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1	Influence of nickel manganese cobalt oxide nanoparticle			
2	composition on toxicity toward Shewanella oneidensis MR-1:			
3	Redesigning for reduced biological impact			
4 5 6 7	Ian L. Gunsolus ^{§,*} , Mimi N. Hang ^{†,*} , Natalie V. Hudson-Smith [§] , Joseph T. Buchman [§] , Joseph W. Bennett [‡] , Daniel Conroy [†] , Sara E. Mason [‡] , Robert J. Hamers [†] , and Christy L. Haynes [§]			
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15	ABSTRACT : Lithium nickel manganese cobalt oxide $(L_{1x}N_{1y}Mn_zCo_{1-y-z}O_2, 0 \le x, y, z \le 1,$			
16	also known as NMC) is a class of cathode materials used in lithium ion batteries. Despite			
17	increasing use of NMC in nanoparticle form for next generation energy storage			
18	applications, the potential environmental impact of released nanoscale NMC is not well			
19	characterized. Previously, we showed released nickel and cobalt ions from nanoscale			
20	$Li_{1/3}Ni_{1/3}Mn_{1/3}Co_{1/3}O_2 \ \text{were largely responsible for impacting growth and survival of the}$			
21	Gram-negative bacterium, Shewanella oneidensis MR-1. ¹ Here, we show the first steps			
22	toward material redesign of NMC to mitigate biological impact and how chemical			
23	composition of NMC can significantly alter biological impact toward S. oneidensis. We			
24	first synthesized NMC with varied stoichiometry, with an aim to reduce Ni and Co			
25	content: $Li_{0.68}Ni_{0.31}Mn_{0.39}Co_{0.30}O_2$, $Li_{0.61}Ni_{0.23}Mn_{0.55}Co_{0.22}O_2$, and			
26	$Li_{0.52}Ni_{0.14}Mn_{0.72}Co_{0.14}O_2$. Then, S. oneidensis were exposed to 5 mg/L of these NMC			
27	formulations, and the impact on bacterial oxygen consumption was analyzed.			

Measurements of the NMC composition by X-ray photoelectron spectroscopy and composition of the nanoparticle suspension aqueous phase by inductively coupled plasma-optical emission spectroscopy showed release of Li, Ni, Mn, and Co ions. Bacterial inhibition due to redesigned NMC exposures can be ascribed largely to the impact of ionic metal species released from the NMC, most notably, Ni and Co. Tuning the NMC stoichiometry to have increased Mn at the expense of the Ni and Co showed lowered, but not completely mitigated biological impact. This study reveals that chemical composition of NMC nanomaterials is an important parameter to consider for sustainable material design and usage. Environmental Significance Statement: Engineered nanomaterials play important roles in the development of sustainable technologies. As a prime example, next generation nano-enabled products for energy storage will utilize complex, multicomponent nanoscale metal oxide systems to give higher performance through its unique sizedependent properties. While these nanomaterials can considerably enhance the sustainability of our society, it is also imperative to understand the environmental implications of these materials upon release into the environment (most likely through disposal, due to the lack of infrastructure and motivation to recycle lithium ion battery materials). This work explores the potential biological impact of a class of lithium ion battery materials and identifies important variables to consider when designing these nanomaterials for sustainable use.

Lithium nickel manganese cobalt oxide ($Li_xNi_yMn_zCo_{1-v-z}O_2$, 0 < x, y, z < 1) is part of a wide class of lithium intercalation compounds with great importance in energy storage

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51	applications. Since the 1990s, lithium cobalt oxide (LCO) has been widely
52	commercialized as a cathode material in lithium-ion batteries. ² With the increasing
53	economic growth related to rechargeable batteries for use in mobile electronics and
54	electric vehicles, replacement of Co in LiCoO ₂ with Ni and Mn to create lithium nickel
55	manganese cobalt oxide (NMC) has emerged as an attractive alternative high
56	performance material with reduced cost and increased stability. ^{3,4,5} Bulk NMC typically
57	consists of nanoparticles sintered into larger, micron-sized aggregates. However, cathode
58	materials such as NMC are increasingly used directly in nanoscale form to enhance
59	battery performance. ⁶ In addition, after micron-sized NMC particles are integrated into
60	complete batteries, free nanoparticles are formed during operation as the aggregates
61	undergo stress-induced fracturing to form smaller nanoscale sheet-like particles. ¹ The use
62	of NMC on a large scale is driven largely by rapidly escalating use in electric vehicles
63	and hybrid vehicles. The amount of energy stored in a lithium ion battery is directly
64	proportional to the mass of the electrodes, with a typical electric vehicle cathode
65	containing approximately 50 kg of NMC. The rapid increase in use of NMC-based
66	batteries in transportation, ⁷ mobile electronics, and other technologies increases the
67	potential for NMC nanomaterial entry into natural environments through manufacture,
68	use, and disposal.

Despite the apparent large-scale use of NMC materials, little is known about the
environmental implications and mechanisms of toxicity for these nanoscale complex
oxides. In previous work, we showed that equistoichiometric Li_{1/3}Ni_{1/3}Mn_{1/3}Co_{1/3}O₂
impacted the growth and survival of the Gram-negative bacterium *S. oneidensis*, and that
this impact arose primarily from released nickel and cobalt ions.¹ Measurements of both

the NMC surface composition and of the released metals in the suspension aqueous phase showed that the material underwent partial dissolution to release Li, Ni, Mn, and Co ions into solution, with an unexpected non-stoichiometric dissolution pattern. While the dissolved Ni and Co ions had negative effects on exposed bacteria, the Mn and Li ions did not have significant impact. That study represented the first study of nanoscale NMC on an organism and provided mechanistic insights into how such a complex nanomaterial might impact biological systems. However, NMC consists of a class of materials with a range of stoichiometries which are chosen for specific requirements for operating voltage, longevity and cost, raising the question of how the composition affects the biological impact.⁸ In principle, this could lead to the ability to re-design lithium-ion battery cathodes based on the environmental impact as well as other factors such as electrical performance and cost. In this work, we report on the stoichiometric redesign of nanoscale NMC and how this redesign influences the mechanism of interaction between NMC and S. oneidensis. S. oneidensis is the model bacterium employed in this work based on its widespread

distribution in the environment and the critical role it plays in global nutrient cycling.⁹

90 Using the mechanistic insight obtained in our previous study,¹ we redesigned nanoscale

91 NMC to contain less Ni and Co with the hypothesis that this change would lower or

92 eliminate the material's toxicity to *S. oneidensis*. To lower the Co and Ni content while

93 maintaining the lithium intercalation properties, the Mn content was increased;

94 accordingly, these materials can be identified as Mn-rich NMC. Following nanomaterial

95 synthesis, the response of *S. oneidensis* to these Mn-rich NMC nanomaterials was

96 measured; in parallel, the physical and chemical transformations of the material were

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97 characterized. This exploration of the relationship between material chemical
98 composition and biological impact yields mechanistic insights about potential routes to
99 mitigate adverse nanoparticle impact and optimize sustainable use of NMC
100 nanomaterials.

