

1 **SUPPLEMENTAL MATERIAL**

2 **for:**

3
4 **Enhanced uranium immobilization and reduction by *Geobacter sulfurreducens* biofilms**

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10 Running Head: Uranium reduction by *Geobacter* biofilms

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18 **TABLES**

19 **Table S1.** U immobilization and reduction by WT and mutant (pilA and pilA+) biofilms grown for
 20 24, 48, and/or 72 h. ^a

Biofilm (strain/age)	Total U immobilized (mM)	U(IV) ^b (mM)
WT/24h	0.183 (0.018)	0.033 (0.01)
WT/48h	0.238 (0.033)	0.161 (0.031)
WT/72h	0.267 (0.024)	0.248 (0.017)
pilA/48h	0.155 (0.009)	0.059 (0.003)
pilA+/48h	0.313 (0.026)	0.238 (0.020)

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 22 ^a Shown are averages and standard deviations or errors (in brackets) of three (WT samples) or
 23 two (pilA and pilA+) independent biofilm assays, respectively.

24 ^b Fraction of the immobilized U reduced to U(IV) (estimated by XANES)

25
 26 **Table S2.** Statistical analyses of biofilm structural parameters determined from confocal
 27 micrographs of the biofilms with the COMSTAT software (1). ^a

Biofilm (strain/age)	Total biomass ($\mu\text{m}^3/\mu\text{m}^2$)	Surface Coverage (%)	Average Thickness (μm)	Roughness coefficient (0 to ∞)	Surface Area ($\times 1000$ μm^2)	Surface/ volume ratio ($\mu\text{m}^2/\mu\text{m}^3$)	Maximum Thickness (μm)
WT/24h	6.35 (0.96)	80.72 (5.15)	7.78 (1.13)	0.26 (0.07)	596 (80)	2.92 (0.27)	17.23 (3.59)
WT/48h	10.63 (1.29)	91.84 (1.66)	13.25 (1.83)	0.18 (0.03)	1233 (155)	3.6 (0.42)	25.95 (2.97)
WT/72h	10.56 (3.27)	92.24 (6.62)	13.93 (3.86)	0.24 (0.06)	1010 (233)	3.05 (0.36)	28.58 (4.84)
pilA/48h	5.21 (0.69)	77.79 (3.55)	6.79 (0.98)	0.32 (0.04)	573 (87)	3.41 (0.33)	17.90 (1.64)
pilA+/48h	43.42 (14.78)	99.53 (0.54)	60.06 (18.86)	0.06 (0.10)	6072 (2456)	1.2 (0.7)	64.02 (17.48)

28 ^a Shown are averages and standard deviations (in brackets) of 16-30 replicate fields for each
 29 strain.

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Table S3. EXAFS modeling results for R and σ^{2**}

Path	CN	R (Å)	σ^2 ($\cdot 10^{-3}$ Å ²)
Oax	Noax	1.80 ± 0.01	1*
Oeq	Noeq	2.38 ± 0.02	**
C1	Nc1	2.86 ± 0.01	2 ± 7
C2	Nc2	3.49 ± 0.03	2 ± 7
Oax1-Oax2	Noax	3.61 ± 0.02	2*
Oax1-U-Oax2	Noax	3.61 ± 0.02	2*
Oax1-U-Oax1	2Noax	3.61 ± 0.02	4*
C3	Nc1	4.54 ± 0.07	2 ± 7
C1-C3	2Nc1	4.54 ± 0.07	2 ± 7
C1-C3-C1	Nc1	4.54 ± 0.07	2 ± 7
Odist	Nc2	4.69 ± 0.08	2 ± 7
C2-Odist	2Nc2	4.72 ± 0.08	2 ± 7
C2-Odist-C2	Nc2	4.74 ± 0.08	2 ± 7

