

Phosphorus Depletion as a Green Alternative to Biocides for Controlling Biodegradation of Metal Working Fluids

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ABSTRACT

Metal working fluids (MWFs) are used as lubricants and coolants in the manufacturing operations. Their biodeterioration, whilst in-operation, is a widespread problem leading to poor performance, and worker health issues. Adding biocides, though effective in reducing microbial growth, leads to the production of recalcitrant wastewaters, which are difficult to dispose or recycle on-site. Increasing environmental concerns have led to robust legislation for reducing/eliminating the use of toxic biocides in MWF, stimulating a growing interest in the development/application of alternative biological preservation strategies. In this study, inducing nutrient imbalance was investigated for controlling microbial growth in MWF by controlling the availability of a key microbial nutrient. Phosphorus was immobilised employing insoluble La_2O_3 , to form $\text{La}(\text{PO}_4)$. Concentrations of La_2O_3 greater than 0.08%w completely inhibited microbial growth (from $1.4\text{E}+7\text{CFU/mL}$ to 0CFU/mL), and hindered biodegradation. Raman spectroscopy analysis suggested that La_2O_3 converted intracellular phosphorus into $\text{La}(\text{PO}_4)$. The growth inhibition potential of a 0.06%w concentration of the three chemicals studied, were ranked as $\text{La}(\text{NO}_3)_3 > \text{La}_2\text{O}_3 > \text{formaldehyde}$, respectively. The antimicrobial property of La_2O_3 was tenable by adding sufficient phosphate to overcome La_2O_3 inhibition. This technology offers the potential to reduce/eliminate the use of biocides in MWFs, make end-of-life treatment more feasible, and close the water-loop.

Keywords: biodegradation, biocide, phosphorus, nutrient starvation, metal working fluid, toxicity, lanthanum, water reuse

1. INTRODUCTION

Metalworking fluids (MWFs) are widely employed as coolants and lubricants in manufacturing operations such as cutting and rolling of metals, and account for 15% of the total cost of machining.^{1,2} Common compounds used in the formulation of synthetic and semi-synthetic MWFs include: glycols, esters, amines, fatty acids, emulsifiers, corrosion inhibitors, polymers, and biocides.^{3,4} Microbial contamination of water-based MWF is a widespread problem leading to premature biodeterioration, functional failure, hygienic concerns and significant economic loss.^{5,6} MWFs may lose their lubrication and anticorrosion properties as a result of microbial contamination and have to be replaced more frequently, leading to elevated operating costs and significant machining down time. Biocides are therefore added to MWFs to reduce microbial growth and biodeterioration. However, the inclusion of biocides in current MWF has been associated with respiratory and skin diseases for workers, environmentally hazardous wastewater discharge, and challenges with stabilizing biocide concentration (due to water evaporation).^{7,8,9} Furthermore, biocides have sub-optimal performance when considering the increased drive to treat and recycle water on-site and employing more favourable and sustainable waste treatment options such as biotreatment.

Once operationally exhausted, MWFs are typically treated using physicochemical and/or biological processes. Biological treatment of MWF is increasingly taken serious as a disposal option and acknowledged as a potentially cost-effective and more sustainable end-of-life treatment strategy, particularly employing indigenous microbial consortia.^{10,11,12} However, the addition of biocides and recalcitrant components to MWFs formulation, particularly in synthetic and semi-synthetic MWFs, reduces the effectiveness of biological processes as stand-alone end-of-life solutions for treating spent

47 MWFs wastewater to regulatory discharge limits.^{13,15} Hence, additional physicochemical treatments such
48 as advanced oxidation (e.g. ozone and Fenton reactions) are often required to eliminate the recalcitrant
49 components prior to discharge.^{14, 15,16} Annually, 20 billion litres of spent MWF wastewater is generated
50 globally, and the annual cost of treatment and disposal of this wastewater in the UK alone is between 8-
51 16 million pounds.¹⁷

52 The key to designing sustainable MWFs is to make them non-biodegradable when in-operation,
53 and predisposed to biodegradation at end-of-life (cradle-to-grave design). Additionally, they should have
54 reduced environmental emissions, and health and safety risks. The most widely used method today for
55 controlling microbial growth in MWF is the addition of antimicrobial chemicals or biocides. Besides
56 biocides, membrane-separation technologies, ultrasonic sound, and ultraviolet light have been suggested
57 for reducing microbial growth and increasing the life-span of MWF.^{6,18,19}

58 One method of manipulating microbial growth, that has received very little attention, is controlling
59 the nutrient ratios (C:N:P).²⁰ Phosphorus limitation, for instance, often constrains microbial growth, as it
60 serves as an essential building block for nucleic acids, proteins, and energy carriers. Phosphorus
61 immobilisation, has been previously suggested as a means to reduce the growth of microorganisms in
62 water bodies.²¹ Lanthanum binds strongly to phosphate (solubility product $pK_{La(PO_4)}=26.15$),²² and has
63 been used in medical and environmental applications for permanently binding phosphate (e.g. Phoslock®
64 for reducing algae overgrowth in lakes, and Fosrenol® for treating Hyperphosphatemia).^{23,24} Greber *et al.*
65 suggested the use of lanthanum oxide nanoparticles as an antimicrobial strategy with toxicity controllable
66 by the addition of phosphate.²⁵ They reported that as lanthanum oxide converts to lanthanum phosphate,
67 toxicity towards microorganisms decreases.

68 In this study, nutrient imbalance was applied to control microbial growth in MWF. Phosphorus
69 immobilisation employing La^{3+} originated from insoluble La_2O_3 or soluble $La(NO_3)_3$ was used to induce
70 imbalance in the C:N:P. In the case of La_2O_3 , physical immobilization onto an inexpensive media and

application as a cartridge in the MWF recycling unit is proposed as a possible means of removing phosphorus and prolonging MWFs life-span. This technology offers the possibility of switching MWF to a biodegradable waste at end-of-life by recovering and adding the immobilised phosphorus to the MWF wastewater. The approach would reduce/eliminate the need to employ environmentally-hazardous biocides in MWFs formulation, and improve the feasibility of end-of-life treatment.

