

Copper isotope fractionation during adsorption and intracellular incorporation by bacteria: Isotopic data

Starting solution for all adsorption experiments $\delta^{65}\text{Cu} = 0.712 \pm 0.14$ unless otherwise noted

n = number of replicate analyses of the same sample, d = number of column replicates included

All adsorption experiments conducted using a 0.01M NaClO_4 electrolyte solution.

Control experiment with 10 mg/L Cu only, no bacteria

| Sample ID | F precipitated | pH | $\delta^{65}\text{Cu}$ solution | n | d | 2 sigma |
|-----------|----------------|------|---------------------------------|---|---|---------|
| 21.2 | 0.40 | 6.44 | 1.077 | 2 | | 0.105 |
| 21.3 | 0.01 | 3.30 | 0.722 | 1 | 2 | 0.193 |
| 21.5 | 0.05 | 6.12 | 0.748 | 1 | | |
| 21.7 | 0.03 | 4.49 | 0.673 | 1 | | |
| 21.9 | 0.58 | 6.67 | 1.130 | 2 | | 0.112 |
| 21.10. | 0.94 | 7.31 | -1.308 | 2 | | 0.049 |

***E. coli* adsorption vs. pH, 5g/L bacteria, 10 mg/L Cu**

| Sample ID | F adsorbed | pH | $\delta^{65}\text{Cu}$ solution | n | d | 2 sigma |
|-----------|------------|------|---------------------------------|---|---|---------|
| Cu.8.1.U | 0.21 | 2.79 | 0.719 | 1 | | |
| Cu.8.3.U | 0.35 | 3.63 | 1.340 | 1 | | |
| Cu.8.5.U | 0.39 | 3.91 | 1.307 | 1 | | |
| Cu.8.1.F | 0.21 | 2.85 | 0.974 | 1 | | |
| Cu.8.3.F | 0.24 | 2.91 | 1.097 | 2 | 2 | 0.152 |
| Cu.8.6.F | 0.84 | 5.61 | 1.580 | 1 | | |
| Cu.8.8.F | 0.45 | 3.94 | 1.891 | 2 | | 0.124 |
| Cu.8.9.F | 0.88 | 5.90 | 2.009 | 1 | | |
| Cu.8.10.F | 0.43 | 3.22 | 1.524 | 1 | | |

***E. coli* adsorption vs. pH, 15g/L bacteria, 2 mg/L Cu**

| Sample ID | F adsorbed | pH | $\delta^{65}\text{Cu}$ solution | n | d | 2 sigma |
|-----------|------------|------|---------------------------------|---|---|---------|
| 4B.1.Cu | 0.95 | 6.45 | 0.943 | 1 | | |
| 4B.2.Cu | 0.64 | 4.22 | 1.276 | 1 | | |
| 4B.3.Cu | 0.90 | 5.30 | 1.333 | 1 | | |
| 4B.4.Cu | 0.93 | 5.03 | 0.770 | 1 | | |
| 4B.5.Cu | 0.45 | 2.49 | 1.253 | 1 | | |
| 4B.6.Cu | 0.72 | 3.76 | 0.873 | 1 | | |
| 4B.7.Cu | 0.80 | 3.96 | 1.565 | 1 | | |

4B.8.Cu 0.67 3.14 1.703 1

B. subtilis adsorption vs. pH, 5g/L bacteria, 10 mg/L Cu

| Sample ID | F adsorbed | pH | δ65Cu solution | n | d | 2 sigma |
|-----------|------------|------|----------------|---|---|---------|
| 20.1 | 0.43 | 4.30 | 0.603 | 1 | | |
| 20.2 | 0.27 | 2.09 | 0.629 | 1 | | |
| 20.3 | 0.27 | 3.15 | 0.932 | 3 | 2 | 0.184 |
| 20.4 | 0.65 | 5.30 | 0.966 | 3 | 2 | 0.164 |
| 20.5 | 0.64 | 6.57 | 1.037 | 2 | 2 | 0.085 |
| 20.6 | 0.43 | 4.44 | 1.171 | 2 | | 0.145 |
| 20.7 | 0.50 | 4.03 | 0.232 | 1 | | |
| 20.8 | 0.17 | 2.90 | 0.704 | 1 | | |

E. coli metal loading experiment, 5g/L bacteria, variable Cu

| Sample ID | F adsorbed | pH | δ65Cu solution | n | d | 2 sigma |
|--------------|------------|------|----------------|---|---|---------|
| 12.1.Cu.Load | 0.43 | 4.00 | 1.046 | 2 | | 0.070 |
| 12.2.Cu.Load | 0.26 | 4.00 | 1.316 | 2 | | 0.009 |
| 12.3.Cu.Load | 0.13 | 4.00 | 1.281 | 2 | 2 | 0.289 |

