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The effects of indigenous microorganisms and water treatment with ion exchange resin on Cu-Ni flotation performance

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ABSTRACT

Mineral processing utilizes large amounts of water and aims to reduce water consumption by recirculation and closing the water loops. This results in accumulation of chemical and biological contaminants in process water that may have adverse outcomes on the process performance. To optimize water quality suitable for each process step and plant, knowledge of both chemical and biological effects are needed as well as techniques to best remove the contaminants. This study focused on the consequences of microorganisms, enriched from the actual process step and plant, knowledge of both chemical and biological effects are needed as well as techniques to best remove the contaminants. This study focused on the consequences of microorganisms, enriched from the actual process earlier, on the flotation performance in the multi-metal Kevitsa mine in Northern Finland and the applicability of ion exchange for the removal of dissolved sulfur species and microorganisms from water. The increase of microbial load from the original 10^6 to added 10^7 16S rRNA copies mL ^ -1 affected positively the flotation selectivity, especially in the case of nickel. Two tested water types, process water (PW) and final tailings water (FT), behaved slightly differently. In the Cu flotation phase added microorganisms did not affect the Cu recovery of FT but decreased significantly the recovery of Cu in PW. With equal Cu grade, the recovery was as high as approximately 25 percentage points lower. However, added microorganisms in both water types decreased notably the recovery of Ni in Cu concentrate (18 to 37 %-points). At the same time the amount of Ni recovered in the Ni concentrate increased by 18 to 33 %-points with added microorganisms. Visually the froth layer was higher and more stable in the Ni flotation in experiments with added microorganisms compared to experiments without added microorganisms. The concentrations of dissolved sulfate and thiosulfate ions were low in the studied waters compared to added 10^6 copies mL ^ -1 affected positively the flotation selectivity, especially in the case of nickel. Two tested water types, process water (PW) and final tailings water (FT), behaved slightly differently. In the Cu flotation phase added microorganisms did not affect the Cu recovery of FT but decreased significantly the recovery of Cu in PW. With equal Cu grade, the recovery was as high as approximately 25 percentage points lower. However, added microorganisms in both water types decreased notably the recovery of Ni in Cu concentrate (18 to 37 %-points). At the same time the amount of Ni recovered in the Ni concentrate increased by 18 to 33 %-points with added microorganisms. Visually the froth layer was higher and more stable in the Ni flotation in experiments with added microorganisms compared to experiments without added microorganisms. The concentrations of dissolved sulfate and thiosulfate ions were low in the studied waters compared to operations treating massive sulfide ores and did not significantly affect the flotation performance. For this reason, the IX water treatment was not required for these ions. However, the IX treatment proved to be effective in removing both sulfur species and microorganisms. The use of dissolved air flotation (DAF) was a successful pretreatment for ion exchange in removal of microorganisms. However, microorganisms are not usually taken into consideration when process performance or water cleaning techniques are designed and optimization could result generally in even better outcome.

1. Introduction

The mining industry aims to close the water loops especially in flotation, which uses the largest amounts of water in the mine site (Kinnunen et al., 2021), as well as to optimize water use by different approaches that include combining weather information and geo-metallurgical modelling, water chemistry simulations and optimization of dewatering systems (Moraga et al., 2023; Michaux et al., 2019; Gálvez et al., 2014). However, the circulation of process water results in the accumulation of contaminants, such as suspended solids, dissolved sulfur species, metal ions and organic flotation reagents as well as microorganisms (Liu et al., 2013a; Meng et al., 2022; Kinnunen et al., 2020) in the water, which may have a detrimental effect on flotation performance (De Mesquita et al., 2003; Mhonde et al., 2020; Liu et al., 2013b). Recirculation of water is expected to increase the accumulation of heat and nutrients that favor the growth of microorganisms and buildup of different organic compounds which could act as surface activators, dispersants or flocculants. These products could interfere with the flotation process (Rao and Finch, 1989; Forssberg and Hallin, 1989; Levay et al., 2001; Liu et al., 2013a). Recycled water needs to be treated to the optimal level, where the process performance is not affected and recycled water is treated just to the right level to fit its purpose. There is

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no need to target complete impurity removal (Kinnunen et al., 2021). For example, 30% removal of contaminants causing problems from recycled process water may be sufficient to ensure an adequate flotation performance.

Microorganisms can have negative, neutral or positive impacts on flotation, but this phenomenon has remained largely unstudied in minerals processing (reviewed by Kinnunen et al., 2020; Asgari et al., 2022; Mishra et al., 2023). Most of the research has focused on the impact of pure cultured bacterial strains on the flotation. However, natural microbial communities existing in processing plants are much more complex. Microbiological diversity and microbiomes in mining processes consist of tens to hundreds of different microbial species (Bomberg et al., 2020; Miettinen et al., 2021). In addition, microbiological signatures are unique for each mine site depending on parameters such as the ore composition, process water quality, seasonal conditions and a multitude of chemical process variables (e.g. Miettinen et al., 2021; Bomberg et al., 2020; Arias et al., 2023; Evdokimova et al., 2012). The presence of considerable amount of microorganisms and large variation in microbial quantities in different steps of minerals processing have been shown in recent studies (Bomberg et al., 2020; Miettinen et al., 2021). However, knowledge on actual impacts of indigenous microorganisms, both positive and negative, on the flotation process performance is still absent.