*value held, **pilA: 25 ± 6, WT: 18 ± 4

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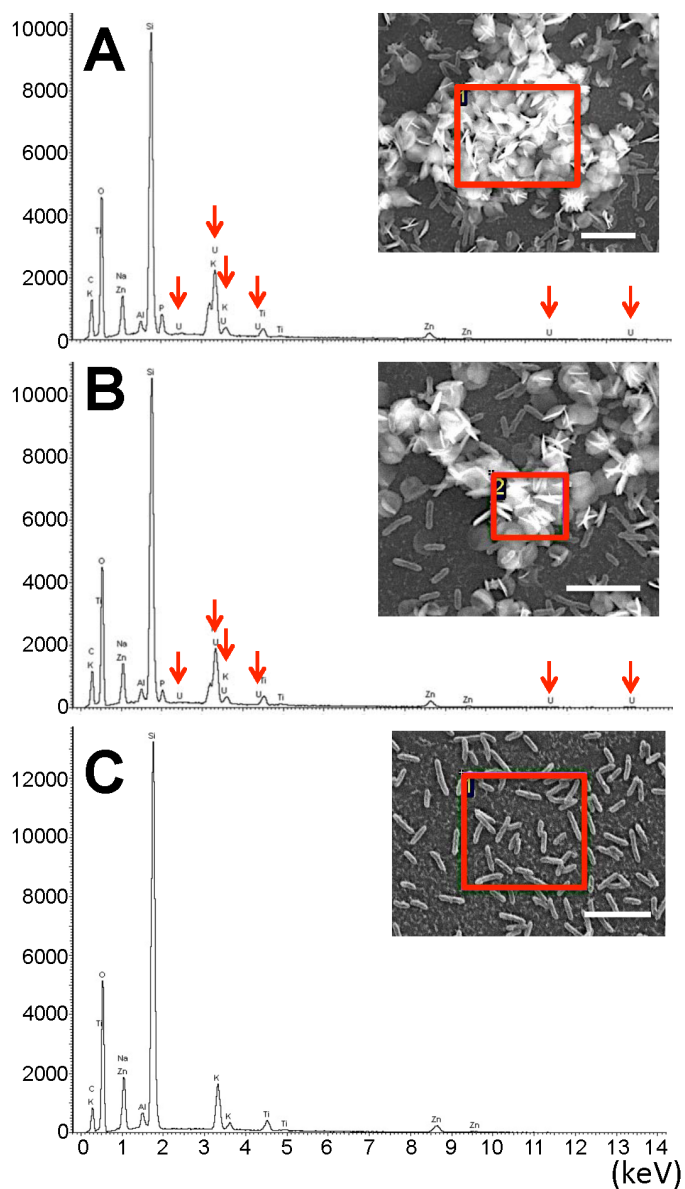
Table S4. EXAFS modeling results for coordination numbers

data set	Noax	Noeq	C1	C2
WT	1.0 ± 0.1	6.3 ± 1.4	1.4 ± 0.7	1.4 ± 0.8
pilA	1.5 ± 0.1	6.9 ± 2.2	1.2 ± 0.7	1.2 ± 0.7

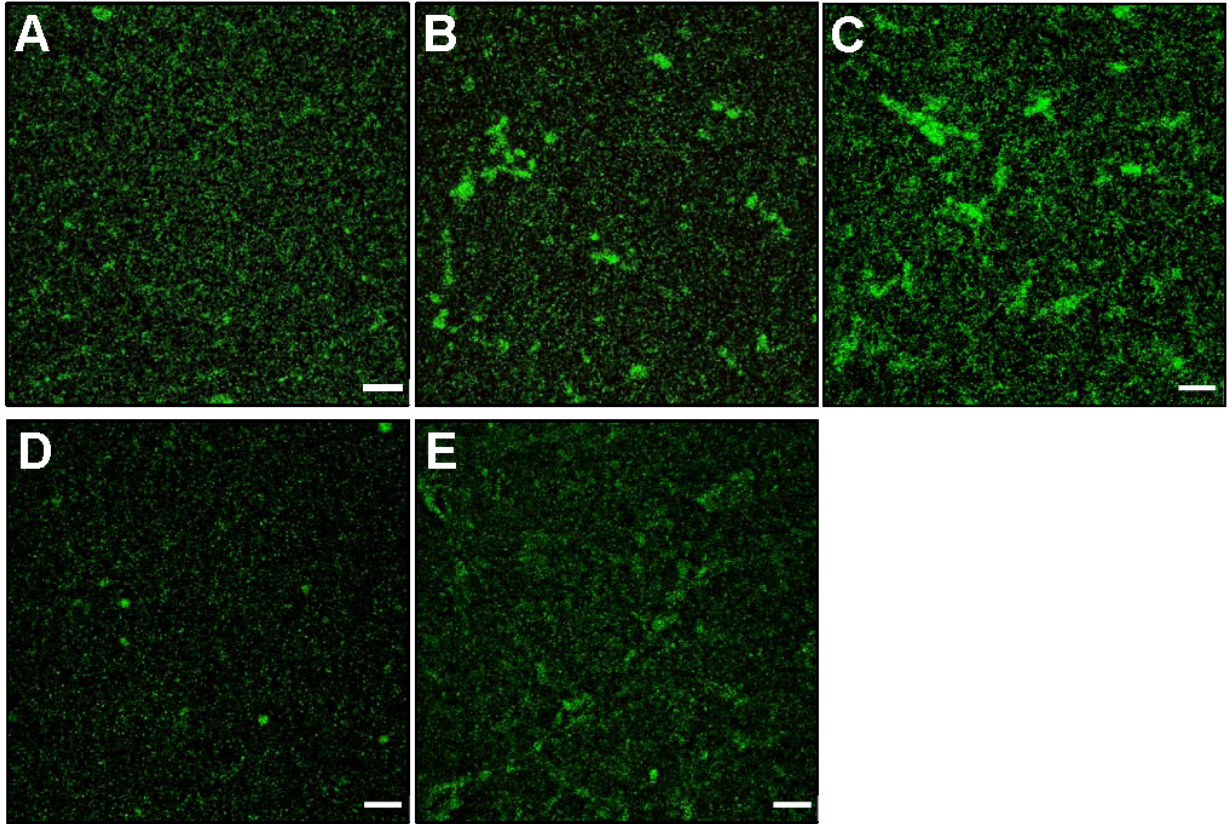
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36 FIGURES



