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RESEARCH ARTICLE

Bioleaching of Manganese from Ferromanganese Nodules Utilizing Acetogenic Bacteria in a Symbiotic Community of Bacteria and Yeast

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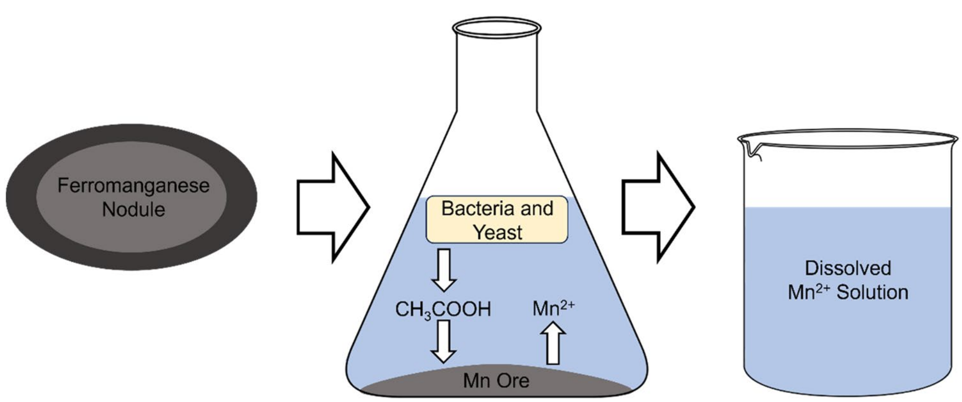
Abstract
Increased global demand for manganese has led to renewed interest in the development of sustainable and cost-effective methods for the utilization of low-grade manganese resources. Bioleaching approaches that use microorganisms to extract metals from ores are an intriguing alternative processing method that has been widely employed for numerous other mineral resources. In this study, we investigated the ability of acetogenic bacteria within a symbiotic community of bacteria and yeast (SCOBY) cultured from commercially available kombucha and vinegar food products to leach manganese from a low-grade ore composed of ferromanganese nodules. We found that kombucha-derived SCOBYs extracted up to 26.3% of total manganese from the ore in twelve days and was selective against the leaching of iron (< 0.66% of total iron leached). Increased extraction rates by kombucha-derived SCOBYs were observed with stirring and staggered addition of ore to the leach media. In contrast, vinegar-derived SCOBYs leached lower concentrations of manganese from the ore (maximum of 3.1% of total manganese leached) under all conditions. Optimal leaching was observed using kombucha-derived SCOBYs with staggered addition of ore. The difference in leaching efficiencies between the SCOBYs is hypothesized to result from differences in the microbial community's ability to withstand harsh environmental conditions present in the media during leaching. Further work to optimize conditions for acetic acid production and ore upgradation processes may also improve leaching efficiencies and rates.

Graphical Abstract



Keywords Bioleaching · Manganese · SCOBY · Acetogenic

Introduction

The utilization of microorganisms to extract metals from ores, or bioleaching, has emerged as a key technique for processing a variety of mineral resources [1–3]. Bioleaching

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Extended author information available on the last page of the article methods are an attractive alternative to traditional metallurgical extraction methods because they remove or reduce the need for the use of hazardous materials and generally have lower energy input requirements making them more environmentally sustainable [4]. The relative simplicity of bioleaching methodologies has also attracted attention for its potential as a more economical extraction method particularly for low-grade ores [5]. Accordingly, developing novel bioleaching methods for additional ore types could have significant impacts on the ability to utilize ores that were previously uneconomical as a resource.

Manganese (Mn) is an important mineral resource globally where it is primarily used in the production of steel as well as emerging uses in other industries such as next generation battery technologies [6, 7]. Manganese ores are generally processed utilizing a combination of pyrometallurgical and hydrometallurgical techniques [8]. However, most of the manganese ore deposits globally are low grade (i.e. < 25% Mn; Mn/Fe < 2.5) and have not been utilized due to an inability to develop economical processing methods with traditional techniques [9]. Increased demand for manganese has led to renewed interest in development of alternative methods for processing of these lower grade ores [10]. Numerous studies have investigated the use of bioleaching to extract manganese from a variety of ore materials including manganese oxide ores, spent batteries, and electrolytic manganese residues and have achieved high (> 80%) recovery rates [11, 12].

