

# Antimicrobial and Oxidative Properties of Sodium Ferrate for the Combined Removal of Arsenic in Drinking Water with Shell Ash of Unio

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**ABSTRACT:** The present study shows arsenic removal and bactericidal efficiency of sodium ferrate with various adsorbents to decontaminate water. Among the tested adsorbents, the ash of Unio (*Lamellidens marginalis*) shells was found to be the best. It reduces 500 ppm arsenic in water to <10 ppb in 40 min by adsorption. As(III) was converted into As(V) by *in situ* prepared  $\text{Na}_2\text{FeO}_4$ , which was then removed by adsorption on to the ash of Unio. Bactericidal potential of sodium ferrate for the bacterium *E. coli* was found better than those of three antibiotics viz. moropenum, vancomycin, and piperacillin tozabactam, and it was found at par with ampicillin. Bactericidal effect was found due to the leakage of protein from the cell wall. Effects of different process variables on the extent of removal of As(V) were studied. Composition of ash and morphological changes after adsorption were determined with Fourier transform infrared (FT-IR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) studies.

## 1. INTRODUCTION

Arsenic is a semi-metal element in the environment that naturally can be found in rocks, soil, water, air, and in plants and animals. Arsenic has no taste or smell. It can also be released into the environment from some agricultural and industrial sources. Health hazards of arsenic are well-known.<sup>1</sup> The United States Environmental Protection Agency (US EPA) also has classified arsenic as a carcinogen.<sup>2</sup> About 200 million people around the world consume water with arsenic levels that exceed the permissible limit established by the World Health Organization (10  $\mu\text{g/L}$ ), and another 50 million people use water with arsenic levels >50  $\mu\text{g/L}$ .<sup>3,4</sup> In many parts of the world, such as west Bengal region in India, Bangladesh, and the inner regions of Mongolia in China, arsenic concentration exceeds the standard value.<sup>5,6</sup> More than 60% of the groundwater in Bangladesh contains greater arsenic concentration than the WHO guideline value,<sup>7</sup> which affects the health of millions of people. Continuous exposure to and drinking of arsenic contaminated groundwater (for >5 years) causes damage to the skin, the central nervous system, and the liver and may cause lung, bladder, liver, skin, renal, and kidney cancers.<sup>8–12</sup> However, most devastating forms of arsenic related cancers have been observed in India and Bangladesh.<sup>13</sup> As(III) is more toxic and mobile than As(V); thus, to enhance immobilization of arsenic, it is highly desirable to oxidize As(III) species.<sup>14</sup> A number of chemicals including gaseous chlorine, hypochlorite, ozone, permanganate, hydrogen peroxide, and Fenton's reagent ( $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ ) can directly oxidize As(III).<sup>15–18</sup> Direct oxidation of As(III) by UV is slow, but the presence of sulfite<sup>19</sup> and ferric ions<sup>15</sup> catalyze the rate of oxidation. Apart from these, ferrate ( $\text{Fe(VI)}$ ) can easily oxidize As(III) and has been proposed to be a green oxidant, coagulant, disinfectant, and antiflocculant; therefore, it is a promising multipurpose water treatment chemical.<sup>20–22</sup> Treatment of infected water with ferrate kills harmful organisms (e.g., bacteria and viruses) and controls/removes the odor precursors. The byproduct of  $\text{Fe(VI)}$  is nontoxic  $\text{Fe(III)}$ , making  $\text{Fe(VI)}$  an

environmentally friendly oxidant. Moreover, the ferric oxide produced from  $\text{Fe(VI)}$  acts as a powerful coagulant that is suitable for the removal of metals, nonmetals, radionuclides, and humic acids.<sup>23–25</sup> Oxidation alone does not remove arsenic from solution and must be coupled with a removal process such as coagulation, adsorption, or ion exchange. Therefore, many conventional arsenic removal procedures involve a pretreatment for As(III) oxidation, followed by the adsorption or coprecipitation of the As(V) formed, using adsorbents or coagulants. Preliminary studies for the oxidation of As(III) by  $\text{Fe(VI)}$  and subsequent removal of arsenic from wastewater using  $\text{Fe(III)}$  as coagulant<sup>26</sup> and the oxidation of As(III) with hexacyanoferrate(III) and subsequent removal of As(V) from water by adsorption have been reported.<sup>27</sup> In the past decade, the use of biosorbents for the removal of contaminants have become increasingly attractive due to high adsorption capacities, low costs, and regenerability of the sorbent,<sup>28,29</sup> but few efforts have been made to remove As(V) by biosorption.<sup>30–34</sup> Biomaterials containing chitin have been recognized as effective biosorbents for metals.<sup>35,36</sup> However, challenges in searching biosorbents with high uptake and low cost as well as in understanding the biosorption mechanisms are still to be solved satisfactorily. Along with arsenic, drinking water is frequently contaminated with microorganisms such as *E. coli*, which is one of the species in the faecal coliform group, and has been used as an indicator for the faecal contamination in a given water sample. The Gram-negative cell wall of *E. coli* is complex and can survive for variable periods in natural waters. Safe drinking water regulation requires inactivation of *E. coli*.  $\text{K}_2\text{FeO}_4$  has been found to effectively inactivate both the bacteria (*Escherichia coli*)<sup>37</sup> and viruses (*f2 coliphage* and *Q $\beta$  coliphage*)<sup>38,39</sup> in synthetic buffer solutions

