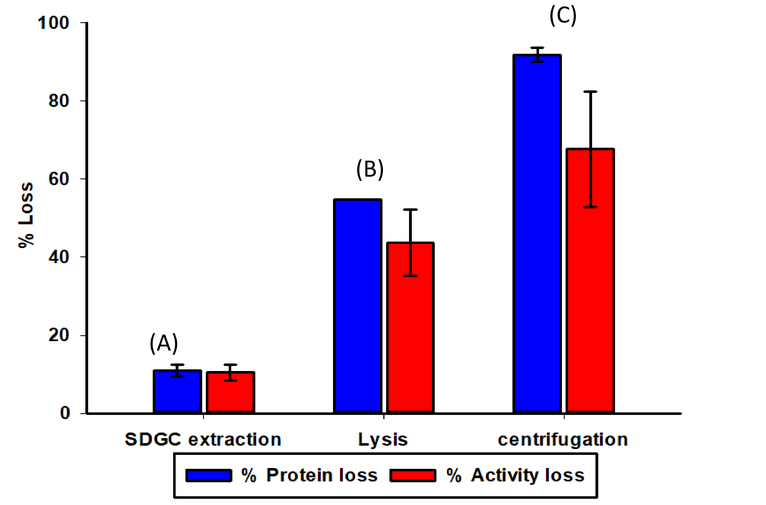
- Extraction was tracked by enzyme activity and protein mass balance by the colorimetric methyl viologen activity assay and the BCA assay, respectively.

RESULTS

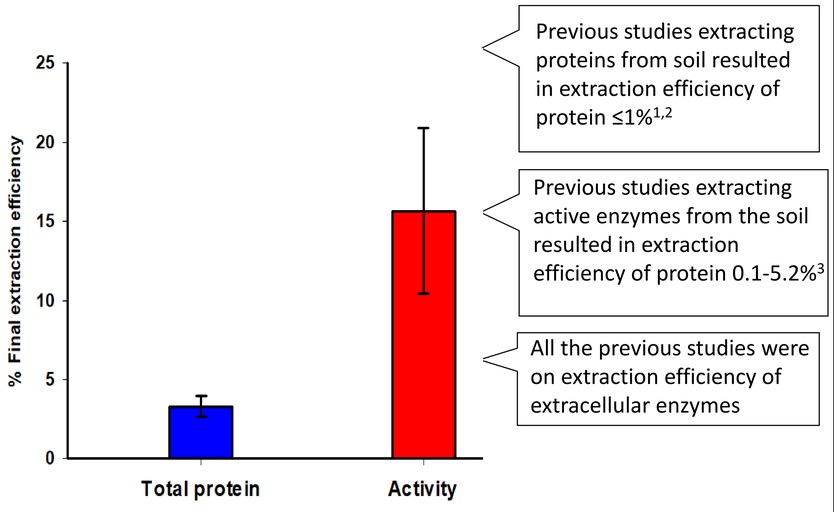
Indirect method resulted in higher activity than the direct method .



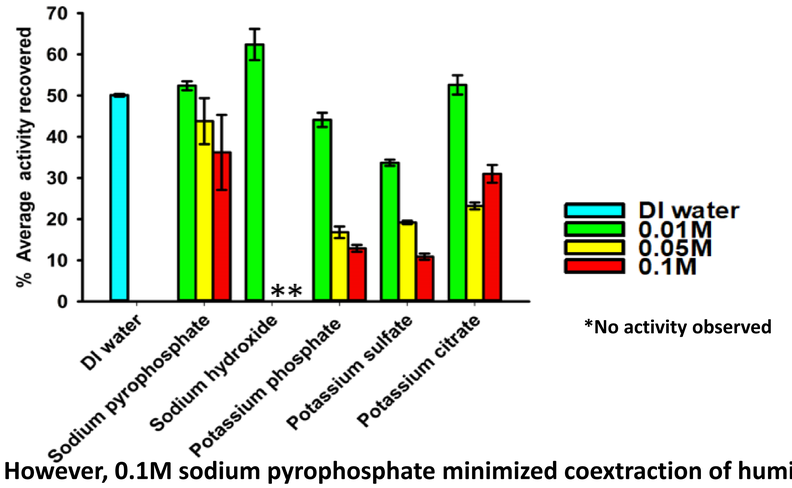
Optimized protocols for the a) SDGC, b) lysis, and c) centrifugation steps improved extraction efficiency.



Overall extraction efficiency is higher as compared to other previous studies

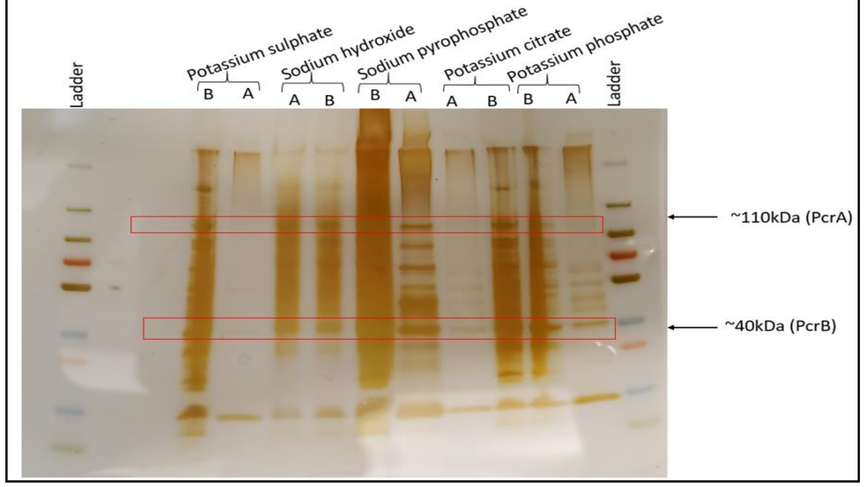


To further improve extraction recovery, different buffers were tested, and 0.1M sodium hydroxide had the highest recovery.



*No activity observed

However, 0.1M sodium pyrophosphate minimized coextraction of humic substances.



CONCLUSIONS

- The indirect method of extracting active enzymes from soil was better than the direct method.
- 0.01M NaOH was the best extractant in recovering active enzymes in the centrifugation step but coextracted humic substances.
- 0.1M sodium pyrophosphate was best extractant in obtaining good quality protein bands for further proteomic analysis.
- Mass spectrometry analysis for identification of the extracted enzymes is ongoing

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