102 Methods

NMC and LiCoO₂ synthesis. Nanosheets of NMC with various stoichiometries were synthesized by adapting methods previously reported for synthesis of LiCoO₂,^{10,11} which we extended to NMC.¹ Reagents were purchased from Sigma-Aldrich, and Barnstead Nanopure water (18 M Ω cm resistivity) was used. A Ni_vMn_zCo_{1-v-z}(OH)₂ precursor was first synthesized via dropwise addition of an aqueous transition salt mixture containing cobalt (II) acetate, nickel (II) acetate, and manganese (II) acetate into 0.1 M aqueous LiOH under magnetic stirring. Specific Ni_xMn_yCo_{1-x-y}(OH)₂ compositions were obtained by varying the molar ratios of Co^{2+} : Ni^{2+} : Mn^{2+} during this step. The resulting precursors were isolated with repeated cycles of centrifugation and suspension in water (1X) and methanol (4X) followed by drying in a desiccator. Each dried mixed metal hydroxide (0.250 g) was then added separately to a 10 g molten salt flux (6:4 molar ratio of LiNO₃:LiOH) at 205 °C with magnetic stirring to form Li_xNi_yMn_zCo_{1-y-z}O₂. After 30 min, the reactions were quenched with water, and the various NMC solids were isolated with repeated cycles of centrifugation and resuspension in water (1X) and methanol (4X) followed by drying in a desiccator. All centrifugation was completed at 4686xg via a Thermo Scientific Sorvall legend X1R Centrifuge with a Thermo TX-400 rotor.

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Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)
Characterization of NMC Composition and of Metal Release into Bacterial Growth
Medium. To characterize the composition of each synthesized NMC sample, freshly
prepared *aqua regia* (3:1 v/v mixture of 37% v/v HCl and 70% v/v HNO₃) was used to
digest each sample. Each digested sample was diluted in nanopure water, and the metal
concentrations of three analytical replicates were determined using a PerkinElmer Optima
2000 ICP-OES.

To determine the metal release into the bacterial growth medium from the different stoichiometries of NMC, suspensions of each NMC composition were magnetically stirred at 30 $^{\circ}$ C in the growth medium to mimic the biological exposure of NMC to S. oneidensis. The aqueous phase was sampled at 72 h. The solution phase measurements were collected with two sample replicates and three analytical replicates per NMC stoichiometry. To isolate the aqueous phase, samples were centrifuged at 4696xg for 20 min to remove the majority of the NMC nanoparticles in the suspension. To ensure removal of the nanoparticles from the aqueous phase, supernatant was collected from the uppermost region of the liquid phase and then ultracentrifuged at 288,000xg for 2 h using a Beckman Coulter Optima Ultracentrifuge with a SW-41 Ti Rotor. Dynamic light scattering (Malvern Zetasizer Nano ZS) was used to ensure effective sedimentation of nanoparticles. The supernatants were analyzed via ICP-OES to determine the concentrations of dissolved metal species.

Assessing ROS formation from NMCs. Aminophenyl flourescein (APF) (Molecular
Probes by Life Technologies) was purchased as a 5 mM solution in dimethylformamide
(DMF) and used as received. In dark amber glass jars, each of the NMC stoichiometries

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were suspended in an aqueous solution of 10 mM HEPES and 1 µM APF, followed by sonication for 10 minutes. Each NMC stoichiometry was prepared in duplicate and were magnetically stirred. Simultaneously, a reference sample was prepared using identical conditions without the NMC. After 23.5 h, both NMC and reference solutions were kept in the dark using foil wrapped centrifuge tubes and centrifuged at 4696xg for 30 minutes to remove the majority of NMC nanoparticles. The top of the supernatant was removed, and an equal volume of 0.5 M NaOH solution was added to the collected supernatant for analysis. An ISS K2 Multifrequency Phase Flourometer was used to analyze sample fluorescence at 24 h (excitation/emission at 490/515 nm). Additionally, a 320 nm cut-on filter (Newport) was placed in the excitation path to eliminate residual 245 nm light from source.

X-ray Photoelectron Spectroscopy (XPS). As-synthesized NMC nanomaterials for XPS analysis were prepared via drop-casting a methanolic solution of NMC onto borondoped SiO₂ wafers. NMC samples for XPS analysis after exposure to bacterial growth medium for 72 h were first rinsed in ultrapure water using centrifugation (5X). The final pellet of NMC obtained after 5 cycles of water rinses was then mixed with methanol and drop-cast onto boron-doped SiO₂. XPS data were acquired using a custom ultra-high vacuum Phi system with a monochromatized Al K_{α} source (1486.6 eV photon energy) and a hemispherical electron energy analyzer. All spectra were obtained using 45° photoelectron takeoff angles. Spectra were fit using CasaXPS Software¹² and inelastic mean free paths (IMFPs) were estimated using the NIST Effective Attenuation Length Database¹³via the TPP-2M equation¹⁴ to obtain IMFPs of 2.4 nm (Co), 2.2 nm (Ni), and 2.8 nm (Mn).

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165 Scanning Electron Microscopy (SEM).

A Leo Supra55 VP scanning electron microscope (SEM) was used to obtain images of
the NMC samples. Samples were prepared by drop-casting a methanolic solution of
NMC onto boron-doped SiO₂. SEM images were obtained using 1 kV incident electron
energy with a standard in-lens detector.

