Supplemental Information for:

Effect of Bacterial Growth Stage on the Response to Two-Dimensional Nanomaterials

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Supplemental Methods:

2D nanomaterial synthesis and characterization

Suspended MoS_2 and $MoSe_2$ nanosheets were synthesized from bulk MoS_2 and $MoSe_2$ powders by a previously established chemical exfoliation method.[1] In that method, 3 mL of 1.6 M n-butyllithium in hexane was added to 300 mg of MoS_2 bulk powder. For $MoSe_2$ nanosheets, 3 mL of n-butyllithium was added to 476 mg $MoSe_2$ bulk powder. Both were left under mild stirring conditions at room temperature in a nitrogen purged glove box for 24 and 48 hrs for $MoSe_2$ and MoS_2, respectively. The resulting intercalation compounds were washed twice via centrifugation of 50 mL samples at 4000 RPM for 30 min with hexane to remove excess organo-lithium reagent and any organic by-products. The intercalation compound was then decomposed in DI water in an ultrasonic bath for 30 min to achieve exfoliation. Any unexfoliated MoS_2 or $MoSe_2$ solids were removed by centrifugation at 1000 RPM for 15 minutes. The final supernatant contained well-dispersed TMD nanosheets from which lithium hydroxide (LiOH) was removed through dialysis (3.5kDa MWCO), using DI water as a receiving fluid. The receiving fluid pH was monitored and refreshed when it became basic approximately 6 times over a 3-day period until the receiving fluid remained neutral. Dialysis was performed in a nitrogen purged glove bag.

GO in suspension was produced using a modified Hummers' method using the protocol from Li et al. 2013.[2] Briefly, 10 g of potassium persulfate and 10 g phosphorus pentoxide were dissolved in 100 mL sulfuric acid that has been pre-heated to 80ºC. This formed a pretreatment acid wash that was applied to 14 g of Bay Carbon Inc. SP-1 grade graphite powder, and the mixture held at 80ºC for 5 hrs. The mixture is rapidly cooled in an ice bath and the acid is slowly dissociated with 200 mL of DI water. The resulting mixture was vacuum filtered through a 0.2 μm filter, rinsed with 1L of DI water, and left to dry overnight. The intercalated graphite was then added to a

mixture of 10 g sodium nitrate dissolved in 500 mL of sulfuric acid. 70 g of potassium permanganate was slowly added while controlling the resulting exothermic reaction by keeping the mixture temperature below 10ºC. Once potassium permanganate was fully dissolved, it was then gently heated to 40º C and held for 3 hrs and then quickly cooled once again in an ice bath. The acid was then dissociated with the addition of 1L of DI water and limiting the temperature to 55ºC. 60 mL of 30% hydrogen peroxide was then added dropwise and then left stirring over night to allow for complete consumption. The resulting solution was then acid washed to remove any residual salts with 1M hydrochloric acid and centrifuged at 4000 RPM for 30 min, which was done 5 times. This was followed by an acetone wash under the same centrifugation conditions. The resulting material was then left to dry for 72 hours in the fume hood. Once fully dried, 4 g of dried sample was dispersed in 1L of DI and sonicated for 40 min and then centrifuged for 5 min at 1000rpm for a total of 3 times.

X-ray diffraction (XRD) analysis was performed on a Bruker D8 Discover X-ray Diffraction System. X-ray photoelectron spectroscopy (XPS) analysis was performed on a Thermo Scientific K-Alpha XPS.