However, the use of bioleaching for processing manganese carbonate ores, which are the dominant mineral in > 40% of manganese deposits globally, has received only limited study [13]. The buffering capacity of carbonate produced as a byproduct of the bioleaching of carbonate minerals presents a particular challenge as it can cause an increase in pH as leaching proceeds causing adverse effects to the microbial communities used during bioleaching [14]. Previous efforts to process manganese carbonate ores with bioleaching utilized *Acidithiobacillus ferroxidans* successfully demonstrated high leaching efficiencies of manganese and iron from a rhodochrosite rich ore, and the ability to directly synthesize Mn–Fe oxides from the leachate [15]. However, this method requires the external adjustment of pH prior to onset of bioleaching and the presence of pyrite in the ore to maintain pH through sulfide oxidation, which may limit its applicability to other carbonate ores particularly those that do not contain pyrite in the gangue. Additionally, the method was not selective for Mn versus Fe which may limit the types of products that can be produced from the leachate [16].

Symbiotic communities of bacteria and yeast (SCOBYs) that are commonly employed in the production of acetic acid-based food products such as kombucha and vinegar produce acetic acid through fermentation of sugars to alcohol by yeast (including *Brettanomyces, Hanseniaspora,* and *Zygosaccharomyces)* followed by conversion of alcohol to acetic acid and other organic acids by bacteria (including *Acetobacteraceae* and *Lactobacteriacea*e) or conversion of alcohol to acetic acid [17–20]. Acetic acid has previously been used to selectively leach carbonate minerals indicating the potential utility for utilizing acetic acid producing SCOBYs to bioleach manganese carbonate ores [21–23]. Furthermore, SCOBYs commonly produce cellulose-based pellicles which provide protection from changes in environmental conditions indicating they may be particularly well suited to withstand the dynamic and harsh chemical environments (e.g. variable pH and high metal content) generated as the result of bioleaching processes [24]. In this study, we investigated the ability of acetogenic bacteria in SCOBYs cultured from commercially available kombucha and vinegar food products to selectively leach manganese from a low-grade manganese carbonate ore. This work provides novel insights into bioleaching of manganese carbonate ores and is to our knowledge the first study to utilize SCOBYs for bioleaching.

Materials and Methods

Description and Characterization of Ore Material

The manganese ore used in this study was composed of ferromanganese nodules from the DeGrey Member of the Pierre Shale near Chamberlain, South Dakota. These nodules outcrop extensively in central South Dakota and the deposit is estimated to contain approximately 5 billion metric tons of manganese placing it among the largest manganese deposits in the world [25]. However, despite the size of these deposits previous efforts to utilize them as a manganese resource have failed due to an inability to develop economical extraction techniques utilizing traditional metallurgical methods [26].

For this study, several kilograms of ore material were collected, crushed, ground, homogenized, and sieved and ore material in the < 200 mesh (74 microns) fraction used for leaching experiments. Bulk elemental concentration of the ore material was determined using an Olympus Vanta M Series X-Ray Fluorescence analyzer and found Mn concentrations of 16.7 weight percent and Fe concentrations of 11.6 weight percent. Previous mineralogical characterization of the nodules has indicated that manganese bearing minerals primarily consists of manganocalcite, rhodochrosite, manganite, and pyrolusite [26].

Experimental Methods

Establishment of Starter Microbial Cultures

Mixed microbial cultures for bioleaching experiments were established utilizing microbial communities derived from commercially available kombucha (Large SCOBY Starter Culture, The Kombucha Shop) and vinegar (Apple Cider Vinegar with the Mother, Bragg) products. Sweetened tea was utilized as media for kombucha cultures and was created by steeping four tea bags (Lipton Classic Black, 0.1 oz per bag) in 710 mL of boiling distilled water for five minutes followed by addition of 55 g of sucrose in a one-gallon glass vessel. Kombucha cultures were prepared by combining 710 mL of tea media with 140 mL of kombucha starter tea and SCOBY. Vinegar cultures were prepared by combining 140 mL of vinegar with 710 mL of hard cider (2 Towns Cosmic Crisp, 8% ABV) as media in a one-gallon glass vessel. Both kombucha and vinegar starter cultures were allowed to grow for one week before use in bioleaching experiments.

