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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

24 so, yeast surface display of biomineralization peptides helped control for size distribution and
25 crystallinity of precipitated metal sulfide nanoparticles. Altogether, this yeast-based platform not
26 only removes heavy metals but also offers a platform for metal re-extraction through
27 precipitation of metal sulfide nanoparticles.
28
29 Growing consumption of electronic goods and raw materials have pushed mining and
30 manufacturing practices to unprecedented levels that the United Nations Environment
31 Programme (UNEP) declared a global waste challenge in 2015 in order to monitor waste risk and
32 waste crimes[1]. Because of the demand for metals, there has been a cumulative 41.8 million
33 metric tonnes (46.1 million tons) of electronic waste (e-waste) globally in 2014 which grew an
34 additional 20-25% in 2018[1,2]. In addition, the United States has more than 13,000 reported active
35 mining sites with an additional 500,000 that are abandoned yet still polluting 16,000 miles of
36 streams[3,4]. Metal contaminates are typically copper, lead, cadmium, mercury and zinc[1,5]. Despite
37 these obvious waste sources, industry still continues to unsustainably mine for raw materials,
38 especially given the growing demand and consumption of batteries and electric vehicles[6]. China
39 alone produces and consumes one of the largest quantities of batteries in the world, and in 2013
40 generated 570 kilotons of battery waste with less than 2% being collected and recycled[7]. The
41 main consequences of battery waste, especially from lithium-ion batteries, is the release of toxic
42 amounts of copper and lead, with other metals such as cobalt, nickel, and chromium leaching
43 into neighboring soils and streams[6].
44
45 Unfortunately the advancement of remediation technologies, in particular heavy metal removal,
46 is slow in comparison to the rise of e-waste and the pace of mining[1]. So far, practical

47 implementation of heavy metal remediation has relied on physicochemical treatments, the most
48 ubiquitous method being chemical precipitation via lime, hydroxides (e.g. NaOH) or sulfides
49 (e.g. FeS or H_2S)[5]. Sulfides have been the more desirable reagent for precipitation as it is more
50 reactive and has a lower rate of leeching than hydroxide precipitates, but the counter is that
51 sulfide gas storage and handling is dangerous and costly making lime and hydroxides the
52 preferred choice despite being less effective[5]. Overall, chemical precipitation is costly, requires
53 dedicated infrastructure, involves handling dangerous compounds and reactive gases, and
54 generates secondary waste in the form of sludge[5,8,9]. Furthermore, sludge is ineffectively
55 eliminated through pyrolysis or physical transport to landfills for burial[8,10]. Because of this,
56 many of the precipitated waste leach back into nearby water sources thereby perpetuating this
57 cycle of inefficient cleaning. Thus, physicochemical treatment via chemical precipitation is not
58 an amenable option for developing countries which typically face the biggest challenge for heavy
59 metal removal[10]. Therefore, there is an urgent need to replace chemical precipitation with an
60 alternative and more sustainable technology.
61
62 In contrast to physicochemical processes, scientists have discovered the benefits of using
63 biological systems to remediate waste as a natural alternative. Bioremediation has gained traction
64 for wastewater treatment due to its natural means to process waste in addition to its autonomous
65 growth and environmentally friendly reactions[11,12]. In addition, there is hope that with the
66 growing toolkit of molecular biology and bioengineering technologies scientist could further
67 augment biology's capability to manipulate and convert waste. Already, scientist have
68 discovered naturally occurring microorganisms which have been observed to tolerate and
69 accumulate toxic metals, for example metal reducing microorganisms, particularly bacteria[13–17].