2. MATERIALS AND METHODS

2.1. Metalworking Fluid and Phosphorus Immobilising Agent

The MWF used in this study was the synthetic Syntilo 9913 (individual components supplied by BP, Lubricants UK Ltd, Swindon, UK), containing eight chemical constituents. Due to commercial sensitivity, the exact identification of the compounds cannot be revealed. In general terms the formulation used in this investigation contained amine, organic acid, benzene derivatives, polymers, and biocide. La_2O_3 , and $\text{La}(\text{NO}_3)_3$ (Sigma Aldrich- Gillingham, UK) were used in this study as phosphorus scavengers. Their mechanism for scavenging phosphorus involves the formation of the highly stable and insoluble lanthanum phosphate ($K_{\text{SPLaPO}_4} = 4\text{E-}23^{26}$).

2.2. Biodegradation and Microbial Growth Measurements

2.2.1. MWF Degradation and Microbial Growth

In order to test the degradation of MWF and extent of microbial growth, a 2% v/v of the synthetic MWF was prepared and 100 mL aliquots were added to 250 mL flasks. La_2O_3 was added to each flask in suspended form to yield concentrations ranging from 0-0.4%w. Half a millilitre of resuscitated mid-exponential acclimated mixed bacterial consortia from a previously prepared freeze dried stock was added to each flask as the inoculum. The mixed bacterial consortia were taken from an active bioreactor treating MWF waste, and freeze-dried to ensure that every experiment was performed with the same starting

93 inoculum.^{27,28} The flasks were kept in a shaking incubator at 20 °C and 120 rpm for 14 days. Chemical
94 oxygen demand (COD) of the mixture was measured (Hach-CODHR-2125915) and used as a
95 biodegradation indicator. Microbial growth in each flask was assessed by the Miles and Misra plating
96 method on Luria-Bertani (LB) agar plates.²⁹

97 The impact of phosphorus (Na₃(PO₄) Sigma Aldrich) addition (10% w of La₂O₃) on controlling
98 the effectiveness of La₂O₃ in quenching biodegradation of MWF was also assessed.

99 2.2.2. Optical Density Measures of Microbial Growth

100 The aerobic growth of single strain *Agrobacterium radiobacter* (designated strain 5-BA-A) was
101 monitored under exposure to test chemicals in a 96-well plate, by measuring absorbance at 630nm over a
102 period of 18 h. *Agrobacterium radiobacter* (designated strain 5-BA-A) has been previously reported as a
103 key microbial strain in the biodegradation of metal working fluids. See supporting information (SI) for
104 detailed procedure.

105 2.2.3. Microbial Consortia Respiration and Post-Exposure Recovery

106 The respiration activity of the mixed bacterial consortia was evaluated under exposure to test
107 compounds using a micro-plate based respiration system, with the trade name of MicroRespTM, which
108 provides a measure of released CO₂. Respiration was determined colourimetrically, based on the change
109 in the colour of a pH indicator dye (cresol red) embedded in agarose gel, upon exposure to CO₂.³⁰ Post
110 exposure recovery tests were conducted using the Miles and Misra method²⁹ on Luria-Bertani (LB) agar
111 plates. Respiration and recovery results were presented as % of control (5g/L glucose, and 815 mg/L
112 NH₄Cl). Furthermore, a dimensionless parameter is defined as “growth prevention potential,” which
113 describes the bacterial inactivation per unit concentration of test compound:

$$114 \text{ growth prevention potential} = \frac{1}{(\text{CFU} / \text{mL as \% of control}) \cdot (\text{concentration of compound \% w})}$$

115

Details of this method can be found in the supporting information (SI) document.

2.3. Toxicity using Biosensors

Two types of biosensors were used in this work to assess the toxicity of selected chemicals: *Acinetobacter baylyi* ADP1_recA_lux, and *Escherichia coli* HB101_pUCD607_lux.

The *A. baylyi* ADP1_recA_lux is a chromosomally based whole cell toxicity-sensing biosensor that is activated to express bioluminescence when exposed to DNA damaging toxicants.³¹ The *lux* operon is fused to the inducible promoter of an essential gene involved in DNA repair, which causes the luminescence to activate when DNA damage occurs.³² Stock suspensions of the *A. baylyi* ADP1_recA_lux biosensor were prepared according to procedure described elsewhere.³¹ In a 96-well microplate, biosensor were exposed to various concentrations of test compounds (La_2O_3 , $\text{La}(\text{NO}_3)_3$, and formaldehyde), and relative luminescence was calculated over 6 h using optical density and luminescence measurements³³

E. coli HB101_pUCD607_lux is an *E. coli* HB101 transformed with the multi-copy pUCD607 plasmid containing the luxCDABE gene cassette from *Vibrio Fischeri*.³⁴ In a healthy cell, with no metabolic impairment, the *lux* reported genes are constitutively expressed leading to the constant production of visible light. When cellular metabolism is disrupted as a result of exposure to test compounds, a decrease is detected in the cellular light output that is proportional to the degree of toxicity.³² In a 96-well microplate, the biosensor luminescence was measured upon 30min exposure to test compounds. See supporting information (SI) document for detailed procedures for both biosensors.