170 Transmission Electron Microscopy (TEM) for Nano-NMC Characterization. To

collect the edge-on images of Mn-rich nanosheets, they were dispersed in Milli-Q water
to a concentration of 1 mg/mL with sonication for 10 minutes. A 10 µL droplet of each
suspension was deposited on 200 mesh copper TEM grids with Formvar and carbon
supports (Ted Pella Inc., Redding, CA). The grids were turned upside down to improve
the chances of the nanoplates drying edge-on during the 2 hour drying period. Images
were collected using a Tecnai T12 transmission electron microscope with an operating
voltage of 120 kV.

Biological Transmission Electron Microscopy (TEM). To prepare the NMC-exposed bacteria for imaging by TEM, they were first embedded in epoxy resin.^{15,16} Briefly. the bacteria were suspended in HEPES buffer at an optical density (OD) of 0.2 and exposed to 5 mg/L Mn-rich NMC for 30 minutes. The cells were pelleted and washed with 0.1 M sodium cacodylate buffer three times before being fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 50 minutes. The samples were again washed in sodium cacodylate buffer and gradually dehydrated using a series of ethanol washes (30%, 50%, 70%, 80%, 95%, and 100% ethanol in water). Following three washes with propylene oxide, the samples were incubated in 2:1 propylene oxide:resin for 2 hours, 1:1 propylene oxide:resin overnight, fresh 1:1 propylene oxide:resin for 4 hours, and then in resin

Environmental Science: Nano

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overnight. The resin was then replaced with fresh resin and the samples were cured in a
40 °C oven for 24 hours followed by a 60 °C oven for 48 hours. Approximately 70-nmthick slices were cut using a Leica EM UC6 Ultramicrotome and stained with uranyl
acetate and lead citrate for improved contrast. The slices were placed on copper grids
(200 mesh) with Formvar and carbon supports and imaged with a Tecnai T12
transmission electron microscope at an operating voltage of 120 kV.

Measuring Bacterial Response to NMC. Bacterial respiration was monitored throughout the exposure to NMC variants and equivalent metal ion concentrations by quantifying $O_2(g)$ consumption using a 32-vessel respirometer system (Respirometry Systems and Applications, Inc., Springdale, AR). Bacterial cell suspensions were made from colonies grown on LB agar plates and were diluted 1:10 v/v into fresh bacterial growth medium containing 11.6 mM NaCl, 4.0 mM KCl, 1.4 mM MgCl₂, 2.8 mM Na₂SO₄, 2.8 mM NH₄Cl, 88.1 µM Na₂HPO₄, 50.5 µM CaCl₂, 10 mM HEPES, and 100 mM sodium lactate. Each 125 mL glass respirometer vessel contained 100 mL of this bacterial suspension. NMC nanoparticles were dispersed in bacterial growth medium at 2000 mg/L by 10 minutes of bath sonication and added to respirometry vessels to achieve a final concentration of 5 mg/L. In vessels with NMC, no modifications were made to prevent aggregation. Neither dynamic light scattering (Table S1) nor macroscopic visualization of the NMC suspensions (Figure S1) showed significant difference in nanomaterial aggregation state as the NMC composition was varied. Stock solutions of NiCl₂, CoCl₂ MnSO₄, and LiOH were prepared in bacterial medium and added to vessels to achieve Ni²⁺,Co²⁺, Mn²⁺ and Li⁺ concentrations equivalent to that of dissolved metal ion concentrations resulting from a 5 mg/L NMC nanoparticle suspension/dispersion as

determined by ICP-OES. In particular, Co³⁺ and Mn⁴⁺ are not stable in those oxidation states in water and are able to oxidize water to form reactive oxygen species (ROS) such as hydroxyl radicals.^{28,29,22} Co³⁺ and Mn⁴⁺ are reduced prior to dissolution in this process and therefore species in the 2+ state were selected for ion controls. The respirometer vessels were placed in a 30°C water bath for 72 hours with constant stirring (500 rotations per minute) and under constant $O_2(g)$ pressure. CO_2 produced by respiring bacteria is removed from the vessel by reaction with 1 mL of 30% KOH aqueous solution resting in an insert in the vessel headspace. By removal of CO₂, the total pressure inside the vessel is decreased. O_2 is resupplied to the vessel at 10 minute intervals such that a constant overall pressure is maintained. O2 additions to vessels are tracked over time to measure bacterial respiration. To quantify the similarity between non-fitted respiration curves, correlation coefficients between curves were calculated in Excel using the correlation feature of the Analysis ToolPak. In all cases, ion equivalents were better correlated to NMC nanoparticle exposure than they were to the controls. All correlation coefficients are available in Table S1. All statistical data are shown in Table S1.

226 Results and Discussion

227 Preparation and Characterization of Nanoscale Mn-rich NMC. The bulk

228 nanomaterial composition was characterized using inductively coupled plasma-optical

- emission spectroscopy (ICP-OES) after the material was acid digested to give a partially
- 230 lithiated NMC material with stoichiometries Li_{0.68}Ni_{0.31}Mn_{0.39}Co_{0.30}O₂,
- $Li_{0.61}Ni_{0.23}Mn_{0.55}Co_{0.22}O_2$, and $Li_{0.52}Ni_{0.14}Mn_{0.72}Co_{0.14}O_2$. For simplicity, these
- 232 compositions will be referred to as NMC ($X_{Mn}=0.39$), NMC ($X_{Mn}=0.55$), and NMC
- 233 (X_{Mn}=0.72), respectively. SEM images (Fig. 1a-c) of the synthesized NMC

Environmental Science: Nano

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nanomaterials show consistent sheet-like morphology, and complementary edge-on TEM images in the insets show these NMC nanosheets to be <5 nm in thickness. Powder X-ray diffraction of the NMC nanosheets were all similarly indexed to the $R\bar{3}m$ space group (Fig. 2).^{17,18,10}

Impact of NMC nanosheet composition on Shewanella oneidensis MR-1. The

biological impact of the NMC nanomaterials with varying transition metal content was assessed by monitoring its effect on the aerobic respiratory activity of S. oneidensis.

