The effect of pyrite on *Escherichia coli* in water: proof-of-concept for the elimination of waterborne bacteria by reactive minerals

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ABSTRACT

We present proof-of-concept results for the elimination of waterborne bacteria by reactive minerals. We exposed *Escherichia coli* MG1655 suspended in water to the reactive mineral pyrite (FeS_2) at room temperature and ambient light. This slurry eliminates 99.9% of bacteria in fewer than 4 hours. We also exposed *Escherichia coli* to pyrite leachate (supernatant liquid from slurry after 24 hours), which eliminates 99.99% of bacteria over the same time-scale. Unlike SOlar water DISinfection (SODIS), our results do not depend on the presence of ultraviolet (UV) light. We confirmed this by testing proposed SODIS additive and known photo-catalyst anatase (TiO_2) for antibacterial properties and found that, in contrast to pyrite, it does not eliminate *E. coli* under our experimental conditions. Previous investigations of naturally antibiotic minerals have focused on the medical applications of antibiotic clays, and thus have not been conducted under experimental conditions resembling those found in water purification. In our examination of the relevant literature, we have not found previously reported evidence for the use of reactive minerals in water sanitization. The results from this proof-of-concept experiment may have important implications for future directions in household water purification research.

Key words | antibacterial minerals, household water treatment, sanitation, sustainable development

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INTRODUCTION

Worldwide, diarrheal diseases are the second most common cause of death for children under five years old (Bhutta *et al.* 2013). Exposure to the four pathogens (rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli* and *Shigella*) most often responsible for moderate-to-severe diarrhea (MDS) in children (Kotloff *et al.* 2013), is preventable, and generally stems from the consumption of unsanitary or fecally contaminated water (Bhutta *et al.* 2013). A complete response to diarrheal disease requires improvements in the availability of medical treatment, water provision, and sanitation (Bhutta *et al.* 2013). However, research shows that interventions addressing water quality (protection or treatment at the source or point-of-use) are significantly more effective at reducing childhood morbidity from MDS than those that improve water supply (improved source or distribution) (Waddington *et al.* 2009). One methodology for point-of-use water treatment involves treating water in the home, instead of centrally or at the source. Such techniques, known as household water treatment and safe storage (HWTS), may provide cost-effective interim approaches for improving water quality and have become an important part of the joint WHO/UNICEF strategy to control diarrhea (WHO & UNICEF 2013). Many currently available HWTS devices have already been shown to provide effective, low-cost methods for improving drinking water quality (Lantagne *et al.* 2011) in the homes of the more than 768 million people who lack access to improved water sources (WHO & UNICEF 2013), and the estimated 1.2 billion additional people who use water from sources with significant sanitary risks (Onda *et al.* 2012).

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Common, currently available HWTS options include: boiling, filtration (biosand and ceramic), chlorination, combinations of filtration or flocculation with chlorination, and SOlar water DISinfection (SODIS). Although these technologies are effective, they are not without their drawbacks. Because SODIS involves exposing water to sunlight in clear plastic or glass bottles, its only associated cost is that of acquiring an appropriate bottle, therefore it is an essentially no-cost intervention (Lantagne *et al.* 2011). However, the efficacy of SODIS depends on highly variable solar intensity (Berney *et al.* 2006), which makes sustained use difficult to encourage and reduces its overall efficacy (Mäusezahl *et al.* 2009). In contrast to SODIS, initial cost is a major drawback of biosand filters (CDC & USAID 2008d), while chlorination (with and without flocculation) and ceramic filtration both require continued investment or replacement (CDC & USAID 2008a, b, 2010). In addition, many ceramic filters contain colloidal silver, which is both expensive and potentially dangerous, as an antibacterial agent. HWTS techniques based on chlorination have the added environmental costs associated with chlorine manufacture (Stringer & Johnston 2001). Chlorination also faces safety concerns about the potential long-term carcinogenic effects of chlorine by-products (CDC & USAID 2008c). Boiling has low monetary costs and has been traditionally practiced for hundreds of years, but it has high fuel, environmental, and human health costs (Gadgil 2008; CDC & USAID 2009). Thus, a novel HWTS technique that addresses some of the drawbacks of the currently available HWTS options may be more effective and attractive to users, saving additional lives.