2.4. Raman spectroscopy

Raman spectroscopy is a vibrational spectroscopy technique that can be used to collect the unique chemical finger print of molecules, as each molecule has a different set of vibrational energy levels, and the photons emitted will have unique wavelength shifts. In this work Raman spectroscopy of *Agrobacterium radiobacter* (strain 5-BA-A) was used to verify the mineralization of phosphate in cells

139 upon exposure to La_2O_3 . This microorganism is a common Gram negative bacillus found in the indigenous
140 MWF-degrading mixed community.³⁵ *Agrobacterium radiobacter* cultures (preparation described in SI)
141 were exposed to La_2O_3 for 3h, and then 2 μL of the cellular suspension was spread on a calcium fluoride
142 (CaF_2) slide and allowed to dry before Raman analysis.³⁶ Raman spectra was acquired for the samples
143 using a confocal Raman microscope (LabRAM HR, HORIBA Scientific, London, UK) equipped with an
144 integrated Olympus microscope (BX41). A 50x magnifying dry objective (Olympus, UK) was used to
145 observe and obtain Raman signals, and each spectra were acquired in the range of $3400\text{-}300\text{ cm}^{-1}$ with a
146 resolution of 1 cm^{-1} . An acquisition time of 10 s was used for each measurement, and the Raman scattering
147 was excited with a 532nm Nd:YAG laser (Torus Laser, Laser Quantum, UK). The samples used for Raman
148 spectroscopy were: $\text{La}(\text{PO}_4)$, La_2O_3 , *Agrobacterium radiobacter*, and *Agrobacterium radiobacter*
149 exposed to La_2O_3 .

150 **2.5. Scanning electron microscopy**

151 Scanning electron microscopy was used to qualitatively examine the effects of lanthanum on the
152 indigenous mixed community cells. Microorganisms were exposed to 0.04% La_2O_3 for 2 h, then washed
153 with buffer solution, and immersed in 2.5% glutaraldehyde for 1 h. Treatment with osmium tetroxide,
154 dehydration, and gold coating were carried out according to procedure detailed in SI. Images were
155 acquired using the JEOL JSM-6390 scanning electron microscope at 5 kV.

156 **2.6. In-Line Filtration Unit Tests**

157 The effectiveness of fixated La_2O_3 in protecting synthetic MWF against microbial contamination
158 (indigenous microbial consortia) was tested in a proof-of-concept cartridge unit. Acrylic beads
159 (polyethylene-co-ethyl-acrylate, Sigma Aldrich) were sprinkle-coated with La_2O_3 powder in aluminium
160 trays, and placed in the oven at $65\text{ }^\circ\text{C}$ (softening temperature of the polymer beads). The beads were then
161 washed three times with distilled water (to wash-away unattached La_2O_3), and packed in a glass column

(diameter 4 cm, height 25 cm). The beads were spherical (diameter of 5 mm), and had $18 \pm 2 \text{ g/m}^2 \text{ La}_2\text{O}_3$ coated on their surface. Two filtration units were established; one with uncoated and the other with coated beads. To simulate active biodegradation conditions, preserved indigenous mixed microbial community was used to contaminate MWF (initial concentration of $5.7\text{E}+5 \pm 5\text{E}+4 \text{ CFU/mL}$). One litre of contaminated synthetic MWF was circulated through each unit using peristaltic pumps set at a 3 mL/min flowrate. Microbial growth and COD were measured over the course of one week. To prevent evaporation, all the open vessels were covered with para film.

2.7. Statistical Analysis

The statistical significance of the results was tested by performing student t-test. Each test was repeated in triplicates on three different occasions, and measurements were also conducted in triplicate. The student t-test was assessed in pairs for COD removal, microbial growth, bioluminescence, respiration, and absorbance with $\alpha=0.05$. Calculated p values less than 0.05 demonstrated statistically significant differences.

3. RESULTS

3.1. Biodegradation of synthetic metal working fluid in the presence of La_2O_3

Figure 1 displays the effects of La_2O_3 on the biodegradation of synthetic MWF, and microbial growth after 14 days. As the concentration of La_2O_3 increased, COD values remained stable throughout the experiment and lower bacterial counts were detected, both implying lower levels of MWF biodegradation. At La_2O_3 concentrations greater than 0.08%w, bacterial count (initially at $1.4\text{E}+7 \text{ CFU/mL}$ at time zero) declined to 0 CFU/mL, and COD values (initially at 13750 mg/L) did not significantly change ($P>0.25$). This suggests that La_2O_3 not only prevented further growth of the inoculum, but also inactivated the introduced microbial inoculum.