Table S1: Microplate layout for respiration and growth assays

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
A																2	4	7	9	12	14	Bac	A
B																3	5	8	10	13	15	DI	B
C																2	4	7	9	12	14	Bac	C
D																3	5	8	10	13	15	DI	D
E																2	4	7	9	12	14	Bac	E
F																3	5	8	10	13	15	DI	F
G																2	4	7	9	12	14	Bac	G
H	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	3	5	8	10	13	15	DI	H
I																2	4	7	9	12	14	Bac	I
J																3	5	8	10	13	15	DI	J
K																2	4	7	9	12	14	Bac	K
L																3	5	8	10	13	15	DI	L
M																2	4	7	9	12	14	Bac	M
N																3	5	8	10	13	15	DI	N
O																2	4	7	9	12	14	Bac	O
P																3	5	8	10	13	15	DI	P
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	

2	Graphene oxide-2.27 µg/mL Column 2-Samples; Column 17 (A, C, E, etc.)-Blanks no bacteria
3	Graphene oxide-1.14 µg/mL Column 3-Samples; Column 17 (B, D, F, etc.)-Blanks no bacteria
4	Graphene oxide-0.57 µg/mL Column 4-Samples; Column 18 (A, C, E, etc.)-Blanks no bacteria
5	Graphene oxide-0.11 µg/mL Column 5-Samples; Column 18 (B, D, F, etc.)-Blanks no bacteria
6	Graphene oxide-0.00 µg/mL Column 6-Samples
7	MoS_2-2.50 µg/mL Column 7-Samples; Column 19 (A, C, E, etc.)-Blanks no bacteria
8	MoS_2-1.14 µg/mL Column 8-Samples; Column 19 (B, D, F, etc.)-Blanks no bacteria
9	MoS_2-0.62 µg/mL Column 9-Samples; Column 20 (A, C, E, etc.)-Blanks no bacteria
10	MoS_2-0.12 µg/mL Column 10-Samples; Column 20 (B, D, F, etc.)-Blanks no bacteria
11	MoS_2-0.00 µg/mL Column 11-Samples
12	$MoSe_2$-2.52 µg/mL Column 12-Samples; Column 21 (A, C, E, etc.)-Blanks no bacteria
13	$MoSe_2$-1.26 µg/mL Column 13-Samples; Column 21 (B, D, F, etc.)-Blanks no bacteria
14	$MoSe_2$-0.63 µg/mL Column 14-Samples; Column 22 (A, C, E, etc.)-Blanks no bacteria
15	$MoSe_2$-0.13 µg/mL Column 15-Samples; Column 22 (B, D, F, etc.)-Blanks no bacteria
16	$MoSe_2$-0.00 µg/mL Column 16-Samples
Bac	Bacteria and DI water
DI	DI water

Table S2: Microplate layouts for membrane permeability assays

Plate 1:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**		
C		**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**		
D		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**DI**	**DI**		
E		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Blank**	**Blank**		
F		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**PBS**	**PBS**		
G		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Stain**	**Stain**		
H												

Plate 2:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**		
C		**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**	**STD**		
D		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**DI**	**DI**		
E		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Blank**	**Blank**		
F		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**PBS**	**PBS**		
G		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Stain**	**Stain**		
H												

STD	Standards for percent membrane permeability
2	Graphene oxide-2.27 µg/mL Plate 1 rows D & E -blanks (no bacteria)
3	Graphene oxide-0.11 µg/mL Plate 1 rows D & E-blanks (no bacteria)
4	MoS_2-2.50 µg/mL Plate 1 rows D & E-blanks (no bacteria)
5	MoS_2-0.12 µg/mL Plate 1 rows D & E-blanks (no bacteria)
6	$MoSe_2$-2.52 µg/mL Plate 1 rows D & E-blanks (no bacteria)
7	$MoSe_2$-0.13 µg/mL Plate 1 rows D & E-blanks (no bacteria)
8	Control-0.00 µg/mL Plate 1 rows D & E-blanks (no bacteria)
DI	DI water (200 µL)
Blank	10% PBS (100 µL) + stain solution (100 µL)
PBS	10 % PBS (100 µL) + DI water (100 µL)
Stain	Stain solution (100 µL) + DI water (100 µL)