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and secondary effluents. Use of potassium ferrate for various end applications is associated with its difficult and multistep separation process in the solid form, the problem which can be solved effectively by using *in situ* prepared sodium ferrate. Potential of *in situ* prepared sodium ferrate<sup>40,41</sup> in the oxidation of various organics has been shown by us, but the combined use of *in situ* prepared sodium ferrate in the removal of arsenic and inactivation of *E. coli* for purification of water has not been reported till now. In this paper, we have shown that *in situ* prepared sodium ferrate may be a potential oxidant for the oxidation of As(III) and a disinfectant for *E. coli*. Moreover, quite high concentration of As(V), produced by the oxidation of As(III), may be reduced to <10 ppb by adsorbing it on the ash of the shell of Unio (fresh water mussels; *Lamellidens marginalis*). Mechanism of adsorption of As(V) by the ash of Unio shell has been explained on the basis of the results of the study of heat treatment of ash, Fourier transform infrared (FT-IR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) of the ash material.

## 2. MATERIALS AND METHODS

**2.1. Materials and Preparation of Sodium Ferrate Solution.** All studies were performed with analytical grade chemicals, which were used as such without further purification. Sodium ferrate was prepared by the oxidation of ferric nitrate (Merck) with sodium hypochlorite (Qualigen) as per stoichiometry of the reaction reported earlier.<sup>40,41</sup> Formation of a clear dark purple colored solution within few minutes (1–3 min) under strongly basic conditions indicates the formation of ferrate dianions.<sup>42</sup> Solutions of As(III) and As(V) were prepared by dissolving sodium arsenite ( $\text{NaAsO}_2$ ) (Sigma-Aldrich) and sodium arsenate ( $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ ) (Sigma-Aldrich), respectively, in double distilled water.

**2.2. Adsorbent Preparation.** Shells of dead Unio (*Lamellidens marginalis*; Lamarck 1819) were collected from the ponds and rivers of eastern Uttar Pradesh, India, where these are abundantly available. Shells were crushed to powder mechanically with the help of pestle and mortar and were heated in a porcelain dish till a white mass was obtained. The white mass was mechanically crushed again to reduce the particle size and to enhance the surface area of ash (adsorbent-I). A similar procedure was followed for getting the ashes of coconut (*Cocos nucifera*: Carl Linnaeus) husk/fiber (adsorbent-II), coconut (*Cocos nucifera*) shell (adsorbent-III), bagasse (*Saccharum barberi*: Jacob Jeswiet) (adsorbent-IV), and groundnut (*Arachis hypogaea*: Carl Linnaeus) shell (adsorbent-V). Commercially available adsorbents such as silica gel (Merck) (adsorbent-VI), montmorillonite K10 (Sigma-Aldrich) (adsorbent-VII), charcoal (Merck) (adsorbent-VIII), and coal (from local market) (adsorbent-IX) were also tested for the adsorption of As(V).

**2.3. Experimental Procedure.** Stoichiometric amount of sodium ferrate solution ( $2.74\text{--}10.96 \times 10^{-3}$  M) was added dropwise with constant stirring into 50 mL of As(III) solution ( $0.25\text{--}1.0 \times 10^{-3}$  M). This ensures complete oxidation of As(III) into As(V). The resultant solution was again stirred for 10 min at room temperature after adding 1.0 g of adsorbent and then the contents were allowed to settle for 30 min. Supernatant was collected by filtration and analyzed for remaining concentration of arsenic as As(V). Complete conversion of As(III) into As(V) was ensured with the help of arsenic test kit (Orlab Pvt. Ltd., Hyderabad, India). Complete conversion was also confirmed with ion-chromatograph (Basic IC 883) in the range of our study at which the highest concentration of arsenic

and lowest concentration of sodium ferrate in the ratio (1:2.74) were taken.

**2.4. Determination of pH Value at Zero Point Charge ( $\text{pH}_{\text{ZPC}}$ ).** The pH value at the point of zero charge ( $\text{pH}_{\text{ZPC}}$ ), which has important effects on adsorption capacity of Unio ash, is determined by the pH drift method.<sup>43</sup> In a typical procedure, a solution of 0.005 M  $\text{CaCl}_2$  was boiled to remove dissolved  $\text{CO}_2$  and then cooled to room temperature. The pH was adjusted to a value between 2 and 11 using 0.5 M HCl or 0.5 M NaOH. Unio ash (0.05 g) was added into 20 mL of the pH-adjusted solution in a capped vial and equilibrated for 24 h. The final pH was measured and plotted against the initial pH. The pH at which the curve crosses the  $\text{pH}_{\text{initial}} = \text{pH}_{\text{final}}$  line is taken as  $\text{pH}_{\text{ZPC}}$ .

**2.5. Analysis and Characterization.** Bruker Vertex-70 FT-IR spectrophotometer was used to investigate the main functional groups involved in arsenic adsorption. XPERT-PRO (PANalytical) instrument was used for powder XRD analysis to find out the structural information of ash of Unio before and after calcination at 900 °C for 1 h. Digital pH meter ( $\mu$ -pH System 361, Systronics) was used to measure pH and temperature of the solution. SEM analysis was carried out using EDAX, FEI Quanta 200 scanning electron microscope after gold coating to investigate the morphological changes in the ash before and after arsenic uptake. As(V) concentrations were checked basically with the help of arsenic test kit (Orlab Pvt. Ltd. Hyderabad, India), and the results obtained under the optimum conditions were confirmed with the help of Basic IC 883 (Metrohm, Switzerland) with a Metrosep A Supp 4-250/4.0A column in which retention time for As(V) was 23.42 min. However, the reported rounded off concentrations in Table 1 are as obtained from the arsenic kit.