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

93

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

114

115 **RESULTS**

116 **Engineering yeast to metabolically produce sulfide species**

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

133

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

138 normal conversion of sulfide to thiol containing biomolecules such as cysteine and methionine
139 was retarded by removing pathway enzymes ΔCYS4, ΔHOM2, ΔMET17, thereby forcing
140 expulsion of the intermediate H_2S. In CSM cultures, ΔCYS4, ΔHOM2, ΔMET17 and ΔHM217
141 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
142 133±3 ppm (3.9±0.09 mM) of sulfide species in a 50 mL flask culture, respectively (**Figure 1c**;
143 **Supplementary Figure 1a**). Sulfide production was optimized by altering the media
144 composition, primarily by removing cysteine and methionine. For ΔMET17, sulfide production
145 was tuned from a negligible amount to over 1000 ppm (approximately 30 mM) with a maximum
146 production rate of 75±18 ppm (2.2±0.53 mM) hr^{-1} in 50 mL CSM cultures lacking methionine
147 (**Figure 1d**; **Supplementary Figure 1b, c**).
148
149 **Using sulfide-producing yeast for chemical precipitation**
150 Cultures of ΔCYS4, ΔHOM2, and ΔMET17 were incubated with 100 μM copper, zinc,
151 cadmium, lead, or mercury and shaken overnight. The amount of metal precipitated correlated to
152 the strain's capacity to produce H_2S (**Supplementary Figure 1a**) which could be tuned by
153 altering culture conditions. Cultures grown in YPD precipitated the least amount of metals,
154 whereas cultures grown in CSM lacking methionine or cysteine precipitated almost twice as
155 much copper, cadmium, mercury, and lead ($p < 0.05$) (**Figure 2a**; **Supplementary Figure 2b**).
156 Culture density (OD) also affected the amount of metal precipitated. ODs at mid-log led to
157 higher amounts of metal precipitation, primarily due to fast yeast growth rates which
158 corresponded to fast sulfide production rates (**Figure 1c**; **Supplementary Figure 2c**). Arsenate
159 (AsO_4^{3-}) and chromate (CrO_4^{2-}) were also tested and were effectively precipitated

160 (**Supplementary Figure 3**). However, the precipitation of arsenate and chromate were mainly
161 due to their reduction in to insoluble oxides rather than by direct sulfide precipitation.
162
163 When metals were mixed together, the preference for precipitation was copper, lead, cadmium,
164 mercury, and zinc in that order; loosely following their trends in solubility products and in line
165 with observations from past physicochemical precipitation experiments[5,20,31] (**Figure 2c,d**).
166 Rounds of precipitation, with unprecipitated metals transferred to fresh cultures, were tested to
167 determine the minimum number of iterations required to completely remove metals from
168 solution, a practice normally implemented in industrial water processing[8,10,32,33]. 2 rounds were
169 required to remove copper and lead below 1% (1 μM or 63 ppb and 207 ppb, respectively), 3
170 rounds for cadmium and mercury (below 1 μM or 112 ppb and 201 ppb, respectively), and 4
171 rounds to remove zinc below 20% (20 μM or 1.31 ppm) (**Figure 2c, d**). These results closely
172 approached EPA standards for potable waters (i.e. tens to hundreds of ppb)[34,35].
173
174 Sulfide-producing yeast were also tolerant to high levels of metal concentrations, some as high
175 as 100 μM cadmium and lead. ΔMET17 showed robust growth curves than compared to WT in
176 metal containing media (**Supplementary Figure 4a**). In addition, cells that underwent metal
177 precipitation were regrown without any significant change in growth rate (**Supplementary**
178 **Figure 4b**).
179

180 Yeast display affects the amount of metal precipitated

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

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

188

189 **Engineered yeast can remove metal waste found in oil sands**

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

198

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

206 precipitating metals in real-world environments. After 3 rounds of yeast mediated metal
207 precipitation, the amount of copper, cadmium, mercury, lead, and zinc levels closely approached
208 zero ($p < 0.05$). Examining the remediated effluent visually, the opacity of effluent dramatically
209 reduced after just one round (**Figure 3d**; **Supplementary Figure 7**).
210
211 **Controlling metal sulfide particle size and morphology**
212 The resultant precipitated mass was another consideration to judge the sustainability of this
213 yeast-based system. Typically in chemical precipitation, precipitates form large amorphous
214 masses which are difficult to separate and are thus routinely dumped into landfills or
215 burned[8,10,32]. Therefore, another consideration was to control the morphology and crystallinity of
216 precipitates as a mean to improve downstream separation, recovery, and possibly recyclability of
217 converted metals.
218
219 Precipitate experiments in CSM lacking both methionine and cysteine with fast H_2S production
220 rates above 50 ppm hr^{-1} led to precipitates characterized by amorphous structures with average
221 sizes exceeding 1 μm and size distribution spanning 2-3 orders of magnitude ($p < 0.05$) (**Figure
222 4a**). The precipitates were also shown to damage the cell wall, as TEM analysis of cell sections
223 showed fragmented cell walls surrounded by large metal sulfide aggregates (**Figure 4a**). As H_2S
224 production rates slowed by supplementing cultures with methionine and cysteine, the average
225 precipitate size began to decrease while uniformly nucleating onto the cell wall as examined
226 under TEM and EDX (**Figure 4b**; **Supplementary Figure 8a**). Cultures in fully supplemented
227 CSM with H_2S production rates below 10 ppm hr^{-1} produced particles with controlled size
228 distributions between 5-50 nm for cadmium sulfide ($p < 0.05$) (**Figure 4c**). In addition, purified