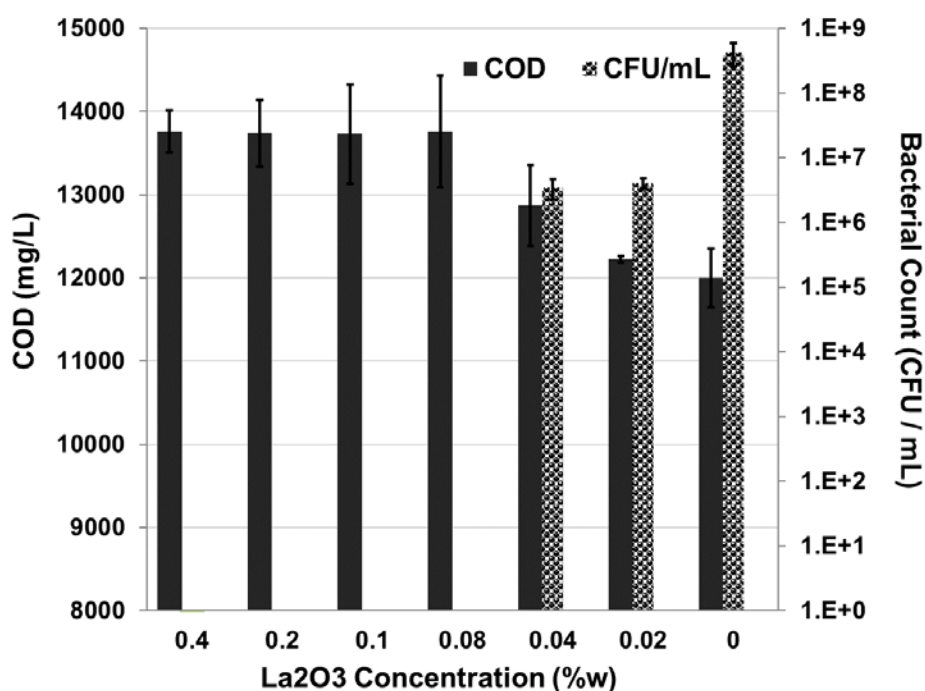


Figure 1. The effect of La₂O₃ concentration on the total culturable microorganisms (CFU/mL) and chemical oxygen demand (COD) reduction of synthetic metal working fluid after 14 days of exposure to bacterial contamination. Bacterial count and COD at time zero were 1.4E+7 CFU/mL and 13750 mg/L, respectively. Error bars represent standard deviation from at least three replicates from three test runs.

Respiration activity and post exposure recovery of indigenous mixed community organisms are shown in Table 1. Both La₂O₃ and La(NO₃)₃ demonstrated inhibitory effects on respiration. It should be noted that high respiration activity (i.e. CO₂ release) could be indicative of both stress (and eventual death) and activity (growth phase).³⁷ It can be seen from this Table that in order to reach over 99% reduction in growth (CFU/mL), the required concentrations of La₂O₃, La(NO₃)₃, and formaldehyde were 0.05, 0.03, and 0.25%w, respectively. In terms of growth prevention potential, between the 0.06%w concentrations of all three compounds, the growth prevention potential of La(NO₃)₃ was almost 4 times greater than that of La₂O₃, and 30 times greater than formaldehyde. This implies that in terms of microbial inactivation per amount of chemical used, La(NO₃)₃ performed best of the three chemicals tested. It should be noted that unlike La₂O₃, which is insoluble, La(NO₃)₃ is a soluble phosphorus scavenger, and that may have contributed to its increased effectiveness in scavenging phosphorus in the aquatic system.

Table 1. Respiration (indicated by % CO₂ of the water control sample), and post exposure recovery (% growth of the water control sample) for indigenous mixed microbial community exposed to La₂O₃, La(NO₃)₃, and formaldehyde.

Compound	Concentration (%w)	Respiration (% CO ₂)	CFU/mL (% of water control)	Growth prevention potential
La ₂ O ₃	0.11	33 ± 11	0	-
	0.06	65 ± 7	0.3 ± 0.08	60.6
	0.027	74 ± 5	3.7 ± 0.5	10.0
	0.006	82 ± 10	9.9 ± 4	16.8
La(NO ₃) ₃	0.14	78 ± 5	0	-
	0.06	84 ± 2	0.06 ± 0.01	238.0
	0.03	82 ± 3	0.6 ± 0.07	55.5
	0.016	80 ± 5	1 ± 0.1	62.5
Formaldehyde	1	78 ± 4	0.05 ± 0.04	20
	0.25	108 ± 6	0.4 ± 0.1	10
	0.06	112 ± 4	2.3 ± 0.7	7.2

3.2. Raman Spectroscopy & Scanning Electron Microscopy

The Raman spectroscopy results demonstrate spectral shifts of *Agrobacterium* (designated strain 5-BA-A), La₂O₃, La(PO₄), and a mixture of La₂O₃ and *Agrobacterium* (Figure 2). It can be seen that *Agrobacterium*, La₂O₃ and La(PO₄) all generated unique Raman spectra. The Raman spectra corresponding to the *Agrobacterium* + La₂O₃ contained peaks associated with pure La₂O₃ and *Agrobacterium* (as expected), but peaks associated with pure La(PO₄) also appeared. As the only source of phosphorus in this sample was *Agrobacterium*, this suggests that La₂O₃ extracted phosphorus from the cells, forming stable La(PO₄). This may be a possible mechanism for the observed killing of exposed bacteria that were in close vicinity to La₂O₃. As represented in Figure 1, La₂O₃ not only prevented further growth of the microorganisms, but also inactivated the initial inoculum.

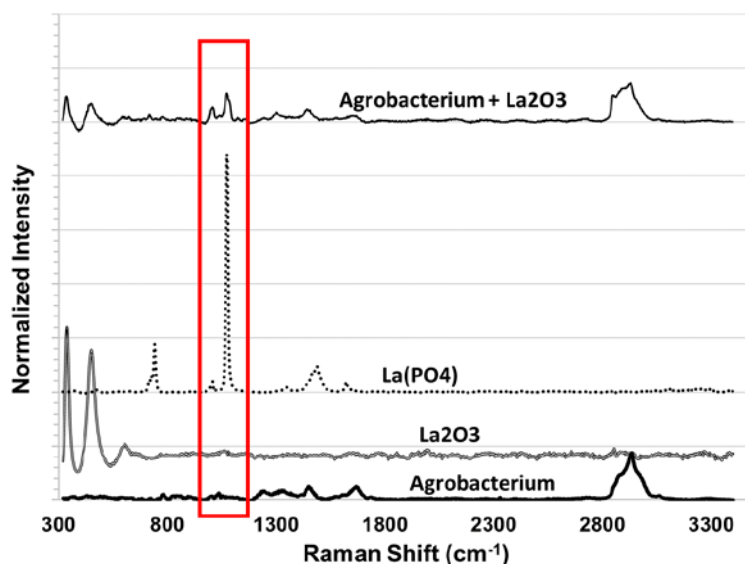


Figure 2. Raman spectrum of individual *Agrobacterium*, La_2O_3 , and $\text{La}(\text{PO}_4)$, as well as *Agrobacterium* mixed with La_2O_3 for two hours. It should be noted that each spectra represents an average of three measurements.

