Using yeast to sustainably remediate and extract heavy metals from wastewaters

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ABSTRACT

Our demand for electronic goods and fossil fuels have challenged our ecosystem with contaminating amounts of heavy metals causing numerous water sources to become polluted. To counter heavy metal waste industry has relied on a family of physicochemical processes with chemical precipitation being one of the most commonly used. However, the disadvantages of chemical precipitation are vast, some of which are the generation of secondary waste, technical handling of chemicals, and need for complex infrastructures. To circumvent these limitations, biological processes have been sought after to naturally manage waste. Here, we show that yeast can act as a biological alternative to traditional chemical precipitation by controlling naturally occurring production of hydrogen sulfide (H_2S). Sulfide production was harnessed by controlling the sulfate assimilation pathway, where strategic knockouts and culture conditions generated H_2S from 0 to over 1000 ppm (~30 mM). These sulfide-producing yeasts were able to remove mercury, lead, and copper from real-world samples taken from the Athabasca Oil Sands. More

so, yeast surface display of biomineralization peptides helped control for size distribution and crystallinity of precipitated metal sulfide nanoparticles. Altogether, this yeast-based platform not only removes heavy metals but also offers a platform for metal re-extraction through precipitation of metal sulfide nanoparticles.

Growing consumption of electronic goods and raw materials have pushed mining and manufacturing practices to unprecedented levels that the United Nations Environment Programme (UNEP) declared a global waste challenge in 2015 in order to monitor waste risk and waste crimes[1]. Because of the demand for metals, there has been a cumulative 41.8 million metric tonnes (46.1 million tons) of electronic waste (e-waste) globally in 2014 which grew an additional 20-25% in 2018[1,2]. In addition, the United States has more than 13,000 reported active mining sites with an additional 500,000 that are abandoned yet still polluting 16,000 miles of streams[3,4]. Metal contaminates are typically copper, lead, cadmium, mercury and zinc[1,5]. Despite these obvious waste sources, industry still continues to unsustainably mine for raw materials, especially given the growing demand and consumption of batteries and electric vehicles[6]. China alone produces and consumes one of the largest quantities of batteries in the world, and in 2013 generated 570 kilotons of battery waste with less than 2% being collected and recycled[7]. The main consequences of battery waste, especially from lithium-ion batteries, is the release of toxic amounts of copper and lead, with other metals such as cobalt, nickel, and chromium leaching into neighboring soils and streams[6].

Unfortunately the advancement of remediation technologies, in particular heavy metal removal, is slow in comparison to the rise of e-waste and the pace of mining[1]. So far, practical

implementation of heavy metal remediation has relied on physicochemical treatments, the most ubiquitous method being chemical precipitation via lime, hydroxides (e.g. NaOH) or sulfides (e.g. FeS or H_2S)[5]. Sulfides have been the more desirable reagent for precipitation as it is more reactive and has a lower rate of leeching than hydroxide precipitates, but the counter is that sulfide gas storage and handling is dangerous and costly making lime and hydroxides the preferred choice despite being less effective[5]. Overall, chemical precipitation is costly, requires dedicated infrastructure, involves handling dangerous compounds and reactive gases, and generates secondary waste in the form of sludge[5,8,9]. Furthermore, sludge is ineffectively eliminated through pyrolysis or physical transport to landfills for burial[8,10]. Because of this, many of the precipitated waste leach back into nearby water sources thereby perpetuating this cycle of inefficient cleaning. Thus, physicochemical treatment via chemical precipitation is not an amenable option for developing countries which typically face the biggest challenge for heavy metal removal[10]. Therefore, there is an urgent need to replace chemical precipitation with an alternative and more sustainable technology.

In contrast to physicochemical processes, scientists have discovered the benefits of using biological systems to remediate waste as a natural alternative. Bioremediation has gained traction for wastewater treatment due to its natural means to process waste in addition to its autonomous growth and environmentally friendly reactions[11,12]. In addition, there is hope that with the growing toolkit of molecular biology and bioengineering technologies scientist could further augment biology's capability to manipulate and convert waste. Already, scientist have discovered naturally occurring microorganisms which have been observed to tolerate and accumulate toxic metals, for example metal reducing microorganisms, particularly bacteria[13–17].

One particular family of interest are sulfate-reducing microorganisms (SRM) which use sulfate as their terminal electron acceptor to generate H_2S as a by-product leading to precipitation of nearby metals. Connecting the dots, it is easy to see that biology has already developed a mechanism for biotic chemical precipitation using H_2S producing SRMs. Interesting use of these organisms have been the design of anaerobic beds or stirred tank reactors for precipitation of metal contaminated effluent[18,19]. However, the limiting piece to this technology is the biology itself. SRMs are obligate anaerobes, require precise handling of culture conditions, and grow slowly. In addition many SRMs are unable to process complex carbon sources and require additional anaerobic microflora to persist[20] creating an additional layer of complexity when managing reactors. To circumvent these stringent culture conditions, scientist have begun to extract and transfer their unique behavior into more tractable organisms, such as *E. coli*, by heterologously expressing enzymes and non-native metal reducing pathways—a growing field of technology that uses genetically modified organisms (GMOs) for bioremediation applications[21]. Examples have been the expression of the mercuric reductase genes from *Thiobacillus ferrooxidans* into *E. coli*[22], or using combinations of protein and metabolic engineering to endow *E.coli* with sulfide generating capabilities much like SRMs[23]. Similar concepts have been developed in plants, such as in *A. Thaliana*, where phytochelatins, reductases, and transporters derived from other species were integrated for heavy metal removal[24]. With the advent of molecular biology there has been studies of several hundred genetic systems that have leveraged GMOs to degrade waste for bioremediation applications[21,25]. Although promising, research up to now has had limited success with GMOs for bioremediation due to the complex reactions involved and the ill-defined environments in which these organisms have to tolerate and remediate in[25].