**2.6. Determination of Antibacterial Activity of *In Situ* Prepared Sodium Ferrate(VI) for Inactivation of *E. coli*.** Bacteria *Escherichia coli* were obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India, through the Department of Biotechnology, University of Allahabad. All experiments to study the bactericidal effect of sodium ferrate on *E. coli* were conducted in triplicate. *E. coli* was incubated at  $37 \pm 1$  °C for 24 h in 50 mL nutrient broth medium (HI-Media, Mumbai). A mother plate was prepared by putting the sample strain of *E. coli* on the plate containing 20 mL nutrient agar (HI-Media, Mumbai) by streaking method and the plate was incubated for 24 h. Numbers of colonies were counted by the spread plate method in which *E. coli* cells, plated on nutrient agar, were incubated at  $37 \pm 1$  °C for 24 h. The population of *E. coli* in the original suspension was in the range of  $(5 \text{ to } 6) \times 10^7$  colony forming unit (CFU)/mL. Experiments were performed in triplicate and the results have been discussed in section 3.8 by taking the average values. Four methods were followed to study the inactivation of *E. coli* by sodium ferrate:

- (1) Efficiency of sodium ferrate (% killing of *E. coli*) was calculated by counting the colonies of *E. coli* in terms of CFU/mL before and after treating the medium with sodium ferrate for a period of 24 h at  $37 \pm 1$  °C.
- (2) Minimum concentration of sodium ferrate for inactivation of *E. coli* was obtained by MIC (minimum inhibitory concentration) test. 100  $\mu\text{L}$  saline inoculum (containing 0.9% NaCl) of *E. coli* was taken in 10 test tubes (TTs) already containing 1 mL nutrient broth, and 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20  $\mu\text{L}$  sodium ferrate, respectively, was mixed in each TT. All the TTs were incubated for 24 h at

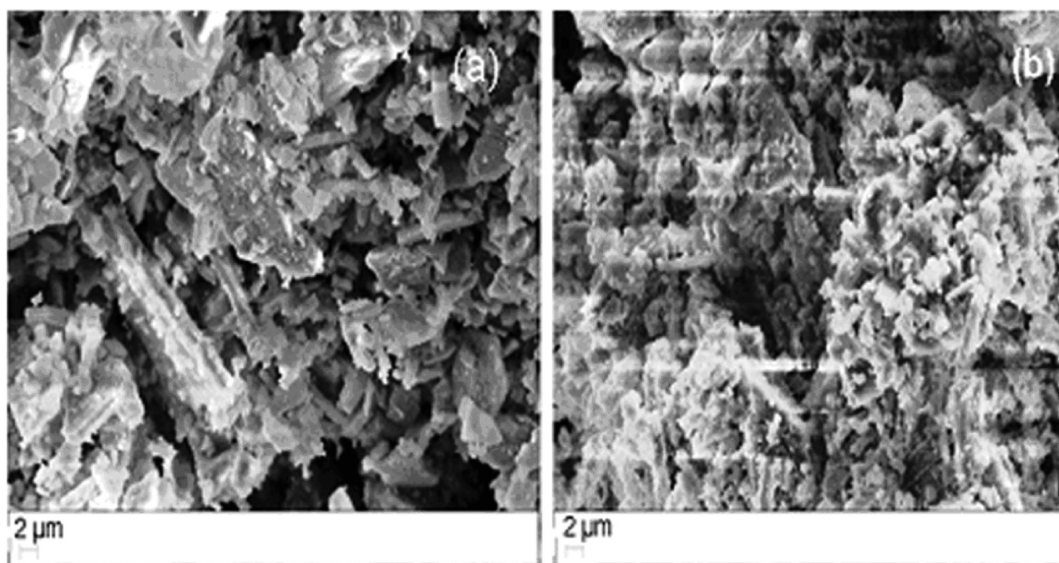
1		2		3		4		5		6		
initial [As(III)] variation		[Na <sub>2</sub> FeO <sub>4</sub> ] variation		adsorbent variation		effect of presence of other metals		effect of pH variation		effect of temp. variation		
[Fe(VI)] = 2.74 × 10 <sup>-3</sup> M; pH = 6.54 ± 0.65; temp. = 30 °C; adsorb. I = 1.0 g		[As(III)] = 0.50 × 10 <sup>-3</sup> M; pH = 6.54 ± 0.65; temp. = 30 °C; adsorb. I = 1.0 g		[As(III)] = 0.50 × 10 <sup>-3</sup> M; [Fe(VI)] = 4.11 × 10 <sup>-3</sup> M; pH = 6.54 ± 0.65; temp. = 30 °C		[As(III)] = 0.50 × 10 <sup>-3</sup> M; [Fe(VI)] = 4.11 × 10 <sup>-3</sup> M; pH = 6.54 ± 0.65; temp. = 30 °C; adsorb. I = 1.0 g		[As(III)] = 0.50 × 10 <sup>-3</sup> M; [Fe(VI)] = 4.11 × 10 <sup>-3</sup> M; temp. = 30 °C; adsorb. I = 1.0 g		[As(III)] = 0.50 × 10 <sup>-3</sup> M; [Fe(VI)] = 4.11 × 10 <sup>-3</sup> M; pH = 6.54 ± 0.65; adsorb. I = 1.0 g		
As <sup>III</sup> × 10 <sup>-3</sup> M	0.25	500	Na <sub>2</sub> FeO <sub>4</sub> × 10 <sup>-3</sup> M	remaining [As] ppb	adsorbents (1.0 g)	remaining [As] ppb	other metals	remaining [As] ppb	pH	remaining [As] ppb	temp. °C	remaining [As] ppb
					adsorb. I	25	Cu <sup>2+</sup>	10	3.54	0.0	18	200
	0.50	500		0.0	adsorb. II	400	Cr <sup>6+</sup>	100	6.08	10	26	100
	1.00	>1000		25	adsorb. III	1000	Pb <sup>2+</sup>	50	7.60	100	30	200
				50	adsorb. IV	>1000	Hg <sup>2+</sup>	100	8.21	200	35	300
				400	adsorb. V	500			9.0	500	40	300
					adsorb. VI	>1000						
					adsorb. VII	>1000						
					adsorb. VIII	500						
					adsorb. IX	500						