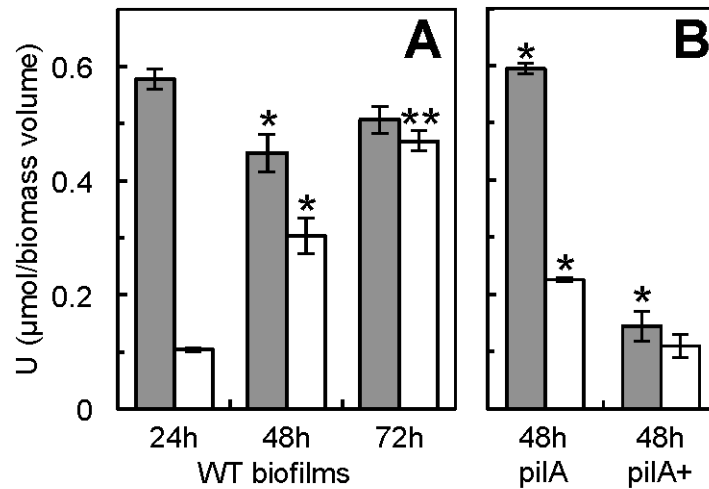
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38 **Fig. S1: Elemental analyses of biofilm-associate precipitates.** EDS spectra of regions of 48-
39 h old biofilms (boxed regions in SEM micrographs shown in insets) showing the elemental
40 composition of the mineral associated to biofilms exposed to 1 mM uranyl acetate for 24 h (**A**
41 and **B**) in reference to unexposed controls (**C**). The U peaks are highlighted with red arrows.
42 Scale bar in insets, 5 μm.
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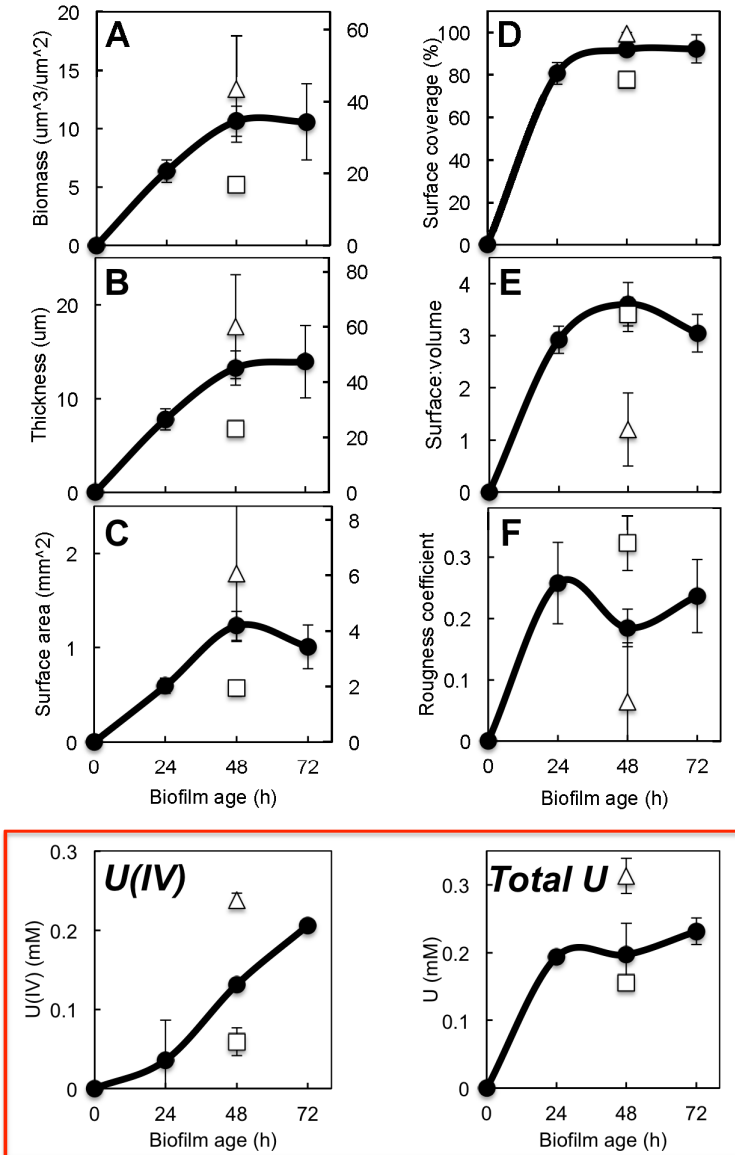
45 **Fig. S2:** CLSM micrographs showing top view projections of 24-, 48- and 72-h WT biofilms (A,
46 B, and C, respectively), and 48-h pilA (D) and pilA+ (E) biofilms stained the BacLight™ viability
47 kit (green, live cells; red, dead cells). Scale bar, 20 μm.