Acetic Acid Production Experiments

Following establishment of starter cultures, experiments were performed to assess acetic acid production of these cultures in the absence of manganese ore, as well as the effects of stirring on acetic acid production. Cultures were prepared in 250 mL polycarbonate shaking flasks with vented caps that allow for gas exchange with the atmosphere (DWK Life Sciences) For kombucha cultures, 20 mL of starter culture was combined with 130 mL of sweet tea media prepared as described above. For vinegar cultures, 20 mL of starter culture was combined with 130 mL of yeast extract and ethanol media prepared by combining 108 mL of distilled water, 12 mL of absolute ethanol, and 0.375 g of yeast extract. Stirred cultures were stirred at 120 rpm using an orbital shaker in ambient light and temperature conditions (~ 25 °C and 15 h of daylight per day). Unstirred cultures were left on the benchtop adjacent to the orbital shaker under the same ambient conditions. One mL samples of the culture media were collected to measure acetic acid production utilizing a sterile 2 mL syringe and filtered with a 0.2 micron PVDF syringe filter at seven and fourteen days. Samples were diluted with deionized water as necessary to ensure all acetic acid concentrations fell within the calibration range.

Acetic acid production was assessed using a Dionex ICS-6000 High Pressure Ion Chromatography (IC) System with suppressed conductivity detection in the Engineering and Mining Experiment Station at South Dakota Mines. Analytes were separated by injecting 25 µL of sample onto an AS-11 column and utilizing a hydroxide gradient elution at a flow rate of 1.5 mL/min. Elution parameters were 1 mM KOH for 6 min, a linear ramp to 30 mM over 5 min, a linear ramp to 60 mM KOH over 1 min, and re-equilibration at 1 mM KOH for 5 min between samples. External calibration was performed using a certified acetate standard (High Purity Standards). Calibration accuracy was assured by analyzing a certified acetate standard (High Purity Standards) from a different production batch at the midpoint of the calibration curve and ensuring recovery of ± 10% of certified values.

Abiotic Acetic Acid Leaching Experiments

Leaching experiments were performed to compare the leaching capacity of acetic acid produced by the kombucha SCOBY and reagent acetic acid at equivalent acid concentrations. A kombucha sample was collected from the starter culture and its acetic acid concentration determined to be 3.6% using the IC method described above. A reagent acetic acid solution of 3.6% acid content was also prepared by dilution of glacial acetic acid (Fisher Scientific) with deionized water. Leaching experiments were performed by combining 15 g of ore material with 150 mL of kombucha or reagent acetic acid in a 250 mL beaker. Solutions were stirred at 350 rpm and 1 mL were samples collected at 0, 5, 15, 30, 60, and 120 min. All samples were immediately filtered with a 0.2 micron PVDF syringe filter to remove any particulates and diluted 2000× with nitric acid (PlasmaPure, SCP Science) and deionized water to a 2% nitric acid concentration.

Manganese and iron concentrations in the solution at each time point were measured utilizing an Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) in the Engineering and Mining Experiment Station at South Dakota Mines. The ICP-MS was operated in helium collision mode and quantification performed by external calibration with a multielement standard solution (SCP28-AES, SCP Science). Internal standardization was used to account for any matrix effects by in-line addition of Sc to all samples and standards at a concentration of 1 mg/L. Instrument performance was assessed by measurement of a second multielement standard solution (ICP-MSCS-PE3-A, High Purity Standards) and recovery was ensured to be ± 10 of certified values.

Bioleaching Experiments

Bioleaching experiments were conducted to determine the capability of kombucha and vinegar cultures to leach manganese from the ore as well as test the effects of stirring and timing of ore additions on leaching capacity. Cultures for bioleaching were created by combining 20 mL of cultures utilized in the acetic acid production experiments with 130 mL of either sweet tea media for kombucha or yeast extract and ethanol media for vinegar in 250 mL polycarbonate Erlenmeyer flasks. Cultures were allowed to grow for two days before addition of ore material to initiate the bioleaching experiments. All bioleaching experiments were performed in duplicate. Baseline bioleaching experiments were performed by adding 15 g of ore and leaving undisturbed on the benchtop under ambient light and temperature conditions in the library. The effects of stirring on leaching capacity were assessed by shaking flasks at 120 rpm in an orbital shaker. The effects of staggering ore additions were assessed by adding 5 g of ore at experiment onset, 5 g on day four of the experiment, and 5 g on day seven of the experiment. For all experiments, 2 mL samples of media were collected after 2, 4, 7, 12, and 19 days to assess manganese and iron concentrations via ICP-MS as described above. All samples were filtered with a 0.2 micron PVDF filter to remove particulates and diluted 2000 × with nitric acid and deionized water to a 2% nitric acid concentration.