Mine sites are different in relation to minerals, flow sheets and end products. As a consequence, also the characteristics of process water and the sensitivity of flotation to process water quality vary to a great extent. The input water quality varies significantly in the mining processes. There are simple and economical widespread physicochemical methods for water treatment such as sedimentation, neutralization and coagulation-flocculation but they often have a low efficiency with recalcitrant pollutants (Jing et al., 2023) and microorganisms. Biodegradation of various refractory flotation reagents has been shown (Chen et al., 2011; Cheng et al., 2012). However, not all reagents are easily treatable and may require long duration. Advanced oxidation process (AOP) technologies that include photocatalytic, ozone and Fenton oxidations are applicable for degradation of organic compounds, such as xanthate that is the most widely used collector for sulfide minerals (Yuan et al., 2023), and also for effective decrease of microbial load. The removal of inorganic compounds impeding flotation demand different treatments. Sulfur species are abundant in sulfide ore flotation. The effects of high concentrations of sulfate, elemental sulfur, thiosulfate and sulfide for different ore flotation can be either positive, negative or negligible (Castellón et al., 2022) emphasizing the need to optimize the water quality flexibly depending on the process.

Ion exchange (IX) has been used in mining industry to remove anions such as sulfate (Feng et al., 2000) and to improve the selective metals recovery (e.g. nickel, copper and cobalt) from process solutions and mining tailings (Botelho Junior et al., 2019). IX is based on exchanging ions (anions or cations) between electrolyte solution (aqueous phase) and resin (solid phase) (Botelho Junior et al., 2019). Microbial cells behave as large ions and thus can adsorb to the ion exchange resins. For example, ion exchange resins have been used as carrier material for iron-oxidizing microorganisms in packed-bed reactors (Grishin and Tuovinen, 1998). Ion exchangers may also be negatively affected by biofouling i.e. by the undesired deposition of microorganisms on surfaces (Flemmings, 2020). Biofilms that have been formed on the surface of ion exchange material have been shown to decrease the ion exchange rate, but not the capacity (Lahay and Green, 1989).

This study focuses on the effect of indigenous microorganisms, enriched from the actual process, on the flotation performance in the Kevitsa plant. In addition, the applicability of ion exchange for the removal of dissolved sulfur species and microorganisms from process water is evaluated. Since water recycling will accumulate microorganisms to process water, novel knowledge on microbial effects on flotation performance is needed. In literature, information is lacking on how the water quality can be maintained in the recycled process water in flotation, and on how the microbial numbers and community in flotation are affected by ion exchange especially in continuous systems. Therefore, the objective is to answer the following questions: (1) Which microorganisms are present in Kevitsa process waters and at what quantities? (2) How the accumulation of indigenous microorganisms affects the flotation performance? (3) Is there a difference in flotation performance between process water and recycled final tailings water? (4) How does ion exchange function in the removal of accumulated dissolved sulfur species and microorganisms from the recycled water? The results are part of the H2020 ITERAMS project (Grant agreement# 730480), which contributes to closing the water loops and minimizing the environmental impacts in mining.

2. Materials and methods

2.1. Flotation experiments

All flotation experiments were carried out at the Bolden Kevitsa mine in Sodankylä, Finland. The flotation tests were conducted to investigate (1) effects of high load of microorganisms on flotation performance and (2) water treatment by IX and DAF. In the microbial tests, two water types were used, process water (PW) that feeds the plant and water from the final tailings (FT). Flotation tests with and without added microorganisms were performed in duplicate in order to analyze the reproducibility. The flotation tests were conducted according to the Bolden Kevitsa laboratory’s standard selective rougher flotation flow sheet and reagents dosage for both Cu minerals (chalcopyrite and cubanite) and Ni mineral (pentlandite) (Table 1). Both rougher Cu and Ni flotations were performed at two stages. The same reagents were used in the flotation experiments as are used in the concentrator plant. Aerophine (3418 A) and xanthate (SIPX) were used as collectors in Cu rougher and Ni rougher flotation stages, respectively. Carboxymethyl cellulose (CMC) was used to depress the naturally floatable gange minerals and Nasfroth was used as frother throughout the circuit. The flotation tests were conducted at a 2 L cell for the microbial tests in an automatic flotation machine (Outotec, Finland) with a solid density of 38% and temperature around 20°C. Temperature, pH, oxidation reduction potential (ORP) with Ag/AgCl reference electrode, specific conductivity (SPC) and dissolved oxygen (DO %) were measured directly in the slurry with the YSI ProDSS multiparameter probe. The sensors were checked before each round of tests and calibrated when needed.

Samples were taken for microbial, chemical and mineralogical analyses from the flotation experiments. Microbial samples were taken from the PW and FT just before the start of flotation experiments by pouring 900 mL into one-liter sterile plastic bottles. Waters with the added microorganisms before the experiments (10 mL) and all the microbial samples at the end of flotation (25 mL) were taken with a single-use sterile syringe into 50 mL sterile tubes and frozen at −20°C before analysis. The flotation products, concentrates and tailings, were dried and splitted on site and analyzed at Labtium Eurofins in Sodankylä. In total 14 elements and compounds were analyzed. S was analyzed with combustion IR technique by an Eltra elemental analyzer. The elemental compositions (K, Ca, Mg, Na, Si, P, Co, Cu, Ni and Fe) of the waters before and after flotation tests were analyzed with ICP-OES, while the anions (SO4²⁻, Cl⁻, SO3²⁻) with ion chromatography.

The second group of flotation tests during IX experiments were conducted using the PW, Pond water and Water reservoir water with and without IX treatment as shown in Table 2. One additional flotation test was also conducted using Water reservoir sample treated with a combination of DAF and IX system. Flotations were performed similarly as in case of microbial flotations but in a 5 L cell. Subsamples from all the water samples were taken to demonstrate the effects of water treatment on removing microorganisms and the flotation performance.
2.2. Water treatment by ion exchange resin and dissolved air flotation

The removal of dissolved sulfur species and microorganisms was determined with a large-scale ion exchange column (Fig. 1) at Kevitsa. The columns were filled with approximately 1.5 kg Selion resin (a gel type, strong, basic, Type II anion resin). The resins were regenerated with 15L/h NaOH (2 %). The tests performed using ion exchange experiments were as described in Table 2. In order to determine the effects of water treatment on the flotation performance, water samples were used without treatment and with different levels of water treatment. The water samples were taken at certain time intervals from the clean water bins. For example, the water sample of 15–30 min represents the bulk water sample collected in a bin from the 15th minute of the water treatment to 30th minute. This was considered as a more accurate approach to collect representative water samples compared to the snapshot sampling approach. The reproducibility of the IX tests was tested using the PW (30 L/h) in the beginning of the test program. The tests had very high reproducibility as shown with the SO_{4} snapshot sampling approach. The reproducibility of the IX tests was achieved by using representative water samples compared to the snapshot sampling approach.