Figure 3 shows cellular oxygen consumption measured over 72 h following exposure to 5

mg/L of NMC (X_{Mn} =0.39), NMC (X_{Mn} =0.55), or NMC (X_{Mn} =0.72). The oxygen

consumption profile of a bacterial population mirrors their well-characterized population growth dynamics, which consists of three distinct phases (Fig. 3, black trace): a short lagphase, wherein population size and oxygen consumption remain approximately constant; an exponential phase, wherein the population size and oxygen consumption increase rapidly; and a stationary phase, wherein the population size and oxygen consumption reach an equilibrium state. Exposure to NMC was observed to delay the exponential

growth phase in a material-dependent manner inversely proportional to the amount of

manganese present in the nanosheets, indicating inhibition of cell population growth as a

function of material composition in the order NMC ($X_{Mn}=0.39$) > NMC ($X_{Mn}=0.55$) >

NMC (X_{Mn} =0.72). This delayed onset of exponential growth, also referred to as increased lag time, is a function of both cell death and cell inhibition.¹ This delay has been shown to occur in multiple species after exposure to heavy metals.¹⁹ Both experimental data such as that presented here and biologically informed mathematical modeling show that observed lag time is proportional to heavy metal concentration.¹⁹ In

acute toxicity studies, recovery would be expected to occur in agreement with a

logarithmic growth model and is observed in Figure 3 and Figure 7.^{20,1}

Figure 4 shows the oxygen uptake rate of the bacterial system as a function of exposure duration, where the oxygen uptake rate is the derivative of the oxygen consumption shown in Figure 3. The peaks in Figure 4 have maxima associated with the inflection point of each exposure condition in Figure 3. Figure 4 shows a decay in the oxygen uptake rate as a function of NMC nanomaterial composition. These data, which we analyze comparatively by a Pearson correlation in the SI, clearly demonstrate that the biological impact of NMC nanomaterials on cells decreases as the NMC composition becomes more enriched with Mn.

Transformation of NMC in Biological Media and Release of Ions: To determine how the observed biological effects were related to metal ions in solution, we used ICP-OES to analyze the chemical stability of each NMC composition in bacterial growth medium in the absence of bacteria. After NMC exposure to bacterial growth medium for 48 and 72 h, the aqueous phase was isolated from the NMC nanosheets to measure the concentration of dissolved Ni, Co, Mn, and Li. Figure 5a shows the concentration of dissolved Li, Ni, Mn, and Co species from 5 mg/L of each NMC composition after exposure to bacterial growth medium for 72 h. These data show that the concentration of dissolved Ni, Mn, and Co is partially dependent on the NMC stoichiometry. NMC nanosheets with higher Mn-content release more Mn and less Ni and Co. However, the ratio of transition metal released is not congruent with the composition of the NMC nanosheet. For all compositions investigated, the materials have a Ni:Co ratio close to 1:1. However, in each case Ni ions are released at a greater rate compared with Co. This

Environmental Science: Nano

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result is consistent with our previous study showing partial, incongruent dissolution with preferential release of Ni from $Li_{1/3}Ni_{1/3}Mn_{1/3}Co_{1/3}O_2$ as compared to Mn or Co.¹ To complement analysis of metal ion concentration in the aqueous phase, we used XPS to measure the change in Ni, Mn, and Co content near the surface region of the nanomaterial after exposure to the bacterial growth medium for 72 h. These NMC nanomaterials were rinsed 5X in ultrapure water before XPS analysis, and the

composition was determined using fractional atomic composition, f_x , which is defined as

$$f_x = \frac{\frac{A_x}{S_x \lambda_x}}{\sum_i \frac{A_i}{S_i \lambda_i}}$$

where x = Ni, Mn, or Co; A_i = area of measured XPS peaks for the element i, S_i = atomic sensitivity factor for element *i*, and λ_i = the inelastic mean free path for element *i* in NMC.¹ Figure 5b summarizes these fractional compositions from the different NMC stoichiometries before and after exposure to growth medium for 72 h. In each case, the exposure to the aqueous medium modifies the surface composition, lowering the surface abundance of Ni and thus increasing the relative abundance of Mn. Thus, our XPS data show that the NMC materials are transformed upon exposure to the medium to leave behind a surface that has been enriched with Mn and deficient in Ni. The complementary results from both XPS and ICP-OES show that all of the studied NMC nanomaterial compositions are chemically unstable in bacterial growth medium and can therefore release metals into the aqueous matrix in a non-stoichiometric manner.

Direct vs. Indirect Interaction: To determine whether the effects of NMC on *S*.

oneidensis arise from direct interaction with the nanoparticles or from ions in solution,

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300	we conducted two sets of experiments. First, we recorded transmission electron
301	micrographs of cells following exposure to NMC. These results (Fig. 6a-c) show no
302	significant interaction of the materials with near neutral electrophoretic mobilities (Table
303	S4) the bacterial cell surface. Overall, there appears to be little affinity between NMC
304	aggregates and the cells To further confirm that NMC induces its effects via indirect
305	processes such as exposure to the dissolved transition metal cations, we measured
306	bacterial respiration of S. oneidensis exposed to different NMC compositions and also
307	measured respiration for S. oneidensis exposed to free metal ions, using the metal
308	concentrations measured by ICP-OES (Figure 5a) from 5 mg/L NMC of the appropriate
309	composition after 72 h. Figures 7 and 8 show respirometry data for S. oneidensis
310	exposed to the three NMC compositions as the Mn fraction increases from 0.39 (Fig. 7a)
311	to 0.55 (Fig. 7b) and then to 0.72 (Fig. 7c). Each figure includes a comparison of the
312	respirometry data with 5 mg/L of NMC, along with free-ion controls that include all
313	cations (Fig. 7a-c) or only Ni and Co cations (Fig. 8a-c). Figures 7 and 8 show that
314	exposure to 5 mg/L NMC induces a delay in bacterial growth when compared to S .
315	oneidensis not exposed to any NMC; this delay is most apparent for the NMC having the
316	lowest Mn fraction (X_{Mn} =0.39, Fig. 7a) and least pronounced when using NMC having
317	the largest Mn fraction (X_{Mn} =0.72, Fig.7c). Figure 7a shows that the total free metal
318	concentration cannot fully account for the bacterial growth inhibition seen with NMC
319	(X_{Mn} =0.39), and their respective respiration curves are statistically distinct (p=0.05) for
320	most of the exposure period. Conversely, Figures 7b and 7c show that there is no
321	statistical difference between the total free metal ion concentrations and NMC respiration
322	curves throughout most of the exposure. This indicates that for NMC (X_{Mn} =0.55) and