Results

Acetic Acid Production and Abiotic Leaching Experiments

Acetic acid production by the kombucha and vinegar cultures with and without stirring are shown in Fig. 1 and online supplementary material Table S1. Both culture types produced acetic acid and acetic acid production increased with stirring for both kombucha and vinegar cultures. Acetic acid concentrations in media increased with time for both stirred and unstirred cultures of kombucha and stirred cultures of vinegar. In contrast, the acetic acid concentration in the media decreased between day seven and day fourteen for the unstirred vinegar culture.

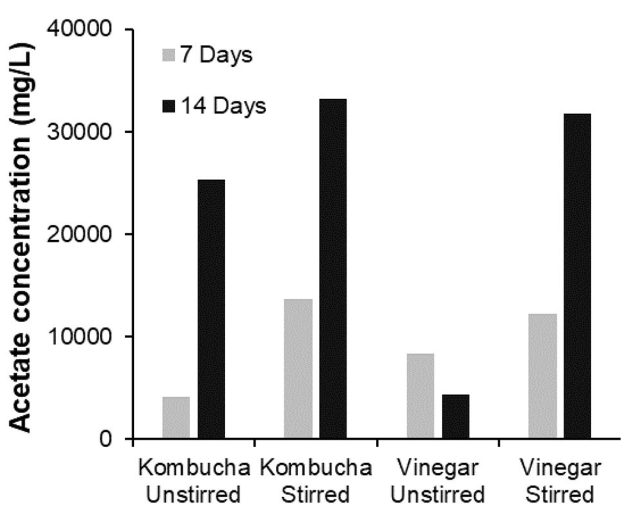

Fig. 1 Acetic acid production by kombucha and vinegar SCOBYs in growth media without the addition of manganese ore. Experiments were performed by inoculating 130 mL of media with 20 mL of starter culture. Stirred cultures were stirred at a rate of 120 rpm

Abiotic leaching experiments demonstrated similar leaching rates and efficiency for kombucha-derived and reagent grade acetic acid at equivalent acid concentrations (Fig. 2 and Table S2). At termination of the experiment after 120 min of leaching kombucha had leached 13.26% of total manganese in the ore and reagent acetic acid had leached 14.9% of total manganese. Both kombucha and reagent acetic acid were highly selective for manganese over iron from the ore material. At termination of the experiment kombucha had leached 0.5% of total iron in the ore and reagent acetic acid had leached 0.6% of total iron.