- (3) Antibiotic sensitivity of sodium ferrate was compared with four antibiotics viz. moropenem, vancomycin, ampicillin, and piperacillin tozabactam by measuring the inhibition zone developed by antibiotics and sodium ferrate. For this purpose, three nutrient agar plates (1, 2, and 3) were infected by the saline inoculum containing *E. coli*. The discs of antibiotics (moropenem, vancomycin, ampicillin and piperacillin tozabactam) along with blank disc (containing no antibiotic) dipped in 20  $\mu$ L sodium ferrate solution, were put at appropriate places. The plates were incubated for 24 h at  $37 \pm 1^\circ\text{C}$  for developing *E. coli* and the inhibition zones.
- (4) Protein leakage efficiency of sodium ferrate for inactivation of *E. coli* was tested by Lowry's method<sup>44</sup> for protein estimation (Table 2). Known concentrations of protein

quantity of sodium ferrate ( $\mu\text{L}$ )	optical density (O.D.) (in triplicate)	mean (O.D.)	estimated protein ( $\mu\text{g}$ )
5	0.087/0.095/0.137	0.106	52
10	0.105/0.120/0.135	0.120	58
15	0.147/0.142/0.162	0.150	72
20	0.055/0.053/0.069	0.059	31

**3.1. Effect of Initial As(III) Concentration on the Removal of Arsenic.** Effect of change of initial concentration of As(III) on the removal of arsenic (as As(V)) from the test solution was studied by changing the initial concentration of As(III) keeping other variables (sodium ferrate concentration, adsorbent, pH, and temperature) constant. Under these conditions, a sufficient amount of oxidant ensures complete conversion of As(III) into As(V) and thus, increases in the initial concentration of As(III) decreased the remaining total arsenic concentration in the test solution. As soon as the concentration of As(III) exceeds the concentration of sodium ferrate in the test solution, the total amount of arsenic in the filtrate starts increasing. This trend is obvious because (i) in the second case sufficient amount of sodium ferrate is not available to convert





**Figure 1.** SEM images (at 5K $\times$ ) of ash of Unio (a) before arsenic uptake (b) after arsenic uptake.

excess As(III) into As(V), which is adsorbed by the ash of Unio shell, and (ii) the arsenic test kit measures total arsenic (As(III) and As(V)) present in the solution and when maximum As(V) has been adsorbed, additional As(III) adds to the total amount of arsenic in the filtrate. It is clear from column 1 in Table 1 that the initial increase in As(III) concentration has a positive effect on the removal of arsenic and after a certain limit total amount of remaining arsenic increases.

**3.2. Effect of Sodium Ferrate(VI) Concentration on the Removal of Arsenic.** Removal of As(V) increased with increase in sodium ferrate concentration up to  $4.11 \times 10^{-3}$  M, after which a decrease in As(V) removal was observed (Table 1, column 2). Decrease in As(V) removal at higher amounts of sodium ferrate is probably due to the fact that sodium ferrate is highly basic in nature which increases pH of the solution. At high pH (above pH 7), surface of the adsorbent may become negatively charged and starts repelling the negatively charged arsenate ions resulting in the decreased adsorption of As(V).

**3.3. Efficiency of Various Adsorbents on the Removal of Arsenic.** Nine adsorbents were tested for the removal of arsenic. Ash of Unio shell (adsorbent-1) was found to be the best for the removal of As(V) (Table 1, column 3). Better efficiency of adsorbent-1 is probably due to the specific ratio in which various metal oxides and carbonates are present in the ash.<sup>45–48</sup>