Efforts to improve existing HWTS options can be found throughout the water treatment and sustainable development literatures. In particular, research to speed up or mitigate the solar dependence of SODIS has revealed both the mechanisms of SODIS (Acra *et al.* 1980; Malato *et al.* 2007), and many possibilities for improving the speed or efficacy of these mechanisms through chemical additives (e.g., Berney *et al.* 2006; Gelover *et al.* 2006; Fisher *et al.* 2008; Heaselgrave & Kilvington 2010; Sciacca *et al.* 2010; Spuhler *et al.* 2010; Harding & Schwab 2012). Unfortunately, many of these experiments assume extensive UV exposure (Gelover *et al.* 2006; Malato *et al.* 2007; Spuhler *et al.* 2010) and thus continue to depend on solar radiation. Several other proposed improvements involve pure chemicals (e.g. Fisher *et al.* 2008; Sciacca *et al.* 2010; Spuhler *et al.* 2010) not readily available in developing countries. However, many proposed SODIS additives are chosen based on the hypothesis that UV-produced reactive oxygen species (ROS) drive bacterial cell death in SODIS (Malato *et al.* 2007; Spuhler *et al.* 2010). This is very similar to the products observed for reactive minerals in solution in the geochemical literature (Schoonen *et al.* 2000, 2006; Borda *et al.* 2001, 2003, 2004; Cohn *et al.* 2004, 2006b; Harrington *et al.* 2012a). Thus, we hypothesized that minerals might provide effective, natural water sanitization that acts via a mechanism similar to that of SODIS, and may address some of its drawbacks.

Recent results from experiments on the efficacy and composition of naturally occurring antibiotic clays (Williams *et al.* 2004, 2008, 2011) further support the potential of minerals as water sanitizers. Clay minerals, however, are impractical for use in water sanitization as they are fine-grained and difficult to remove from water. Further, natural variation in clay mineral deposits makes the identification of potentially antibacterial clays difficult, and hinders the understanding of the drivers of their antibacterial properties (Haydel *et al.* 2008; Williams *et al.* 2008).

We tested the effectiveness of a natural (Huanzala, Peru) pyrite sample of high purity (Harrington *et al.* 2012a) at the reduction of culturable *E. coli* in water as a proof-of-concept experiment demonstrating the potential of the elimination of waterborne bacteria by minerals. We chose pyrite based on observations of ROS production by pyrite in solution in the geochemical literature (Schoonen *et al.* 2000; Borda *et al.* 2001, 2003; Cohn *et al.* 2004, 2006c; Harrington *et al.* 2012a), the presence of pyrite in one particularly effective antibiotic natural clay (Williams *et al.* 2011), and compelling results that iron-based additives significantly improve the speed and efficacy of SODIS (Sciacca *et al.* 2010; Spuhler *et al.* 2010). As a comparison, we also tested the ability of pure, synthetic anatase (TiO_2), a previously proposed SODIS additive (Gelover *et al.* 2006) to reduce culturable *E. coli* bacteria in water in the absence of UV light. Finally, we combined pyrite leachate with the iron-chelator ethylenediamenetetraacetic acid (EDTA) to reduce the reactivity of the dissolved iron, as well as the enzyme catalase to remove hydrogen peroxide, a precursor to other

ROS. The results of these experiments confirmed that cell death is driven by a combination of dissolved iron and ROS, a mechanism similar to that hypothesized for SODIS.

Our experiments involved exposing exponential phase *E. coli* MG1655 bacteria to one pure mineral, thus ensuring that any observed bactericidal effects would be attributable only to the tested mineral (or leachate). We conducted our experiments in conditions similar to actual water purification: bacteria suspended in water, room temperature, and ambient light. This contrasts with previous research on antibiotic clays, in which cells are usually incubated with the mineral slurry or leachate and retained (at least partially) in growth media (Williams *et al.* 2004, 2008, 2011; Haydel *et al.* 2008; Cunningham *et al.* 2010; Otto *et al.* 2010); and also with previous research on SODIS additives (Gelover *et al.* 2006; Fisher *et al.* 2008; Heaselgrave & Kilvington 2010; Sciacca *et al.* 2010; Spuhler *et al.* 2010; Harding & Schwab 2012), which usually involves UV exposure. We also present time-dependent bacterial survival, which has not been extensively studied in mineral slurries.

MATERIALS AND METHODS

Tested anti-bacterial minerals and materials

We tested the anti-bacterial properties of the reactive mineral pyrite (FeS_2) in comparison with the photo-catalyst anatase (TiO_2) by exposing *E. coli* MG1655 bacteria to slurries of these minerals. To understand the chemical drivers of cell death in pyrite slurry and to eliminate physical bacteria–mineral interactions as a potential cause of cell death, we also tested bacterial survival in pyrite leachate. We then used the iron-chelator EDTA and enzyme catalase to chemically modify pyrite slurry and test potential chemical drivers for cell death. Finally, we tested the survival of *E. coli* in acid solution to confirm that acidity was not a factor in bacterial elimination by pyrite.