To avoid the technical hurdles of engineering SRMs or expressing foreign pathways in either bacteria or plants, a more tractable biological platform was used in this study to develop a bioremediation system for heavy metal removal. More so, an organism that could easily be used by both scientist and non-scientists, in addition to having an established presence in industrial and consumer settings was prioritized. Therefore, yeast was chosen. The common baker's yeast, *S. cerevisiae*, is widely used in both scientific and consumer settings and by using yeast advantages beyond the biotechnology, such as infrastructure to scale, cost, packaging and transport are already in place[26–28]. The goal of this work was to transform yeast into a bioremediation platform for heavy metal removal and tap into the available resources for translating yeast into a usable system for practical waste remediation and recycling in real-world settings. Rather than assembling complex metabolic circuits or introducing foreign genes, yeasts' natural metabolic pathways were engineered to endogenously generate H_2S to concentrations similar to those produced by SRMs. However, unlike SRMs sulfide production was controlled both in rate and overall production by modifying the sulfate assimilation pathway. These engineering steps endowed these yeasts with metal sulfide precipitation capabilities. More so, controlling sulfide production helped control for precipitate size distribution and crystallinity which could potentially improve downstream filtration and recycling processes. Overall these results show that yeast, an already environmentally friendly and sustainably grown organism conventionally used for food and beverages, could be used as an agent for heavy metal detoxification.

RESULTS

Engineering yeast to metabolically produce sulfide species

The metabolic transformation of sulfide to sulfate, sulfite, and thiol functional groups require complex multi-step reactions. Fortuitously, the wine-industry was key in elucidating much of the fundamental insights in controlling sulfide production, specifically H_2S. Good wine makers have known that over-fermenting yeast can produce an off-putting egg smell, with scientist identifying the build-up of H_2S gas as the primary cause[29]. Wine researchers identified that the yeast sulfate assimilation pathway driven under fermentation conditions drove the production of H_2S gas (**Figure 1a**)[29,30]. From there, yeast wine-strains were engineered to suppress the production of H_2S for better quality wine. However, by performing the opposite modifications yeasts' natural sulfide production was harnessed for heavy metal sulfide-induced precipitation. During this investigation it was shown that single gene knockouts in the sulfate assimilation pathway promoted H_2S production in a controllable manner. Knockout strains that produced detectable amounts of H_2S were ΔMET2, ΔMET6, ΔMET17, ΔHOM2, ΔHOM3, ΔSER33 and ΔCYS4 (**Figure 1a**). Specifically, ΔHOM2, ΔMET17, and ΔCYS4 were chosen as experimental strains due to their consistently high levels of sulfide production and normal growth characteristics in complete synthetically defined media (CSM) compared to the other strains. From ΔHOM2, and ΔMET17 a double deletion was performed to obtain ΔHOM2 and ΔMET17 (ΔHM217).

Despite the metabolic complexities of the sulfate assimilation pathway, yeast H_2S production was observed to follow Le Chatelier's Principle. Supplying the necessary nutrients such as nitrogen sources and sulfate, while limiting the amount of 'products', i.e. cysteine and methionine, stimulated the yeast sulfate assimilation pathway to produce H_2S (**Figure 1b**). The

normal conversion of sulfide to thiol containing biomolecules such as cysteine and methionine was retarded by removing pathway enzymes ΔCYS4, ΔHOM2, ΔMET17, thereby forcing expulsion of the intermediate H_2S. In CSM cultures, ΔCYS4, ΔHOM2, ΔMET17 and ΔHM217 produced 99±3 ppm (2.9±0.09 mM), 62±3 ppm (1.8±0.09 mM), 54±5 (1.6±0.15 mM), and 133±3 ppm (3.9±0.09 mM) of sulfide species in a 50 mL flask culture, respectively (**Figure 1c**; **Supplementary Figure 1a**). Sulfide production was optimized by altering the media composition, primarily by removing cysteine and methionine. For ΔMET17, sulfide production was tuned from a negligible amount to over 1000 ppm (approximately 30 mM) with a maximum production rate of 75±18 ppm (2.2±0.53 mM) hr^{-1} in 50 mL CSM cultures lacking methionine (**Figure 1d**; **Supplementary Figure 1b, c**).

Using sulfide-producing yeast for chemical precipitation

Cultures of ΔCYS4, ΔHOM2, and ΔMET17 were incubated with 100 μM copper, zinc, cadmium, lead, or mercury and shaken overnight. The amount of metal precipitated correlated to the strain's capacity to produce H_2S (**Supplementary Figure 1a**) which could be tuned by altering culture conditions. Cultures grown in YPD precipitated the least amount of metals, whereas cultures grown in CSM lacking methionine or cysteine precipitated almost twice as much copper, cadmium, mercury, and lead ($p < 0.05$) (**Figure 2a**; **Supplementary Figure 2b**). Culture density (OD) also affected the amount of metal precipitated. ODs at mid-log led to higher amounts of metal precipitation, primarily due to fast yeast growth rates which corresponded to fast sulfide production rates (**Figure 1c**; **Supplementary Figure 2c**). Arsenate (AsO_4^{3-}) and chromate (CrO_4^{2-}) were also tested and were effectively precipitated

(**Supplementary Figure 3**). However, the precipitation of arsenate and chromate were mainly due to their reduction in to insoluble oxides rather than by direct sulfide precipitation.