Water samples after IX were taken to analyse the changes in sulfur chemistry and in the size of microbial populations before and after IX treatments. Triplicate samples from different treatments (from 60 mL to 300 mL) were filtered on 0.22 µm pore-size Sterivex™ polyethersulfone (PES) filter units using sterile 100 mL syringes (KD-JET III, KDM). Filter units were placed in sterile 50 mL plastic test tubes equipped with screw caps (BD Falcon). Samples from ion exchange column resin materials (50 mL) were taken from the top of the columns for microbial analysis after completion of the adsorption stage and were kept frozen at −20 °C before analysis.

2.3. Enrichment of microorganisms for flotation experiment

Microbial samples for enrichment were obtained from Kevitsa mine waters on site in August 2017 (process water PW, final tailings FT, nickel thickener overflow NiTo) (Bomberg et al., 2020) and February 2018 (PW, NiTo, Cu thickener overflow CuTo) (Bomberg et al., 2023), into 1 L sterile plastic bottles (Nalgene). Samples were chilled to 4 °C and shipped in a cooling box to Espoo, Finland. Two parallel enrichment cultures were established immediately after arrival for each sample and culture medium, containing 9 mL of water sample amended with 1 mL of sterile 0.05 % (w/v) yeast extract and 0.15 % (w/v) tryptic soy broth (Ye + TSB) or 0.03 % (w/v) R2A or 20 mM NH_{4}HCO_{3} and 10 mM FeSO_{4}·7H_{2}O or left without iron addition. In addition, DSMZ medium 486 (DSMZ, 2022) containing thiosulfate and bromocresol purple as indicator for thiosulfate oxidation, was used at 1/10 strength by adding 1 mL of medium to 9 mL sample water. Cultures were incubated for 7 days at 25 °C with 140 rpm, protected from light. After the growth period, several 1.0 mL aliquots were frozen with glycerol (15 %) at −80 °C.

To enrich the microorganisms further, culture media based on a mixture of three filtered (0.45 µm Corning) sample waters (PW, FT, NiTo) from the Kevitsa minerals processing plant were used as base for the culture media, to which the carbon sources and ferrous iron were added as described above before inoculation with 1 mL of pre-enriched
incubation for 6 days at 25 °C with 140 rpm, protected from light, all samples grown in the same culture media were combined and enrichments were continued with fresh enrichment media (25 mL of combined enrichment with 50 mL of new medium). In addition, 1 g Kevitsa ore, which had been ground and immediately vacuum packed and stored in a freezer at −20 °C, was added to all media, except to Medium 486. Enrichment cultures were refreshed four times (1:10) every second or third week, whereafter they were transferred to larger culture volumes (300 or 520 mL) and incubated for 10 days. Culture liquids from all seven enrichment culture types, a total of 1 L of each medium type, were harvested by centrifugation in sterile centrifugation tubes for 30 min at 3000g (Heraeus Multifuge X3 FR, Thermo Scientific). Supernatants were moved to new sterile centrifuge tubes and centrifuged for an hour at 3000g. All pelleted mass was combined and washed with sterile distilled water three times with the same volume of water that was removed after centrifugation in between washings (60 min, 3000g). The final deposit was dissolved and mixed in sterile distilled water and two 0.3 g aliquots were frozen for further DNA analyses. The rest of the biomass was divided into four equal lots (3.3 g wet weight) in sterile 50 mL falcon tubes for freeze drying (Christ Alpha 2–4, B.Braun Biotech International) for three days before storing dry at room temperature until the use in the flotation experiments. Microbial cultures were re-activated for 20 h before the use in flotation experiments by mixing 40 mL of distilled sterile water into the biomass tubes and incubated at room temperature for 19 h covered from light. One hour before the use, the microorganisms were mixed with the rest of the of the water used in the flotation experiment in a cleaned and dried container. 2.4. Live/dead staining and microscopy

Freeze dried biomass was moistened thoroughly with sterile filtered (0.22 μm pore-size) Kevitsa mine process water and incubated for one or 16 h. Microbial cells were then stained with LIVE/DEAD® BacLight™ Bacterial Viability (Thermo Fisher Scientific) stain for 15 min at room temperature in dark according to the manufacturer protocol (3 μL of mixture of equal volumes of both dyes, SYTO 9 and propidium iodide, for 1 mL of microbial suspension). Stained cells were examined on microscopy slides using an Axio Imager M2 epifluorescence microscope (Carl Zeiss Microscopy) equipped with a digital camera (AxioCam MRm, Carl Zeiss). The share of green (active) and red (inactive) cells was estimated.

2.5. DNA extraction

Two DNA extraction protocols for flotation experiment samples were used as part of the samples were challenging due to the high mineral and particle amount. NucleoSpin Soil DNA extraction kit (Macherey-Nagel, Germany) using lysis buffer SL1 with Enricher solution SX according to the manufacturer’s instructions was used for the enriched biomass before freeze drying and for the PW and FT samples before flotation with microbial additions (2 mL). These samples were first centrifuged in a table-top Eppendorf 5417R microcentrifuge at 16 000g for 2 h, at 4 °C and most of the supernatant was removed from the tubes before DNA extraction with the kit.