Pyrite (FeS_2)

Natural pyrite from Huanzala, Peru was purchased from Wards Natural Science and prepared for use in our experiments according to methods previously described by Harrington *et al.* (2012a). Two batches of pyrite were prepared, one for use during the slurry exposure experiments and a later, additional batch used in our leachate exposure experiments. The specific surface areas (SSA) of both samples were determined by analysis with a Quantachrome NOVA 5-point BET analyzer. The SSA of the pyrite used in the slurry experiments was found to be 2.434 m^2/g, while that for the pyrite used in the leachate exposure experiments was 4.354 m^2/g. We added appropriate amounts of pyrite from each batch to achieve mineral loadings of 0.10 m^2/mL. All mineral loadings were normalized to SSA because previous mineral toxicity research has shown that toxicity between materials is most easily compared when normalized with respect to exposed surface area (e.g., Harrington *et al.* (2012a) and citations therein).

Anatase (TiO_2)

We purchased reagent-grade (99%) synthetic anatase powder from Fisher Scientific and used it as received in our anatase slurry exposure experiments. The SSA was again analyzed using a Quantachrome NOVA 5-point BET analyzer and found to be 9.471 m^2/g. To allow for direct comparisons between our pyrite and anatase experiments, we maintained a surface area-normalized mineral loading of 0.10 m^2/mL.

Mineral slurry preparation

We added 0.26 g of the <38 μm size-fraction (specific surface area = 2.434 m^2/g) of our prepared pyrite sample to 5 mL bacterial solution, for a total surface-area normalized pyrite loading of 0.10 m^2/mL. We used the same surface area-normalized loading for our anatase exposure experiments; 0.0106 g of anatase at specific surface area = 9.471 m^2/g added to 1 mL bacterial solution gives a 0.10 m^2/mL TiO_2 loading.

Pyrite leachate preparation

Our pyrite leachate consisted of 0.11 g of the <38 μm size-fraction (specific surface area = 4.354 m^2/g) of our second prepared pyrite sample added to 5 mL deionized water at

a surface area-normalized loading of 0.10 m^2/mL. We left this slurry on a shaker for 24 hours, and then filtered it through a 0.2 μm filter to remove any suspended mineral particles. This leachate can be considered representative of the most extreme amounts of potentially bactericidal chemicals released by pyrite in our mineral slurry experiments.

Stock EDTA solution preparation

We used reagent-grade disodium ethylenediameneteraacetate dihydrate (Sigma-Aldrich) to make a stock solution of 0.5 M EDTA, which we diluted to a 250 mM concentration. We then treated the pyrite leachate with 20 μL of EDTA solution per 1 mL leachate, based on our measurements of dissolved Fe_{total}.

Catalase treatment

To remove hydrogen peroxide, a precursor to OH radical, from our pyrite leachate, we added 10 mg of culture-suitable solid catalase (Sigma-Aldrich) directly to 1 mL leachate, based on our measurements of the total ROS production in pyrite leachate after 24 hours.

Chemical analysis of pyrite leachate

We measured the dissolved Fe_{total} (both ferrous and ferric) of the leachate using Ferrozine reagent (Schoonen *et al.* 2006). We also measured the production of ROS with an OH-detection protocol using 3′-(*p*-aminophenyl)fluorescein (APF), which converts to a fluorescent species in the presence of OH radical. We measured the fluorescence of APF after 24 hours of exposure to our mineral slurries using a HACH4000 bench-top spectrophotometer equipped with a fluorometer setting and compared our measured values to empirical calibration curves to determine the OH-radical concentration in pyrite slurry after 24 hours. This method has previously been shown to effectively determine the concentrations of ROS in mineral slurries (Cohn *et al.* 2009).

Acid solution

We diluted stock 12 M HCl (Sigma-Aldrich) to a 1 mM concentration with de-ionized water to produce a solution at pH = 3.0, which we used to test *E. coli* MG1655 survival in acid. pH = 3.0 was the lowest pH value we observed in either our pyrite leachates or slurries over 24 hours.

Bacterial culture

We performed all of our experiments using *E. coli* MG1655 bacteria obtained from the laboratory of Dr. Wali Karzai in the Department of Biochemistry and Cell Biology and Center for Infectious Diseases at Stony Brook University. Fresh cultures were prepared in advance of each replicate by subculturing from a liquid culture incubated overnight in Luria–Bertrani (LB) broth at 37 °C. Subcultures were incubated in LB at 37 °C to approximately log-phase, an optical density at 600 nm (OD_{600}) of about 1, or 4–5 hours. The bacteria were collected by centrifugation, rinsed twice in de-ionized water by centrifugation and suspended in de-ionized water. The bacterial suspension was diluted to produce a 10 mL bacterial solution at an OD_{600} of 0.05. This dilute bacterial solution was then separated into two 5 mL samples, one of which received treatment (pyrite or anatase) and the other served as control. For leachate exposure, the test bacterial population was collected by centrifugation after the final rinse and re-suspended in pyrite leachate, chemically modified (by the addition of either EDTA or catalase) pyrite leachate or HCl. The control population again remained in water.