When metals were mixed together, the preference for precipitation was copper, lead, cadmium, mercury, and zinc in that order; loosely following their trends in solubility products and in line with observations from past physicochemical precipitation experiments[5,20,31] (**Figure 2c,d**). Rounds of precipitation, with unprecipitated metals transferred to fresh cultures, were tested to determine the minimum number of iterations required to completely remove metals from solution, a practice normally implemented in industrial water processing[8,10,32,33]. 2 rounds were required to remove copper and lead below 1% (1 μM or 63 ppb and 207 ppb, respectively), 3 rounds for cadmium and mercury (below 1 μM or 112 ppb and 201 ppb, respectively), and 4 rounds to remove zinc below 20% (20 μM or 1.31 ppm) (**Figure 2c, d**). These results closely approached EPA standards for potable waters (i.e. tens to hundreds of ppb)[34,35].

Sulfide-producing yeast were also tolerant to high levels of metal concentrations, some as high as 100 μM cadmium and lead. ΔMET17 showed robust growth curves than compared to WT in metal containing media (**Supplementary Figure 4a**). In addition, cells that underwent metal precipitation were regrown without any significant change in growth rate (**Supplementary Figure 4b**).

Yeast display affects the amount of metal precipitated

Yeast display technology was used to modify the yeast surface to test whether changes in cell surface chemistry would promote further precipitation. Thiol and metal-binding moieties such as

histidine increased precipitation of cadmium, zinc, and mercury by 5-10%, but were negatively affected by more hydrophobic residues such as valine and leucine (**Figure 2e, f**; **Supplementary Figure 5**). Precipitation of copper and lead were not as affected. A hypothesis was that the fast copper/lead sulfide reaction rates favored precipitation in solution rather than the diffusion-limited process of nucleating onto the cell surface.

Engineered yeast can remove metal waste found in oil sands

Effluent from the Athabasca Oil Sands in Canada was received and subjected to yeast induced metal precipitation. The Athabasca Oil Sands is a well-known deposit of bitumen and crude oil, and for almost a hundred years the area has been a key resource for oils and fossil fuels which still drives the global economy today[36]. Due to this, the area has been heavily mined and contaminated with human-driven excavations, drilling, and mining leading to erosion, pollution, and ecological damage making the Athabasca Oil Sands an area in need of major remediation[37]. A sample of the effluent was obtained (**Figure 3a**) and fractionated with gentle centrifugation to separate the liquid phase from the solid debris (**Figure 3b**).

ICP analysis revealed that the liquid phase from the Athabasca Oil Sands contained appreciable amounts of copper, cadmium, mercury, lead, and zinc with the more toxic cadmium, mercury, and lead being more abundant per weight (1-2 ppm or mg/L) (**Supplementary Figure 6**). One round of yeast induced chemical precipitation showed greater than 85% removal of copper, mercury, and lead, and between 30-50% removal of cadmium and zinc (**Figure 3c**). These results were consistent with past metal uptake experiments at 100 μM (10-20 times more concentrated) and support the idea that these engineered yeasts can be just as effective at

precipitating metals in real-world environments. After 3 rounds of yeast mediated metal precipitation, the amount of copper, cadmium, mercury, lead, and zinc levels closely approached zero ($p < 0.05$). Examining the remediated effluent visually, the opacity of effluent dramatically reduced after just one round (**Figure 3d**; **Supplementary Figure 7**).

Controlling metal sulfide particle size and morphology

The resultant precipitated mass was another consideration to judge the sustainability of this yeast-based system. Typically in chemical precipitation, precipitates form large amorphous masses which are difficult to separate and are thus routinely dumped into landfills or burned[8,10,32]. Therefore, another consideration was to control the morphology and crystallinity of precipitates as a mean to improve downstream separation, recovery, and possibly recyclability of converted metals.

Precipitate experiments in CSM lacking both methionine and cysteine with fast H_2S production rates above 50 ppm hr^{-1} led to precipitates characterized by amorphous structures with average sizes exceeding 1 μm and size distribution spanning 2-3 orders of magnitude ($p < 0.05$) (**Figure 4a**). The precipitates were also shown to damage the cell wall, as TEM analysis of cell sections showed fragmented cell walls surrounded by large metal sulfide aggregates (**Figure 4a**). As H_2S production rates slowed by supplementing cultures with methionine and cysteine, the average precipitate size began to decrease while uniformly nucleating onto the cell wall as examined under TEM and EDX (**Figure 4b**; **Supplementary Figure 8a**). Cultures in fully supplemented CSM with H_2S production rates below 10 ppm hr^{-1} produced particles with controlled size distributions between 5-50 nm for cadmium sulfide ($p < 0.05$) (**Figure 4c**). In addition, purified

particles had a 1:1 metal to sulfide stoichiometry (**Supplementary Figure 8b**). A hypothesis for this phenomenon could be that slower H_2S production rates allowed metals time to diffuse and nucleate on to the yeast cell surface. Given that the cell wall consists of negatively charged polysaccharides and proteins, a reason could be that the electronegative environment allowed for somewhat size-controlled nucleation.

Recycling cadmium into cadmium sulfide nanoparticles

Metal nucleation was further explored by displaying nucleating peptides to facilitate metal sulfide growth, a concept that has been successfully employed in other biological organisms such as viruses and bacteria[16,23,38,39]. Without any displayed motifs, precipitated cadmium sulfide examined under high resolution TEM (HRTEM) produced large amorphous structures (**Figure 5a**). Crystalline structures indicated by lattice fringes were first observed with the hexa-cysteine motif, CCCCCC. More prominent lattice fringes were observed with GGCGGC and GCCGCC displayed peptides, glycine-cysteine motifs generally conserved in metal-binding proteins such as metallothioneins[40] (**Figure 5a,b**; higher resolution images in **Supplementary Figure 9** and **Supplementary Figure 10**). Slowing the rate of sulfide production below 10 ppm hr^{-1} while displaying glycine-cysteine motifs generated cadmium sulfide quantum dot-like nanoparticles in the 10-50 nm range (**Figure 5c, d**). With more crystalline features these cadmium sulfide particles gave a strong excitation peak at 330 nm and an emission peak at 480 nm (**Figure 5e**). Industrially, cadmium sulfide nanoparticles are routinely used for their optical properties in LEDs and photocells. Therefore, these results encourage the idea that there may be potential to convert precipitated metal sulfides into recyclable and useful materials. In addition, the ability to

control for precipitate size and crystallinity, and developing a direct method for metal re-extraction through cell wall removal could simplify downstream extraction and recycling[41].