Environmental Science: Nano

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323	NMC ($X_{Mn}=0.72$), release of free metal ions from the nanomaterial is responsible for
324	bacterial growth inhibition. All statistical data are presented in Table S1.
325	Ni ²⁺ and Co ²⁺ account for most, but not all, of NMC's impact: To specifically explore
326	how Ni and Co affected bacterial growth, we exposed S. oneidensis to a mixture of only
327	Ni^{2+} and Co^{2+} (without Li^{+} or Mn^{2+}) at the concentrations we determined were produced
328	by NMC dissolution after 72 h from the different NMC stoichiometries. Previous studies
329	have demonstrated that Li^+ concentrations approximately 7-fold higher than those
330	observed in this study. ¹ Figure 8a shows that a mixture of free Ni^{2+} and Co^{2+} (orange
331	trace) could not fully account for the exponential growth delay caused by NMC
332	$(X_{Mn}=0.39)$ nanoparticles (red trace). For the majority of the duration of the experiment,
333	the respiration profile produced by exposure to Ni^{2+} and Co^{2+} is statistically distinct
334	(p=0.05) from that produced by NMC nanosheets. These differences indicate that while
335	free Ni ²⁺ and Co ²⁺ are a major source of bacterial growth inhibition, there may be
336	additional mechanisms of toxicity for NMC ($X_{Mn}=0.39$). In contrast, figures 8b-c show
337	that for NMC with higher Mn content (X_{Mn} =0.55 and 0.72), the respiration curves from <i>S</i> .
338	oneidensis exposed to a mixture of free Ni ²⁺ and Co ²⁺ were not statistically different from
339	those produced by exposure to NMC nanoparticles. This similarity in respiration curves
340	indicates that for higher Mn content NMC, (X_{Mn} =0.55 and 0.72) the mixture of free Ni
341	and Co metal ions fully accounts for the magnitude of bacterial growth inhibition.
342	Therefore, for NMC with high Mn content (X_{Mn} =0.55) and NMC (X_{Mn} =0.72) the primary
343	mechanism of inhibition of <i>S. oneidensis</i> growth is through dissolved Ni^{2+} and Co^{2+}
344	species. However, for NMC (X_{Mn} =0.39), our data indicate that additional mechanisms of
345	toxicity are likely important.

Environmental Science: Nano

Environmental Science: Nano Accepted Manuscript

In summary, the respirometry studies show that when S. oneidensis is exposed to various compositions of NMC, cell growth and reproduction are differentially inhibited. Specifically, the magnitude of inhibition of cell population growth varies as a function of material composition, in the order NMC ($X_{Mn}=0.39$) > NMC ($X_{Mn}=0.55$) > NMC $(X_{Mn}=0.72)$ (lowest inhibition). Dissolution and solution-phase speciation of NMC: To better understand the role of metal ions in disrupting bacterial respiration, we must characterize both the kinetics of nanoscale NMC dissolution and the distribution of products formed. Our data show that all NMC compositions dissolve incongruently in bacterial growth media, with preferential release of Li^+ , Ni^{2+} and Co^{2+} . The incongruent dissolution of NMC, with release rates in the order $Li^+>Ni^{2+}>Co^{2+}>Mn^{2+}$, is likely driven both by kinetics and thermodynamics and is closely connected with the oxidation states of metals in NMC, which are best described as Li⁺, Ni²⁺, Co³⁺, and Mn^{4+,21} For transition metal cations in solution, Pourbaix diagrams show that the thermodynamically stable hydrated cation are those with low valence charge: Li⁺, Ni²⁺, Co²⁺, and Mn²⁺, ^{22,23} Consequently, Li⁺ and Ni²⁺ can be released directly into solution without changing oxidation state, while Co³⁺ and Mn⁴⁺ must initiate an oxidation process such as water oxidation.^{24,25} The incongruent dissolution can be rationalized by a thermodynamic analysis considering dissolution as a two-step process in which (1) a metal atom is first removed from the lattice as a neutral atom, and (2) this neutral metal atom is oxidized to the 2+ hydrated ion. In Supporting Information (Figure S1), we plot the literature values of bond dissociation energies (BDE, step 1) and the free energy

Environmental Science: Nano

View Article Online DOI: 10.1039/C6EN00453A

369	changes associated with oxidation to the 2+ hydrated ions (ΔG^{0}_{SHE} , step 2). When
370	both factors are considered, the expected trend in dissolution is Li > Co \sim Ni > Mn.
371	This trend is similar to the experimental results. One reason for the variation could
372	be that the metal-oxygen bond dissociation energy (M -O BDE) values are averaged
373	over a number of different chemical environments, with no details about the crystal
374	structure, orientation of bonds, or oxidation state. The preferential release of Ni can
375	also be understood chemically on the basis of crystal field theory. Ni $^{2+}$ typically
376	prefers a four-fold square planar arrangement in oxide materials (over an
377	octahedral arrangement which is associated with Ni^{3+} or Ni^{4+}) to minimize unpaired
378	electron spins. In NMC, all of the transition metal cations are in an octahedral
379	environment, and thus there is a driving force to form either an O vacancy to
380	alleviate the six-fold coordination of Ni, or for the Ni to migrate out of the octahedral
381	cage all together. This preference for reduced coordination is less for Co and Mn, as
382	the spins associated with their higher oxidation states are stable in an octahedral
383	oxide environment. ^{26,27}

Based on this reasoning, it is clear that the preferential dissolution of Ni and Co can be rationalized. Once released into the aqueous growth medium, there are additional interactions that may also play a role. ROS species are known to induce cellular damage on a molecular level.³⁰ While these processes may be occurring in all the considered NMC stoichiometries, we expect that ROS production would be highest for NMC having the highest cobalt fraction (X_{Mn}=0.39), since aqueous nickel species should not generate significant ROS and manganese species, which could generate ROS, are not as soluble. Indeed, it is this composition, with the largest cobalt fraction for which