Pyrite and anatase slurry exposure

Both the mineral-treated and control bacterial solutions were maintained in suspension on a bench-top shaker at room temperature for 24 hours. Aliquots were taken immediately after mineral addition, after 1 hour, 4 hours, and 24 hours to measure time-dependent bacterial elimination. Each of these experiments was repeated in triplicate with independent replicates.

Pyrite leachate exposure experiments

Both the test *E. coli* bacterial population (suspended in pyrite leachate), and the control population (suspended in water) were retained in suspension on a bench-top shaker at room temperature for 24 hours. Aliquots were taken

immediately before treatment, immediately after treatment, at 30 minutes (leachate only), 1 hour, 2 hours (leachate only), and 4 hours. We replicated these experiments in triplicate.

Acid exposure experiments

The test *E. coli* population (suspended in acid) and the control population (suspended in water) were retained in suspension on a bench-top shaker at room temperature for 24 hours. Aliquots were taken immediately before treatment, at 2 and 24 hours after exposure. The experiment was replicated in triplicate.

Mitigation of pyrite leachate to understand the drivers of bacterial elimination

Based on our measurements of Fe_{total}, we added 20 μL of stock EDTA solution to 1.0 mL total leachate. In a separate experiment, we added 10 mg catalase to 1.0 mL total leachate. We then exposed exponential phase *E. coli* MG1655 to either water (control), untreated leachate (positive control), EDTA-treated, or catalase-treated leachate. We measured viable bacterial populations (CFU/mL) immediately before the final suspension in leachate (pre-treatment), at 0 minutes (immediately after treatment with leachate) and at 2 hours. Cell death at these time-points was then compared to that observed in slurry and unmodified leachate. Each of these experiments was independently replicated in triplicate.

Quantifying *E. coli* elimination

To measure time-dependent bacterial survival, aliquots of each bacterial suspension were taken at the specified time-points throughout each experiment. These aliquots were then serially diluted 10^{-2} to 10^{-4}-fold and 10 μL spots were placed on dry LB agar plates, which were incubated for 12 hours. For each time-point, we spotted each dilution twice, on two separate dry LB agar plates to account for variability in the dilute bacterial suspensions. We used standard colony counting techniques to detect viable bacteria (CFU/mL) after the techniques described in Zuberer (1994). Colony counts were first estimated by using the 10 μL spots and accounting for serial dilution. These were confirmed in later replicates by plating 1 mL of the lowest dilution in which visible colonies occurred.

RESULTS

Bactericidal effects of pyrite slurry

We found pyrite to be extremely detrimental to bacterial viability (Figure 1(a)), and observed a steady decrease in viability over the 24-hour slurry exposure period. Four

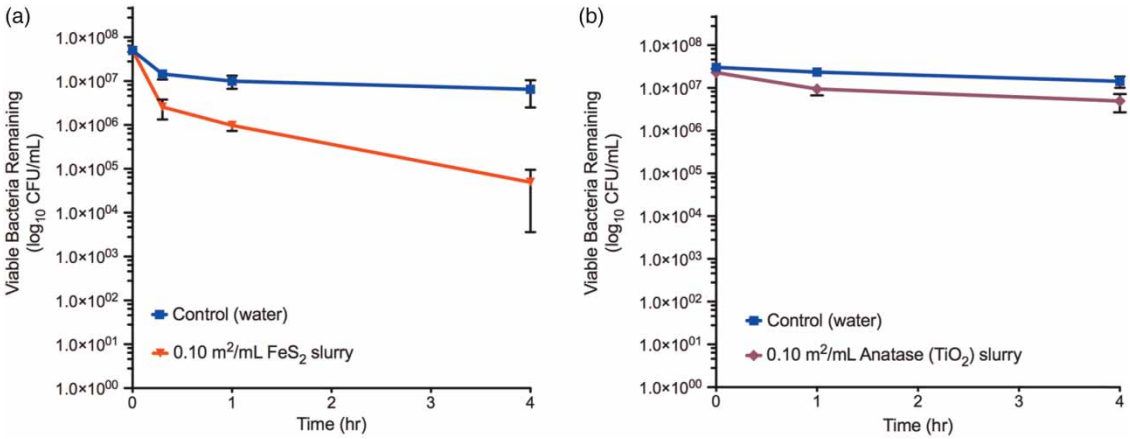

Figure 1 | *E. coli* MG1655 cell death in pyrite (FeS_2) and anatase (TiO_2) slurry. Pyrite slurry causes rapid cell death in water with 99.9% of original bacteria eliminated after 4 hours (a). In contrast, anatase slurry does not cause significant culturable *E. coli* bacterial reduction after 4 hours (b). Both pyrite and anatase exposures were conducted in the absence of UV light. Displayed values represent mean CFU ($N = 3$, SEM) of viable bacteria at each time point.