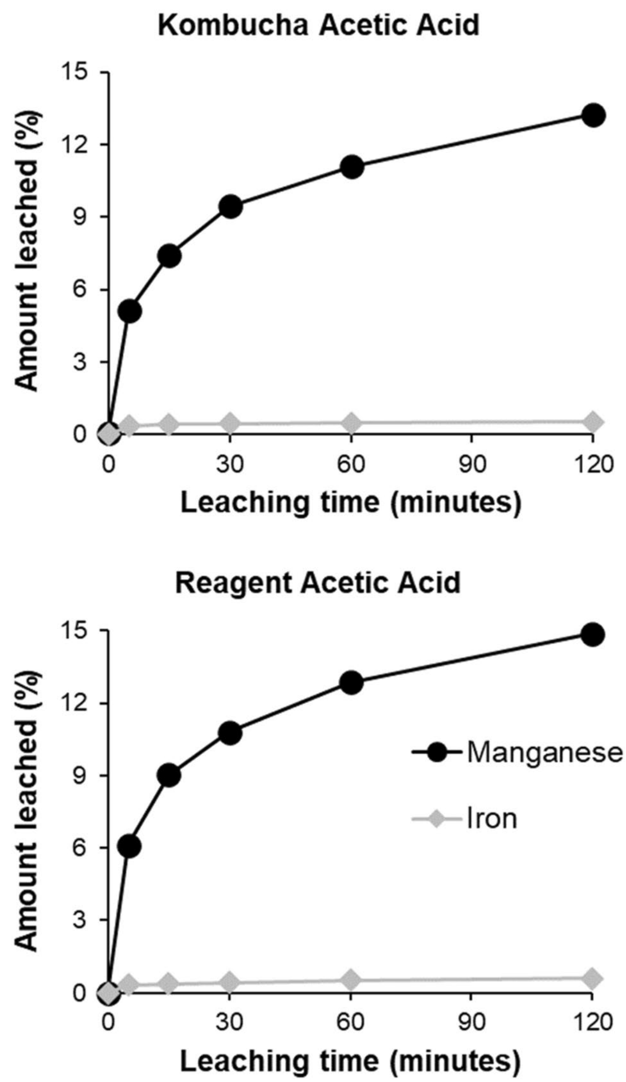

Fig. 2 Comparison of leaching rates between kombucha-derived acetic acid in culture media and reagent acetic acid at equivalent acid concentrations. Leaching experiments were performed by combining 150 mL of 3.6% acetic acid solutions with 15 g of ore at a stir rate of 350 rpm

Bioleaching Experiments

Both kombucha and vinegar cultures produced acetic acid under the experimental growth conditions (Fig. 1). Acetic acid production was higher when stirred versus unstirred for both culture types after both seven and fourteen days. Acetic acid concentrations in the culture media increased with time for all experimental conditions except for the unstirred vinegar cultures which had a decrease in acetic acid concentration between seven and fourteen days.

Kombucha cultures under all experimental conditions (i.e. unstirred, unstirred with staggered ore addition, and stirred) all leached > 24% of total manganese in ore (Fig. 3 and Table S3). Maximum manganese concentrations were observed after 12 days for the staggered ore addition and stirred conditions before decreasing at day 19. For the unstirred condition maximum leaching rates were observed after 19 days. The stirred and staggered ore addition conditions had higher manganese leaching rates compared to the unstirred condition. All kombucha cultures leached comparatively low amounts of iron (< 0.6% of total iron in the ore; Fig. 3 and Table S3). The stirred and staggered ore conditions showed similar temporal trends with a maximum iron concentration measured at day 7 of the experiment.

Vinegar cultures leached comparatively lower amounts of manganese from the ore compared to kombucha cultures across all experimental conditions (Fig. 4 and Table S3). The maximum amount of manganese leached from ore was 10% in one replicate of the staggered ore addition condition which had maximum manganese concentrations at day 19 of the experiment. All other experiments leached < 4% of the manganese within the ore. The amount of iron leached from the ore was similarly low (< 0.2% of total iron in the ore; Fig. 3 and Table S3) for all experimental conditions.