Considerations and feasibility in industrial settings

Yeast culture compositions are chemically defined and standard among scientists, with yeasts being able to survive on several carbon sources at varying temperatures and at pH's as low as 3-4. In addition, yeasts grow in defined culture environments in both aerobic and anaerobic conditions. These factors have made yeast one of the most understood and appreciated organisms not only to scientists, but also for bakers, beer makers, and everyday consumers[27,28]. A typical laboratory only needs 3 dollars to produce 1 L of yeast with respects to the cost of consumables such as glucose, extracts, and buffers[42]. Industrially, the infrastructure to scale and bioreactor optimization done by both the beer and pharmaceutical industries have reduced the cost to 16 cents per liter or lower[26,42,43]. These factors allowed a global production of more than one million tons of yeast by weight in 2015[44]. More so, packaging and delivery of yeast through freeze-dried and active-dried packets have allowed the yeast market to touch all areas of the globe, allowing both high tech industries as well as rural villages the power to brew their own yeast[28,44]. If the scale and breadth of the yeast market can be tapped for bioremediation purposes, specifically the precipitation and conversion of heavy metals, then the potential impact on heavy metal waste management can be significant and profound.

DISCUSSION

Future work will investigate more complex displayed biomineralization peptides in order to improve metal sulfide formation and capture. Further design of biomineralization peptides could

have two major applications: selective precipitation of metals and the creation of unique metal sulfide alloys that mimic doped metal sulfide compounds. Highly toxic elements such as cadmium and mercury in potable waters should be preferentially removed than less toxic elements such as sodium or calcium. With engineered biomineralization peptides, it may be possible to selectively precipitate highly toxic metals such as mercury versus calcium even at disproportionate concentrations by using known heavy-metal binding motifs found in nature[16,38,39,45]. Another application is the ability to create useful metal sulfides in a ratiometric manner. Many metal sulfides used industrially are doped with other divalent metals to enhance their physicochemical properties in semiconductors, solar cell, and magnetic materials[46–48]. Therefore, engineering yeast to facilitate ratiometric precipitation of multi-metal sulfides is a concept that is especially interesting if the dopant metals are already present in the effluent.

More work is needed to design a pipeline for real-world bioremediation at scale. There are at least two primary strategies. The first is to grow yeast and securely package them into cartridges through size-exclusion filters or chemical cross-linking. These cartridges would maintain the optimal microenvironment for yeast to thrive and produce H_2S, e.g. salt, pH, nutrients, etc. The cartridges could then be fitted to a larger vessel that would enter a waste-contaminated area. As gaseous H_2S is produced, the surrounding environment would begin to precipitate heavy metals. Thorough investigation would be required to determine a cartridges' efficacy over time, in which a new cartridge would replace it and the precipitates within the old cartridge removed and recycled. An alternative solution would be to bring effluent to a treatment plant where waste is added to a yeast bioreactor. In this system technologies from large scale yeast fermentation could be leveraged to determine optimal fluid control to move waste between multiple yeast beds for

rounds of remediation[26,42,43]. Similarly, these reactors would have separate controls to replenish reacted yeast and supply fresh cultures when needed. These processes are no different than traditional abiotic processes for mine effluent treatment. Current treatments use an assortment of chemical beds containing lime, iron, etc. that have high pH to precipitate heavy metals[4,33]. Rather than relying on externally sourced chemicals for waste treatment, it would be more advantageous to use a renewable biological system such as yeast to control the reaction and by-products from treated waste waters.

Having yeast naturally produce sulfides is an attractive solution for curbing industry's reliance for mined sulfide gas. Currently, sulfide is produced from petroleum, natural gas, and related fossil fuel activities with China, US and Canada being leading producers[49,50]. Sulfate however, the metabolic precursor to H_2S in the yeast sulfate assimilation pathway[29,30], is generally more accessible through natural oxidation of ores, shales, and agricultural runoff[51] making sulfate more readily accessible than sulfide gas. Therefore, feeding yeast a low value resource such as sulfate, and generating a higher value product such as H_2S could be a tremendous benefit for industry. These engineered yeasts provide a natural, environmentally responsible, low-cost H_2S source while also simplifying H_2S storage and transportation. Currently H_2S storage is hazardous and costly, but with a yeast-based system storing H_2S is equivalent to storing yeast themselves.

In conclusion, this work used yeast to generate H_2S to precipitate heavy metals from contaminated waters. Furthermore, production of H_2S was tuned through gene knockouts and modulating media conditions, thereby controlling the quantity of metal precipitation and precipitate morphology. Crystallinity of metal sulfides was also controlled through displayed

biomineralization peptides, making these particles easier to extract. This work ultimately showed that yeast could be a viable platform for heavy metal waste remediation and metal re-extraction and invites the exploration of other yeast-facilitated bioremediation processes.

METHODS

Yeast strain and culture

Yeast strain W303α was obtained from the Amon Lab at MIT. Synthetically defined dropout media (SD) was made by combing 1.7 g/L yeast nitrogen base without amino acid and ammonium sulfate (YNB) (Fischer), 5 g/L ammonium sulfate (Sigma), 1.85 g/L drop-out mix without methionine and cysteine (US Biological), 20 g/L glucose (Sigma), and 10 mL/L 100X adenine hemisulfate stock (1 g/L) (Sigma). Complete synthetically defined media (CSM) was made by adding cysteine and methioneine amino acids at a final concentration of 50 mg/L (Sigma). Both SD and CSM were pH'd to 7 with NaOH. Mixtures were stirred and filtered through a .22 μm filter top (EMD). YPD media was made by adding 10 g/L yeast extract, 20 g/L peptone (Fisher), and 20 g/L glucose (Sigma) and filtered sterilized. Plates were made by adding 20 g/L Bacto Agar (Fisher) and autoclaving.