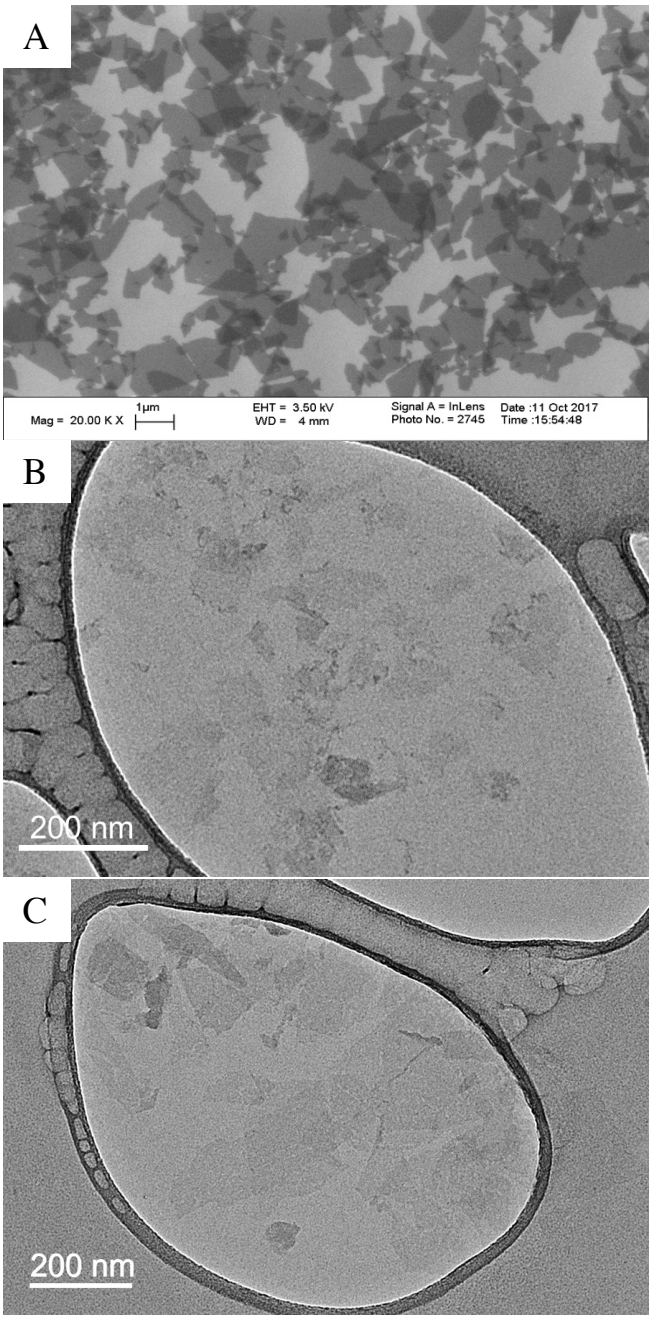

Figure S1: Nanosheets used in this study (A) graphene oxide (GO), (B) MoS_2, and (C) $MoSe_2$. The GO image was acquired using scanning electron microscopy and the Mo materials were imaged with transmission electron microscopy.