The same protocol was used also for the ion exchange filter and resin samples. However, first, the filter cylinders in sterile plastic bags were opened by smashing with a hammer whereafter the filters were cut from the cylinders with sterile scalpels in a laminar flow hood and placed in sterile 5 mL centrifugation tubes containing the beads from one bead tube of the DNA extraction kit together with two-fold reagent volumes. Triplicate 1 g resin samples were directly transferred to 5 mL centrifugation tubes together with the beads and reagents as described for the filters. In order to release the microorganisms from the filters and resins the tubes were vortexed for 5 min horizontally with the lysis buffer SL1 and enhancer solution SX. The tubes were centrifuged in an Eppendorf 5810R benchtop at 3184g for 5 min, after which the supernatant continued in the DNA extraction according to the manufacturer’s protocol.

Another DNA extraction protocol was used for microbial flotation experiment PW and FT samples without added microbes before flotation and all samples after the microbial flotation experiments as described in Bomberg and Miettinen (2023). Frozen samples were first thawed at 4 °C, mixed well and triplicate 17 mL aliquots were further used for DNA extraction.

2.6. Estimation of microbial community size with quantitative PCR

The number of bacterial 16S rRNA and fungal 5.8S rRNA genes was estimated with quantitative PCR (qPCR) and used as a proxy for biomass in the mineral processing water samples and enrichment cultures. The bacterial and fungal community sizes were detected according to Miettinen et al. (2021). All bacterial and fungal amplifications were performed in triplicate in the LightCycler 480 instrument (Roche Applied Science). The bacterial and fungal amplification results were compared to that of a plasmid standard dilution series containing the 16S rRNA gene insert of Escherichia coli or the 5.8S rRNA gene of Aspergillus versicolor for fungi.

2.7. Amplicon library preparation, sequencing and sequence analysis

The bacterial, archaeal and fungal communities were characterized using amplicon sequencing of three bacterial and archaeal 16S RNA genes and the fungal ITS1 region. The bacteria and archaea were targeted using the Bact_0341F/Bact_805R (Herlemann et al., 2011) and S-D-Arch-0349-a-S-17/S-D-Arch-0787-a-A-20 (Klindworth et al., 2013) primers, respectively. The fungal communities were targeted using the primer pair ITS1 and ITS2 (Gardes and Bruns, 1993; White et al., 1990). The amplicon library preparation and sequencing was performed similarly as in Bomberg and Miettinen (2023).

The sequence analysis was done using the DADA2 (Callahan et al., 2016) pipeline in R (R Core Team, 2021) adapted for single reads. First, adapters and primers were removed from the sequence reads and shorter than 200 nt long reads were removed using cutadapt version 3.4 (Martin, 2011). Using the DADA2 workflow, the sequence reads were quality filtered (maxEE = 2, maxN = 0, truncQ = 2, minLen = 200), sequencing errors and chimeric sequences removed, and sequence reads grouped into ASVs. Taxonomical classification of the ASVs was performed using DECIPHER (Wright et al., 2012) in R against the Silva nr99 v 138.1 training set (Quast et al., 2012; Yilmaz et al., 2014) for bacteria and archaea and the UNITE sh_general_release_dynamic_10.05.2021 database (Kölgalj et al., 2013; Nilsson et al., 2019; UNITE Community, 2017) for fungal ITS1 sequences. The sequences have been submitted to the European Nucleotide Archive (ENA) at European Molecular Biology Laboratory - European Bioinformatics Institute (EMBL-EBI) under accession number PRJEB664423 (https://www.ebi.ac.uk/ena).

Alpha- and beta-diversity calculations were performed with Phyloseq (McMurdie and Holmes, 2013) in R (Computing, R, 2013), excluding singleton ASVs. The PcoA and balloonplots were visualized in R using ggpubr (Kassambara and Kassambara, 2020), ggplot2 (Villanueva and Chen, 2019), RcolorBrewer (Neuwirth and Neuwirth, 2014), viridis (Garnier et al., 2021) and tidyverse (Wickham et al., 2019).

3. Results

3.1. Effect of added microorganisms on the solution chemistry

Table 3 shows the effect of added microorganisms on the PW and FT waters in terms of physico-chemical parameters. The presence of added
microorganisms decreased redox potential both in PW and FT around 30 to 40 mV. However, as the level of DO was not reduced, the drop at redox potential was not due to a consumption of oxygen but likely an effect of the microbial surfaces or/and some compounds that they secreted. In addition, conductivity was increased due to microbial addition. Reproducibility of the experiments was quite good as the duplicate experiments showed similar trends, except for the DO measurement with PW with added microorganisms that showed the highest and the lowest result of all measurements, likely indicating some problem in either measurement.

In order to ensure that the addition of microorganisms did not change the water matrix, both water with and without microorganism addition were sent to analysis (Table 4). Overall, the addition of microorganisms did not affect the water matrix in terms of major compounds/elements. However, the addition of microorganisms increased the amount of P from 0.06 up to 0.12 mg L⁻¹ in the PW before flotation. This shows that the phosphorus from the cultures was detectable. In the case of FT water before flotation, there was an increase in concentration of metals (e.g. Co, Cu, Ni) and thiosulfate. Results from the duplicate experiments showed high reproducibility of the analysis.

### 3.2. Effect of microorganisms on the Cu and Ni grade and concentrate recovery

The addition of microorganisms in FT water did not have any clear effect on the recovery and grade of Cu all four experiments showing quite similar curves (Fig. 2 B). However, the addition of microorganisms in PW decreased significantly the recovery of Cu in the Cu concentrates (Fig. 2 A). The decrease in the recovery with microorganisms producing an equal Cu grade with the water without microorganisms could be as high as approximately 25 percentage points. However, the Cu grade slightly increased. The replicate experiments with or without added microorganisms showed the same trends even though with the added microorganisms the curves had clear difference.