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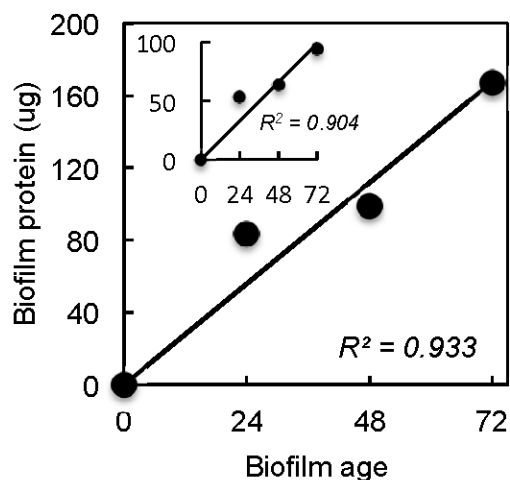
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51 **Fig. S3: U immobilization and reduction per biofilm biomass unit.** Total U immobilized
 52 (gray) and fraction reduced to U(IV) (white) per biomass volume unit by WT biofilms grown for
 53 24, 48 and 72 h (**A**) and by 48-h old biofilms of the pilin-deficient pilA mutant and the
 54 hyperpilated pilA+ strains alone (**B**). The biofilms were exposed to 1 mM of uranyl acetate for
 55 24 h. Shown are averages and standard deviation/error of triplicate (WT) or duplicate (pilA and
 56 pilA+) biofilm samples, respectively, normalized by the biomass volume estimated with the
 57 COMSTAT software analysis (Table S2). Significant differences ($p < 0.05$ and $p < 0.005$) in *t*-
 58 test pairwise comparisons with the 24-h (**A**) or 48-h (**B**) WT biofilms are indicated with one or
 59 two stars, respectively.

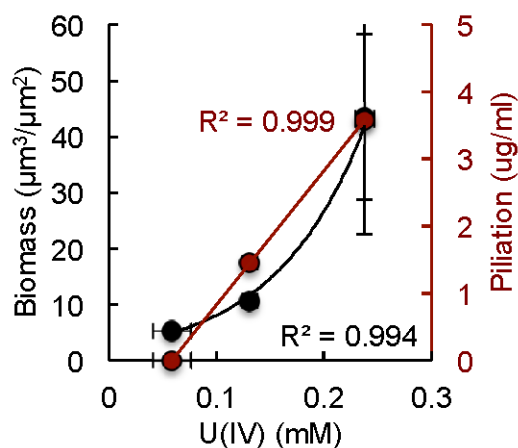


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61 **Fig. S4:** Correlation between the age of the WT (circles), pilA (squares), and pilA+ (triangles)
 62 biofilms and biofilm structural parameters such as biomass (A), thickness (B), surface area (C),
 63 surface coverage (D), surface to volume ratio (E), and roughness coefficient (F). The secondary
 64 Y axis in some of the plots is the same as the primary axis but at a larger scale to accommodate
 65 to the higher values of the pilA+ biofilm values. The structural parameters showing similar
 66 trends have been aligned vertically (left and right). For comparisons, the time course plots of U
 67 immobilization (total U) and reduction U(IV) by the biofilms are also provided (boxed in red,
 68 plotted from data shown in Tables S1).



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 70 **Fig. S5:** Linear correlation between the total protein content of biofilms and biofilm age in plastic
 71 wells of 6-well plate (main) and coverslip assembly (inset) assays.
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 74 **Fig. S6:** Correlation between U reduction (fraction of the immobilized U reduced to U(IV)) by 48-
 75 h old pilA, WT, and pilA+ biofilms and the biomass volume (left axis) or piliation (right axis,
 76 piliation levels as reported by (2)). The correlation with piliation is linear whereas but exponential
 77 with biomass.

78 **REFERENCES**

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