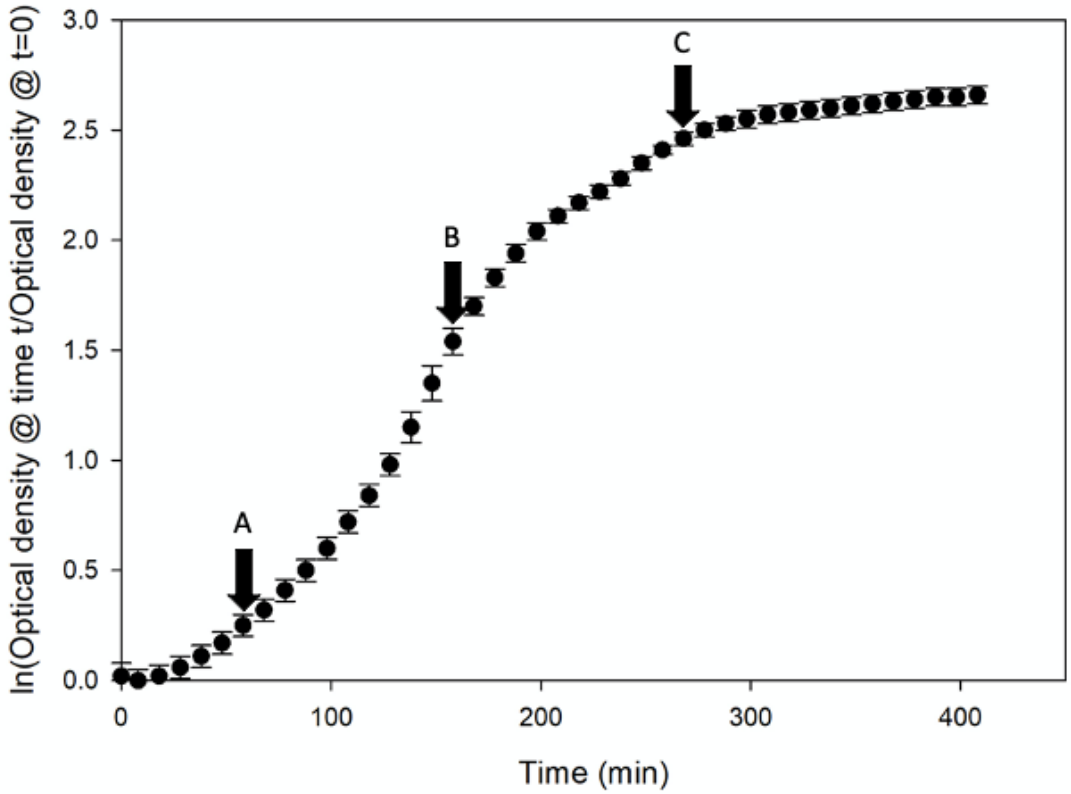

Figure S2: Growth curve of BTF 132 *E. coli* with no nanomaterial addition. Arrows indicate the points in the growth curve where nanosheets were added in later experiments. Each arrow marks the start of a different phase of bacterial growth: exponential phase (A), transition to stationary phase (B), and stationary (C). N=240.

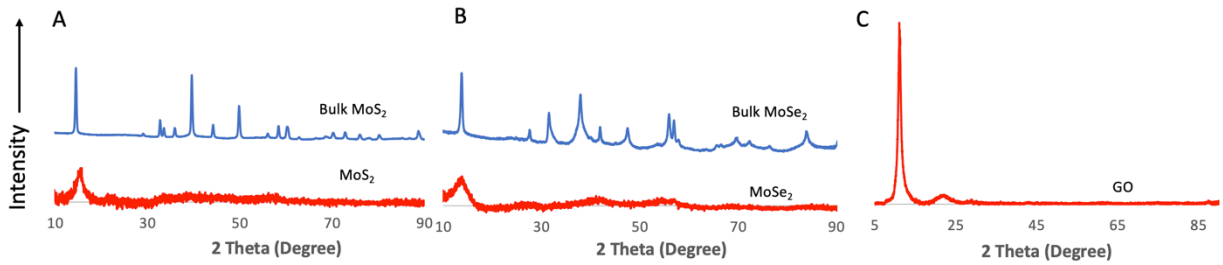

Figure S3: XRD analysis of (A) bulk MoS_2 and MoS_2 nanosheets confirming primary peak at 15.12º (interlayer spacing ~0.59 nm), (B) bulk $MoSe_2$ and $MoSe_2$ nanosheets confirming primary peak at 13.76º (interlayer spacing ~0.64 nm), and (C) GO confirming primary peak at 11.02º (interlayer spacing ~0.80 nm).