hours of pyrite slurry exposure eliminated 99.9% of the original viable bacteria. This is 2 hours faster than the recommended SODIS exposure time (CDC & USAID 2008e). However, the *E. coli* is not completely eliminated until after 4 hours. Thus, exposure to pyrite slurry will not make drinking water EPA compliant (US EPA 2012) until after exposures longer than 4 hours. However, three-fold viable *E. coli* reduction after only 4 hours is a large reduction of bacterial load. With fewer active bacteria, the risk of exposure to diarrheal disease may be reduced.

We observed an inconsistent initial reduction in bacterial viability immediately after the addition of pyrite. Viability is not consistently reduced to below 10% until after 1 hour of exposure. These results are consistent with previous experiments exposing live epithelial lung cells to reactive earth materials (Harrington *et al.* 2012a, b), although the rate of cell death is significantly slower for bacteria. A similar trend has been observed for bacteria exposed to bactericidal clay and clay leachates (Cunningham *et al.* 2010; Williams *et al.* 2011). The quick and effective sanitization of water by pyrite demonstrates the potential of mineral water sanitization. After 24 hours, culturable *E. coli* is reduced to zero in pyrite slurry. No colonies form, even after plating a full 100 μL of slurry. Bacterial elimination by pyrite proceeds in the absence of UV light.

In the absence of UV light anatase is not anti-bacterial

In contrast to previous experiments on anatase in the presence of a UV light source, we found only a minimal difference between bacterial survival in water (control) and anatase slurry (Figure 1(b)). These results reinforce the known dependence of photoactive bactericides on UV light. Although such additives make SODIS faster and more effective (Gelover *et al.* 2006; Fisher *et al.* 2008; Heaselgrave & Kilvington 2010; Sciacca *et al.* 2010; Spuhler *et al.* 2010; Harding & Schwab 2012), their dependence on photo-activation means end-user results will still be weather dependent and potentially inconsistent.

Bactericidal effects of pyrite leachate

We then conducted a series of experiments to understand the interactions between *E. coli* MG1655 and pyrite. Understanding what leads to the elimination of *E. coli* by pyrite is necessary for determining which minerals may make effective future water sanitization aids. *E. coli* bacterial elimination occurs more quickly in 24-hour pyrite leachate than in pyrite slurry (Figure 2). These results eliminate physical grain–bacteria interactions as the driver of pyrite's bactericidal properties.

Chemical analysis of pyrite leachate

The 24-hour pyrite leachate contains 100 ± 10 (SD) mg/L total Fe and produces 4.39 ± 0.2 (SD) μmol/mL OH radical. Based on these observations, we surmised that the dissolution of iron into solution drives both bacterial elimination and a steady production of ROS. This hypothesis is supported by previous work on the oxidation of pyrite and its production of OH radical in solution (Borda *et al.* 2003, 2004). The pyrite-produced ROS may disrupt the cell membrane while $Fe_{(aq)}$ infiltrates and overwhelms the cell. A lag between mineral addition and dissolution in slurry might explain the quicker bacterial elimination observed in leachate. The greater importance of chemical, rather than physical, interactions in bacterial elimination by pyrite reinforces the potential for using these findings as a starting point to find and test other mineral bactericides. It may be possible to find commonly available rocks and/or minerals that will be more appropriate for use in HWTS

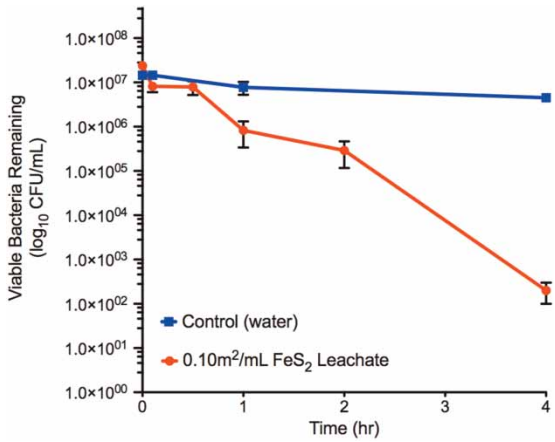

Figure 2 | *E. coli* MG1655 cell death in 24-hour pyrite leachate. *E. coli* bacteria die more rapidly in pyrite leachate than slurry. Leachate eliminates 99.99% of original bacteria after 4 hours. Displayed values represent mean CFU ($N = 3$, SEM) of viable bacteria at each time point.