Scanning electron microscopy images of indigenous mixed community cells are shown in Figure 3. Exposure to La_2O_3 caused morphological changes in the microorganisms shifting from rod- to doughnut-shape. Morphological changes in bacterial cells due to environmental stress such as nutrient limitation and oxidative stress have been previously reported in the literature.³⁸ Specifically, conversion from rod- to doughnut-shape has been observed previously in *Campylobacter* species, and believed to be indicative of near-death state.³⁹ Besides nutrient limitation, the nucleation and formation of $\text{La}(\text{PO}_4)$ on the cell-wall may have caused the bacteria to curl as a response to the attached solid. High affinity and binding of rare earth ions to phosphates and carboxyl groups in bacterial cell wall has been previously reported.⁴⁰

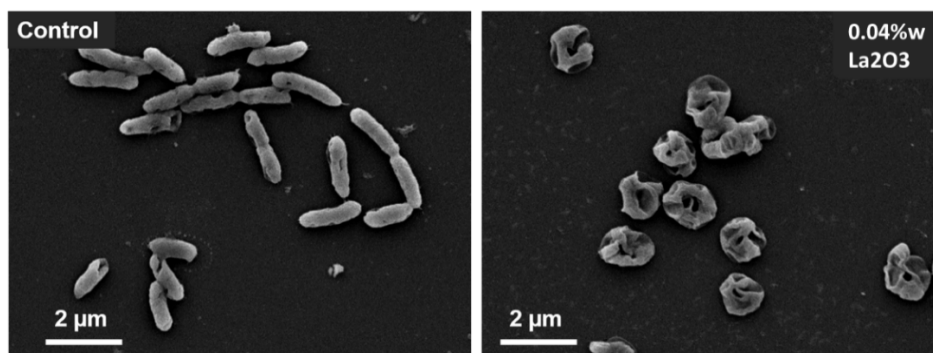


Figure 3. Scanning electron microscopy images of MWF mixed community culture mixed with La_2O_3 , after two hours.

3.3. Metabolic Inhibition and Genotoxicity

The metabolic impairment of *E.coli* HB101_pUCD607_lux upon exposure to La_2O_3 , $\text{La}(\text{NO}_3)_3$ and formaldehyde is represented in Figure 4. It can be seen that the metabolic impairment, exhibited as reduction in cellular light output, caused by La_2O_3 was significantly ($p < 0.005$ at 0.22 and 0.05 %w, and $p < 0.02$ at 0.027 %w) less than that detected for formaldehyde and $\text{La}(\text{NO}_3)_3$. Between formaldehyde and $\text{La}(\text{NO}_3)_3$, however, no statistically significant difference (t-test, $p > 0.25$) was detected in the concentration range examined.

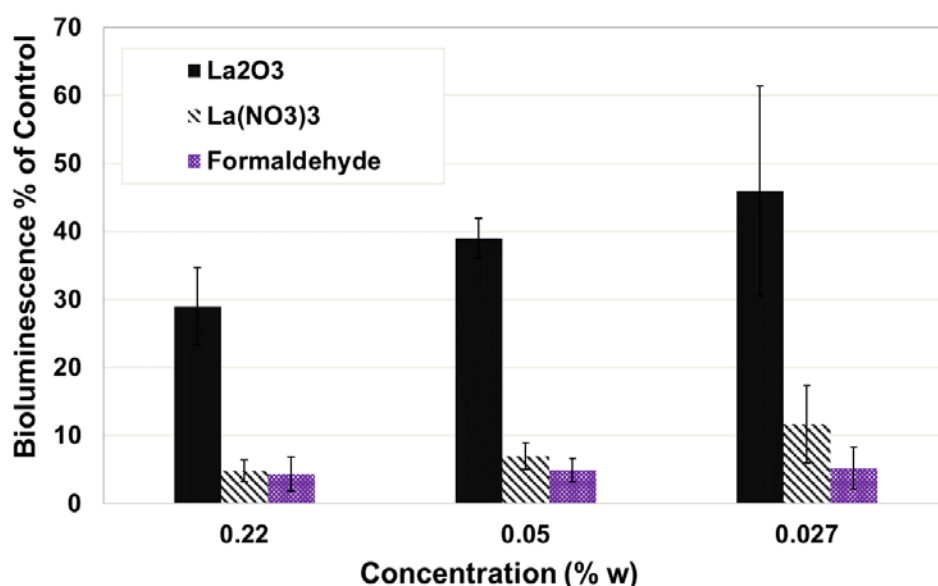
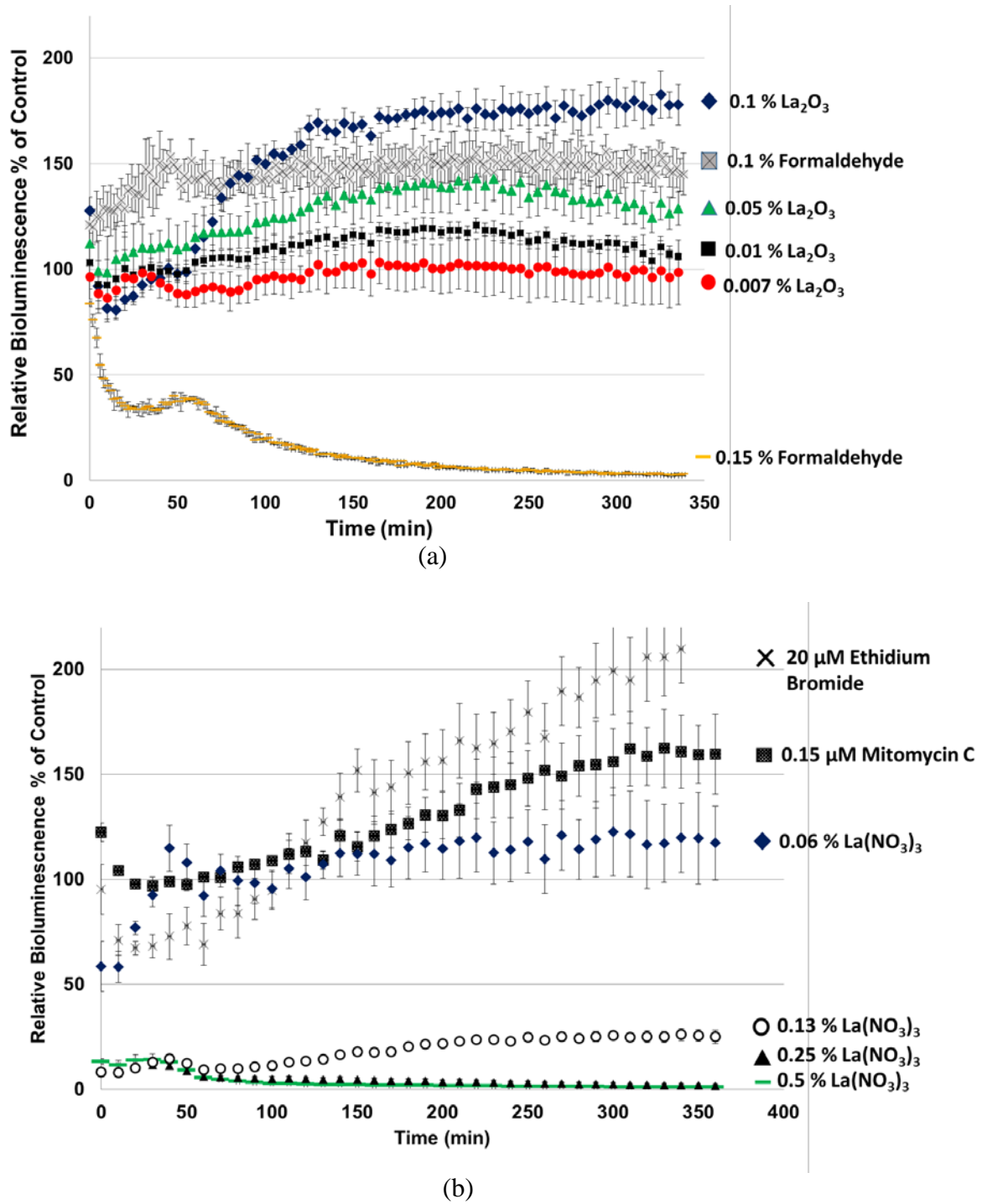