229 particles had a 1:1 metal to sulfide stoichiometry (**Supplementary Figure 8b**). A hypothesis for
230 this phenomenon could be that slower H_2S production rates allowed metals time to diffuse and
231 nucleate on to the yeast cell surface. Given that the cell wall consists of negatively charged
232 polysaccharides and proteins, a reason could be that the electronegative environment allowed for
233 somewhat size-controlled nucleation.
234

235 **Recycling cadmium into cadmium sulfide nanoparticles**

236 Metal nucleation was further explored by displaying nucleating peptides to facilitate metal
237 sulfide growth, a concept that has been successfully employed in other biological organisms such
238 as viruses and bacteria[16,23,38,39]. Without any displayed motifs, precipitated cadmium sulfide
239 examined under high resolution TEM (HRTEM) produced large amorphous structures (**Figure**
240 **5a**). Crystalline structures indicated by lattice fringes were first observed with the hexa-cysteine
241 motif, CCCCCC. More prominent lattice fringes were observed with GGCGGC and GCCGCC
242 displayed peptides, glycine-cysteine motifs generally conserved in metal-binding proteins such
243 as metallothioneins[40] (**Figure 5a,b**; higher resolution images in **Supplementary Figure 9** and
244 **Supplementary Figure 10**). Slowing the rate of sulfide production below 10 ppm hr^{-1} while
245 displaying glycine-cysteine motifs generated cadmium sulfide quantum dot-like nanoparticles in
246 the 10-50 nm range (**Figure 5c, d**). With more crystalline features these cadmium sulfide
247 particles gave a strong excitation peak at 330 nm and an emission peak at 480 nm (**Figure 5e**).
248 Industrially, cadmium sulfide nanoparticles are routinely used for their optical properties in
249 LEDs and photocells. Therefore, these results encourage the idea that there may be potential to
250 convert precipitated metal sulfides into recyclable and useful materials. In addition, the ability to

251 control for precipitate size and crystallinity, and developing a direct method for metal re-
252 extraction through cell wall removal could simplify downstream extraction and recycling[41].
253
254 **Considerations and feasibility in industrial settings**
255 Yeast culture compositions are chemically defined and standard among scientists, with yeasts
256 being able to survive on several carbon sources at varying temperatures and at pH's as low as 3-
257 4. In addition, yeasts grow in defined culture environments in both aerobic and anaerobic
258 conditions. These factors have made yeast one of the most understood and appreciated organisms
259 not only to scientists, but also for bakers, beer makers, and everyday consumers[27,28]. A typical
260 laboratory only needs 3 dollars to produce 1 L of yeast with respects to the cost of consumables
261 such as glucose, extracts, and buffers[42]. Industrially, the infrastructure to scale and bioreactor
262 optimization done by both the beer and pharmaceutical industries have reduced the cost to 16
263 cents per liter or lower[26,42,43]. These factors allowed a global production of more than one million
264 tons of yeast by weight in 2015[44]. More so, packaging and delivery of yeast through freeze-dried
265 and active-dried packets have allowed the yeast market to touch all areas of the globe, allowing
266 both high tech industries as well as rural villages the power to brew their own yeast[28,44]. If the
267 scale and breadth of the yeast market can be tapped for bioremediation purposes, specifically the
268 precipitation and conversion of heavy metals, then the potential impact on heavy metal waste
269 management can be significant and profound.
270
271 **DISCUSSION**
272 Future work will investigate more complex displayed biomineralization peptides in order to
273 improve metal sulfide formation and capture. Further design of biomineralization peptides could

