

Role of Ferrate VI (K_2FeO_4) as an Iron VI Compound in Water Treatment

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Abstract: In this study, Iron VI compounds show an incredible progression from being powerful super oxidants and antibacterial, which indeed shows their interest as a green and versatile chemical product in water treatment. This article therefore presents the inactivation efficiencies of these pathogenic microorganisms (*E. coli*, Missouri Bacteria, *Streptococcus*, *Shigella*, *Proteus*, *Pseudomonas* sp.) by ferrate (VI) and evaluates their resistant power for the first time against ferrate VI (K_2FeO_4) of purity > 97% in order to optimize their concentration under specific conditions, as well as to study the effect of the contact time of ferrate VI on the different bacterial strains. 4mg/l, 5mg/l, 5mg/l, 6mg/l, 6mg/l and 6mg/l respectively represent the optimum doses to inhibit these microorganisms within a contact time of 20 minutes at pH = 8. The water purification of the Fez wadi (Fez – Morocco) was used as an application to establish our results. Due to their pollution by different indigenous bacteria and their location in the city, the dose of K_2FeO_4 has been optimized at 12 mg/l.

Keywords: iron(VI) compounds, oxidation; clotting; flocculation, disinfectant, bactericide.

1. Introduction

Disinfection is a pathway that serves to inhibit harmful microorganisms (bacteria and viruses) and control odor precursors. However, to reduce the harmful effects of the products resulting from chlorination on health, specialists have been led to minimize their concentrations during the disinfection of drinking water with chlorine.

Recently the iron of oxidation state VI ($Fe^{VI}O_4^{2-}$) has become an incredible Green Technology by their wide application as an environment-friendly oxidant, disinfectant and coagulant in water treatment, [1], [2], [3], [4], [5], [6], [7], [8], [9]. Several examples include inactivation of microorganisms such as fecal and total coliforms, *Escherichia coli*, viruses as well as oxidation of pharmaceuticals and organics, personal care and removal of toxic metals [10], [11], [12], [13], [14], [15], [16], [17], [18], [19], [20], [21].

Several studies made on the bactericidal power of K_2FeO_4 on pathogenic microorganisms such as *E. coli*, *Salmonella*, *Shigella*, coliforms, viruses [22-26]. Gilbert and al. [23] limited the field of effectiveness of the FeO_4^{2-} ion as a powerful bactericide to more alkaline media and noted that the reactivity of ferrate is maximal for pH below 8.

According to Waite [24] at pH=7, the effect of K_2FeO_4 is rapid on *E. coli*, *Salmonella*, *Shigella*.

Previous work has established the effectiveness of ferrate VI oxidation of various synthetic organic materials (benzene) [27] and for decolorizing [28, 29] and for removing contaminating inorganic compounds (cyanide and hydrogen sulfide) [30].

Other research has been done on the disinfectant power of ferrates (VI) to inhibit bacteria [31, 32]. Murmann and al [33] have shown that between 0-50 ppm of FeO_4^{2-} is sufficient to destroy bacteria complementarily.

Other studies have shown that the destruction of 99.9% of *Escherichia coli* requires only a dose of 6 mg/l of iron VI at a pH of 8.2 for a contact time of seven minutes and that the capacity disinfection of FeO_4^{2-} increases remarkably, when the pH exceeds 8.0 [23].

Studies by Shink and waite (1980) [34], Kazama (1995) [35] revealed 99.9% deactivation of coliphage ϕ_2 virus in water using 10 mg/l K_2FeO_4 at pH 7.8 for a contact time of 30 minutes.

This work consists in studying the bactericidal power of ferrate (VI) (K_2FeO_4) on some harmful bacteria and determining their resistant capacity to limit the dose required to destroy them. The test was carried out on water from Oued Fez in Fez, Morocco.