techniques than pyrite. For example, laterite, a soil type rich in iron oxides that is common in the tropics and is an effective arsenite adsorbent (Maiti *et al.* 2007), may be an effective treatment for bacterially contaminated water as well, but has not yet been specifically tested for antibacterial properties. However, a laterite-based constructed soil filter was reported to cause a 3-log reduction in culturable fecal coliform over the course of a season at an Indian water treatment plant, which is encouraging (Kadam *et al.* 2009).

Chemical drivers of pyrite leachate's reduction of culturable *E. coli* in water

To better understand the drivers of pyrite's bactericidal properties, we systematically blocked the chemical interactions of the three major products of pyrite dissolution: dissolved iron, ROS, and acidity. We first added the iron-chelator EDTA to pyrite leachate to reduce the reactivity of the dissolved iron. We then added the enzyme catalase to remove hydrogen peroxide, a precursor to OH radical. Finally, we compared bacterial survival in acid to that observed in acidic pyrite leachate. The addition of EDTA (Figure 3(a)) and catalase (Figure 3(b)) both significantly reduced pyrite leachate's bactericidal efficacy. Bacterial survival is higher after EDTA addition than catalase addition (Figure 3). However, iron chelation also reduces the capacity for ROS production (Cohn *et al.* 2006a), so there may be combined effects between ROS and dissolved iron for which these experiments cannot account. To address these combination effects, we attempted to test bacterial survival in leachate to which we simultaneously added both EDTA and catalase. However, this produces an unidentified precipitate and does not prevent bacterial elimination (Supplemental information, Figures S1 and S2, available online at http://www.iwaponline.com/wh/013/013.pdf). Despite this, results showing increased *E. coli* colony forming units in pyrite leachate after either dissolved iron or ROS have been mitigated, indicate that ROS and dissolved iron may act in combination to eliminate *E. coli* cells in water. Such a mechanism is similar to that proposed for bactericidal natural clays (Williams *et al.* 2008, 2011), and has also been suggested as an explanation of the enhanced bacterial reduction of SODIS in the presence of a natural, iron-rich clay from Burkina Faso (Sciacca *et al.* 2010).

Pyrite dissolution in the presence of oxygen produces sulfuric acid via Reaction (1)

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+}_{(aq)} + 4SO^{2-}_{4\ (aq)} + 4H^{+}_{(aq)} \quad (1)$$

Dissolving pyrite rapidly achieves pH = 3 and then stabilizes. To test whether low solution pH drives cell death, we exposed *E. coli* MG1655 to 1 mM HCl (pH = 3.09). After 24 hours of exposure, *E. coli* MG1655 viability is slightly reduced (Figure 4), but this reduction is not at all comparable to the bacterial elimination observed in either leachate or slurry. This implies that pH is not the

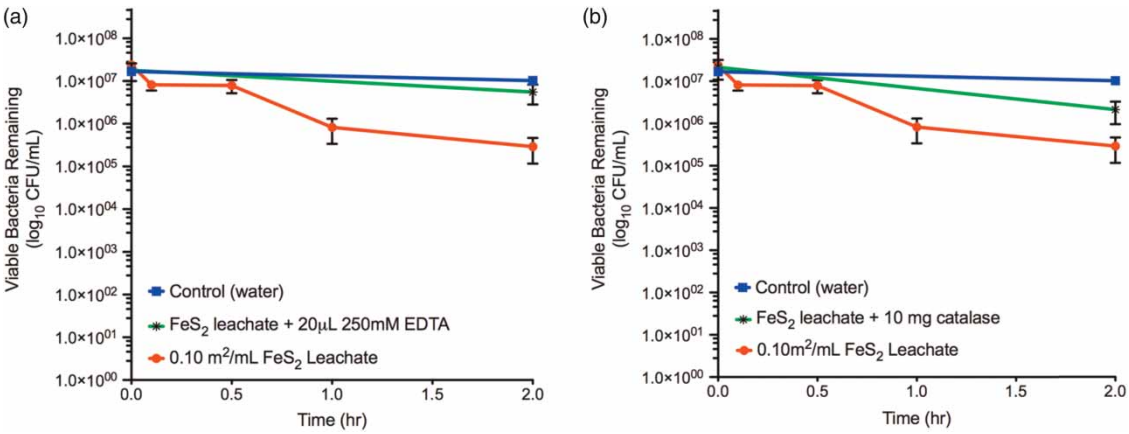

Figure 3 | Comparison of *E. coli* MG1655 survival in pyrite leachate with added EDTA versus added catalase. (a) Bacterial survival in 24-hour pyrite leachate with added EDTA. (b) Bacterial survival in 24-hour pyrite leachate with added catalase. EDTA more effectively prevents bacterial cell death, confirming $Fe_{(aq)}$ as a driver of pyrite's bactericidal properties. Plots display mean CFU ($N = 3$, SEM) of viable bacteria at each time point.