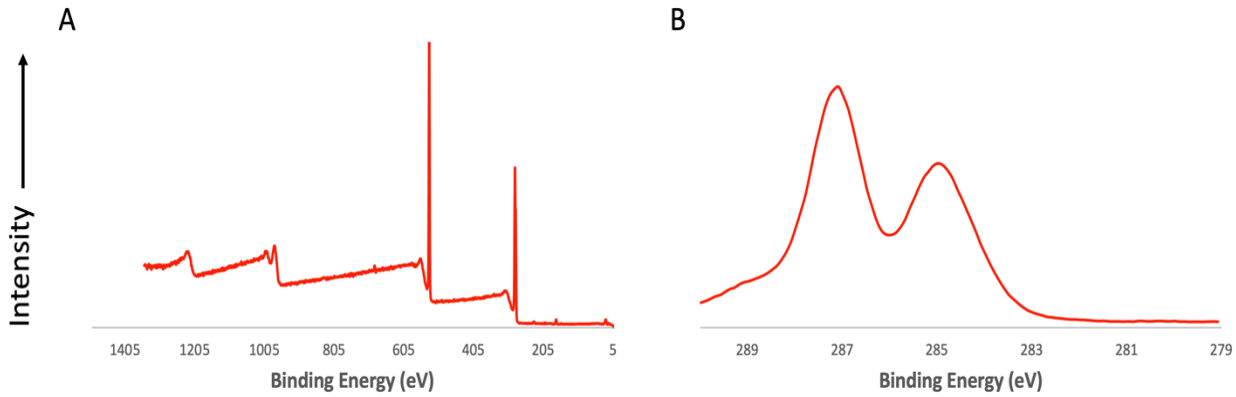

Figure S4: XPS analysis of graphene oxide. (A) Survey scan quatifying carbon:oxygen ratios of 1.94:1. (B) High resolution carbon scan confirming the presence of the 5 main bondings reported as base characteristics of GO (C=C, C-C, C-O, C=O, and O-C=O).

Table S3: Maximum, mean, and minimum values for respiration slopes (Figure 1 in main paper)

	Maximum	Mean	Minimum
GO 0.00 μg/mL	0.0016	0.0012	0.0007
GO 0.11 μg/mL	0.0017	0.0012	0.0008
GO 0.57 μg/mL	0.0018	0.0012	0.0007
GO 1.14 μg/mL	0.0016	0.0011	0.0005
GO 2.27 μg/mL	0.0015	0.0010	0.0005
MoS_2 0.00 μg/mL	0.0021	0.0012	0.0008
MoS_2 0.12 μg/mL	0.0019	0.0012	0.0008
MoS_2 0.62 μg/mL	0.0018	0.0012	0.0007
MoS_2 1.14 μg/mL	0.0019	0.0012	0.0007
MoS_2 2.50 μg/mL	0.0030	0.0012	0.0003
$MoSe_2$ 0.00 μg/mL	0.0017	0.0012	0.0008
$MoSe_2$ 0.13 μg/mL	0.0019	0.0012	0.0008
$MoSe_2$ 0.63 μg/mL	0.0022	0.0012	0.0008
$MoSe_2$ 1.26 μg/mL	0.0023	0.0013	0.0008
$MoSe_2$ 2.52 μg/mL	0.0027	0.0012	0.0008

Table S4: Maximum, mean, and minimum values for exponential phase growth rates normalized to average control (Figure 2 in main paper)

	Maximum	Mean	Minimum
GO 0.11 µg/mL	1.21	1.02	0.87
GO 0.57 µg/mL	1.32	1.04	0.88
GO 1.14 µg/mL	1.36	1.13	0.95
GO 2.27 µg/mL	1.52	1.22	1.01
MoS_2 0.12 µg/mL	1.17	1.01	0.85
MoS_2 0.62 µg/mL	1.11	0.99	0.85
MoS_2 1.14 µg/mL	1.11	0.98	0.83
MoS_2 2.50 µg/mL	1.10	0.96	0.80
$MoSe_2$ 0.13 µg/mL	1.12	1.00	0.85
$MoSe_2$ 0.63 µg/mL	1.14	1.02	0.87
$MoSe_2$ 1.26 µg/mL	1.11	1.02	0.89
$MoSe_2$ 2.52 µg/mL	1.14	1.02	0.88