2. Materials and Methods

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2.1. Bacterial cultures and enumeration

The bacterial culture and count was prepared as follows according to the protocol of El maghraoui and al [36]. After preparation of a nutrient broth (5 g of tryptone, 3 g of bacteriological meat extract, 5 g of sodium chloride per litre) and its sterilization at 121°C for 20 min, it was distributed in Erlenmeyer flasks at the rate of 100ml/erlen. Then each Erlenmeyer flask was inoculated with one of the strains to be studied, and incubated at 37°C for 48 h. Then, the bacterial culture was transferred into a sterile falcon tube and centrifuged at 5000 rpm for 15 min (3 times), performing successive washes with a PBS buffer (phosphate buffer solution).

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

The results were read by measuring the optical density and by counting on nutrient agar (5 g of tryptone, 3 g of bacteriological meat extract, 5 g of sodium chloride, 20 g of agar per litre).

The calculation of the percentages of bacterial destruction is done by the relationship:

$$\% \text{ of bacteria destroyed} = d_2 - d_1 / d_1$$

d_1 et d_2 : Optical densities of bacterial suspensions

$$\text{Bacterial concentration} = (N \text{ colonies} / V_{\text{seeded}}) * F_d$$

(avec: $F_d = 1/d$)

F_d : dilution factor

The choice of water to be treated was won over the water of the Fez ravine, because of their pollution by various wastes and indigenous bacteria, which led us to treat with VI ferrates as a powerful bactericide. To do this, we proceeded as follows:

Sterilization of the bottle to avoid any external contamination, Sampling and bacteriological analysis before and after treatment with different concentrations of Ferrate, by measuring the Optical Density (O.D) and by counting on agar, to optimize the dose and the contact time. Therefore, we tried to optimize the dose of ferrate and the appropriate contact time for disinfection.

The count of total coliforms was carried out by the technique of filtration on Tergitol TTC agar.

2.2. Materials and chemicals

Potassium ferrate (K_2FeO_4) of purity > 97% used in the treatment of different bacterial strains and in the disinfection of wadi water was prepared by oxidation of ferric nitrate with hypochlorite, according to the Delaude method and Laszlo (1996) [37], within the laboratory: processes, materials and environment (LPME)- FST of Fez.

Fe(VI) stock solutions were prepared by dissolving K_2FeO_4 in distilled water, then filtered through filter paper to

remove traces of colloidal Fe(III) and other particulate impurities.

In order to avoid the problem of degradation of ferrate VI with time during storage, we have chosen during this work to do:

The synthesis of ferrate VI is permanently analyzed by the volumetric method, in order to determine the rate of iron oxidation according to the method of Delaude and Laszlo (1996) [37].

During this work we used the pH of the medium equal to 8, following the results found by Gilbert and al. [23], who limited the area of effectiveness of the FeO_4^{2-} ion as a powerful bactericide to more alkaline media.

All glassware used in these experiments was washed with distilled water and then autoclaved at 121°C for 15 min to ensure sterility and avoid any kind of unwanted contamination.

3. Results

3.1. Evaluation of Ferrate (VI) on the inactivation of different bacterial strains

According to the values of the optical density obtained, there is a clear reduction in the bacterial suspensions of the different strains studied, which testifies to a subsequent reduction in the bacterial concentration after treatment with 6 ml/l of iron (VI) (Table 1 and Figure 1).

Table 1: Values of the optical density obtained at a wavelength of 660 nm before and after treatment of dilute solutions of different strains (diluted with PBS at pH=8) with 6mg/l of iron VI after 24 h of incubation at 37°C.

Strains	Before adding iron VI	After adding iron VI
<i>E. coli</i>	0,233	0,000
<i>Proteus</i>	0,215	0,000
<i>Streptococcus</i>	0,321	0,000
<i>Shigella</i>	1,102	0,000
<i>Pseudomonas sp</i>	1,362	0,000
<i>Missouri Bactéries</i>	1,120	0,000

The evaluation of the survival of different bacterial strains is illustrated in Figures 1 and 2 according to the concentration of iron VI added (24h incubation at 37°C at pH=8).