274 have two major applications: selective precipitation of metals and the creation of unique metal
275 sulfide alloys that mimic doped metal sulfide compounds. Highly toxic elements such as
276 cadmium and mercury in potable waters should be preferentially removed than less toxic
277 elements such as sodium or calcium. With engineered biomineralization peptides, it may be
278 possible to selectively precipitate highly toxic metals such as mercury versus calcium even at
279 disproportionate concentrations by using known heavy-metal binding motifs found in
280 nature[16,38,39,45]. Another application is the ability to create useful metal sulfides in a ratiometric
281 manner. Many metal sulfides used industrially are doped with other divalent metals to enhance
282 their physicochemical properties in semiconductors, solar cell, and magnetic materials[46–48].
283 Therefore, engineering yeast to facilitate ratiometric precipitation of multi-metal sulfides is a
284 concept that is especially interesting if the dopant metals are already present in the effluent.
285
286 More work is needed to design a pipeline for real-world bioremediation at scale. There are at
287 least two primary strategies. The first is to grow yeast and securely package them into cartridges
288 through size-exclusion filters or chemical cross-linking. These cartridges would maintain the
289 optimal microenvironment for yeast to thrive and produce H_2S, e.g. salt, pH, nutrients, etc. The
290 cartridges could then be fitted to a larger vessel that would enter a waste-contaminated area. As
291 gaseous H_2S is produced, the surrounding environment would begin to precipitate heavy metals.
292 Thorough investigation would be required to determine a cartridges' efficacy over time, in which
293 a new cartridge would replace it and the precipitates within the old cartridge removed and
294 recycled. An alternative solution would be to bring effluent to a treatment plant where waste is
295 added to a yeast bioreactor. In this system technologies from large scale yeast fermentation could
296 be leveraged to determine optimal fluid control to move waste between multiple yeast beds for

297 rounds of remediation[26,42,43]. Similarly, these reactors would have separate controls to replenish
298 reacted yeast and supply fresh cultures when needed. These processes are no different than
299 traditional abiotic processes for mine effluent treatment. Current treatments use an assortment of
300 chemical beds containing lime, iron, etc. that have high pH to precipitate heavy metals[4,33]. Rather
301 than relying on externally sourced chemicals for waste treatment, it would be more advantageous
302 to use a renewable biological system such as yeast to control the reaction and by-products from
303 treated waste waters.
304
305 Having yeast naturally produce sulfides is an attractive solution for curbing industry's reliance
306 for mined sulfide gas. Currently, sulfide is produced from petroleum, natural gas, and related
307 fossil fuel activities with China, US and Canada being leading producers[49,50]. Sulfate however,
308 the metabolic precursor to H_2S in the yeast sulfate assimilation pathway[29,30], is generally more
309 accessible through natural oxidation of ores, shales, and agricultural runoff[51] making sulfate
310 more readily accessible than sulfide gas. Therefore, feeding yeast a low value resource such as
311 sulfate, and generating a higher value product such as H_2S could be a tremendous benefit for
312 industry. These engineered yeasts provide a natural, environmentally responsible, low-cost H_2S
313 source while also simplifying H_2S storage and transportation. Currently H_2S storage is hazardous
314 and costly, but with a yeast-based system storing H_2S is equivalent to storing yeast themselves.
315
316 In conclusion, this work used yeast to generate H_2S to precipitate heavy metals from
317 contaminated waters. Furthermore, production of H_2S was tuned through gene knockouts and
318 modulating media conditions, thereby controlling the quantity of metal precipitation and
319 precipitate morphology. Crystallinity of metal sulfides was also controlled through displayed

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

323 **METHODS**

324 **Yeast strain and culture**

325 Yeast strain W303α was obtained from the Amon Lab at MIT. Synthetically defined dropout
326 media (SD) was made by combing 1.7 g/L yeast nitrogen base without amino acid and
327 ammonium sulfate (YNB) (Fischer), 5 g/L ammonium sulfate (Sigma), 1.85 g/L drop-out mix
328 without methionine and cysteine (US Biological), 20 g/L glucose (Sigma), and 10 mL/L 100X
329 adenine hemisulfate stock (1 g/L) (Sigma). Complete synthetically defined media (CSM) was
330 made by adding cysteine and methioneine amino acids at a final concentration of 50 mg/L
331 (Sigma). Both SD and CSM were pH'd to 7 with NaOH. Mixtures were stirred and filtered
332 through a .22 μm filter top (EMD). YPD media was made by adding 10 g/L yeast extract, 20 g/L
333 peptone (Fisher), and 20 g/L glucose (Sigma) and filtered sterilized. Plates were made by adding
334 20 g/L Bacto Agar (Fisher) and autoclaving.
335