B. subtilis metal loading experiment, 1g/L bacteria, variable Cu (δ65 of Cu(ClO4)2 starting soln = 0.88‰)

| Sample ID | F adsorbed | pH | mg/L Cu added | δ65Cu solution | n | d | 2 sigma |
|-----------|------------|------|---------------|----------------|---|---|---------|
| 31.1 | 0.19 | 4.00 | 4.98 | 1.254 | 1 | | |
| 31.2 | 0.14 | 4.00 | 10.09 | 1.462 | 1 | | |
| 31.3 | 0.07 | 4.00 | 25.49 | 1.387 | 1 | | |

Heat-killed E. coli adsorption vs. pH, 5g/L bacteria, 10 mg/L Cu(II)

| Sample ID | F adsorbed | pH | δ65Cu solution | n | d | 2 sigma |
|-----------|------------|------|----------------|---|---|---------|
| 40.2.Cu | 0.27 | 2.80 | 0.417 | 2 | 2 | 0.108 |
| 40.3.Cu | 0.38 | 3.40 | 0.750 | 1 | | |
| 40.4.Cu | 0.45 | 3.90 | 0.851 | 2 | | 0.372 |
| 40.5.Cu | 0.44 | 4.00 | 0.816 | 1 | | |
| 40.6.Cu | 0.45 | 4.10 | 0.399 | 2 | | 0.260 |
| 40.8.Cu | 0.75 | 5.11 | 0.341 | 2 | 2 | 0.014 |

48 hour kinetics experiment with E. coli, 5g/L bacteria, 10 mg/L Cu(II)

| Sample ID | F adsorbed | pH | sample time (hours) | δ65Cu solution | n | d | 2 sigma |
|-----------|------------|------|---------------------|----------------|---|---|---------|
| 39.1.Cu | 0.22 | 4.30 | 0.08 | 1.265 | 1 | | |
| 39.2.Cu | 0.23 | 4.30 | 0.08 | 1.361 | 1 | | |
| 39.3.Cu | 0.28 | 4.30 | 0.25 | 1.811 | 1 | | |
| 39.4.Cu | 0.32 | 4.30 | 0.50 | 1.019 | 1 | | |
| 39.5.Cu | 0.36 | 4.30 | 1.00 | 1.370 | 1 | | |

| | | | | | | | |
|---------|------|------|-------|-------|---|--|-------|
| 39.6.Cu | 0.37 | 4.30 | 1.33 | 1.229 | 1 | | |
| 39.7.Cu | 0.52 | 4.30 | 25.00 | 1.325 | 1 | | |
| 39.8.Cu | 0.55 | 4.30 | 48.00 | 1.618 | 2 | | 0.131 |

***E. coli* kinetics and reversibility experiment**
5g/L bacteria, 10 mg/L Cu(II)

| Sample ID | F adsorbed | pH | sample time (hours) | $\delta^{65}\text{Cu}$ solution | n | d | 2 sigma |
|--------------|------------|------|---------------------|---------------------------------|---|---|---------|
| 11.1.Cu.Kin | 0.17 | 4.00 | 0.08 | 0.669 | 2 | 2 | 0.098 |
| 11.2.Cu.Kin | 0.18 | 4.00 | 0.08 | 0.981 | 2 | 2 | 0.150 |
| 11.3.Cu.Kin | 0.20 | 4.00 | 0.25 | 0.860 | 2 | | 0.486 |
| 11.4.Cu.Kin | 0.19 | 4.00 | 0.28 | 0.762 | 1 | | |
| 11.6.Cu.Kin | 0.18 | 4.00 | 0.50 | 0.511 | 2 | | 0.072 |
| 11.7.Cu.Kin | 0.21 | 4.00 | 1.00 | 1.478 | 3 | 2 | 0.063 |
| 11.8.Cu.Kin | 0.22 | 4.00 | 2.00 | 0.525 | 2 | | 0.088 |
| 11.9.Cu.Kin | 0.20 | 4.00 | 3.00 | 0.948 | 1 | | |
| 11.10.Cu.Kin | 0.44 | 5.00 | 3.12 | 0.903 | 2 | | 0.170 |
| 11.11.Cu.Kin | 0.41 | 5.00 | 3.28 | 0.947 | 1 | | |
| 11.12.Cu.Kin | 0.44 | 5.00 | 3.53 | 0.955 | 1 | | |
| 11.14.Cu.Kin | 0.45 | 5.00 | 5.00 | 1.028 | 1 | | |
| 11.15.Cu.Kin | 0.45 | 5.00 | 5.00 | 1.130 | 1 | | |
| 11.16.Cu.Kin | 0.35 | 4.00 | 5.02 | 0.894 | 2 | | 0.032 |
| 11.17.Cu.Kin | 0.31 | 4.00 | 5.13 | 0.672 | 1 | | |
| 11.18.Cu.Kin | 0.31 | 4.00 | 5.45 | 1.203 | 1 | | |
| 11.19.Cu.Kin | 0.29 | 4.00 | 5.55 | 1.373 | 1 | | |
| 11.20.Cu.Kin | 0.28 | 4.00 | 5.55 | 0.862 | 1 | | |
| 11.21.Cu.Kin | 0.29 | 4.00 | 6.00 | 0.988 | 1 | | |
| 11.22.Cu.Kin | 0.29 | 4.00 | 6.50 | 0.652 | 1 | | |
| 11.23.Cu.Kin | 0.35 | 4.00 | 24.00 | 1.179 | 1 | | |