Cloning strategy and yeast transformations

The pRS303 and pRS305 vectors were used to clone the HIS and LEU markers for gene deletions in W303α via homologous recombination. Single gene deletions of SER33, SER1, SER2, HOM2, HOM6, MET2, MET6, MET17, CYS3, and CYS4 were deleted by amplifying the LEU marker using PCR with 30 bp of the appropriate up and downstream overlaps to their respective gene target (**Supplementary Table 1**). Double mutants were created by amplifying the HIS marker with 30 bp of the appropriate overlap to the target gene and transformed into the single deletant strains (**Supplementary Table 2**).

A constitutive yeast display vector constructed in the Belcher lab named pYAGA contains the AGA1 and AGA2 gene downstream of a GAP promoter and upstream of a CYC1 terminator. Single stranded sequences coding for hexa-peptide repeats were ordered from IDT and annealed with sticky ends matching the BamHI and PmeI cloning sites of pYAGA (**Supplementary Table 3**). Hexa-peptide sequences were phosphorylated with T4 PNK prior to ligation using T4 ligase (NEB). Circularized plasmids were transformed into chemically competent NEBα following the recommended NEB protocol and selected using ampicillin.

Yeast transformations were performed using Frozen-EZ Yeast Transformation Kit II (Zymo). For deletions, transformed cells were plated onto YPD for 1-2 days and replica plated onto drop out media (either HIS, LEU, or both) to select for positive transformants. Otherwise, plasmid transformations were grown directly onto plates with the appropriate drop-out media. Plasmid or genomic DNA was isolated by using silica bead beating and phenol/chloroform (Sigma) extraction. Sequences were confirmed by amplifying the isolated DNA using PCR and sequencing the DNA fragment using QuintaraBio sequencing services.

Screening and quantifying H_2S gas production

Cultures were initially screened in 5 mL CSM cultures in 14 mL BD culture tubes with taped lead acetate hydrogen sulfide indicator strips (VWR). Cultures were grown at 30ºC over 1-2 days and H_2S was detected when strips became darkened. Quantitative sulfide detection was monitored using Draeger hydrogen sulfide detection columns (VWR). 50 mL cultures in 250 mL Erlenmeyer flasks were corked with a single-hole rubber stopper in which hydrogen sulfide columns were fitted. Cultures grew for 1-2 days and were visually inspected at specific time-

points to measure sulfide production. Knockouts ΔSER33 and ΔCYS4 became auxotrophic to cysteine while ΔHOM3 and ΔMET2 became slow growers on synthetically defined (SD) media. Combination knockouts with ΔCYS4 produced extremely slow growers.

OD_{600} culture density measurements

Discrete time point optical density measurements were performed using 2 mL non-frosted cuvettes (VWR) and a table-top DU800 Beckman Coulter spectrophotometer measuring at 600 nm. Continuous growth curve studies were performed on a shaking 96 well BioTek Synergy 2 plate reader held at 30ºC with 100 μL cultures. Cultures were first diluted from overnights to < 0.1 OD_{600} and aliquoted into a 96-well round bottom plate (Cellstar) with the appropriate metal and concentration.

Quantifying metal precipitation

Liquid stocks of copper (II) chloride, zinc chloride, cadmium nitrate, lead nitrate, and mercury (II) chloride (Sigma) were made at 100 mM in water. Metal precipitation studies were performed by diluting overnight cultures to varying culture densities in 5 mL of fresh culture containing 100 μM of metal. Cultures were grown overnight, spun down at 900x*g* for 3 min in a swinging bucket rotor and supernatant collected for metal measurement. Metal content was measured on an Agilent ICP-AES 5100 following standard operating procedures. Trace concentrations of metal below 10 μM were measured on an Agilent ICP-MS 7900. If samples were to be diluted, they were diluted in 3% HNO_3 (Sigma) to fit within the dynamic range of ICP detection.

For all experiments, a sample of just media with spiked metal (e.g. 100 μM) was measured to act as a reference for the initial metal content of copper, zinc, cadmium, lead, and mercury in the media. Metal removal measurements were calculated by taking the ICP measurements of the supernatant and subtracting from this reference to give the quantity of metal precipitated.

Multiple uptake experiments were performed by resuspending 1 OD_{600} of fresh yeast grown the previous day with the equivalent volume of supernatant from the current metal precipitation experiment. For example, after the first round, the supernatant was collected and transferred to a freshly spun down culture inoculated the day before to a final OD of 1. The precipitation experiment was performed again, making this the second round of precipitation. This process was repeated at most up to 4 times, with each iteration sampled for ICP measurement.

Quantifying metal removal from oil sand samples

Samples of effluent were taken from the Athabasca Oil Sands in Canada. Liquid was gently centrifuged at 1000xg for 30 minutes to fractionate liquid, oil and solid phases. The liquid phase was used as the waste medium to test for yeast-induced metal precipitation. Although not thoroughly investigated in this study, the oil phase contained many organics, aromatics, and oils contributed from mined runoff. The solid phase contained a heterogeneous mixture of large debris, rocks, and precipitates that were easily spun down during centrifugation or through size-exclusion filtration.

To prepare the precipitation experiments, an overnight of ΔMET17 was grown in CSM-M and spun down. 1 OD_{600} per mL of cells was added to a 1 to 1 mixture of 2X CSM-M (prepared by

doubling all ingredients) and liquid phase extracted from the effluent. The mixture was incubated overnight for 12 hours, spun down, and visualized for precipitation. The supernatant was taken for ICP measurement for copper, cadmium, mercury, lead, and zinc following the procedures explained above.