Environmental Science: Nano Accepted Manuscript

To assess ROS formation from the various NMC nanoparticles upon exposure to water, a fluorescence assay using APF was used. APF is nonfluorescent until it reacts with a hydroxyl radical to form fluorescein. This oxidative process produces a bright green fluorescence (excitation/emission at 490/515 nm). Figure 9 shows that after 24 h of NMC exposure to APF, the NMC materials with higher manganese content (X_{Mn}) result in reduced fluorescence intensity compared to those with lower X_{Mn}. This indicates that NMC compositions with higher manganese content produce less hydroxyl radicals. Overall, Figure 9 suggests composition-dependent formation of ROS may be an additional route of toxicity toward S. oneidensis that is not accounted for in the ion control studies using only M²⁺ ions. A more extensive investigation of ROS in this system will be the subject of future work.

We used Visual Minteg equilibrium modeling software^{3132,} to calculate the distribution of species in growth medium upon addition of NiCl₂, CoCl₂, MnCl₂, and LiCl at the concentrations observed from 5 mg/L of each NMC composition). The resulting detailed speciation accounting for growth medium components is included in Supporting Information. No solid precipitates were expected to form from any of the NMC compositions investigated, and none were visible during experiments. However, a significant fraction of total metal ions in solution are in the form of metal-lactate complexes and/or phosphate complexes. Metal-lactate complexes account for 67% of total Ni, 44% of total Co, and 9.0% of total Mn. Metal hydrogen phosphate complexes

View Article Online DOI: 10.1039/C6EN00453A

415 (M-HPO₄, where M=Ni, Mn, or Co) represent 23% of total Ni, 41% of total Co, and 416 79% of total Mn. Notably, for all NMC compositions, only a relatively small fraction of 417 the metals are present as free 2+ ions: (approximately 9.8 % Ni^{2+} , 12% Mn^{2+} , and 14.2% 418 Co^{2+}).

In addition to aforementioned possible explanations for the nanoscale NMC toxicity, one other potential contributing factor that is not accounted for in the presented experiments lies in the biological function of *S. oneidensis*. Specifically, the genus *Shewanella* has the most diverse set of respiratory pathways currently known for any organism, including respiratory pathways that process chelated Mn³⁺, Mn³⁺ in solids and Mn⁴⁺ in solids, ^{32,33} but not Ni, Li, or Co ions in any oxidation state.³⁴ It is possible that *S. oneidensis* interaction with NMC nanosheets may contribute to the dissolution of NMC in bacterial media based on the ability of S. oneidensis to recognize mineral surfaces³⁵ along with the aforementioned ROS production hypothesis. In this case, the ion balance and oxidation states may differ from those predicted using measurements of NMC in media (without bacteria) or Minteq modeling and will be tested in future work.

431 Conclusions

Previously, our study of the impact of $Li_{1/3}Ni_{1/3}Mn_{1/3}Co_{1/3}O_2$ on *S. oneidensis* showed that release of Ni²⁺ and Co²⁺ acted as the primary source of bacterial toxicant.¹ In this paper, we demonstrate how altering the chemical composition of NMC by varying the ratio of the transition metal species changes the magnitude of the nanomaterials' toxicity toward bacteria. Specifically, we showed that by lowering the Ni and Co content in NMC, we

Environmental Science: Nano Accepted Manuscript

were able to lower but not completely mitigate the biological impact. The higher the Mn content in the NMC nanomaterials, the lower the biological impact. This study provides insight into how chemical transformations of cathode materials such as NMC can potentially occur in the environment. This is also the first study to address material redesign of a cathode material toward being benign-by-design. While we maintain that more effort is necessary to obtain benign-by-design nanoscale cathode materials, the insight from these fundamental studies can contribute to our understanding of how to design environmentally safe and sustainable materials for energy storage applications.

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Environmental Science: Nano Accepted Manuscript