The presence of added microorganisms in both PW and FT affected the Ni concentrate both in terms of recovery and grade (Fig. 2 C,D). The replicate tests showed similar trends. In the presence of added microorganisms, the recovery of Ni in the Cu concentrate was much lower than without added microorganisms. This results in a higher value product, since Ni in the Cu concentrates results in a penalty for the mine.

Table 3 summarizes the recovery of Cu and Ni concentrates and their distribution in the produced concentrates. The high microbial load had a major effect on metals recovery in the concentration stage, particularly for Ni. Addition of microorganisms in PW decreased the recovery of Cu in the Cu concentrate, while it did not have as clear effect in the FT medium. The increased amount of microorganisms in PW and FT decreased notably the recovery of Ni in the Cu concentrate by 37 and 18 percentage points, respectively. At the same time, the amount of Ni recovered in the Ni concentrate increased by 33 and 18 percentage points, respectively. At the same time the mass pull was lower in the Cu flotation in case of added microorganisms but slightly higher in the Ni flotation. Reproducibility of the replicate experiments based on the recovery results was good as the deviations were lower than the difference in the results between compared experiments. The same was true in case of mass pull in Cu flotation but in Ni flotation the deviations were bigger than the differences in the mass pull results.

The water recoveries in the first nickel scraping phase with added microorganisms both with PW and FT experiments were higher than without microorganisms by 5.8 and 4.3 percentage points, respectively. In other flotation phases the differences between experiments with or without microorganisms were minor. The presence of added microorganisms appeared to stabilize the froth in the nickel flotation which was also confirmed by the froth height during the flotation visually (Fig. 4). The froth height in the presence of added microorganisms was two to three times larger than without added microorganisms. The froth height was increased in the conditions with added microorganisms, the recovery of Ni in the Cu concentrate was much lower than without added microorganisms. This results in a higher value product, since Ni in the Cu concentrates results in a penalty for the mine.

### Table 4

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Under detection limit (DL).
three times higher than without added microorganisms especially in case of Ni-flotation.

3.3. Microorganisms in flotation experiments

The enriched biomass before freeze drying was analyzed for bacterial 16S rRNA and fungal 5.8S rRNA genes and based on these results it was estimated that around $10^7$ microbial cells mL$^{-1}$, mainly bacteria, were added to the PW and FT waters that were used for the flotation experiments. Based on live-dead staining, the share of active cells was small after one hour, but already around 75–80% after 16 h. Therefore, the majority of the added microorganisms were expected to be viable, as they were activated for 20 h in water before addition to the flotation experiments. The main bacterial genera of the additions were *Burkholderia-Caballeronia-Paraburkholderia* and unclassified Burkholderiaceae and the fungal groups consisted mainly of unclassified Fungi and the genus *Cadophora* (Fig. 5).

The detected initial bacterial gene copy count in the original PW was
Based on the gene copy counts of the enriched biomass it was estimated that the microbial additions to the PW and FT were $1.0 \times 10^7$ cells mL$^{-1}$. The actual detected bacterial gene copy counts in samples with added microorganisms were $3.8 \times 10^7$ and $2.2 \times 10^7$ genes mL$^{-1}$ in PW and in FT water samples, respectively. This equals one to one and a half order of magnitude higher bacterial counts in microorganisms added (M) samples before flotation. When the same waters were studied after the flotation, the bacterial counts were increased especially in samples with added microorganisms. The increase was clear but smaller (less than half an order of magnitude) in samples without added microorganisms and up to one order of magnitude in samples with microorganisms added.

In the case of fungal gene copy counts the results varied more than with the bacteria (Fig. 6). The fungal counts were in general lower, from only a few hundreds in a mL in samples without added microorganisms after the flotation, and up to $4.8 \times 10^4$ 5.8S rRNA genes mL$^{-1}$ in PW. The addition of microorganisms increased the fungal gene copy count by around one order of a magnitude. The flotation process had significant decreasing effects on the fungal counts in waters without added microorganisms (up to two orders of magnitude). In samples with added microorganisms the effect was lesser, the fungal gene copy counts being at the same level or slightly lower (less than half an order of magnitude).

The bacterial communities in the enrichment cultures, PW and FT to which enrichment culture had been added, both before and after the flotation experiment, were all similar according to principal coordinate analysis and clustered tightly to the left of the PCoA-plot (Fig. 7). The original FT and PW samples differed from each other and from the samples containing enrichment culture, forming their own distinct clusters. There was no great difference in the bacterial community composition between the different DNA extraction methods within the same sample type. The main bacterial genera (Fig. 6) found with higher relative abundance in the original PW than in FT were *Polaromonas* before flotation and *Thiobacillus* after flotation. In FT *Pseudomonas* was the most dominant family and unclassified Microbacteriaceae dominated after flotation. The PW and FT with added microorganisms both before and after flotation were dominated with the same genera as were found from the enrichment cultures i.e. *Burkholderia-Caballeronia-Paraburkholderia* and to lesser extent unclassified Burkholderiaceae. No archaeal communities were detected in the enriched biomass or in any of the flotation samples.

The fungal community in the enrichment cultures was separated into two clusters, with Enrichment 2 and 3 falling close together in the upper left corner of the plot and Enrichment 1 falling to the far right of the plot together with the flotation samples to which microorganisms had been added (Fig. 7). The original PW and FT waters after flotation with no added microorganisms fell mostly into one cluster to the lower left of the plot. The fungal community of the original PW was similar to that of Enrichment 2 and 3, dominated by unclassified fungi, but differed from the PW after flotation with higher fungal diversity. Without added microorganisms, the fungal community composition of the FT and PW samples after flotation became very similar, as was the case also when microorganisms were added to these samples and *Cadophora* genus was the dominant fungal group (Fig. 9).
Fig. 6. The number of bacterial 16S rRNA and fungal 5.8S rRNA genes in process water and final tailings water with or without added microorganisms before (BF) and after (AF) flotation in duplicate flotation experiments (1, 2) according to quantitative PCR.