Figure 4. The effect of La_2O_3 , $\text{La}(\text{NO}_3)_3$, and formaldehyde on metabolic activity (represented as the bioluminescence of *E. coli* HB101_pUCD607_lux). Lower bioluminescence % of control represents higher metabolic inhibition. It should be noted here that the control is LB with 50 $\mu\text{g/L}$ Ampicillin. Note that error bars represent standard deviation of three measurements

Toxicity represented by DNA damage in *A. baylyi* ADP1_recA_lux as a result of exposure to various concentrations of La_2O_3 , and $\text{La}(\text{NO}_3)_3$ are shown in Figure 5. This shows the relative bioluminescence of biosensor under various concentrations of the test compounds. DNA damage increased as the concentration of La_2O_3 increased from 0.007% to 0.1% (Figure 5-a). Furthermore, La_2O_3 demonstrated greater toxicity (DNA damage) compared to formaldehyde at 0.1%w (after 150 min), which

245 is consistent with the growth prevention data summarized in Table 1. $\text{La}(\text{NO}_3)_3$, in contrast, demonstrated
 246 greater DNA-damage potential, and at concentrations greater than 0.06% caused death leading to
 247 immediate loss of biosensor luminescence (Figure 5-b).

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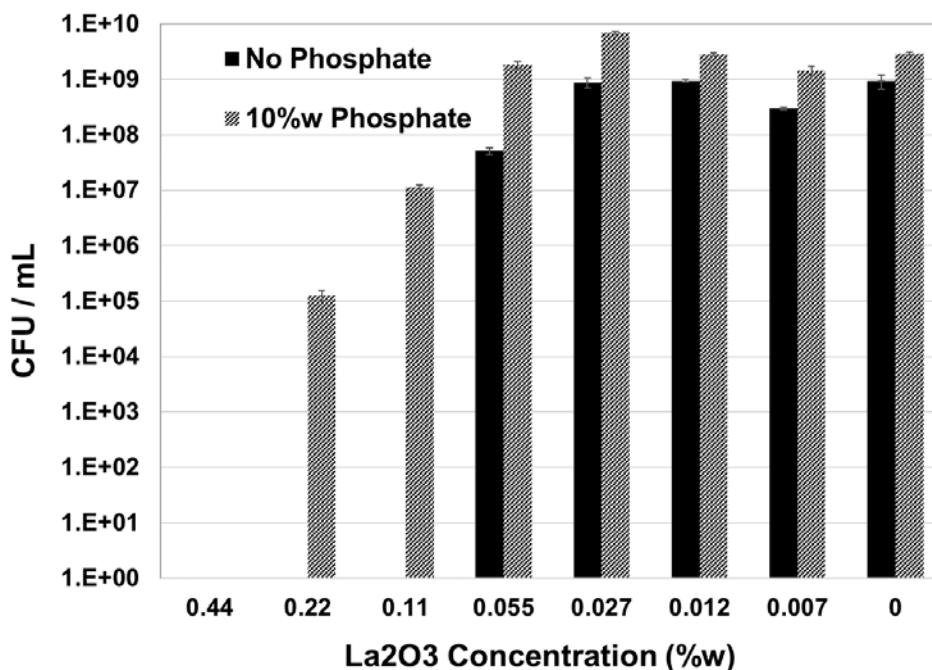
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252 **Figure 5. Effects of La_2O_3 (a), $\text{La}(\text{NO}_3)_3$ (b), formaldehyde (a), ethidium bromide (b), and Mitomycin C (b) on the**
 253 **relative bioluminescence of *ADP1_recA_lux*. It should be noted that control was LB broth with 10 $\mu\text{g/L}$ kanamycin,**

254 and that the ADP1_recA_lux biosensor activates to express bioluminescence in the presence of DNA-damaging events.
 255 The error bars represent standard deviation of triplicate experiments from two different runs.