Table S5: Maximum, mean, and minimum values for transitional phase growth rates normalized to average control (Figure 2 in main paper)

	Maximum	Mean	Minimum
GO 0.11 µg/mL	1.33	0.98	0.35
GO 0.57 µg/mL	1.28	0.97	0.29
GO 1.14 µg/mL	1.24	0.93	0.31
GO 2.27 µg/mL	1.12	0.91	0.46
MoS_2 0.12 µg/mL	1.46	1.02	0.36
MoS_2 0.62 µg/mL	1.44	1.01	0.35
MoS_2 1.14 µg/mL	1.42	1.01	0.35
MoS_2 2.50 µg/mL	1.46	1.01	0.35
$MoSe_2$ 0.13 µg/mL	1.37	1.02	0.36
$MoSe_2$ 0.63 µg/mL	1.48	1.07	0.43
$MoSe_2$ 1.26 µg/mL	1.52	1.07	0.43
$MoSe_2$ 2.52 µg/mL	1.54	1.06	0.39

Table S6: Maximum, mean, and minimum values for stationary phase growth rates normalized to average control (Figure 2 in main paper)

	Maximum	Mean	Minimum
GO 0.11 µg/mL	1.89	0.94	-1.26
GO 0.57 µg/mL	1.90	0.93	-1.44
GO 1.14 µg/mL	1.84	0.77	-1.32
GO 2.27 µg/mL	1.61	0.12	-1.86
MoS_2 0.12 µg/mL	2.27	1.05	-1.21
MoS_2 0.62 µg/mL	2.08	1.00	-1.67
MoS_2 1.14 µg/mL	2.07	0.99	-1.64
MoS_2 2.50 µg/mL	2.12	0.96	-1.73
$MoSe_2$ 0.13 µg/mL	2.03	1.02	-1.61
$MoSe_2$ 0.63 µg/mL	2.07	1.09	-1.61
$MoSe_2$ 1.26 µg/mL	2.02	1.01	-1.62
$MoSe_2$ 2.52 µg/mL	1.93	1.01	-1.40

Table S7: Skewness, Kurtosis, and Ryan-Joiner test results for the respiration curve slopes (Figure 1)

Variable	Skewness	Kurtosis	Ryan-Joiner statistic	P-value for Ryan-Joiner
GO 0.00 µg/mL	0.610	-0.660	0.964	<0.01
GO 0.11 µg/mL	0.600	-0.700	0.965	<0.01
GO 0.57 µg/mL	0.520	-0.250	0.982	<0.01
GO 1.14 µg/mL	0.220	-0.550	0.986	0.034
GO 2.27 µg/mL	0.580	-0.620	0.968	<0.01
MoS_2 0.00 µg/mL	1.040	0.910	0.964	<0.01
MoS_2 0.12 µg/mL	0.600	-0.380	0.978	<0.01
MoS_2 0.62 µg/mL	0.490	-0.400	0.980	<0.01
MoS_2 1.14 µg/mL	0.540	-0.510	0.979	<0.01
MoS_2 2.50 µg/mL	0.860	2.540	0.972	<0.01
$MoSe_2$ 0.00 µg/mL	0.620	-0.640	0.971	<0.01
$MoSe_2$ 0.13 µg/mL	0.510	-0.330	0.985	0.022
$MoSe_2$ 0.63 µg/mL	0.900	0.750	0.971	<0.01
$MoSe_2$ 1.26 µg/mL	1.040	1.240	0.968	<0.01
$MoSe_2$ 2.52 µg/mL	1.600	4.690	0.943	<0.01