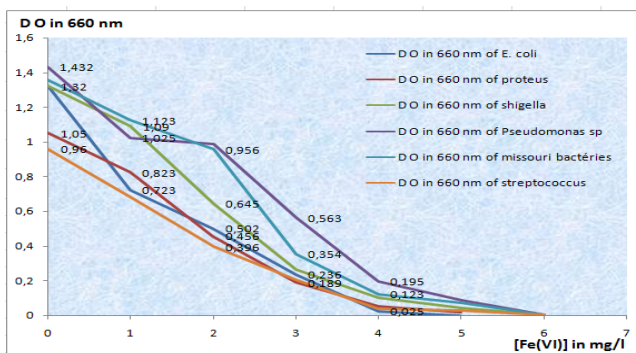


Fig. 1: Optical density of bacterial strains before and after addition of ferrate VI (K_2FeO_4) in alkaline medium at pH=8.

According to the results of the optical density drawn up in Fig. 1, an irregular decrease is observed between the different bacterial strains studied. This is why we can attribute this interference to the resistant capacity of each bacterium and to confirm this hypothesis we proceeded to optimize the mass of iron VI, ask to disinfect each strain in isolation, using the method of enumeration on agar and depending on the pH of the medium.

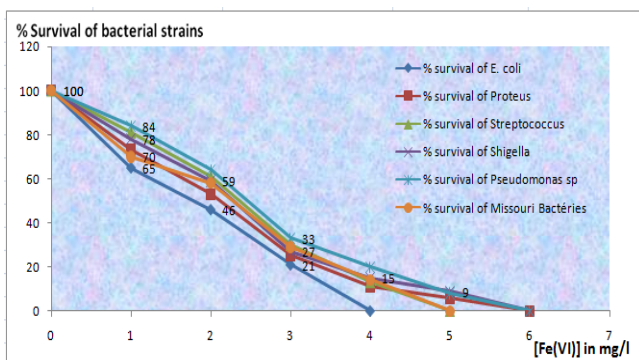


Fig. 2: Percentage of survival at 100 ml according to the concentration of ferrate VI pH=8.

According to Fig. 2 it can be seen that the mass of ferrate necessary to inhibit and eliminate the different bacterial strains it as depends on their nature and respective resistance. This result coincides with that found by the measurements of the optical density as a function of the concentration of ferrate VI added (figure. 1), which proved the difference in resistance between the bacterial strains.

In order to optimize the quantity of ferrate VI necessary for the inactivation of the different strains, a count was carried out according to the method of counting on agar (testimonial solutions and treated with different doses of ferrate) after 24 h of incubation at 37°C.

The results of this count are shown in the following figures (3, 4, 5, 6, 7, 8).

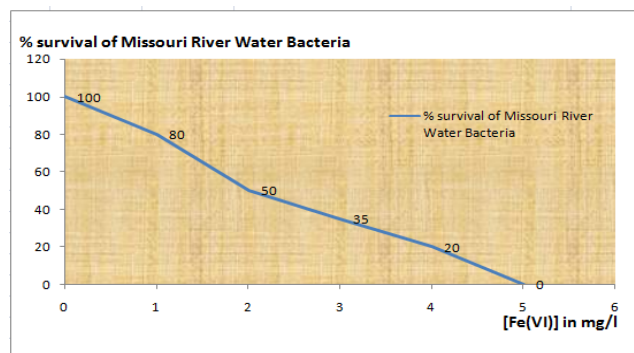


Fig. 3: Survival percentage of Missouri Bacteria from river water in 100 ml as a function of the concentration of K_2FeO_4 in pH=8.

The complete inactivation of Missouri bacteria from river water requires only 5 mg/l of ferrate VI (Fig. 3), which confirms the previous results.

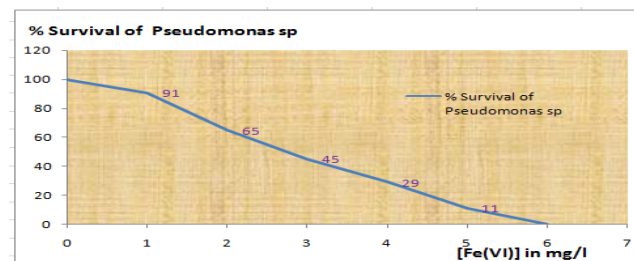


Fig. 4: Survival percentage of Pseudomonas sp in 100 ml as a function of the quantity of K_2FeO_4 in pH=8.