**3.4. Effect of the Presence of Other Heavy Metals on the Removal of Arsenic.** Efficiency of adsorbent-I to remove As(V) from water was also tested in the presence of  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Cr}^{6+}$  metals ions (Table 1, column 4). Maximum removal of As(V) even in the presence of  $\text{Cu}^{2+}$  ions suggests that surface precipitation was absent. As it was not possible to measure active sites of the adsorbent in the sample, it may be presumed that probably  $\text{AsO}_4^{3-}$  and  $\text{Cu}^{2+}$  ions bind at different sites on the adsorbent, and thus, changes in average surface potential caused by  $\text{Cu}^{2+}$  adsorption do not affect the adsorption of As(V). As(III) solution becomes turbid immediately on adding  $\text{Cr}^{6+}$  or  $\text{Pb}^{2+}$  ions, which indicates the formation of complex between As(III) and  $\text{K}_2\text{Cr}_2\text{O}_7$  or  $\text{Pb}(\text{NO}_3)_2$ . Formation of complex decreases the conversion of As(III) into As(V) resulting in the decrease in adsorption of As(V). Turbidity was not observed on adding  $\text{Hg}^{2+}$  ions to the As(III) solution; thus, complex formation in this case may be ruled out. Absence of

turbidity and increase in total remaining concentration of As(V) on adding  $\text{Hg}^{2+}$  ions indicate competition between As(V) and  $\text{Hg}^{2+}$  ions for the same adsorbent sites. This competition reduces the available sites for adsorption of As(V) and in turn the active surface area of the adsorbent. Interactions between the adsorbed ions may be direct (e.g., chemical reaction between two ions at the surface) or indirect, through weak van der Waal's forces on the adsorbent surface.<sup>49</sup>

**3.5. Effect of pH of Solution on the Removal of Arsenic.** All experiments were performed in the near neutral range of pH ( $6.54 \pm 0.65$ ), except the pH variation in which pH of the solution was changed from 3.54 to 9.0. It was observed that removal of arsenic as As(V) by adsorption on the ash of Unio increases with increase in acidity of the medium. It was interesting to note that the admissible limit of arsenic in drinking water as suggested by WHO (10 ppb) was crossed only when pH of the solution was increased above 6.08, while 100% removal was observed at pH 3.54 (Table 1, column 5). At lower pH, the oxidative nature of ferrate is increased.<sup>50</sup> Moreover, protonation of the surface of adsorbent at low pH also facilitates the adsorption of negatively charged arsenate ( $\text{AsO}_4^{3-}$ ) ions through electrostatic attraction. At higher pH, the adsorbent surface carries more negative charges which results in decreased adsorption of As(V). The effect of pH on As(V) removal is further supported by  $\text{pH}_{\text{ZPC}}$  (pH value at zero point charge) value of 8.3 for the ash of Unio.

**3.6. Effect of Temperature on the Removal of Arsenic.** Large differences in temperature particularly in Asian countries in different seasons increases the importance of the study of effect of temperature. The effect of change of temperature on the removal of arsenic was investigated at  $\text{pH } 6.54 \pm 0.65$  (Table 1, column 6). Decrease in As(V) removal on increasing the temperature from 26 to 35  $^\circ\text{C}$  indicated that the adsorption process was exothermic in nature.

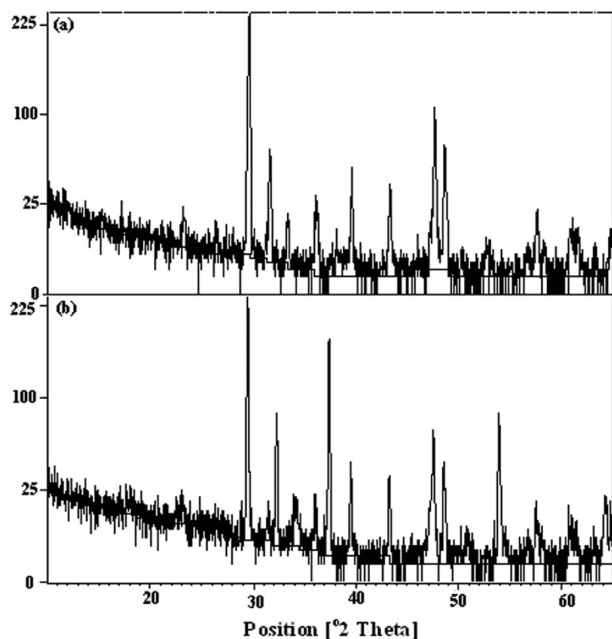
**3.7. Analysis of Adsorbent and Probable Adsorption Mechanism.** The mechanism of adsorption of arsenic on the ash of the shell of Unio has been suggested on the basis of experimental results and the data reported by other workers.<sup>51–54</sup> The initial loss of 0.2597% in weight on heating the ash at 110  $^\circ\text{C}$  for 1 h indicated the presence of superficially adsorbed moisture or hygroscopic nature of the ash. On further heating the

ash to 900 °C for 1 h, the loss in weight was found to be 25.80%, which gave a rough indication of the percentage of carbonate in the sample. Efficiency of the ash of Unio to remove arsenic from

