The role of ferrates (VI) as a disinfectant: Quantitative and qualitative evaluation for the inactivation of pathogenic bacteria

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Iron compounds in the oxidation state (VI) have the advantage of being powerful antioxidants and bactericides, which explains their particular interest in water treatment. The aim of this work was to investigate the ability of inhibition of pathogenic microorganisms (Escherichia coli, Salmonella, Staphylococcus aureus, Bacillus sp., Pseudomonas sp., Enterococcus fecalis) by ferrate and for the first time, to evaluate their capacity to resist ferrate Na₂FeO₄ in order to optimize its concentration in specific conditions. The doses required to inhibit these microorganisms are respectively: 5 mg / l, 8 mg / l, 6 mg / l, 4.5 mg / l, 6.3 mg / l and 7.6 mg / l. The application to establish our results was made through the sanitation of water of the river Mehraz (Fez - Morocco). Because of the resistance of indigenous bacteria and the influence of different parameters, we optimized the concentration of Na₂FeO₄ to 30 mg / l.

Key words: Potassium Ferrate (VI), water sanitation, disinfection, coagulation, wastewater treatment, bactericidal.

INTRODUCTION

Disinfection is a process which aims to eliminate harmful microorganisms (bacteria and viruses) and control odor precursors. However, to reduce the adverse effects of products resulting from the chlorination on health, professionals were conducted to minimize their concentrations in drinking water’s disinfection by chlorine.

Recently, the interest for ferrate as an oxidizing agent in water treatment has been growing in comparison with the other agents used (Sharma et al., 2011; Sharma, 2010; Jiang, 2007).

Sharma (2010) demonstrated the feasibility of Fe VI treatment of polluted waters of various origins (waste waters and industrial effluents). He showed that a lot of compounds, containing inorganic or organic nitrogen can be degraded by ferrate with the formation of non-hazardous products.

The bactericidal power of K₂FeO₄ has been studied by various authors (Cho et al., 2006; Gilbert et al., 1976; Waite, 1979; Farooq and Bari 1986; Jiang and Lloyd, 2002) who worked on different pathogenic microorganisms such as E. coli (colibacillosis), Salmonella (typhoid fever), Shigella (dysentery) as well as coliforms. Gilbert et al. (1976) followed the speed of action of K₂FeO₄ (5.10-5 M) in buffered medium and noted that the reactivity of ferrate is maximal at pH less than 8, and the ion FeO₄²⁻ remains an effective bactericide for more alkaline environ-
mments
At pH = 7, Waite (1979) found a rapid effect of K$_2$FeO$_4$ on E. coli, Shigella and Salmonella. Previous studies focused on the use of ferrate (VI) to oxidize various synthetic organic materials (benzene) (Waite and Gilbert, 1978), to discolor (White and Franklin, 1998; Jiang and Wang, 2003) and to remove inorganic contaminants (cyanide and hydrogen sulfide) (Sharma et al., 1999). Other studies have been conducted on the ferrate (VI) as a disinfectant to kill bacteria (Jessen et al., 2008; Jiang et al., 2002). The bacteria were completely eliminated at a dose range between 0-50 ppm of FeO$_4$$^{2-}$ (Murnann and Robinson, 1974). Gilbert et al. (1976) have shown that a dose of 6 mg iron / l had a 99.9% bactericidal effect on Escherichia coli, at a pH of 8.2 and a contact time of seven minutes. The results of this study also showed that the disinfection capability of FeO$_4$$^{2-}$ increases remarkably when the pH exceeds 8.0. Studies by Schink and Waite (1980) and Kazama (1995) showed a 99.9% deactivation effect of the coliphage f$_2$ virus in water by using 10 mg / l K$_2$FeO$_4$ at pH=7.8 for a contact time of 30 minutes.

The purpose of this work was to study the bactericidal power of ferrate (VI) (Na$_2$FeO$_4$) on some pathogenic microorganisms and to evaluate their resistance in order to optimize the lethal concentration. The application was made on the water of the river Mehraz in Fez city (Morocco).