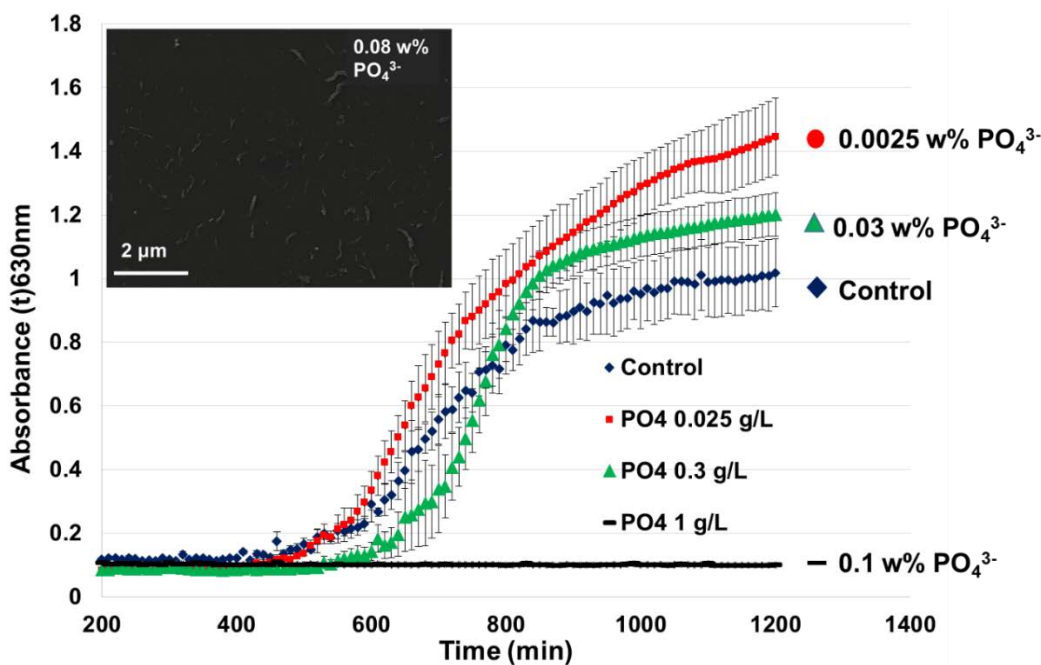
256 3.4. The Effect of Phosphate

257 The antimicrobial property of La_2O_3 were found to be controllable through the addition of
 258 phosphate (Figure 6). In Figure 6-a microbial growth in synthetic MWF is demonstrated at different La_2O_3
 259 concentrations, in the presence and absence of 10%w phosphate. The antimicrobial property of La_2O_3
 260 decreased in the presence of phosphate, suggesting that the La_2O_3 -phosphate combination could be
 261 applied for controlled inhibition of microbial growth in other products susceptible to biodeterioration.
 262 However, there was a limit in term of controlling the toxicity of La_2O_3 with phosphate, since
 263 concentrations greater than 0.08w% were found to inhibit the growth of the indigenous microbial
 264 community (Figure 6-b), and this was most likely due to cell membrane rupture. Figure 6-b represents
 265 microbial growth curves (based on optical density) at various phosphate concentrations. It can be seen that
 266 at 0.1w% phosphate, the growth of indigenous microbial community was inhibited. The SEM of the
 267 indigenous microbial community exposed to 0.08%w appeared as cell debris compared to the healthy
 268 organisms shown in Figure 3.



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(a)



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(b)

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Figure 6. The impact of 10%w phosphate addition on the effectiveness of La₂O₃ for inhibiting microbial growth of indigenous microbial community in synthetic MWF (a). Growth curves of indigenous microbial community exposed to various concentrations of PO₄³⁻ and an SEM image of exposure to 0.8 g/L showing cell debris (b). It should be noted that PO₄³⁻ was added in the form of Na₃(PO₄) and no form of lanthanum was added. Error bars represent standard deviations of triplicate measurements.

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4. DISCUSSION

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Uncontrolled microbial growth in MWF and biodeterioration leads to operational problems including: loss of lubricity, generation of odours, decrease in pH, and microbially induced corrosion.⁴¹ In extreme cases, MWF must be disposed and machines decontaminated, leading to costly down time. Biocides reduce biodeterioration and increase the operational life of MWFs, but cause environmental and health issues.^{7,9} The accelerated degradation and increased global demand of water has highlighted the value of the closed-loop economy, particularly in water-intensive industrial processing.⁴² Closed-loop or circular economy of water is a model in which water is reused repeatedly, ideally on-site, whilst retaining its full value. The integration of biocides in MWFs leads to the formation of recalcitrant and toxic wastewaters that require energy intensive treatment for water discharge/reuse. The European Biocidal

288 Product Directive (BPD) has proposed restrictions on the use of toxic biocides, particularly formaldehyde-
289 releasers, which is driving the MWF industry trends towards less or no biocide usage.⁴³ The US
290 Environmental Protection (EPA) has set the maximum permissible dose for formaldehyde-releasing
291 biocides as 500 ppm, compared to the current limit of 2500 ppm.⁴⁴ The incentive of reducing/eliminating
292 the use of toxic biocides in MWF has stimulated interest in the development of new biological preservation
293 strategies. The aim of this study was to investigate an alternative strategy for reducing microbial growth
294 and the consequent biodeterioration of MWFs, thus avoiding the need of adding biocide.