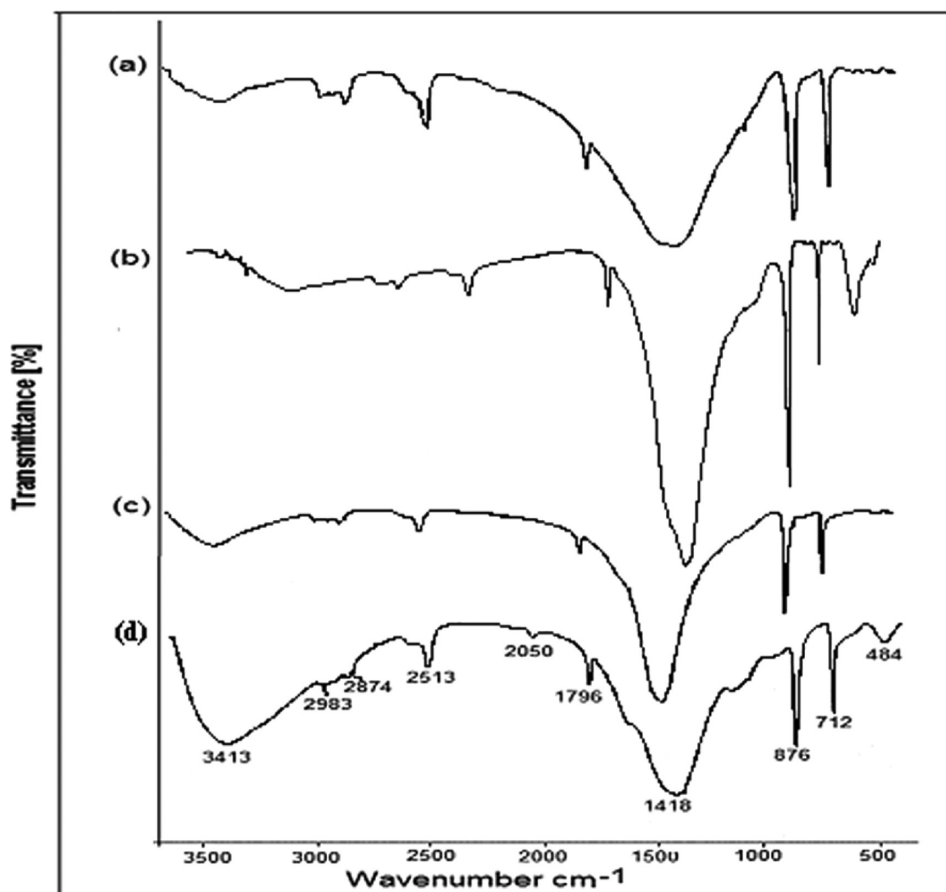
aqueous solution under optimum conditions does not change to a significant extent whether the ash was used before or after calcination. Comparison of the constituents and their ratios in the ashes obtained from different sources as reported by other workers, shows that the constituents in various ashes remain more or less the same although their ratios may change. In the absence of the quantitative/qualitative study of various metal oxides and carbonates present in all the nine ashes used in the present study, it can be suggested to a good approximation that probably the specific ratio of various metal oxides present in the ash of Unio shell is mainly responsible for the excellent arsenic removal in this particular case.

SEM micrographs of the ash of Unio shell before and after arsenic adsorption are given in Figure 1. The pattern in Figure 1a shows the presence of flakes with irregular, rough, and porous surface morphology, indicating high surface area available for adsorption. After adsorption of arsenic in Figure 1b, the decrease in flaked structure and notable changes in the surface of adsorbent clearly indicate that adsorption is a surface phenomenon.

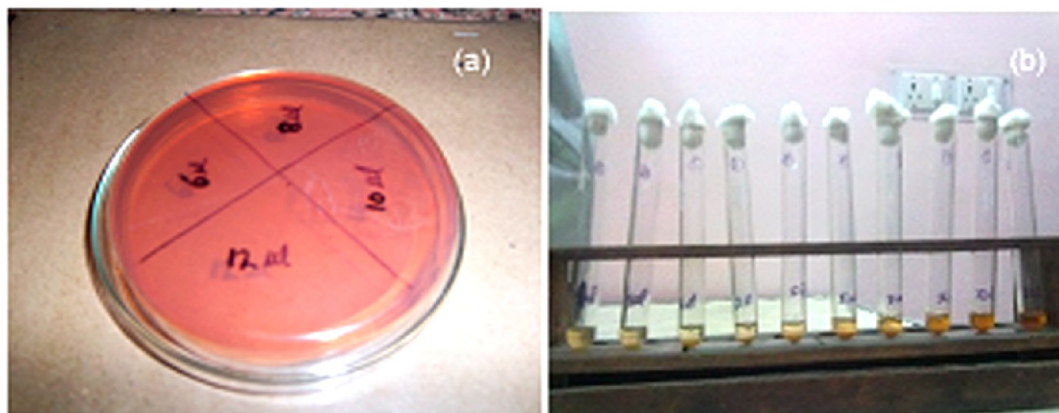
XRD patterns of the ash before and after calcination at 900 °C for 60 min in air are shown in Figure 2. Three peaks at  $2\theta$  values of 10.1836, 15.2669, and 17.2915 disappear on calcination which indicates the collapse of ash structure. No change in the PXRD pattern for mixed oxide reflection was observed before and after calcinations while the carbonate reflection disappears after calcination, which was present before. The disappearance of carbonate reflection pattern in calcined ash is consistent with the



**Figure 2.** XRD pattern of ash of Unio (a) pure ash (b) ash after calcination at 900 °C for 1 h.



**Figure 3.** FT-IR spectra of ash of Unio: (a) pure ash; (b) ash after calcination at 900 °C for 1 h; (c) ash after adsorption of arsenic(V) at neutral pH; (d) ash after adsorption of arsenic(V) at acidic pH.