**MATERIALS AND METHODS**

After preparing a nutrient broth (5 g of tryptone, 3 g of bacterial meat extract, 5 g of sodium chloride per liter) and sterilizing it at 121°C for 20 min, it was distributed in flasks at a rate of 100 ml/flask. Then each flask was inoculated with one of the studied strains, and incubated at 37°C for 48 h. Then, the bacterial culture was transferred into a sterile falcon tube and centrifuged at 5000 tr / min for 15 min (3 times), with successive washes with PBS (phosphate buffer solution).

The pellets were resuspended in PBS and dilutions were prepared and ferrate added at different concentrations and incubated at 37°C for 24 h.

The reading of the results was carried out by measuring the optical density and numeration on nutrient agar (5 g of tryptone, 3 g of bacterial meat extract, 5 g sodium chloride, 20 g of agar per liter).

The calculation of the percentages of bacterial destruction was performed according to the following relationship:

\[
\% \text{ of destroyed bacteria} = \frac{d_2-d_1}{d_1}
\]

Bacterial concentration = (Colony number V inoculated) x Fd (Fd=1/d)

The water of the river Mehraz in Fez is polluted by waste and various indigenous bacteria, which led us to treat it by ferrate as powerful bactericide. To achieve this goal, we proceeded as follows:

After the sterilization of the bottle, to avoid external contamination, sample collection was done and bacteriological analyses were performed before and after treatment with different concentrations of Ferrate, by measuring the D. O. and by numerating on agar plates. Hence, we tried to optimize suitable ferrate dose.

The total coliform count was performed by the technique of filtration, using Tergitol TTC agar. The sodium ferrate (Na$_2$FeO$_4$) has been prepared by electrochemical and characterized by X-ray, IR, and Mössbauer (El Maghraoui et al., 2013).

**RESULTS**

The results of the optical density measurement’s of the bacterial suspensions of different strains studied showed a marked decrease in the D.O, reflecting a subsequent decrease in bacterial concentration after the addition of 8.5 mg / L of Ferrate (Table 1 and Figure 1).

The differences in optical densities obtained between different bacterial strains studied could be explained by their resistance power. This enabled us to optimize the amount of ferrate VI required for each strain according to the pH of the medium by numeration on agar plates.

The Figures 1 and 2 show the variation in survival according to the concentration of Ferrate VI (Na$_2$FeO$_4$) after 24 h of incubation at 37°C in alkaline medium (pH = 8). According to Figure 3, the amount of ferrate VI required for complete inactivation of Enterococcus faecalis is 7.6 mg / l which confirms the previous results.

A concentration of 6.3 mg/l of Na$_2$FeO$_4$ is necessary to inhibit Pseudomonas sp. (Figure 4).

6 mg / l of Na$_2$FeO$_4$, at pH = 8, was required to cause the mortality of Staphylococcus aureus (Figure 5). From Figures 6 and 7 show that Na$_2$FeO$_4$ concentrations of 4.5 mg / l and 5 mg / l are sufficient for complete inactivation of Bacillus sp and E. coli respectively. While the required amount of ferrate IV to completely inactivate Salmonella sp. is 8 mg/l (Figure 8).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Before addition of ferrate VI</th>
<th>After the addition of ferrate VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0.171</td>
<td>0.002</td>
</tr>
<tr>
<td>Salmonella sp</td>
<td>0.376</td>
<td>0.022</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.236</td>
<td>0.008</td>
</tr>
<tr>
<td>Bacillus</td>
<td>1.011</td>
<td>0.001</td>
</tr>
<tr>
<td>Pseudomonas sp</td>
<td>1.551</td>
<td>0.052</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1.398</td>
<td>0.072</td>
</tr>
</tbody>
</table>
Application on the water of the river Mehraz in Fez

On the basis of studies devoted to the river Mehraz in Fez (Koukala et al., 2004), we have chosen this water to deprive it from its pollution. First, we must note that tests for optimizing the amount of ferrate needed for the treatment of this water were conducted (Data not shown). These tests allowed us to determine the amount of Na$_2$FeO$_4$ needed to disinfect the water to 30 mg / l.

The results obtained are shown in the Tables 2, 3 and 4. After various tests, the results for the DO and numeration on agar are similar, showing that the (30 mg/l) concentration of ferrate is sufficient for cleaning this water.