336 **Cloning strategy and yeast transformations**

337 The pRS303 and pRS305 vectors were used to clone the HIS and LEU markers for gene
338 deletions in W303α via homologous recombination. Single gene deletions of SER33, SER1,
339 SER2, HOM2, HOM6, MET2, MET6, MET17, CYS3, and CYS4 were deleted by amplifying
340 the LEU marker using PCR with 30 bp of the appropriate up and downstream overlaps to their
341 respective gene target (**Supplementary Table 1**). Double mutants were created by amplifying
342 the HIS marker with 30 bp of the appropriate overlap to the target gene and transformed into the
343 single deletant strains (**Supplementary Table 2**).
344

345 A constitutive yeast display vector constructed in the Belcher lab named pYAGA contains the
346 AGA1 and AGA2 gene downstream of a GAP promoter and upstream of a CYC1 terminator.
347 Single stranded sequences coding for hexa-peptide repeats were ordered from IDT and annealed
348 with sticky ends matching the BamHI and PmeI cloning sites of pYAGA (**Supplementary**
349 **Table 3**). Hexa-peptide sequences were phosphorylated with T4 PNK prior to ligation using T4
350 ligase (NEB). Circularized plasmids were transformed into chemically competent NEBα
351 following the recommended NEB protocol and selected using ampicillin.
352
353 Yeast transformations were performed using Frozen-EZ Yeast Transformation Kit II (Zymo).
354 For deletions, transformed cells were plated onto YPD for 1-2 days and replica plated onto drop
355 out media (either HIS, LEU, or both) to select for positive transformants. Otherwise, plasmid
356 transformations were grown directly onto plates with the appropriate drop-out media. Plasmid or
357 genomic DNA was isolated by using silica bead beating and phenol/chloroform (Sigma)
358 extraction. Sequences were confirmed by amplifying the isolated DNA using PCR and
359 sequencing the DNA fragment using QuintaraBio sequencing services.
360
361 **Screening and quantifying H_2S gas production**
362 Cultures were initially screened in 5 mL CSM cultures in 14 mL BD culture tubes with taped
363 lead acetate hydrogen sulfide indicator strips (VWR). Cultures were grown at 30^oC over 1-2 days
364 and H_2S was detected when strips became darkened. Quantitative sulfide detection was
365 monitored using Draeger hydrogen sulfide detection columns (VWR). 50 mL cultures in 250 mL
366 Erlenmeyer flasks were corked with a single-hole rubber stopper in which hydrogen sulfide
367 columns were fitted. Cultures grew for 1-2 days and were visually inspected at specific time-

368 points to measure sulfide production. Knockouts ΔSER33 and ΔCYS4 became auxotrophic to
369 cysteine while ΔHOM3 and ΔMET2 became slow growers on synthetically defined (SD) media.
370 Combination knockouts with ΔCYS4 produced extremely slow growers.
371
372 **OD_{600} culture density measurements**
373 Discrete time point optical density measurements were performed using 2 mL non-frosted
374 cuvettes (VWR) and a table-top DU800 Beckman Coulter spectrophotometer measuring at 600
375 nm. Continuous growth curve studies were performed on a shaking 96 well BioTek Synergy 2
376 plate reader held at 30ºC with 100 μL cultures. Cultures were first diluted from overnights to
377 < 0.1 OD_{600} and aliquoted into a 96-well round bottom plate (Cellstar) with the appropriate metal
378 and concentration.
379
380 **Quantifying metal precipitation**
381 Liquid stocks of copper (II) chloride, zinc chloride, cadmium nitrate, lead nitrate, and mercury
382 (II) chloride (Sigma) were made at 100 mM in water. Metal precipitation studies were performed
383 by diluting overnight cultures to varying culture densities in 5 mL of fresh culture containing 100
384 μM of metal. Cultures were grown overnight, spun down at 900x*g* for 3 min in a swinging
385 bucket rotor and supernatant collected for metal measurement. Metal content was measured on
386 an Agilent ICP-AES 5100 following standard operating procedures. Trace concentrations of
387 metal below 10 μM were measured on an Agilent ICP-MS 7900. If samples were to be diluted,
388 they were diluted in 3% HNO_3 (Sigma) to fit within the dynamic range of ICP detection.
389