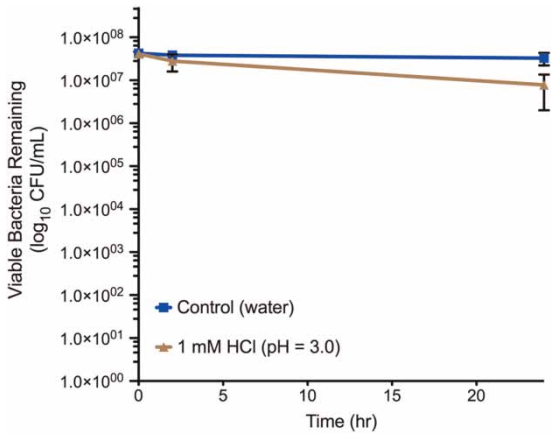

Figure 4 | *E. coli* MG1655 survival in 1 mM HCl (pH = 3.0) after 24 hours. *E. coli* MG1655 cell death in 1 mM HCl is not comparable to that in pyrite leachate or slurry. Plot displays mean CFU ($N = 4$, SEM) of viable bacteria at each time point.

driving factor of pyrite's bactericidal properties. This finding is consistent with previous research on *E. coli* survival in acidic solutions (Small *et al.* 1994; Cunningham *et al.* 2010).

These results confirm that the major drivers of pyrite's bactericidal properties are chemical and result from the release of ROS and dissolved ferrous iron into solution.

DISCUSSION

In this study, we investigated the ability of one highly reactive mineral, pyrite, to reduce culturable *E. coli* in water in order to understand the potential of reactive minerals to eliminate waterborne bacteria for use in possible HWTS techniques. Our experiments demonstrate the hitherto untapped potential of minerals as sustainable point-of-use techniques to eliminate common waterborne pathogens in the water of people who lack access to appropriate water treatment and sanitation. Worldwide, 1.2 billion people lack access to clean water and must use compromised water sources with high sanitary risks (Onda *et al.* 2012; WHO & UNICEF 2013). Effective methods for addressing this global problem must be low-cost, simple to implement, locally available, and involve familiar materials (Lantagne & Clasen 2012). Ideally, such methods will also be environmentally responsible and themselves sustainable. The use of inexpensive and readily available minerals as a water sanitation tool may be a promising approach. However, all HWTS technologies require ongoing operation and maintenance efforts in addition to community investment and behavioral change to promote sustained usage (Figueroa & Kincaid 2010). Thus, developing mineral-based water treatment options is only a start and must be followed by engagement with the social and cultural issues surrounding sanitation as well.

Possible implementation options for mineral water sanitization

HWTS interventions with the highest rates of effective use target high-risk households, offer an effective technique, present this technique to a population familiar with it, and provide appropriate materials for use (Lantagne & Clasen 2012). Minerals and earth materials are ubiquitous, cheaper than metals and pure chemicals, and already in use globally as homeopathic remedies for infection (Williams *et al.* 2011). Therefore, minerals may make appropriate materials for HWTS options because they are familiar, can be provided sustainably and may be available locally (depending on regional geology).

We envision two possible approaches for implementing mineral water sanitization. The first involves identifying safe, effective bactericidal minerals and distributing them in a form optimized for water purification, with relevant instructions. This approach is similar to that practiced by providers of dilute sodium hypochlorite and PUR Purifier of Water™ sachets for household chlorination. Thus, we anticipate that this implementation may suffer from similar drawbacks including: distribution and supply-chain issues, continuous costs to users to replace and replenish their chemicals, and risks of negative user experiences through improperly used sachets. However, mineral-based interventions would have the advantage of being naturally derived and would not incur the environmental costs of either manufacturing pure chemicals or exposing people to the risks associated with prolonged chlorine exposure (US EPA 1999; Stringer & Johnston 2001). An alternative approach may be to distribute a guide for identifying local bactericidal minerals, along with information on possible ways to use them for water purification. With training and technical assistance, local leaders could then teach mineral water purification to their communities. This approach is most