The liquid phase metal profile was studied using ICP. Commercial ICP multi-element standards was used to multiplex measurements in parallel (VWR or Agilent). Multiple dilutions of the liquid phase in 3% HNO_3 was performed (such as 1 to 1, 1 to 10, etc.) to determine the level of matrix effect, as the liquid phase contained other contaminants not accounted for in the standards and skewed readings. A 1 to 5 dilution gave consistent results and was used to calculate the concentrations of Na, Mg, K, Ca, Sr, Ba, Mn, Fe, Cu, Zn, Si, Cd, Pb, Hg, Cr, As.

Quantifying yeast display expression using flow cytometry

Displayed peptides were first cloned with a C-terminus V5 tag followed by a stop codon in a constitutive AGA1 and AGA2 vector which was called pYAGA. Cultures were grown to saturating OD and 0.5 OD_{600} were taken for flow cytometry. Cells were washed and pelleted at 900x*g* with PBS+1% BSA. Primary antibodies against V5 (Life Technologies) were diluted 1:500 in PBS+1% BSA and incubated at room temperature for 1 hour. Secondary antibodies with AlexaFluor 488 were diluted 1:2000 in PBS+1% BSA and incubated at room temperature for 1 hour. Cells were then washed and diluted to 1e6 cells per mL for flow cytometry. Flow cytometry was performed on a BD FACS Canto or LSR II following standard operating procedure provided by the Koch Flow Cytometry Core. Yeast cell gating strategy followed: FSC-A and SSC-A was used to gate on cells. FSC-W and FSC-H was used to gate vertically

oriented single cells (vertical singlets). SSC-W and SSC-H was used to gate horizontally oriented single cells (horizontal singlets). After gating on these 3 plots, single cells were measured based on fluorescence (**Supplementary Figure 11**). Cell counts were plotted against binned fluorescent intensity (x-axis) creating a population distribution histogram of fluorescence (y-axis).

Extraction and purification of precipitated metal sulfides

Overnight cultures of metals added to yeast were pelleted at 900x*g* for 3 min. Cultures were washed and resuspended in 1 mL sorbitol citrate. 100T Zymolyase (Zymo) was diluted 1 to 100 and added to the suspension and incubated for >1 hour at 30^{o}C while shaking. Digested cells were pelleted at 900x*g* for 3 min, and supernatant was removed or kept for later analysis of dislodged metal sulfide particles. Cells were resuspended with 1:1 water and oleic acid (organic layer; Sigma). Mixtures were spun down at 900xg for 3 min to pellet cellular debris while allowing insoluble metal sulfide particles to remain in the organic layer. The organic layer was removed and fresh oleic acid was introduced to further extract metal sulfide particles. This process was performed between 1-3 times until coloration was completely transferred into the organic layer. Most organic solvents were observed to work (phenol:chloroform, hexane, octonal, etc.), however oleic acid was more cost effective, easier to handle, and safer to use. Samples could be used immediately for analysis or concentrated by spinning down particles at max speed for 15 min and resuspended in a lower volume in either oleic acid or water.

Excitation and emission measurements using fluorometry

An Agilent Cary Eclipse Fluorescence Spectrophotometer was used to measure the fluorescence of the isolated metal sulfide particles using disposable PMMA acrylic cuvettes (VWR). Excitation and emission scans were performed following standard operating procedures provided by the Center of Material Science Engineering, MIT.

TEM sample prep

Cells were not digested with zymolayse in order to preserve the cell wall for imaging. Cell fixation, dehydration, embedding, and sectioning followed yeast OTO processing provided by the WhiteHead Institute, MIT[52]. The yeast cells were grown to an appropriate optical density and fixed with 2% glutaraldehyde, 3% paraformaldehyde, 5% sucrose in 0.1 M sodium cacodylate buffer (EMS) for 1 hour. Pelleted cells were washed and stained for 30 minutes in 1% OsO_4, 1% potassium ferocyanide, and 5 mM $CaCl_2$ in 0.1 M cacodylate buffer. Osmium staining was followed by washing and staining in 1% thiocarbohydrazide. Pellets were washed and stained again in the reduced osmium solution. The cells were then stained in 2% uranyl acetate (EMS) overnight, serially dehydrated with ethanol, and embedded in EMBED-812 (EMS). Sections were cut on a Leica EM UC7 ultra microtome with a Diatome diamond knife at a thickness setting of 50 nm, stained with 2% uranyl acetate, and lead citrate. The sections were examined using a FEI Tecnai Spirit at 80KV and photographed with an AMT CCD camera.

TEM and EDX analysis

TEM samples of purified metal sulfide particles were prepared on 400 mesh nickel Formvar grids (EMS) by dropping 10 μL of sample onto the grids for 5 min and wicking dry. TEM images were acquired on a FEI Tecnai at 120V. Samples were also monitored by energy-

dispersive x-ray (EDX) spectroscopy to qualitatively determine the relative amounts of sulfide and metal. When necessary, for example with copper, the signal background was corrected by subtracting the spectrum with a region without any metal sulfide particles to deconvolve overlapping peaks from the copper grid. High resolution TEM (HRTEM) images were acquired on a JEOL2010F at 200V to observe crystal spacing. A JEOL2010F was used for more resolved EDX elemental mapping of metal sulfide particles that nucleated on the cell wall.

Purified metal sulfide particles were analyzed for size distribution and morphology using TEM. Size distribution data was determined by imaging 40 random locations on 3 separate samples of isolated metal precipitates using TEM. Particles below 100 nm were imaged on the higher resolution JEOL2010F at greater than 100,000x magnification. Sizes were quantitatively measured using ImageJ, and distributions plotted as histograms.