Fig. 7. Principal coordinates analysis of the bacterial and fungal communities of microbial additions (enrichment) and tested process water (PW) and final tailings (FT) samples before and after flotations with or without microbial additions characterized by iSeq amplicon sequencing.
Fig. 8. The relative abundance of bacterial genera identified in the different samples. The samples are presented on the left of the graph. All bacterial genera present at relative abundances of less than 1 % in all samples were grouped into the category Other, <1 %. BF before flotation, AF after flotation, 1,2 duplicate flotation experiments.
3.4. Removal of dissolved sulfur species and microorganisms with ion exchange

Ion exchange resins effectively removed sulfates (Fig. 10), thiosulfates and polythionates from process waters of Kevitsa. DAF is generally used for removal of colloidal size particles from water. Pre-treatment with DAF was applied on the Water reservoir sample. The IX treatment could be done only for 120 min due to insufficient amount of water obtained from the DAF process.

The thiosulfate concentration was around 20–30 mg/L in the water samples and its concentration decreased below the detection limit (<5 mg/L) after resin treatment. The water treatment tests were conducted at different water flowrates to determine the kinetics of ion adsorption. There were no sulfate ions detected in the PW at 30 L/h even after 120...
min. The higher water flowrate (40 L/h) left more sulfate ions in water and after 90–120 min treatment low concentration of sulfate ions was detected (160 mg/L) in PW. This was totally as a result of the balance min. The higher water flowrate (40 L/h) left more sulfate ions in water and after 90–120 min treatment low concentration of sulfate ions was detected (160 mg/L) in PW. This was totally as a result of the balance between the resin capacity (amount of resin used in the columns) and the amount of sulfate ions fed to the water treatment system as a function of treatment time. The flowrate of water had a significant effect on the performance of adsorption. Regeneration of resins with 2 % NaOH was successful and the resin after regeneration could be used for water treatment. The details about the use of IX for water treatment are discussed in detail elsewhere (Oztürk and Ekmekçi, 2020; Can et al., 2020).

The sulfate content of the three water samples was similar at around 600 mg/L. The best sulfate removal performance was observed with the PW and the lowest performance with the water from the Water reservoir in spite of pre-treatment with DAF. There was high flow resistance through the resin columns with water reservoir sample, which probably was the cause for lower cleaning efficiency. The chemical analysis of the three water samples were similar and it was not possible to clearly identify the reason(s) for flow resistance. Some sort of gel formation on the resins was observed during the tests and it is possible that there were microorganisms present that were able to produce substances that clog and slime whereas in other studied water types this did not happen at least to this extent. DAF pre-treatment did not improve the efficiency of the IX treatment in case of sulfur removal and it can be regarded as a duplicate for IX in case on sulfur species treatment.

Ion exchange resin was also efficient in decreasing the bacterial gene copy counts (Fig. 11), as the effect in all IX-experiments was over two orders of magnitude in three different process water streams. The use time of the resin had some effect on its performance, but after 175 min the resin still removed two orders of magnitude of the bacteria according to the 16S rRNA gene copy counts in Pond water. The greatest decrease in bacterial gene copy counts was obtained with the successive treatment first with DAF as a pretreatment followed by ion exchange resin treatment, where a decrease in the bacterial load of three orders of magnitude was obtained. Resins were studied after the use (2 to 3 h) and they contained from $3 \times 10^5$ up to $10^8$ bacterial 16S rRNA gene copies per g of resin that was significantly (One-way ANOVA analyses Tukey’s pairwise, $p < 0.05$ after Bonferroni correction) more than could be found from the unused resin ($3 \times 10^3$ copies g$^{-1}$). Clearly the highest bacterial 16S rRNA gene copy numbers were detected from the used resin of Water reservoir water even though the bacterial gene copy numbers were at the same level in all untreated waters. This can be related to the clogging that was detected in DAF treatment of Water reservoir water. Ion exchange resins used for cleaning of different water streams removed microorganisms and acted as a carrier material for the microorganisms.

3.5. Effects of ion exchange water treatment on flotation performance

Rougher kinetic flotation tests were conducted using the untreated water and IX treated water samples. The tests with untreated water were performed in duplicate and the average values with standard deviation are presented in Fig. 12. The copper concentrate recovery with untreated PW was higher than that obtained with Water reservoir and Pond water. Pond water treatment by IX after 30 min did not significantly affect the copper concentrate recovery, but improved the Cu/Ni selectivity. This was attributed to the lower mass pull without affecting the copper concentrate recovery. It is likely that the nickel concentrate recovery by entrainment was reduced by water treatment. The mass pull and recoveries of copper and nickel concentrates in Cu-flotation were lower with waters after 120 min treatment or DAF-IX treatment compared to untreated waters. However, better nickel depression in the copper flotation stage resulted in higher recovery of nickel concentrate in nickel flotation stage that followed the copper flotation. Fig. 12(B) shows the mass pull and recoveries of copper and nickel concentrates in the nickel flotation stage. The recoveries were directly affected by the performance of the copper flotation. The highest nickel concentrate recovery was obtained with the test performed using Water reservoir treated by DAF and IX.