similar to that taken by SODIS promoters, but is otherwise a radical departure from previous HWTS interventions because it relies on enhanced behavior modification; all HWTS interventions require some level of behavioral change. One possible drawback of this approach is that it will require significant investment in follow-up and ongoing technical assistance. However, the availability of technical assistance has been shown to be important for the success of nearly all HWTS interventions (Lantagne *et al.* 2011). In this way, mineral-based HWTS interventions do not differ from currently available options. To ensure that water treated by mineral-based HWTS interventions is of a reliable quality, users will need to be trained and supported in their proper use. However, like chemical-based HWTS interventions, our results indicate that reactive minerals eliminate bacteria in a consistent and predictable way. This is an important advantage over SODIS whose efficacy changes significantly with solar flux and weather (Berney *et al.* 2006; du Preez *et al.* 2011; McGuigan *et al.* 2011).

Mineral-based modifications for current HWTS options

Research on mineral water sanitization might help address barriers to adoption for other HWTS options. For example, replacing the colloidal silver currently used in ceramic filters with an antibacterial mineral may significantly reduce initial costs of the filters, and simplify their manufacture. With appropriate research to optimize and implement it, mineral water sanitization may provide a lower-cost alternative to chemical HWTS interventions (e.g., chlorination, PUR Purifier of Water™ sachets) that does not require the manufacture (Stringer & Johnston 2001) and transport of pure chemicals (CDC & USAID 2008b, c). The addition of a bactericidal mineral to the sand column in biosand filters (CDC & USAID 2008d) may improve their bacterial elimination. As we have previously discussed, mineral water purification may be appropriate for replacing SODIS because it works via a similar mechanism.

Potential drawbacks and further research

Although these initial results are encouraging, more research is needed to address existing and potential drawbacks to mineral water purification, as well as determine the best methods for implementation. One primary drawback to mineral water sanitization is the 4-hour wait time. This is still prohibitively long (although faster than SODIS), and may limit future adoption of mineral-based HWTS techniques. However, we have not yet optimized our mineral water sanitization techniques for implementation. Our ultimate goal is to find the optimal mineral, or combination of minerals, that can provide safe drinking water on-demand, without having to wait hours. Thus, future research to optimize bacterial elimination by mineral exposure should be conducted. In addition, future research may discover other minerals with more rapid bacterial elimination than pyrite. The methods we have outlined here can be used to test their efficacy.

We selected pyrite as our proof-of-concept mineral because of its well established reactivity and the extensive geochemical research on its production of ROS in solution. As our results show, pyrite is a highly effective bactericide. We do not, however, recommend that pyrite itself be used for water sanitization. Pyrite acidifies water, may contain heavy metals and other dangerous contaminants, and pyrite dust is dangerous if repeatedly inhaled (Harrington *et al.* 2012a, b). Thus, we do not propose pyrite itself for use in HWTS techniques and we present it here only as a demonstration of the potential of mineral-based water sanitization. Previous research has shown that other iron-bearing minerals may be similarly (if less) reactive in solution (Schoonen *et al.* 2006). Future research should focus on the bactericidal potential of these minerals in particular. We intend to build on our results, conducting further research to identify other minerals and geologic materials better suited than pyrite for use in implemented HWTS techniques. Finally, *E. coli* is only one of four pathogens commonly associated with MDS in children under five (Kotloff *et al.* 2013). Further research is needed to test the efficacy of mineral water sanitization on rotavirus, *Shigella*, and *Cryptosporidium*.

Despite these drawbacks, water purification with minerals presents a compelling research direction for the development of future HWTS techniques. In addition, several barriers to implementation and adoption that currently limit the efficacy of HWTS options (Schmidt & Cairncross 2009; Lantagne & Clasen 2012) might be addressed by mineral-based modifications. Additional

research on mineral water purification is necessary to know which of these might be effective and to optimize mineral use as a stand-alone HWTS option.

To arrive at the concept of mineral bactericides as a low-cost HWTS technology, we applied previous research from three disciplines to a seemingly unrelated challenge. Not only do our results support the potential of mineral water purification, they also emphasize the possibilities of interdisciplinary approaches to sustainable development. This paper is intended as a base, providing new research directions that may eventually lead to innovative solutions for the ongoing global challenge of sustainably providing equitable access to clean water and sanitation.

Lack of access to improved sanitation and clean drinking water is a global crisis. The most effective methods for addressing this crisis must be low-cost, quickly effective, and simple to implement. The use of cheaply available minerals and earth materials as water sanitation aids is based on decades of previous research in complementary fields that have not previously been considered in concert or applied in this context.

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