Figure creation, analysis, and plotting

Raw data was collected and stored as csv or Excel file formats. Data was imported and analyzed with Python using modules such as numpy, pandas, and scipy. Plots were graphed with matplotlib.

Statistical analysis

Statistical parameters including the definitions and values of *n*, SDs, and/or SEs are reported in the figures and corresponding figure legends. When reporting significance, a two-tailed unpaired t-test was performed between observations and p-values reported in the text. The significance threshold was set to $p < 0.05$ for all experiments, or as specified in the text.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon request. The source data underlying Figures 1c, 1d, 2a, 3c, 4a-c, 5e, and Extended Data 2a, 2c, and 3b are provided as a Source Data File.

REFERENCES

1. Rucevska, I., United Nations Environment Programme & GRID--Arendal. *Waste crime - waste risks: gaps in meeting the global waste challenge : a rapid response assessment*. (2015).
2. Balde, C. P., Forti, V., Gray, V., Kuehr, R. & Stegmann, P. *The global e-waste monitor 2017: quantities, flows and resources*. (United Nations University, International Telecommunication Union, and International Solid Waste Association, 2017).
3. Center for Disease Control and Prevention. Statistics: all mining. (2018). Available at: https://www.cdc.gov/niosh/mining/statistics/allmining.html.
4. DeGraff, J. V. Addressing the toxic legacy of abandoned mines on public land in the western United States. in *Reviews in Engineering Geology Volume XVII: Understanding and Responding to Hazardous Substances at Mine Sites in the Western United States* **17**, 1–8 (Geological Society of America, 2007).
5. Fu, F. & Wang, Q. Removal of heavy metal ions from wastewaters: a review. *J. Environ. Manage.* **92**, 407–418 (2011).
6. Kang, D. H. P., Chen, M. & Ogunseitan, O. A. Potential environmental and human health impacts of rechargeable lithium batteries in electronic waste. (2013). doi:10.1021/es400614y
7. Song, X., Hu, S., Chen, D. & Zhu, B. Estimation of waste battery generation and analysis of the waste battery recycling system in China. *J. Ind. Ecol.* **21**, 57–69 (2017).
8. Kurniawan, T. A., Chan, G. Y. S., Lo, W.-H. & Babel, S. Physico–chemical treatment techniques for wastewater laden with heavy metals. *Chem. Eng. J.* **118**, 83–98 (2006).
9. Barakat, M. A. New trends in removing heavy metals from industrial wastewater. *Arab. J. Chem.* **4**, 361–377 (2011).

10. Kumar Gupta, V., Ali, I., A. Saleh, T., Nayak, A. & Agarwal, S. Chemical treatment technologies for waste-water recycling—an overview. *RSC Adv.* **2**, 6380–6388 (2012).
11. Gavrilescu, M., Demnerová, K., Aamand, J., Agathos, S. & Fava, F. Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *New Biotechnol.* **32**, 147–156 (2015).
12. Singh, A. & Prasad, S. M. Remediation of heavy metal contaminated ecosystem: an overview on technology advancement. *Int. J. Environ. Sci. Technol.* **12**, 353–366 (2015).
13. Gadd, G. M. Microbial influence on metal mobility and application for bioremediation. *Geoderma* **122**, 109–119 (2004).
14. Wiatrowski, H. A., Ward, P. M. & Barkay, T. Novel reduction of mercury (II) by mercury-sensitive dissimilatory metal reducing bacteria. *Environ. Sci. Technol.* **40**, 6690–6696 (2006).
15. Silver, S. & Phung, L. T. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Appl. Environ. Microbiol.* **71**, 599–608 (2005).
16. Picard, A., Gartman, A., Clarke, D. R. & Girguis, P. R. Sulfate-reducing bacteria influence the nucleation and growth of mackinawite and greigite. *Geochim. Cosmochim. Acta* **220**, 367–384 (2018).
17. Gartman, A. *et al.* Microbes facilitate mineral deposition in bioelectrochemical systems. *ACS Earth Space Chem.* **1**, 277–287 (2017).
18. Jong, T. & Parry, D. L. Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. *Water Res.* **37**, 3379–3389 (2003).

554 19. Kieu, H. T. Q., Müller, E. & Horn, H. Heavy metal removal in anaerobic semi-continuous
555 stirred tank reactors by a consortium of sulfate-reducing bacteria. *Water Res.* **45**, 3863–3870
556 (2011).
557 20. Neculita, C.-M., Zagury, G. J. & Bussière, B. Passive treatment of acid mine drainage in
558 bioreactors using sulfate-reducing bacteria. *J. Environ. Qual.* **36**, 1–16 (2007).
559 21. Lovley, D. R. Cleaning up with genomics: applying molecular biology to bioremediation.
560 *Nat. Rev. Microbiol.* **1**, 35–44 (2003).
561 22. Shiratori, T., Inoue, C., Sugawara, K., Kusano, T. & Kitagawa, Y. Cloning and expression of
562 *Thiobacillus ferrooxidans* mercury ion resistance genes in *Escherichia coli*. *J. Bacteriol.*
563 **171**, 3458–3464 (1989).
564 23. Wang, C. L., Maratukulam, P. D., Lum, A. M., Clark, D. S. & Keasling, J. D. Metabolic
565 engineering of an aerobic sulfate reduction pathway and its application to precipitation of
566 cadmium on the cell surface. *Appl. Environ. Microbiol.* **66**, 4497–4502 (2000).
567 24. Krämer, U. & Chardonnens, A. The use of transgenic plants in the bioremediation of soils
568 contaminated with trace elements. *Appl. Microbiol. Biotechnol.* **55**, 661–672 (2001).
569 25. Sayler, G. S. & Ripp, S. Field applications of genetically engineered microorganisms for
570 bioremediation processes. *Curr. Opin. Biotechnol.* **11**, 286–289 (2000).
571 26. Vieira, É. D., da Graça Stupiello Andrietta, M. & Andrietta, S. R. Yeast biomass production:
572 a new approach in glucose-limited feeding strategy. *Braz. J. Microbiol.* **44**, 551–558 (2013).
573 27. Barth-Haas Group. Worldwide beer production, 2016. (2018). Available at:
574 https://www.statista.com/statistics/270275/worldwide-beer-production/.