A concentration of 6 mg/l of K_2FeO_4 is necessary to inhibit Pseudomonas sp. (Figure 4) which reveals the optimum dose of ferrate VI required for the disinfectant.

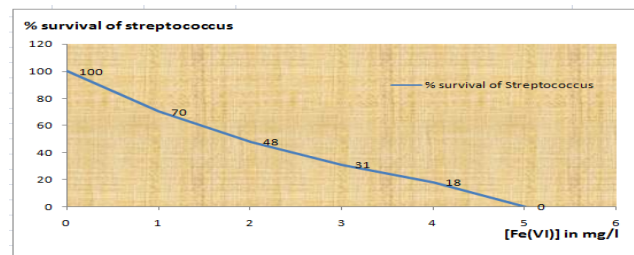


Fig. 5: Percentage of Streptococcus survival in 100 ml as a function of the quantity of K_2FeO_4 added in an alkaline medium at pH=8.

5 mg/l of K_2FeO_4 , at pH = 8, was sufficient to cause Streptococcus mortality (Figure 5), therefore the efficacy of ferrate VI seems very satisfactory and courageous to become a powerful and less expensive alternative to the usual disinfectant deferents.

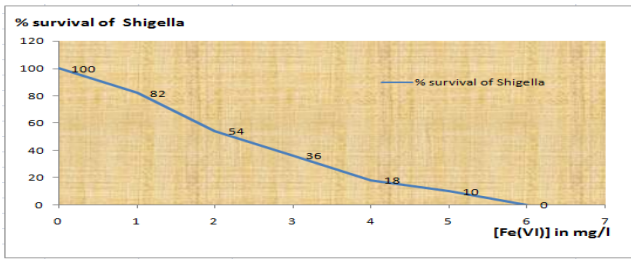


Fig. 6: Percentage of *Shigella* survival as a function of the amount of K_2FeO_4 added at pH=8.

The resistance of *Shigella* bacteria to the various usual disinfectants only requires 6 mg/l of ferrate VI to inactivate it at pH = 8 (Fig. 6).

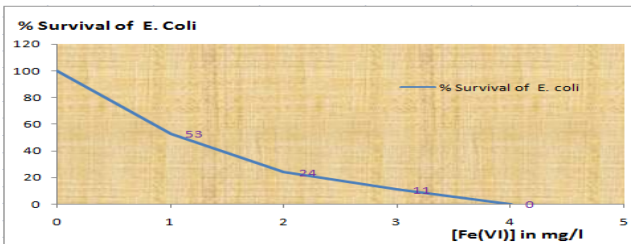


Fig. 7: Percentage of *E.coli* survival according to the amount of K_2FeO_4 added at pH=8.

The figure.7 shows that the K_2FeO_4 concentration of 4 mg/l at pH=8 is sufficient for complete inactivation of *E. coli*.

Table 2: Optical density measured at 660 nm as a function of the contact time of the different strains with K_2FeO_4 after the addition of (4 mg/l for *E. coli*; 5 mg/l for *Streptococcus* and *Missouri Bacteria*; 6 mg/l for *Proteus*, *Shigella* and *Pseudomonas sp*) in solutions diluted with PBS at pH = 8.

Strains	Optical density at t = 0 minutes (Before addition of ferrate VI)	Optical density After t=5 minutes of contact	Optical density After t= 10 minutes of contact	Optical density After t= 15 minutes of contact	Optical density After t= 20 minutes of contact
<i>E. coli</i>	0,243	0.112	0.055	0.021	0.000
<i>Proteus</i>	0,235	0.186	0.091	0.092	0.000
<i>Streptococcus</i>	0,381	0.211	0.097	0.073	0.000
<i>Shigella</i>	1,115	0.452	0.069	0.081	0.000
<i>Pseudomonas sp</i>	1,263	0.532	0.101	0.093	0.000
<i>Missouri Bactéries</i>	1,231	0.321	0.056	0.061	0.000