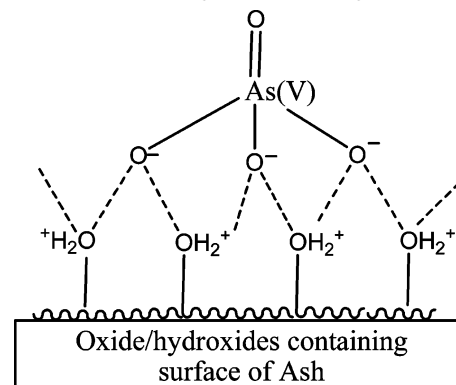
**Figure 4.** Measurement of sodium ferrate concentration for (a) minimum inhibitory concentration (MIC) and (b) minimum bactericidal concentration (MBC) (color figure on the Web only).

weight loss during ignition. Crystallinity of the ash is notably enhanced after calcination but appearance of new peaks was not observed. Average crystalline size of the ash was found to increase from 70.02 to 72.58 nm after calcination as calculated by Scherrer equation,<sup>55</sup> which indicates sintering and thereby aggregation of the ash particles. High adsorption capacity of the ash was due to the nano-size of the particles. An average of 83% particles in precalcinated ash and 76% particles in postcalcinated ash were found to be in the nano scale region (between 10 and 100 nm).

Figure 3 shows FT-IR spectra of (a) pure Unio ash, (b) ash after calcination, (c) ash after adsorption of As(V) at neutral pH, and (d) ash after adsorption of As(V) at acidic pH. Carbonate ion of  $\text{CaCO}_3$ , the main content of Molluscan shell, belongs to a point group  $D_{3h}$  and exhibits generally four normal modes of vibration in infrared region. Out of four normal modes of vibration, the symmetric vibration  $\nu_1$  ( $A_1$ ) is reported to be IR-inactive; hence, only three fundamental bands are generally observed.<sup>56</sup> Two intense and sharp bands observed in the spectrum at 876.18 and 712.29  $\text{cm}^{-1}$  for ( $-\text{OCO}$  bending) of  $\text{CO}_3^{2-}$ , may be assigned to  $\nu_2$  ( $A_2$ ) mode (out of plane deformation band) and  $\nu_4$  (E) mode (in plane deformation band), respectively. Another broad and intense band observed at 1416.60  $\text{cm}^{-1}$  ( $-\text{CO}$  stretching of  $\text{CO}_3^{2-}$ ) may be assigned to  $\nu_3$  (E) mode of  $\text{CO}_3^{2-}$  (a doubly degenerate asymmetric stretching) and is a confirmatory band for carbonate materials. The band at 1083.53  $\text{cm}^{-1}$  ( $-\text{CO}$  stretching of  $\text{CO}_3^{2-}$ ) corresponds to the  $\nu_1$  ( $A_1$ ) fundamental mode (symmetric stretching vibration that is generally IR-inactive) of carbonate molecule. The disappearance of this band after calcination indicates the transformation of aragonite to calcite. The bands observed at 1796.88 and 2514.49  $\text{cm}^{-1}$  are in good agreement with bands observed for  $\text{CO}_3^{2-}$  in  $\text{CaCO}_3$  lattice reported in literature.<sup>57</sup> The peak at wave numbers 3550 to 3200  $\text{cm}^{-1}$  ( $-\text{OH}$  stretching) represents hydroxyl ion stretching of interlayer water molecule,<sup>58</sup> which disappears after calcination at 900 °C for 60 min. The band observed at 477.45  $\text{cm}^{-1}$  before adsorption, due to substrate-surface vibration which intensifies with a small shift in band position, is assigned to the O–As–O bending vibration and confirms the loading of As(V).<sup>59</sup> A change in intensity and position of band after adsorption at acidic pH indicates adsorption of As(V). More adsorption at acidic pH is indicative of nonspecific adsorption of As(V) through coulombic attraction between anions and the surface of adsorbent.<sup>60</sup>

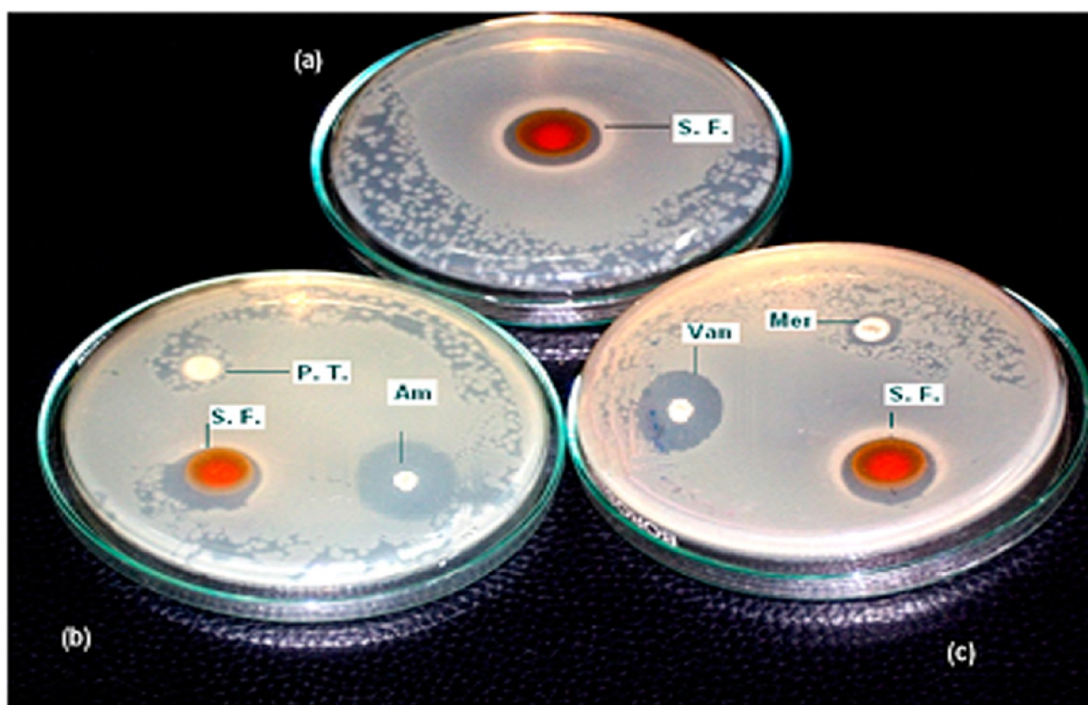
Surface chemistry is important in arsenic removal by metal oxides. The surfaces of metal oxides are collections of unfilled