390 For all experiments, a sample of just media with spiked metal (e.g. 100 μM) was measured to act
391 as a reference for the initial metal content of copper, zinc, cadmium, lead, and mercury in the
392 media. Metal removal measurements were calculated by taking the ICP measurements of the
393 supernatant and subtracting from this reference to give the quantity of metal precipitated.
394

395 Multiple uptake experiments were performed by resuspending 1 OD_{600} of fresh yeast grown the
396 previous day with the equivalent volume of supernatant from the current metal precipitation
397 experiment. For example, after the first round, the supernatant was collected and transferred to a
398 freshly spun down culture inoculated the day before to a final OD of 1. The precipitation
399 experiment was performed again, making this the second round of precipitation. This process
400 was repeated at most up to 4 times, with each iteration sampled for ICP measurement.
401

402 **Quantifying metal removal from oil sand samples**
403 Samples of effluent were taken from the Athabasca Oil Sands in Canada. Liquid was gently
404 centrifuged at 1000xg for 30 minutes to fractionate liquid, oil and solid phases. The liquid phase
405 was used as the waste medium to test for yeast-induced metal precipitation. Although not
406 thoroughly investigated in this study, the oil phase contained many organics, aromatics, and oils
407 contributed from mined runoff. The solid phase contained a heterogeneous mixture of large
408 debris, rocks, and precipitates that were easily spun down during centrifugation or through size-
409 exclusion filtration.
410

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

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

417

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

424

425 **Quantifying yeast display expression using flow cytometry**

426 Displayed peptides were first cloned with a C-terminus V5 tag followed by a stop codon in a
427 constitutive AGA1 and AGA2 vector which was called pYAGA. Cultures were grown to
428 saturating OD and 0.5 OD_{600} were taken for flow cytometry. Cells were washed and pelleted at
429 900x*g* with PBS+1% BSA. Primary antibodies against V5 (Life Technologies) were diluted
430 1:500 in PBS+1% BSA and incubated at room temperature for 1 hour. Secondary antibodies with
431 AlexaFluor 488 were diluted 1:2000 in PBS+1% BSA and incubated at room temperature for 1
432 hour. Cells were then washed and diluted to 1e6 cells per mL for flow cytometry. Flow
433 cytometry was performed on a BD FACS Canto or LSR II following standard operating
434 procedure provided by the Koch Flow Cytometry Core. Yeast cell gating strategy followed:
435 FSC-A and SSC-A was used to gate on cells. FSC-W and FSC-H was used to gate vertically

436 oriented single cells (vertical singlets). SSC-W and SSC-H was used to gate horizontally oriented
437 single cells (horizontal singlets). After gating on these 3 plots, single cells were measured based
438 on fluorescence (**Supplementary Figure 11**). Cell counts were plotted against binned
439 fluorescent intensity (x-axis) creating a population distribution histogram of fluorescence (y-
440 axis).
441
442 **Extraction and purification of precipitated metal sulfides**
443 Overnight cultures of metals added to yeast were pelleted at 900x*g* for 3 min. Cultures were
444 washed and resuspended in 1 mL sorbitol citrate. 100T Zymolyase (Zymo) was diluted 1 to 100
445 and added to the suspension and incubated for >1 hour at 30°C while shaking. Digested cells
446 were pelleted at 900x*g* for 3 min, and supernatant was removed or kept for later analysis of
447 dislodged metal sulfide particles. Cells were resuspended with 1:1 water and oleic acid (organic
448 layer; Sigma). Mixtures were spun down at 900xg for 3 min to pellet cellular debris while
449 allowing insoluble metal sulfide particles to remain in the organic layer. The organic layer was
450 removed and fresh oleic acid was introduced to further extract metal sulfide particles. This
451 process was performed between 1-3 times until coloration was completely transferred into the
452 organic layer. Most organic solvents were observed to work (phenol:chloroform, hexane,
453 octonal, etc.), however oleic acid was more cost effective, easier to handle, and safer to use.
454 Samples could be used immediately for analysis or concentrated by spinning down particles at
455 max speed for 15 min and resuspended in a lower volume in either oleic acid or water.
456
457 **Excitation and emission measurements using fluorometry**