Table S8: Skewness, Kurtosis, and Ryan-Joiner test results for the *E. coli* growth rates after nanomaterial introduction in the exponential phase (Figure 2)

Variable	Skewness	Kurtosis	Ryan-Joiner statistic	P-value for Ryan-Joiner
GO 0.11 µg/mL	0.210	-0.210	0.990	>0.1
GO 0.57 µg/mL	0.610	1.800	0.971	0.032
GO 1.14 µg/mL	0.200	0.050	0.995	>0.1
GO 2.27 µg/mL	0.630	0.500	0.982	>0.1
MoS_2 0.12 µg/mL	-0.350	-0.270	0.984	>0.1
MoS_2 0.62 µg/mL	-0.480	-0.810	0.976	0.053
MoS_2 1.14 µg/mL	-0.450	-0.920	0.974	0.045
MoS_2 2.50 µg/mL	-0.390	-1.170	0.961	<0.01
$MoSe_2$ 0.13 µg/mL	-0.720	0.100	0.972	0.036
$MoSe_2$ 0.63 µg/mL	-0.560	0.020	0.977	0.068
$MoSe_2$ 1.26 µg/mL	-0.490	-0.730	0.979	0.088
$MoSe_2$ 2.52 µg/mL	-0.510	-0.490	0.973	0.040

Table S9: Skewness, Kurtosis, and Ryan-Joiner test results for the *E. coli* growth rates after nanomaterial introduction in the transitional phase (Figure 2)

Variable	Skewness	Kurtosis	Ryan-Joiner statistic	P-value for Ryan-Joiner
GO 0.11 µg/mL	-0.550	0.070	0.986	>0.1
GO 0.57 µg/mL	-0.870	0.690	0.970	0.030
GO 1.14 µg/mL	-0.730	-0.150	0.961	<0.01
GO 2.27 µg/mL	-0.850	-0.300	0.942	<0.01
MoS_2 0.12 µg/mL	-0.380	1.040	0.984	>0.1
MoS_2 0.62 µg/mL	-0.450	0.610	0.987	>0.1
MoS_2 1.14 µg/mL	-0.530	0.630	0.986	>0.1
MoS_2 2.50 µg/mL	-0.510	0.630	0.988	>0.1
$MoSe_2$ 0.13 µg/mL	-0.670	0.440	0.976	0.055
$MoSe_2$ 0.63 µg/mL	-0.520	0.740	0.988	>0.1
$MoSe_2$ 1.26 µg/mL	-0.400	0.460	0.991	>0.1
$MoSe_2$ 2.52 µg/mL	-0.230	0.310	0.993	>0.1

Table S10: Skewness, Kurtosis, and Ryan-Joiner test results for the *E. coli* growth rates after nanomaterial introduction in the stationary phase (Figure 2)

Variable	Skewness	Kurtosis	Ryan-Joiner statistic	P-value for Ryan-Joiner
GO 0.11 µg/mL	-1.300	1.050	0.929	<0.01
GO 0.57 µg/mL	-1.350	1.360	0.931	<0.01
GO 1.14 µg/mL	-1.120	0.770	0.950	<0.01
GO 2.27 µg/mL	-0.850	1.340	0.971	0.032
MoS_2 0.12 µg/mL	-1.160	0.760	0.942	<0.01
MoS_2 0.62 µg/mL	-1.490	1.980	0.924	<0.01
MoS_2 1.14 µg/mL	-1.250	1.210	0.943	<0.01
MoS_2 2.50 µg/mL	-1.270	1.180	0.939	<0.01
$MoSe_2$ 0.13 µg/mL	-1.460	1.810	0.924	<0.01
$MoSe_2$ 0.63 µg/mL	-1.510	1.860	0.916	<0.01
$MoSe_2$ 1.26 µg/mL	-1.410	1.370	0.920	<0.01
$MoSe_2$ 2.52 µg/mL	-1.310	0.940	0.919	<0.01