metal–oxygen bonds that are hydrated with water. At the pH of zero point of charge ( $\text{pH}_{\text{ZPC}}$ ), an equal number of positively and negatively charged surface sites exist, and proportionally more positively charged surface sites exist at pH levels below  $\text{pH}_{\text{ZPC}}$ . In the present case  $\text{pH}_{\text{ZPC}}$  of the ash of Unio was found to be 8.3. Below this value, the surface of the ash will be positively charged and the electrostatic attraction between the positively charged surface and the negatively charged arsenic species will be prominent. Electrostatic attraction of anionic species is favored onto positively charged surface sites. Anionic arsenic species are generally removed more effectively than nonionic arsenic species by most metal-oxide adsorbents. Coulombic forces favor association of anionic arsenate with positively charged sites on metal oxides (e.g.,  $\text{M}-\text{OH}^{2+}$ ). Electrostatic bonds are formed rapidly (within seconds) and depend largely on the charge difference between arsenate and the adsorbent surface. On the basis of the facts obtained from experimental studies and the studies of surface chemistry of metal oxides, As(V) adsorption by the ash of dead Unio containing metal oxides in majority may be represented by the following schematic diagram:



**3.8. Inactivation of *E. coli* by *In Situ* Prepared Sodium Ferrate(VI).** An average of 88.11%, 94.70%, and 97.72% killing efficiency was obtained with respectively 0.6, 0.8, and 1.0 mL of sodium ferrate solution, as observed by the CFU counting method. The results clearly show that sodium ferrate has sufficient capability to kill *E. coli* even at very low doses. Efficiency of sodium ferrate to kill *E. coli* at very low concentrations was further confirmed by MIC (Figure 4a) and MBC (Figure 4b) tests, which shows that 6, 8, 10, 12  $\mu\text{L}$  sodium ferrate solution was sufficient to inhibit the growth of *E. coli*. Figure 5 shows antibiotic sensitivity of sodium ferrate and four antibiotics. In duplicate





**Figure 5.** Antibiotic sensitivity of sodium ferrate (S.F., sodium ferrate; P. T., piperacillin tozabactam; Am, ampicilin; Mer, meropenum; Van, vancomycin (color figure on the Web only).

experiments in the case of sodium ferrate (S.F.), diameters of the inhibition zones were found to be 29/30 mm (Figure 5a). In duplicate experiments in case of different antibiotics the diameters of inhibition zones were found to be 30/31 mm and 10/00 mm for ampicilin (Am) and piperacillin tozabactam (P.T.) (Figure 5b) and 10/14 mm, 25/27 mm in case of meropenum (Mer) and vancomycin (Van) (Figure 5c), respectively (parts b and c contain the inhibition zones of sodium ferrate also for comparison). Protein leakage test was performed to find out the possible inactivation mechanism for inactivation of *E. coli* by sodium ferrate, which shows that 72  $\mu\text{g}$  protein was leaked by *E. coli* at very low concentration of sodium ferrate (15  $\mu\text{L}$ ) (Table 2). The results collectively suggest that cell wall structure is damaged by the application of sodium ferrate leading to the release of its major constituents (i.e., protein and potential cell wall disintegration).

#### 4. CONCLUSION

A simple and economical process is reported for the removal of arsenic and inactivation of *E. coli* for purification of water. As(III) present in water was first oxidized into As(V) with the help of sodium ferrate, and the resultant As(V) was removed by adsorption. Out of nine adsorbents tested, the ash of the dead unio shell was found to be the most effective adsorbent for As(V). It was found that pH of the medium strongly affects the adsorption, and maximum efficiency for As(V) removal was found to be at pH  $3.54 \pm 0.05$ . It has been suggested that, at lower pH, surface activity of ash of Unio (*Lamellidens marginalis*) to adsorb the negatively charged As(V) ions was increased due to protonation of the surface, which becomes positively charged. The present study explores the application of sodium ferrate as a bactericidal agent also in water purification. The bactericidal effect of *in situ* prepared sodium ferrate for *E. coli* is probably due to rupture of cell wall of bacteria that causes leakage of the cell protein and finally death of bacteria.

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##### Notes

The authors declare no competing financial interest.

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