295 The evidence from this study suggests that nutrient imbalance, specifically phosphorus starvation,
296 as a means of reducing MWF biodegradation and microbial growth has the potential of being very
297 effective. Phosphate in MWF could originate from impurities in the original commercial components, or
298 from the make-up water. The suggested antimicrobial technology in this study only suits MWFs that
299 contain non-phosphorus containing compounds as key lubricating and extreme pressure additives.
300 Lanthanum is an effective phosphate scavenger as it can bind strongly to form stable LaPO_4 . It is likely
301 that the microbial growth inhibition and inactivation in the MWF occurred through direct toxicity of
302 lanthanum compounds, and by nutrient limitation through scavenging cellular and bulk phosphate. The
303 formation of $\text{La}(\text{PO}_4)$ upon contact of microorganisms and La_2O_3 (as shown in the Raman spectra in
304 Figure 2) demonstrates this phenomena. Insoluble La_2O_3 and soluble $\text{La}(\text{NO}_3)_3$ were both demonstrated
305 to be effective in preventing microbial growth in MWF and biodeterioration (as demonstrated in Table 1),
306 and both induced cellular damage (toxicity) to the indigenous microbial consortium (as shown in Figure
307 5). It should be noted that in terms of molarity of La, 1g of La_2O_3 is equivalent to 2.6 g of $\text{La}(\text{NO}_3)_3$,
308 which combined with growth inhibition data reflects the superiority of La in the form of $\text{La}(\text{NO}_3)_3$ in
309 inactivating microorganisms. The ability to reduce the toxicity of La_2O_3 by a second factor (phosphate)
310 promises the possibility of temporal control of MWF antimicrobial activity, inhibiting bio-deterioration
311 when in operation then enabling end-of-life biotreatment method. In terms of microbial growth prevention

312 per amount of compound used, the three test compounds ranked as $\text{La}(\text{NO}_3)_3 > \text{La}_2\text{O}_3 > \text{formaldehyde}$. As
313 far as we are aware, this is the first reported study in which the longevity of MWF, or indeed any other
314 similar product, has been manipulated by inducing a nutrient imbalance rather than adding a toxic
315 ingredient.

316 Two possible application modes are suggested for this technology: integration in the formulation
317 of MWF (for both La_2O_3 and $\text{La}(\text{NO}_3)_3$), and integrated as an external cartridge (for La_2O_3). In case of
318 direct addition, $\text{La}(\text{NO}_3)_3$ can be integrated in soluble form, and the $\text{La}(\text{PO}_4)$ precipitate can be separated
319 by filtration (e.g. membrane, etc.) from MWF. The excess $\text{La}(\text{NO}_3)_3$ can be converted to $\text{La}(\text{PO}_4)$ and
320 separated at end-of life by the addition of phosphate, which in excess could positively influence
321 biodegradation of the waste. In the case of La_2O_3 , direct integration into the MWF formulation as a
322 suspension may cause issues of metal staining. However, coating La_2O_3 on an inexpensive media and
323 integration as an in-line cartridge in the MWF circulation-loop offers the opportunity to apply the
324 technology external to the MWF formulation. The proof-of-concept in-line cartridge unit used in this study
325 containing La_2O_3 -coated acrylic beads reduced microbial contamination to zero within two days of its
326 application (Table 2). As the cartridge test were performed as proof of concept, future work should include
327 assessing the capacity for total phosphorus capture by the cartridge, and experimenting with the acid/base
328 wash to determine phosphorus recovery and regeneration of La_2O_3 .

329 **Table 2. Microbial growth (CFU/mL) as a result of contamination in the in-line cartridge filled with La_2O_3 -**
330 **coated and uncoated acrylic beads.**

	Control-Flask	Cartridge with Uncoated Beads	Cartridge with La_2O_3 -coated Beads
Day 0	$5.7\text{E}+5 \pm 5\text{E}+4$	$5.7\text{E}+5 \pm 5\text{E}+4$	$5.7\text{E}+5 \pm 5\text{E}+4$
Day 2	$6.5\text{E}+4 \pm 5\text{E}+3$	$4\text{E}+4 \pm 1\text{E}+4$	0
Day 7	$3.8\text{E}+5 \pm 2.7\text{E}+4$	$8.4\text{E}+4 \pm 7.6\text{E}+3$	0

331
332 With this approach the cartridge immobilises phosphorus from the bulk MWF solution and
333 concentrate it in the form of $\text{La}(\text{PO}_4)$, which can be removed from the system physically, once saturated
334 with phosphorus. Acid/base treatment using 0.5M HCl, or 12.5M NaOH for approximately 5 h, have been

335 reported as effective methods for the recovery (over 90%) of phosphate from $\text{La}(\text{PO}_4)$.⁴⁵ As a phosphorus
336 fixating technology, immobilised La_2O_3 on plastic media can serve as a platform technology for the
337 capture and recovery of this finite resource. Examples of other applications include phosphorus capture
338 from nutrient-rich media such as anaerobic digestion effluents, and reducing phosphate in surface waters
339 for preventing algal blooms. Key advantages to the proposed technology for preventing microbial growth
340 in MWF are: adjustable toxicity, physical mobility (can be removed physically from system), no dose
341 adjustment requirements, and potential for regeneration of active compound. The next steps of this study
342 include developing processes for the recovery of phosphate and La_2O_3 from $\text{La}(\text{PO}_4)$, and determining
343 the capacity of regenerated La_2O_3 for further use to capture phosphorus.

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