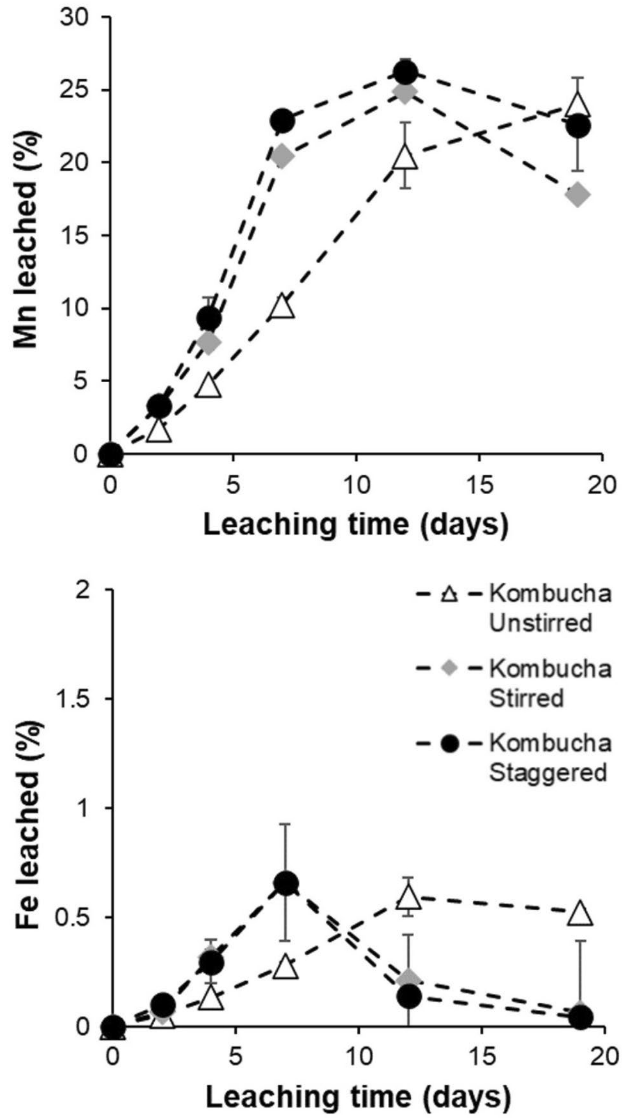

Fig. 3 Leaching efficiencies for manganese and iron with SCOBYs derived from kombucha cultures. Points represent averages of replicate analyses and error bars one standard deviation values. Experiments were confirmed by combining 15 g of ore with 130 mL of media inoculated with 20 mL of starter cultures. Stirred cultures were stirred at a rate of 120 rpm. Staggered additions were performed by adding 5 g of ore at initiation of the experiment, 5 g on day four of the experiment, and 5 g on day seven of the experiment

Discussion

These results demonstrate the ability of acetogenic bacteria within a SCOBY to selectively bioleach manganese from a low-grade manganese carbonate ore. Interestingly, despite kombucha and vinegar cultures producing similar concentrations of acetic acid under stirred conditions in the absence of manganese ore material, kombucha cultures leached substantially higher concentrations of manganese compared to vinegar cultures (Figs. 3 and 4). These results suggest that kombucha cultures are more robust to the changing conditions in solution chemistry that occur during bioleaching (e.g. increased metal concentration and increasing pH). This may be due to either the structure or function of the microbial consortia in kombucha cultures compared to vinegar cultures or differences between the cultures in the production of cellulose-based pellicles which can provide protection for the microbial community from changing environmental conditions. Additional work is needed to characterize shifts in microbial community structure and function during the bioleaching process, and this information could assist in designing optimal environmental conditions for bioleaching.

For the kombucha cultures, the rate of manganese leaching increased with both stirring and staggered addition of manganese ore (Fig. 3). Increased leaching rates under stirred conditions are likely due to enhanced acetic acid production as a result of increased oxygenation of