The sulfate ion concentration of the water samples was in the low range for a typical sulfide flotation operation (Fig. 10). Water treatment by IX removed almost all of the sulfates and significant part of microorganisms in the first stage of the treatment. However, it must be noted that pH of the water after IX treatment increased up to 11 due to the ion exchange reaction. Given that the pulp pH in flotation was higher than in the plant operation, the high pH might affect the flotation performance more than the removal of the dissolved ions or microorganisms and improve the selectivity.
In this study, we show that indigenous mixed microorganisms enriched from the same mineral processing plant water streams affect both the yield and the grade of metal concentrates in flotation applications, which is of increasing importance when the demand for process water recycling increases and mines strive to close the water loops. Our batch flotation tests showed that higher microbial load in PW and FT had

Fig. 11. Number of bacterial 16S rRNA gene copies mL$^{-1}$ water or g$^{-1}$ resin in Process water, Water reservoir, Pond water and resin samples before and after ion exchange resin treatment. Dissolved air flotation (DAF) pretreatment was performed for one Water reservoir sample. Gene copy numbers were determined by qPCR from the DNA extracted from three replicate samples run in triplicate reaction.

Fig. 12. Copper and nickel concentrate recoveries and mass pull in the copper (A) and nickel (B) flotation stages and Cu and Ni grades in Cu (C) and Ni (D) concentrates.

4. Discussion
In this study, we show that indigenous mixed microorganisms enriched from the same mineral processing plant water streams affect both the yield and the grade of metal concentrates in flotation applications, which is of increasing importance when the demand for process water recycling increases and mines strive to close the water loops. Our batch flotation tests showed that higher microbial load in PW and FT had
a positive effect on the flotation selectivity of Kevitsa ore, as well as a slight positive effect on the grade. The added microorganisms in the starting water slightly modified the water matrix of the PW but had no effect on the FT matrix. As the effect of the high microbial load in both the FT and PW on the flotation performance appeared to be mostly similar in all four experiments with added microorganisms, the changes in flotation performance were due to the increased amount of microorganisms reproducibly. The amount of microorganism increased during flotation regardless of the initial amount of microorganisms (Fig. 6) indicating that the conditions during flotation favour microbial growth.

The added microorganisms changed both the composition of the slurry and the froth phase in the flotation tests. The amount of recoverable metals increased in the slurry during the flotation with added microorganisms, which could be a consequence of an increase of oxidation from the ore due to microbial activities, release of substances from the microorganisms or the change of microbe-mineral particle behaviour in flotation. The change in the froth could be explained by the added microorganisms and/or due to the different water matrices used. However, the latter is not likely the main reason as the changes in water matrix were only seen as changes in the concentration of minor elements. Microorganisms could act like a surfactant due to the cell surface itself or due to its secreted products. Interestingly, most of the bacterial groups present at elevated relative abundances in the flotation experiments with added microorganisms (Burkholderiaceae, Xanthomonadaceae, Acinetobacter, Brevundimonas, Fig. 5) possess the ability to produce biosurfactants (Abdel-Mawgoud et al., 2010; Nayak et al., 2009; Mujumdar et al., 2019; Ray et al., 2021). Some of the biosurfactants produced by microorganisms are known to remove metals from soil due to their anionic nature and complexation ability (Herman et al., 1995; Elouzi et al., 2012) and bacterial cells and microbial metabolites such as biosurfactants, extracellular polymeric substances (EPS) and nucleic acids have been used as surface modifiers and collectors in mineral flotation processes (Behera and Mulaba-Bafubiandi, 2017). It is logical that the added microorganisms were already adapted to the mineral processing environment from which they originated and were prepared to respond readily to the flotation conditions likely inducing metabolic activity involved in surfactant or EPS synthesis. Microorganisms produce these compounds to achieve e.g., better bioavailability of prospective nutrients or to precipitate toxic compounds such as heavy metals. The presence of increased amount of microorganisms and/or their metabolism stabilized the froth, especially in the case of Ni flotation (Fig. 9), and increased selectivity and the mass pull. The Cu circuit was less affected by the increased number of microorganisms compared to the Ni circuit. The total recovery of Cu remained constant. However the Ni amount in Cu concentrate was reduced when high microbial load was present. Possibly the microbial attachment to minerals or major metabolism affected the flotation selectivity but the exact phenomenon needs further research. Microbial attachment to mineral surfaces is a strain and mineral specific feature as selective but the exact phenomenon needs further research. Microbial attachment to minerals or major metabolism affected the flotation performance both positively and negatively. However, the added dominant microbial genera originally enriched from the same environment, were different from those naturally dominant in the PW and FT at the time, even though present in these samples at low relative abundances (Fig. 5, Fig. 6). The added microorganisms were able to dominate and multiply in the PW and FT also after flotation implying their competitiveness and adaptation to this environment that would not likely be the case when the added microorganisms would not be from the same environment (Hosokawa et al., 2009). The successful multiplication of these bacteria originating from the same process opens up the possibility to utilize the so called bioaugmentation procedure i.e. addition of pre-grown microorganisms to enhance certain microbial process such as degradation of spilled oil in water/soil or degradation of recalcitrant compounds in wastewater treatment (reviewed by Rahmati et al., 2022; Niza et al., 2016). Consequently, it would be highly interesting to study the addition of autochthonous flotation process bacteria optimizing their numbers and characteristics, ultimately aiming to manage the flotation process more efficiently and in detail to utilize/diminish the impact of microorganisms.

When PW is recycled back to the flotation process, part of dissolved sulfur compounds typically need to be removed. The removal of microorganisms may also be necessary, when the microbial effects are negative on process performance. Ion exchange enabled the removal of dissolved sulfide compounds, such as sulfate, but also microorganisms. In addition, the use of DAF as a pretreatment for ion exchange was successful to remove the colloidal size particles and to avoid contamination and blocking of the resin. However, the Kevitsa ore is a low grade Cu-Ni sulfide ore (Musuku et al., 2016) and contains only a small amount of sulfide minerals that may release sulfur ions to the pulp. Besides, the reagent scheme is not aggressive i.e. no use of sodium metabisulfite or thioulate, and does not significantly increase the dissolution of sulfur species into the process water so it is likely that the impact of water chemistry on flotation performance may not be as significant as in the flotation of massive sulfide ores with high pyrite contents. However, in Kevitsa seasonal variations in recycled water and accumulation of compounds impacts flotation performance (Muzinda and Schreithofer, 2018), as well as indications of seasonality of microbial numbers (Bomberg et al., 2020; Bomberg et al., 2023) has been shown. Therefore IX treatment, which targets specific ions and microorganisms in the process water, could be an effective solution for water treatment and stabilization the flotation performance.