28. PR Newswire. Value of the yeast product market worldwide from 2016 to 2022 (in billions U.S. dollars). (2017). Available at: https://www.grandviewresearch.com/industry-analysis/yeast-market.
29. Swiegers, J. H. & Pretorius, I. S. Modulation of volatile sulfur compounds by wine yeast. *Appl. Microbiol. Biotechnol.* **74**, 954–960 (2007).
30. Linderholm, A. L., Findleton, C. L., Kumar, G., Hong, Y. & Bisson, L. F. Identification of genes affecting hydrogen sulfide formation in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **74**, 1418–1427 (2008).
31. Rickard, D. & Luther, G. W. Metal sulfide complexes and clusters. *Rev. Mineral. Geochem.* **61**, 421–504 (2006).
32. Bolong, N., Ismail, A. F., Salim, M. R. & Matsuura, T. A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination* **239**, 229–246 (2009).
33. Johnson, D. B. & Hallberg, K. B. Acid mine drainage remediation options: a review. *Sci. Total Environ.* **338**, 3–14 (2005).
34. US EPA, O. Table of Regulated Drinking Water Contaminants. (2019). Available at: http://www.epa.gov/your-drinking-water/table-regulated-drinking-water-contaminants#Inorganic.
35. US EPA, O. Secondary Drinking Water Standards: Guidance for Nuisance Chemicals. (2019). Available at: https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals.
36. Sparks, B. D., Kotlyar, L. S., O'Carroll, J. B. & Chung, K. H. Athabasca oil sands: effect of organic coated solids on bitumen recovery and quality. *J. Pet. Sci. Eng.* **39**, 417–430 (2003).

37. Kelly, E. N. *et al.* Oil sands development contributes elements toxic at low concentrations to the Athabasca River and its tributaries. *Proc. Natl. Acad. Sci.* **107**, 16178–16183 (2010).
38. Sakimoto, K. K., Wong, A. B. & Yang, P. Self-photosensitization of nonphotosynthetic bacteria for solar-to-chemical production. *Science* **351**, 74–77 (2016).
39. Sweeney, R. Y. *et al.* Bacterial biosynthesis of cadmium sulfide nanocrystals. *Chem. Biol.* **11**, 1553–1559 (2004).
40. DeSilva Tara M., Veglia Gianluigi, Porcelli Fernando, Prantner Andrew M. & Opella Stanley J. Selectivity in heavy metal- binding to peptides and proteins. *Biopolymers* **64**, 189–197 (2002).
41. Kim, J. *et al.* Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. *J. Biosci. Bioeng.* **95**, 271–275 (2003).
42. Harrison, R. G., Todd, P., Todd, P. W., Rudge, S. R. & Petrides, D. P. *Bioseparations Science and Engineering*. (Oxford University Press, 2015).
43. Hoek, P. V., Dijken, J. P. V. & Pronk, J. T. Effect of specific growth rate on fermentative capacity of baker's yeast. *Appl. Environ. Microbiol.* **64**, 4226–4233 (1998).
44. BCC Research. Yeasts, yeast extracts, autolysates and related products: the global market. (2017). Available at: https://www.bccresearch.com/market-research/chemicals/yeast-yeast-extracts-autolysates-products-chm053c.html.
45. Rugh, C. L. *et al.* Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene. *Proc. Natl. Acad. Sci.* **93**, 3182–3187 (1996).
46. Arai, T. *et al.* Cu-doped ZnS hollow particle with high activity for hydrogen generation from alkaline sulfide solution under visible light. *Chem. Mater.* **20**, 1997–2000 (2008).

47. Parveen, A., Agrawal, S. & Azam, A. Band gap tuning and fluorescence properties of lead sulfide Pb0.9A0.1S (A: Fe, Co, and Ni) nanoparticles by transition metal doping. *Opt. Mater.* **76**, 21–27 (2018).
48. Bhattacharya, S. & Chakravorty, D. Electrical and magnetic properties of cold compacted iron-doped zinc sulfide nanoparticles synthesized by wet chemical method. *Chem. Phys. Lett.* **444**, 319–323 (2007).
49. Selim, H., Gupta, A. K. & Al Shoaibi, A. Effect of reaction parameters on the quality of captured sulfur in Claus process. *Appl. Energy* **104**, 772–776 (2013).
50. US Department of the Interior. USGS minerals information: sulfur. (2018). Available at: https://minerals.usgs.gov/minerals/pubs/commodity/sulfur/.
51. Little, B. J., Ray, R. I. & Pope, R. K. Relationship between corrosion and the biological sulfur cycle: a review. *CORROSION* **56**, 433–443 (2000).
52. Seligman, A. M., Wasserkrug, H. L. & Hanker, J. S. A new staining method (OTO) for enhancing contrast of lipid-containing membranes and droplets in osmium tetroxide-fixed tissue with osmiophilic thiocarbohydrazide (TCH). *J. Cell Biol.* **30**, 424–432 (1966).

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AUTHOR CONTRIBTUIONS

G.L.S, E.E.R, and A.M.B. conceived the study and designed experiments; G.L.S. performed and E.E.R. helped with experiments; G.L.S. analyzed the data and assembled figures; G.L.S, E.E.R, and A.M.B wrote the manuscript.

COMPETING INTEREST

The authors declare no competing interests.