***B. subtilis* kinetics and reversibility experiment**
bacteria, 10 mg/L Cu(II)

5g/L

| Sample ID | F adsorbed | pH | sample time (hours) | $\delta^{65}\text{Cu}$ solution | n | d | 2 sigma |
|-----------|------------|------|---------------------|---------------------------------|---|---|---------|
| 30.1 | 0.42 | 3.93 | 0.08 | 1.034 | 1 | | |
| 30.2 | 0.43 | 3.93 | 0.42 | 1.140 | 1 | | |
| 30.3 | 0.43 | 3.90 | 0.87 | 1.263 | 2 | 2 | 0.047 |
| 30.4 | 0.43 | 3.89 | 1.00 | 1.065 | 1 | | |
| 30.5 | 0.72 | 4.96 | 1.08 | 1.332 | 1 | | |
| 30.6 | 0.72 | 4.80 | 1.18 | 1.452 | 1 | | |
| 30.7 | 0.73 | 5.05 | 1.87 | 1.622 | 2 | 2 | 0.190 |

| | | | | | | |
|--------|------|------|------|-------|---|-------|
| 30.8 | 0.65 | 4.60 | 3.00 | 1.267 | 1 | |
| 30.9 | 0.41 | 3.90 | 3.03 | 1.236 | 1 | |
| 30.10. | 0.45 | 3.90 | 3.13 | 1.351 | 2 | 0.127 |

Starting solution (Cu-citrate) for all intracellular incorporation experiments

$\delta^{65}\text{Cu} = -0.451 \pm 0.122$

| <i>E. coli</i> intracellular Cu | | | | | |
|--|------------|------|------------------------------|---|-------|
| | F Cu SOLID | pH | $\delta^{65}\text{Cu}$ SOLID | n | |
| EC 1 | 0.01 | 7.00 | -3.045 | 1 | |
| EC 2 | 0.02 | 7.00 | -2.493 | 1 | |
| <i>B. subtilis</i> intracellular Cu | | | | | |
| | F Cu SOLID | pH | $\delta^{65}\text{Cu}$ SOLID | n | |
| BS 1 | 0.01 | 7.00 | -1.680 | 1 | |
| BS 2 | 0.02 | 7.00 | -1.658 | 1 | |
| Rio Grande Reservoir consortia intracellular Cu | | | | | |
| | F Cu SOLID | pH | $\delta^{65}\text{Cu}$ SOLID | n | |
| RGB 1 | 0.40 | 7.00 | -3.085 | 1 | |
| Cement Creek consortia intracellular Cu | | | | | |
| | F Cu SOLID | pH | $\delta^{65}\text{Cu}$ SOLID | n | |
| NB 9/24 2 | 0.13 | 7.00 | -2.222 | 2 | 0.027 |
| NB 9/26 1 | 0.27 | 7.00 | -1.804 | 1 | |

| Atomic adsorption standard | | | | | |
|------------------------------------|----|---|------------------------|---------|--|
| | n | d | $\delta^{65}\text{Cu}$ | 2 sigma | |
| AA std | 15 | 5 | 0.712 | 0.140 | |
| Copper rod standard | | | | | |
| | n | d | $\delta^{65}\text{Cu}$ | 2 sigma | |
| Cu ROD | 12 | 3 | 0.716 | 0.166 | |
| Copper-citrate | | | | | |
| | n | d | $\delta^{65}\text{Cu}$ | 2 sigma | |
| Cu-citrate | 4 | 2 | -0.451 | 0.122 | |
| Copper perchlorate | | | | | |
| | n | d | $\delta^{65}\text{Cu}$ | 2 sigma | |
| Cu(ClO ₄) ₂ | 3 | | 0.883 | 0.139 | |