With ion exchange, high concentrations of $SO_4^{2-}$ in regeneration solutions should be removed for example by precipitation, so that regeneration solution can be re-used again in the system. The water treatment with ion exchange resins is based on exchange of hydroxyl ions from the resin and dissolved sulfide species and bacteria from the solution. Hence, the $pH$ of the clean water increased to about $pH$ 11, which could significantly affect flotation behaviour of the sulfide minerals in Kevitsa ore and mask to some extent the influence of removing the microorganisms from the process water. That brings about the importance of developing a mine-site water management model specific for each mine site. The waters from the treatment plant and different section of the mine site could be mixed to produce the process water with the best specifications and at low cost.

Furthermore, only chemical and physical factors have been monitored in flotation water treatment, and information about microorganisms and their removal efficiency has been lacking. Based on the microbial results, ion exchange functioned effectively for physico-chemical water cleaning and also efficiently removed microorganisms from mineral processing waters. The range of reduction in bacterial gene copy counts was steadily over two orders of magnitude, which is a considerable decrease. At most, bacterial target gene numbers decreased from $7 \times 10^6$ down to $1 \times 10^5$ copies mL$^{-1}$, which is a significant decrease (>99%).
Despite the efforts to remove microorganisms, some still remain in the water. The experiments were performed in various process conditions and for three water types aiming to improve the cleaning effect regarding the physico-chemical parameters and not focusing on the removal of microorganisms. Optimization of water cleaning techniques taking into account also microbiological aspects could be used to produce the best water quality suitable for each application and mineral processing plant. Water treatment pilot was operated in continuous mode to evaluate the performance with process disturbances. Continuous pilots demonstrated that the ion exchange water treatment technologies performed well also in the conditions where feed water quality varied in the actual industrial application.

5. Conclusion

Increasing the indigenous microbial load in the flotation from $10^6$ to $10^7$ 16S rRNA gene copies mL$^{-1}$ with microorganisms originating from the process itself had a positive effect on the flotation selectivity of Kevitsa ore especially in Ni flotation in the batch tests. The froth layer height was visually increased and stabilized in the Ni flotation in experiments with added microorganisms. The two tested water types, behaved slightly differently. In the Cu flotation phase added microorganisms did not affect the Cu concentrate recovery of final tailings water but decreased significantly the recovery of Cu concentrate in process water. With equal Cu grade the recovery was up to 25 percentage points lower when comparing extremities of individual experiments. However, added microorganisms in both water types decreased notably the recovery of Ni in Cu concentrate (18 to 37 % points) in average. At the same time the amount of Ni recovered in the Ni concentrate increased by 18 to 33 percentage points with added microorganisms in average. All these changes were likely related to the addition of microorganisms and not to the water matrix change as the addition of microorganisms did not change the major compounds of the water matrix, and had only minor effects on the phosphorus and metal concentrations. Ion exchange treatment proved to be effective in removing both sulfur compounds and simultaneously microorganisms. The range of reduction in bacterial gene copy counts was over two orders of magnitude. DAF was a successful pretreatment strategy for ion exchange and assisted in removal of more microorganisms and colloidal particles. When microorganisms have a negative effect on the process performance, the removal of microorganisms is desired. However, if the accumulation of microorganisms enhances the flotation performance, their removal is undesired. The impacts of microorganisms on the process performance and the need to remove them thus depend on the case. However, microorganisms are not usually taken into consideration when water cleaning techniques are designed and optimization could result in even better outcome for water quality of each application and plant. For future studies different fields of expertise in minerals processing such as chemistry, analytics, process control, metallurgy, mineralogy and microbiology would benefit of cooperation between all parties. E.g. microbiological activity is directly connected to chemical and process control parameters which again are affected by microbial activity such as degradation of chemicals, oxidation of metals or slime formation. Measuring and finding the connections between multitude of parameters and disciplines would help in understanding minerals processing and result in focusing on the main parameters to most effectively utilize and control processes. Inclusion of microbiological analysis may explain previously conflicting and unclear situations in flotation performance. A starting point for a future study could be to find out which microorganisms have the most effect (either positive or negative) on flotation and their effective doses. Understanding the actual mechanisms how microorganisms affect the process is the base for managing microorganisms in flotation.

- High microbial load in copper flotation phase decreased the Cu concentrate recovery by 13 % points in average with process water but with the final tailings water the decrease was only 4 % points.
- High microbial load in copper flotation phase decreased the Ni recovery in Cu concentrate with 37 % points in process water and 18 % points in final tailings waters in average.
- High microbial load in the nickel flotation phase increased the Ni concentrate recovery in process and final tailings waters with 33 and 18 % points, respectively.
- Ion exchange treatment efficiently removed sulfur species and simultaneously microorganisms from real variable flotation plant water. When the microorganisms have a negative effect on flotation performance, ion exchange would be one alternative for the microbial control.

CRediT authorship contribution statement

Hanna Miettinen: Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing, Visualization. Malin Bomberg: Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Visualization. Özlem Bıçak: Conceptualization, Methodology, Investigation, Writing – review & editing. Zafir Ekmeçki: Conceptualization, Methodology, Writing – review & editing. Paivi Kinnunen: Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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