1		
2		
4	458	1. Hang, M. N.; Gunsolus, I. L.; Wayland, H.; Melby, E. S.; Mensch, A. C.; Hurley,
5	459	K. R.; Pedersen, J. A.; Haynes, C. L.; Hamers, R. J., Impact of Nanoscale Lithium Nickel
6	460	Manganese Cobalt Oxide (NMC) on the Bacterium Shewanella oneidensis MR-1.
7	461	Chemistry of Materials 2016 , 28 (4), 1092-1100.
8	462	2. Whittingham, M. S., <i>Lithium batteries and cathode materials</i> . Chemical Reviews
	463	2004 , 104 (10), 4271-4301.
U21	464	3. Kang, K. S.; Meng, Y. S.; Breger, J.; Grey, C. P.; Ceder, G., <i>Electrodes with high</i>
H 2	465	power and high capacity for rechargeable lithium batteries. Science 2006, 311 (5763),
ସ୍ଥି3	466	977-980.
34	467	4. Kang, D. H. P.; Chen, M.; Ogunseitan, O. A., <i>Potential Environmental and</i>
ਰ੍ਹੇ5	468	Human Health Impacts of Rechargeable Lithium Batteries in Electronic Waste.
ଶ୍ <u></u> କ6	469	Environmental Science & Technology 2013, 47 (10), 5495-5503.
) ad ort	470	5. Goodenough, J. B.; Kim, Y., <i>Challenges for Rechargeable Li Batteries</i> .
40 49	471	Chemistry of Materials 2010 , 22 (3), 587-603.
20	472	6. Tang, M.: Huang, H. Y.: Meethong, N.: Kao, Y. H.: Carter, W. C.: Chiang, Y. M.
21	473	Model for the Particle Size. Overpotential, and Strain Dependence of Phase Transition
22	474	Pathways in Storage Electrodes: Application to Nanoscale Olivines Chemistry of
23	475	Materials 2009 21 (8) 1557-1571
<u>9</u> 4	476	7 Agency I F Global FV Outlook: Understanding the Electric Vehicle Landscape
- <u>7</u> 25 26	477	to 2020: April 2013
20 307	470	8 Mo I · Nie M V · Yie I · Dohn I P A systematic study on the reactivity of
28	470	different grades of charged Li Ni Mn Co O, with electrolyte at elevated temperatures
29	479	uniformating rate calorimetry, L Power Sources 2016, 227, 145, 150
<u>(</u> 30	400	0 Bolicov A S: Vlincomon D M: Vlannenhach I A: Wy I: Pomine M E:
[a]1	401	9. Denaev, A. S., Kinigeman, D. W., Kiappenoach, J. A., Wu, L., Kohnne, W. F., Tiodia I. M. Naalaan, K. H. Fradrickaan, I. K. They, I. Clabel Tuguage interna
32 30	402	An alusia of Change alla an aidensis MD 1 France adda Different Terminal Electron
າມ ຊ_∠	483	Analysis of Snewanella oneldensis MIR-1 Exposed to Different Terminal Electron
35	484	Acceptors. Journal of Bacteriology 2005, 187 (20), 7158-7145.
5 	485	10. Qian, D.; Hinuma, Y.; Chen, H.; Du, LS.; Carroll, K. J.; Ceder, G.; Grey, C. P.; Mana V. S. Electronic Science in $N_{\rm env}$ is $N_{\rm env}$ in $S(z, z)$
<u>.</u> 37	486	Meng, Y. S., Electronic Spin Transition in Nanosize Stoicniometric Lithium Cobalt
fa8	487	Oxide. Journal of the American Chemical Society 2012, 134 (14), 6096-6099.
39	488	11. Dogangun, M.; Hang, M. N.; Iroiano, J. M.; McGeachy, A. C.; Melby, E. S.;
40 41	489	Pedersen, J. A.; Hamers, R. J.; Geiger, F. M., Alteration of Membrane Compositional
42	490	Asymmetry by L_1CoO_2 Nanosheets. Acs Nano 2015, 9 (9), 8/55-8/65.
43	491	12. CasaXPS, software version 2.3.16.
44	492	13. Powell, C. J.; Jablonski, A., NIST electron effective-attenuation-length database,
45	493	version 1.3 Standard Reference Database 82; National Institutes of Standards and
46	494	Technology: Gaithersburg, MD, 2011. 2011.
47	495	14. Tanuma, S.; Powell, C. J.; Penn, D. R., <i>Calculation of electron inelastic mean free</i>
48 40	496	paths (IMFPs) VII. Reliability of the TPP-2M IMFP predictive equation. Surface and
50	497	Interface Analysis 2003 , 35 (3), 268-275.
51	498	15. Schrand, A. M.; Schlager, J. J.; Dai, L. M.; Hussain, S. M., Preparation of cells
52	499	for assessing ultrastructural localization of nanoparticles with transmission electron
53	500	<i>microscopy</i> . Nature Protocols 2010 , 5 (4), 744-757.
54	501	16. Feng, Z. V.; Gunsolus, I. L.; Qiu, T. A.; Hurley, K. R.; Nyberg, L. H.; Frew, H.;
55 56	502	Johnson, K. P.; Vartanian, A. M.; Jacob, L. M.; Lohse, S. E.; Torelli, M. D.; Hamers, R.
50 57	503	J.; Murphy, C. J.; Haynes, C. L., Impacts of gold nanoparticle charge and ligand type on
58		

Environmental Science: Nano

1

2		
3	504	surface binding and toxicity to Gram-negative and Gram-positive bacteria Chemical
4	505	Science 2015 6 (9) 5186-5196
5	506	17 Lu Z H · MacNeil D D · Dahn J R Lavered Li NixCol-2xMnx O-2 cathode
7	507	<i>materials for lithium-ion batteries</i> Electrochemical and Solid State Letters 2001 4 (12)
8	508	$\Delta 200-\Delta 203$
,a	500	18 Okuba M · Hasana E · Kim I · Enomata M · Kajima N · Kuda T · Zhau H
<u>کل</u> اًی	509 E10	S: Honma I. Nanosiza effect on high rate Li ion intervalation in LiCoO, electrode
<u>9</u> 1	510	S., Hollind, I., Nanosize effect on high-rate Li-ton intercatation in LiCoO ₂ electrode.
T12	511	Journal of the American Chemical Society 2007, 129 (25), 7444-7452.
93 94	512	19. Gikas, P., Sengor, S. S., Ginn, T., Moderly, J., Peyton, B., <i>The effects of neavy</i>
715	513	metal and temperature on microbial growth and lag. Global Nest Journal 2009, 11 (3),
10 11 11 11	514	325-332
ឡើ7	515	20. Young, J. C.; Cowan, R. M., Respirometry for Environmental Science and
₹ <mark>1</mark> 8	516	Engineering. SJ Enterprises. Springdale, AR. 2004.
.g 19	517	21. Dolotko, O.; Senyshyn, A.; Muhlbauer, M. J.; Nikolowski, K.; Ehrenberg, H.,
<u>Ž</u> 0	518	Understanding structural changes in NMC Li-ion cells by in situ neutron diffraction. J.
21 90	519	Power Sources 2014 , 255, 197-203.
27 23	520	22. Takeno, N. Atlas of E-pH diagrams, Geological Survey of Japan Open File
94	521	Report No. 419 2005 .
25	522	23. A. Jain*, S.P. Ong*, G. Hautier, W. Chen, W.D. Richards, S. Dacek, S. Cholia, D.
26	523	Gunter, D. Skinner, G. Ceder, K.A. Persson (*=equal contributions) The Materials
<u>-</u> 27	524	Project: A materials genome approach to accelerating materials innovation
28	525	APL Materials, 201 3, 1(1), 011002.
29 29	526	24. B. J. Hwang and Y. W. Tsai and D. Carlier and G. Ceder, <i>A Combined</i>
ای 120	527	Computational/Experimental Study on LiNi _{1/3} Co _{1/3} Mn _{1/3} O ₂ , Chem. Mater., 15 (2003)
32	528	3676-3682
33	529	25. K. Kang and Y. S. Meng and J. Breger and C. P. Grev and G. Ceder, " <i>Electrodes</i>
¹² 34	530	with High Power and High Capacity for Rechargeable Lithium Batteries". Science, 311
<u>3</u> 5	531	(2006) 977-980
- 3 26	532	26 B Douglas D McDaniel and I Alexander "Concepts and Models of Inorganic
.337	533	Chemistry 3rd Ed." 1994 I Wiley and Sons
30 90	534	27 G I Miessler P I Fischer and D A Tarr "Inorganic Chemistry 5th Ed." 2014
40	535	Dearson
41	536	28 I i W: Dahn I R Lithium ion calls with aquaous electrolytes Iournal of the
42	527	Electrochemical Society 1005 1/2 (6) 17/2 17/6
43	537	20 Zhang M: do Dogninia M: Eroi H. Time resolved observations of water
44	530	29. Zhang, W., de Respinis, W., Fiel, H., <i>Hime-resolved observations of water</i>
45	539	oxidation intermediates on a cobali oxide nanoparticle calalyst. Nature Chemistry 2014,
40 47	540	0(4), 302-307.
47	541	30. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. I. D.; Mazur, M.; Telser, J., Free
49	542	radicals and antioxidants in normal physiological functions and human disease. Int. J.
50	543	Biochem. Cell Biol. 2007, 39 (1), 44-84.
51	544	31. Gustafsson, J. P. Visual MINTEQ chemical equilibrium model, 3.1; 2013 .
52	545	32. Marsili, E.; Baron, D. B.; Shikhare, I. D.; Coursolle, D.; Gralnick, J. A.; Bond, D.
53	546	R., Shewanella Secretes flavins that mediate extracellular electron transfer. Proceedings
54 55	547	of the National Academy of Sciences of the United States of America 2008, 105 (10),
55 56	548	3968-3973.
57		
58		