Moreover, tests on total coliforms showed that the amount of 30 mg/l ferrate VI (Na$_2$FeO$_4$) was sufficient to complete disinfection of bacteria.
**DISCUSSION**

According to our results, the amount required to inactivate *E. coli* is 5 mg/l at pH = 8 (Figure 7, Table 1). This is consistent with the results of various previous studies Gilbert et al. (1976) and Jiang et al. (2007) which showed that the optimum conditions to achieve 100% efficiency of inactivation of *E. coli* are 4 to 6 mg/l of Ferrate VI for a contact time between 20 and 30 min at pH = 8.2. Gilbert et al (1976) also showed that the disinfection capability of FeO$_4^{2-}$ increases remarkably when the pH exceeds 8.

This low dose of ferrate necessary for the elimination of *E. coli* (5 mg/l at pH = 8), is due to the optimization of the pH of the medium as well as to the stability of the used Ferrate (Na$_2$FeO$_4$).

According to our results, the amount needed to completely inactivate Pseudomonas sp. was 6.3 mg/l at pH = 8. For the other strains tested (*Bacillus, Staphylococcus aureus, Entrecoccus feacalis, Salmonella sp.*), the doses required to inactivate these bacteria are respectively 4.5 mg/l, 6 mg/l, 7.6 mg/l and 8 mg/l at pH = 8 (Figures 3, 5, 6 and 8; Table 1).

These results are in concordance with those found in the literature by Murmann and Robinson (1974).

For *Pseudomonas*, a preliminary study showed that the dose of Ferrate VI and the pH of water influence its disinfection such that the bacteria were completely eliminated.
at FeO$_4^{2-}$ doses between 0 and 50 mg/l (Murmann and Robinson, 1974), confirming the specificity of their resistance.

The results of this study showed that each strain has a specific resistance to Na$_2$FeO$_4$, which explains the different doses used for inactivation under the same conditions.

According to the performance of Iron VI on different strains previously treated at pH = 8 and the results of various previous studies, we deduce that there is a real environmental problem concerning the water of the river Mehrez Fez. This requires further study to fully determine the pollutant factors (bacteria, viruses) having high resistance to the Ferrate VI in order to reduce the required dose of Na$_2$FeO$_4$ to clean the water and optimize the parameters responsible of this high dose (30 mg/l).

The required dose of Iron VI (Na$_2$FeO$_4$) for a bacterial inactivation higher than 99.9% is 30 mg/l. This concentration is consistent with those found in the literature by Murmann and Robinson (1974).

According to the literature, the dose of 10 mg/l of iron (VI) destroys 99.70% of total coliforms and 99.90% fecal coliforms (Aubertin et al., 1996), which is comparable with what we found when treating the water of wadi Mahraz Fez in 30 mg/l ferrate VI (Na$_2$FeO$_4$) (Table 4).

Hence, the ferrates tested in our study showed a good purifying potential of water of the oued Mehrez, which agrees with the prior studies. Indeed, Jiang and Lloyd (2002) reported that the ferrates eliminate the reactive micropolluants contained in waste waters, and makes possible the precipitation of phosphates like ozone thanks to the capacity of the ion ferrate to attack the
Survival percentage of **E. coli**

**Figure 7.** Percentage of *E. coli* survival according to the required quantity of alkali Na$_2$FeO$_4$ at pH = 8.

Survival percentage of **Salmonella sp.**

**Figure 8.** Percentage of *Salmonella* survival in 100 ml according to the required quantity of alkali Na$_2$FeO$_4$ at pH = 8.

<table>
<thead>
<tr>
<th>The addition of ferrate at different dilutions</th>
<th>before treatment</th>
<th>After treatment with 30 mg / l ferrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial sample</td>
<td>0.474</td>
<td>0.002</td>
</tr>
<tr>
<td>Dilution $10^{-1}$</td>
<td>0.352</td>
<td>0.002</td>
</tr>
<tr>
<td>Dilution $10^{-2}$</td>
<td>0.315</td>
<td>0.001</td>
</tr>
<tr>
<td>Dilution $10^{-3}$</td>
<td>0.310</td>
<td>0.010</td>
</tr>
<tr>
<td>Dilution $10^{-4}$</td>
<td>0.06</td>
<td>0.002</td>
</tr>
<tr>
<td>Dilution $10^{-5}$</td>
<td>0.282</td>
<td>0.001</td>
</tr>
<tr>
<td>Dilution $10^{-6}$</td>
<td>0.269</td>
<td>0.001</td>
</tr>
<tr>
<td>Dilution $10^{-7}$</td>
<td>0.253</td>
<td>0.001</td>
</tr>
</tbody>
</table>