458 An Agilent Cary Eclipse Fluorescence Spectrophotometer was used to measure the fluorescence
459 of the isolated metal sulfide particles using disposable PMMA acrylic cuvettes (VWR).
460 Excitation and emission scans were performed following standard operating procedures provided
461 by the Center of Material Science Engineering, MIT.
462

463 **TEM sample prep**

464 Cells were not digested with zymolayse in order to preserve the cell wall for imaging. Cell
465 fixation, dehydration, embedding, and sectioning followed yeast OTO processing provided by
466 the WhiteHead Institute, MIT[52]. The yeast cells were grown to an appropriate optical density and
467 fixed with 2% glutaraldehyde, 3% paraformaldehyde, 5% sucrose in 0.1 M sodium cacodylate
468 buffer (EMS) for 1 hour. Pelleted cells were washed and stained for 30 minutes in 1% OsO_4, 1%
469 potassium ferocyanide, and 5 mM $CaCl_2$ in 0.1 M cacodylate buffer. Osmium staining was
470 followed by washing and staining in 1% thiocarbohydrazide. Pellets were washed and stained
471 again in the reduced osmium solution. The cells were then stained in 2% uranyl acetate (EMS)
472 overnight, serially dehydrated with ethanol, and embedded in EMBED-812 (EMS). Sections
473 were cut on a Leica EM UC7 ultra microtome with a Diatome diamond knife at a thickness
474 setting of 50 nm, stained with 2% uranyl acetate, and lead citrate. The sections were examined
475 using a FEI Tecnai Spirit at 80KV and photographed with an AMT CCD camera.
476

477 **TEM and EDX analysis**

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

481 dispersive x-ray (EDX) spectroscopy to qualitatively determine the relative amounts of sulfide
482 and metal. When necessary, for example with copper, the signal background was corrected by
483 subtracting the spectrum with a region without any metal sulfide particles to deconvolve
484 overlapping peaks from the copper grid. High resolution TEM (HRTEM) images were acquired
485 on a JEOL2010F at 200V to observe crystal spacing. A JEOL2010F was used for more resolved
486 EDX elemental mapping of metal sulfide particles that nucleated on the cell wall.
487
488 Purified metal sulfide particles were analyzed for size distribution and morphology using TEM.
489 Size distribution data was determined by imaging 40 random locations on 3 separate samples of
490 isolated metal precipitates using TEM. Particles below 100 nm were imaged on the higher
491 resolution JEOL2010F at greater than 100,000x magnification. Sizes were quantitatively
492 measured using ImageJ, and distributions plotted as histograms.
493
494 **Figure creation, analysis, and plotting**
495 Raw data was collected and stored as csv or Excel file formats. Data was imported and analyzed
496 with Python using modules such as numpy, pandas, and scipy. Plots were graphed with
497 matplotlib.
498
499 **Statistical analysis**
500 Statistical parameters including the definitions and values of *n*, SDs, and/or SEs are reported in
501 the figures and corresponding figure legends. When reporting significance, a two-tailed unpaired
502 t-test was performed between observations and p-values reported in the text. The significance
503 threshold was set to $p < 0.05$ for all experiments, or as specified in the text.

504

505 **Data availability**

506 The datasets generated and analyzed during the current study are available from the

507 corresponding author upon request. The source data underlying Figures 1c, 1d, 2a, 3c, 4a-c, 5e,

508 and Extended Data 2a, 2c, and 3b are provided as a Source Data File.

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636

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638

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650 **AUTHOR CONTRIBTUIONS**

651 G.L.S, E.E.R, and A.M.B. conceived the study and designed experiments; G.L.S. performed and
652 E.E.R. helped with experiments; G.L.S. analyzed the data and assembled figures; G.L.S, E.E.R,
653 and A.M.B wrote the manuscript.
654

655 **COMPETING INTEREST**

656 The authors declare no competing interests.