1		
1 2 3 4 5 6 7 8 925T5.91 210710705 mostper	549 550 551 552 553 554 555 556 557	 33. Hau, H. H.; Gralnick, J. A., <i>Ecology and biotechnology of the genus Shewanella</i>. In Annual Review of Microbiology, Annual Reviews: Palo Alto, 2007; Vol. 61, pp 237-258. 34. Nealson, K. H.; Scott, J., <i>Ecophysiology of the Genus Shewanella</i>. Springer: New York, 2006; p 1133-1151. 35. Lower, S. K.; Hochella, M. F.; Beveridge, T. J., <i>Bacterial recognition of mineral surfaces: Nanoscale interactions between Shewanella and alpha-FeOOH</i>. Science 2001, 292 (5520), 1360-1363.
1.000000000000000000000000000000000000		
12 23 4 25 6 7 8 9 13 24 5 6 7 8 9 13 24 5 6 7 8 9		

Environmental Science: Nano



Figure 1. Size and morphology analysis of NMC nano-sheets. Scanning electron
microscopy images and transmission electron microscopy images in the inset for a.)

Environmental Science: Nano





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$$Li_{0.61}Ni_{0.23}Mn_{0.55}Co_{0..22}O_2$$
, or 5 mg/L of $Li_{0.52}Ni_{0.14}Mn_{0.72}Co_{0.14}O_2$.



574 Figure 4. Oxygen uptake rate for Shewanella oneidensis MR-1 in growth medium

575 containing no NMC, 5 mg/L of $Li_{0.68}Ni_{0.31}Mn_{0.39}Co_{0.30}O_2$, 5 mg/L of

 $Li_{0.61}Ni_{0.23}Mn_{0.55}Co_{0..22}O_2$, or 5 mg/L of $Li_{0.52}Ni_{0.14}Mn_{0.72}Co_{0.14}O_2$.



Figure 5. Analysis of NMC transformation via a.) ICP-OES measurements of
dissolved Li, Ni, Co, and Mn species from 5 mg/L NMC upon exposure to bacterial
growth medium for 72 h and b.) XPS measurements of NMC before (darker shades) and
after (lighter shades) exposure to bacterial growth medium for 72 h. Red bars indicates
Li_{0.68}Ni_{0.31}Mn_{0.39}Co_{0.30}O₂, blue bars indicate Li_{0.61}Ni_{0.23}Mn_{0.55}Co_{0..22}O₂, and green bars
indicate Li_{0.52}Ni_{0.14}Mn_{0.72}Co_{0.14}O₂. Two replicates are shown with error bars representing
standard deviation.

Environmental Science: Nano

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588 Figure 6. Transmission electron micrographs of *Shewanella oneidensis* MR-1 exposed

589 to A.) $Li_{0.68}Ni_{0.31}Mn_{0.39}Co_{0.30}O_2$, B.) $Li_{0.61}Ni_{0.23}Mn_{0.55}Co_{0..22}O_2$, or C.)

 $590 \qquad Li_{0.52}Ni_{0.14}Mn_{0.72}Co_{0.14}O_{2.}$

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593	Figure 7. Oxygen consumption vs. time for Shewanella oneidensis MR-1 in growth
594	medium in the presence of no NMC, 5 mg/L of NMC of different stoichiometries a.)
595	$X_{Mn}=0.39$, b.) $X_{Mn}=0.55$, and c) $X_{Mn}=0.72$, as well as the respective ion controls.
596	Respective ion controls are a.) 12.9 μ M Ni ²⁺ ,10.3 μ M Co ²⁺ , 11.5 μ M Mn ²⁺ , 38.3 μ M Li ⁺
597	for NMC (X _{Mn} =0.39), b.) 9.56 μ M Ni ²⁺ , 7.35 μ M Co ²⁺ , 16.0 μ M Mn ²⁺ , 40.7 μ M Li ⁺ for
598	NMC (X _{Mn} =0.55), and c.) 5.99 μ M Ni ²⁺ , 4.51 μ M Co ²⁺ , 20.5 μ M Mn ²⁺ , 41.9 μ M Li ⁺ for
599	NMC (X _{Mn} =0.72).

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 $607 \quad \ \ Co^{2+} for \ NMC \ (X_{Mn} = 0.72).$



608

609 Figure 9. ROS Formation. An aminophenyl flourescein assay was used to detect

610 hydroxyl radicals produced from the various NMC in 10mM HEPES buffer after 24 h.

611 Two replicates are shown with error bars representing standard deviation.