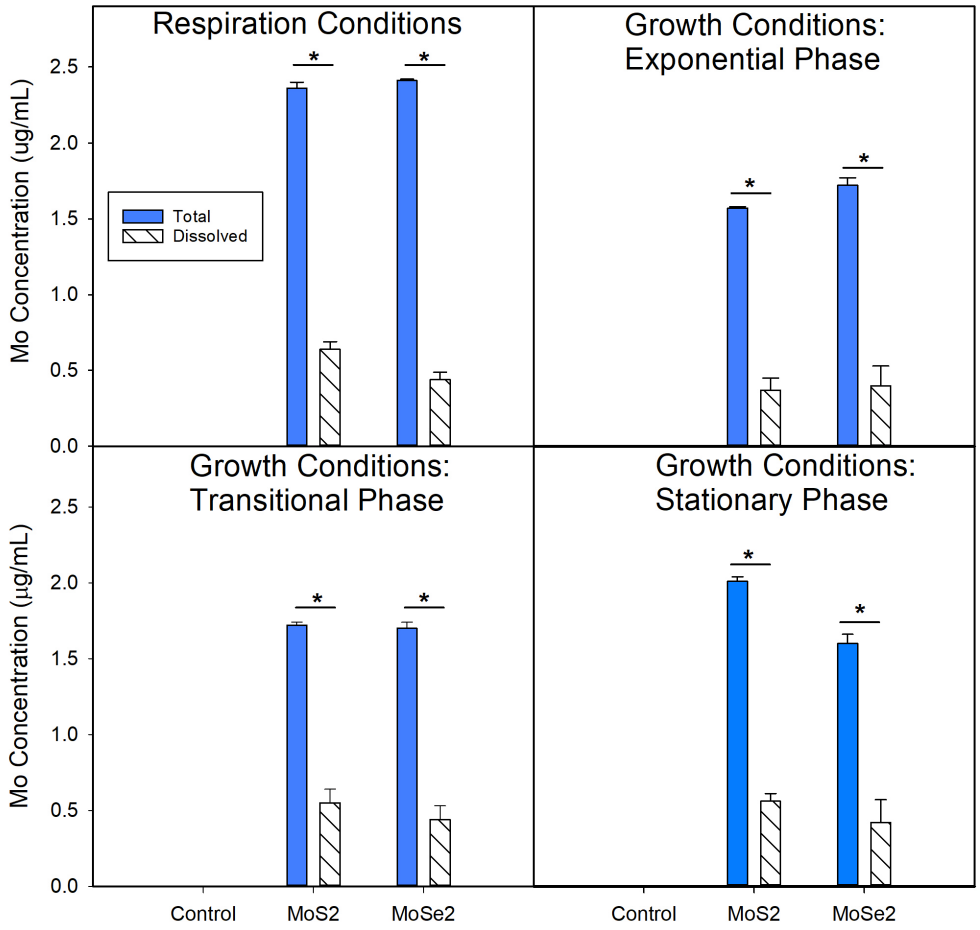

Figure S5: Total (solid blue) vs. dissolved (black striped) fractions of MoS_2 and $MoSe_2$ after exposure to bacteria in the respiration and growth media for 2 hours. Asterisks (*) indicate statistically significant differences (p<0.05) in the concentration of molybdenum (T test, n=4).

References:

1 Z. Wang, W. Zhu, Y. Qiu, X. Yi, A. von dem Bussche, A. Kane, H. Gao, K. Koski and R. Hurt, *Chemical Society Reviews,* 2016, **45**, 1750-1780 (DOI:10.1039/c5cs00914).

2 Y. Li, H. Yuan, A. von dem Bussche, M. Creighton, R. H. Hurt, A. B. Kane and H. Gao, *Proceedings of the National Academy of Sciences - PNAS,* 2013, **110**, 12295-12300 (DOI:10.1073/pnas.1222276110).