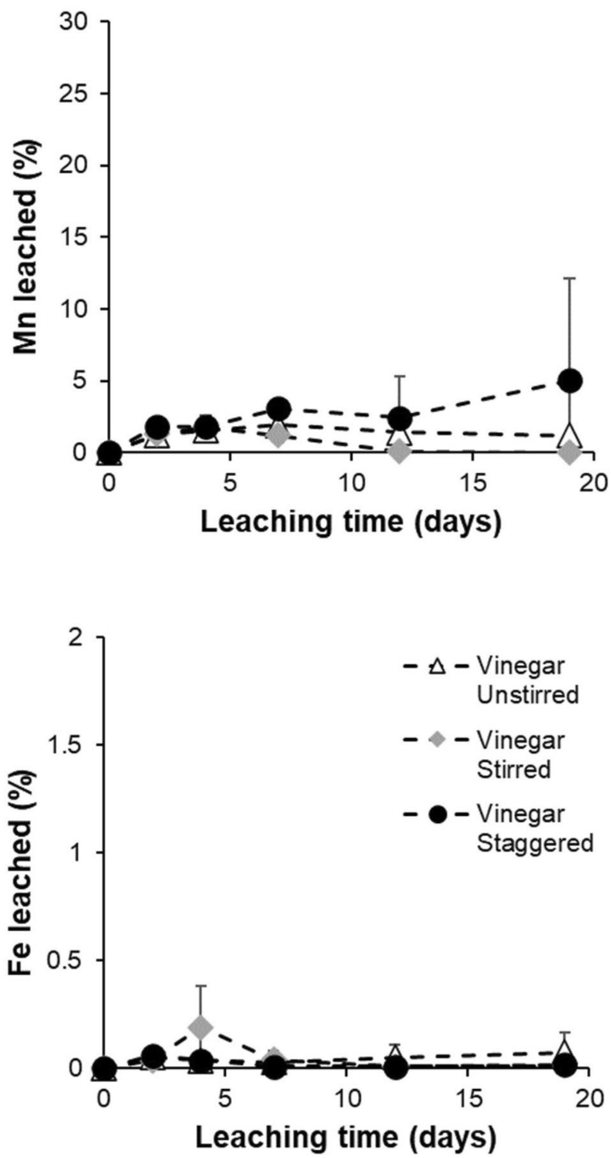

Fig. 4 Leaching efficiencies for manganese and iron with SCOBYs derived from vinegar cultures. Points represent averages of replicate analyses and error bars one standard deviation values. Experiments were confirmed by combining 15 g of ore with 130 mL of media inoculated with 20 mL of starter cultures. Stirred cultures were stirred at a rate of 120 rpm. Staggered additions were performed by adding 5 g of ore at initiation of the experiment, 5 g on day four of the experiment, and 5 g on day seven of the experiment

the media, which has been identified as a key parameter for acetic acid production rates by acetic acid bacteria [27]. Likewise, stirring may also provide increased diffusion rates for acetic acid to the mineral surface providing enhanced leaching rates, which has previously been identified as the rate limiting step for abiotic acid leaching of manganese carbonate ores [28]. Increased leaching rates with staggered addition of ore materials has previously been observed in other bioleaching applications and is hypothesized to result from providing more stable environmental conditions for establishment of cell cultures [29–31]. Further research optimizing stir rate and timing of manganese additions as well as other parameters (e.g. temperature, media composition, pulp density) may also help to improve leaching rates.

Comparison of leaching efficiency between kombucha growth media and reagent acetic acid in abiotic leaching experiments provides insight into leaching mechanisms (Fig. 2). The observed leaching in the absence of microbial biomass indicates that bioleaching by SCOBYs is an indirect process resulting from the action of microbial metabolites rather than direct conversion by microorganisms [32]. Additionally, the similarity in leaching efficiency and leaching rates between the kombucha media and reagent acid indicate that other organic acids or microbial metabolites that may be produced by kombucha SCOBY neither hinder nor enhance manganese leaching. While further work is necessary to fully characterize leaching mechanisms, we hypothesize that it is primarily occurring due to SCOBY produced acetic acid leaching manganese carbonate minerals in the ore following the general reaction:

$$MnCO_3(s) + 2CH_3COOH(aq) \rightarrow Mn^{2+}(aq) + 2CH_3COO^-(aq) + CO_2(g) + H_2O(l) \quad (1)$$

Maximum leaching efficiency in these experiments is thus likely to be controlled by the mineralogy of the ore utilized and it is hypothesized that higher leaching efficiencies by SCOBYs may be possible for ore types that are richer in manganese carbonate versus manganese oxides. This may explain the relative low leaching efficiencies observed when compared to previous bioleaching studies of ores with manganese only contained within manganese carbonate minerals [15].

Another intriguing aspect of the utilization of SCOBY communities to bioleach manganese is the development of cellulose-based pellicles as a byproduct. Bacterial cellulose has uses in numerous industrial, biomedical, and agricultural applications, however the high costs of bacterial cellulose production have limited its utilization over traditional cellulose sources [33]. Further work is necessary to characterize the cellulose produced during the bioleaching experiments and its utility for industrial applications, but cellulose derived as a byproduct of kombucha production has previously been investigated as a sustainable cellulose resource [34]. The potential utilization of bacterial cellulose as a value-added co-product indicates additional potential for bioleaching with SCOBYs as a sustainable and cost-effective method for bioleaching of low-grade manganese ores.

Conclusions

This study highlights the potential for utilization of acetogenic bacteria within a SCOBY to bioleach manganese from a low-grade manganese ore. Differences in the capacity to leach manganese between kombucha-derived and vinegar-derived SCOBYs despite similar acetic acid production rates in abiotic experiments were also observed and are hypothesized to be the result of differences in the ability of SCOBYs to withstand harsh environmental conditions resulting from the leaching. Increased leaching rates were observed with stirring and staggered addition of ore material with a maximum leaching rate of 26.3% of total manganese in the ore extracted after 12 days by kombucha SCOBYs with staggered addition of ore. Production of cellulose pellicles as a co-product may also enhance the cost-effectiveness and environmental benefits of bioleaching over traditional leaching methods. Collectively, this research highlights the potential for the repurposing of SCOBYs used for food production for bioleaching of manganese ores and potentially other carbonate ore materials.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40831-024-00945-9.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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