functional groupings rich in electrons of the micropollutant's molecules. Moreover, it has been proved that Ferrate(VI) (Fe$^{VI}$O$_{4}^{-4}$, Fe(VI)) is a strong oxidizing agent and produces a non-toxic by-product Fe(III), which acts as a coagulant and that Ferrate(VI) is also an efficient disinfectant and can inactivate chlorine resistant microorganisms (Sharma, 2010). He also showed that several compounds can be degraded in seconds to minutes by ferrate (VI) with the formation of non-hazardous products (Sharma, 2010).

Other studies showed that Fe (III) produced after Ferrate decomposition is an excellent coagulant for the removal of metals and radionuclides from contaminated water (Jiang et al., 2001; Jiang and Wang, 2003; Lee et al., 2009; Yngard et al., 2008, 2007; Sharma and Sohn, 2009; Sharma et al., 2007; Jain et al., 2009).

Ferrate ions are more advantageous, in water treatment as disinfectants, to sodium hypochloride, ferric sulphate and aluminium sulphate, because it does not generate any hazardous products during different compounds
degradation in the period of treatment (Jiang et al., 2006).

Conclusion

This manuscript shows the Na₂FeO₄ concentration necessary to inactivate the different studied strains at pH = 8. The results are as follows: *E. coli*: 5 mg/l; *Salmonella*: 8 mg/l; *Staphylococcus aureus*: 6 mg/l; *Bacillus*: 4.5 mg/l; *Pseudomonas*: 6.3 mg/l; *Enterococcus faecalis*: 7.6 mg/l.

Whatever the initial number of indigenous bacteria and the composition of secondary municipal wastewater, the required Na₂FeO₄ dose is around 30 mg/l, at pH = 8, to get a percentage of inactivation of bacteria (of the total aerobic mesophilic flora and total coliforms) higher than 99.9% in the water of the river Mehraz Fez. These results show the promising disinfection capacity of ferrate VI for water treatment and agree with its superior performance as oxidant/disinfector for environmental sanitation which have been demonstrated by several authors (Sharma, 2010), although there is still need for more technical and economic improvements, regarding the application of industrial policy.

Table 3. Bacterial concentration found during the treatment of the Mehraz river’s water by ferrate VI (Na₂FeO₄), determined by agar plate numeration after incubation at 37° C for 24 h.

<table>
<thead>
<tr>
<th>The addition of ferrate different depending on dilution</th>
<th>Before the addition of ferrate</th>
<th>After the addition of 30 mg of ferrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution 10⁻³</td>
<td>2.40 x 10⁵</td>
<td>3</td>
</tr>
<tr>
<td>Dilution 10⁻⁴</td>
<td>2.3 x 10⁵</td>
<td>1</td>
</tr>
<tr>
<td>Dilution 10⁻⁵</td>
<td>1.3 x 10⁶</td>
<td>2</td>
</tr>
<tr>
<td>Dilution 10⁻⁶</td>
<td>1.1 x 10⁶</td>
<td>2</td>
</tr>
<tr>
<td>Dilution 10⁻⁷</td>
<td>1.3 x 10⁷</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4. Bacterial concentration of total coliforms found during the treatment of the Mehraz river’s water by ferrate VI (Na₂FeO₄), determined by agar plate numeration after incubation at 37° C for 24 h.

<table>
<thead>
<tr>
<th>The addition of ferrate different depending on dilution</th>
<th>Before the addition of ferrate</th>
<th>After treatment with 30 mg/l ferrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution 10⁻¹</td>
<td>852 x 10⁵</td>
<td>0</td>
</tr>
<tr>
<td>Dilution 10⁻²</td>
<td>9.4 x 10⁵</td>
<td>0</td>
</tr>
<tr>
<td>Dilution 10⁻³</td>
<td>7.3 x 10⁵</td>
<td>0</td>
</tr>
<tr>
<td>Dilution 10⁻⁴</td>
<td>2 x 10⁴</td>
<td>0</td>
</tr>
</tbody>
</table>