4. Application of ferrate VI in the water treatment of wadi Fez

4.1. Evaluation of the optimum dose of Fe(VI) to disinfect the water of Oued Fez

According to Koukala and al. [38] the river named Wadi Fez (one of the tributaries of the Sebou River – Morocco) in the city of Fez presents a challenge to clean up this water thanks to its heavy pollution downstream of the city of Fez as well as the The most polluted sites are those directly under the influence of domestic and industrial

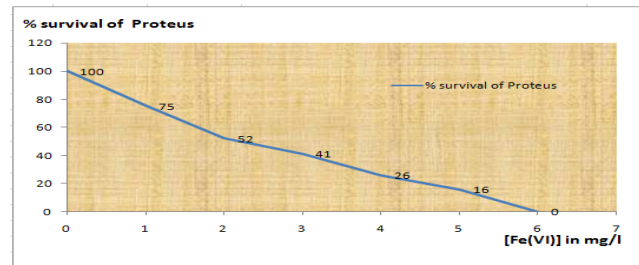


Fig. 8: Percentage of survival of proteus in 100 ml according to the quantity of K_2FeO_4 in an alkaline medium pH=8. The necessary quantity of ferrate VI to completely inactivate the proteus is fixed at 6 mg/l at pH=8 (fig. 8).

3.2. Evaluation of the effect of Fe(VI) contact time on the inactivation of different bacterial strains

According to the results (Table. 2) of the evaluation of the contact time of ferrate VI with the different bacterial strains studied, it is observed that reductions do not exceed 15 minutes of contact time under specific conditions (6 mg / l of K_2FeO_4 at pH = 8), even for that most resistant to ferrate VI. This result is compatible with that found by various previous studies [23].

waste water, in particular effluents from tanneries. Indeed, it should be noted that the various tests carried out to find the dose of iron VI required to disinfect the water of Oued Fez were carried out under different experimental conditions (results not introduced). This work led us to limit the dose of ferrate to 12 mg/l only to kill the various bacteria in this water. Tables 3, 4 and 5 summarize the results found.

The similar results for the optical density and the agar count show that the necessary dose of ferrate VI to disinfect the water of Oued Fez is set at 12 mg/l.

Table 3: Optical density at 660 nm of Fez wadi water after 20 minutes of addition of VI ferrates (K₂FeO₄).

Treatment of different dilution by ferrate	Before addition of ferrate	After hearing 12 mg / l of ferrate
Master sample	0.574	0.002
Solution to 10 ⁻¹	0.352	0.002
Solution to 10 ⁻²	0.302	0.001
Solution to 10 ⁻³	0.290	0.003
Solution to 10 ⁻⁴	0.226	0.002
Solution to 10 ⁻⁵	0.208	0.001
Solution to 10 ⁻⁶	0.198	0.001
Solution to 10 ⁻⁷	0.153	0.000

Table 3 shows a clear decrease in the values of the optical density measured at 660 nm after 20 minutes of the addition of 12 mg / l of ferrate VI to the water of Oued Fez. which means that this small dose is sufficient to disinfect this water after just 20 minutes of contact, therefore, this result proves the effectiveness of this product in the treatment of wastewater as a powerful and courageous disinfectant.

Table 4: Results obtained from the treatment of wadi Fez water with ferrate VI (K₂FeO₄), determined by enumeration on agar (24h incubation at 37°C).

Treatment of different dilution	Before addition of	After hearing 12 mg / l of

Table 6: Results obtained in coliforms after treatment of the water of Oued Fez with different doses of ferrate VI (K₂FeO₄), determined by counting on agar (24 hours of incubation at 37°C).

Treatment of different dilution by ferrate	Before addition of ferrate	After the addition of 4 mg/l of the ferrate	After hearing 6 mg / l of ferrate	After hearing 8 mg / l of ferrate	After hearing 10 mg / l of ferrate	After hearing 12 mg / l of ferrate
Dilution 10 ⁻¹	742 × 10 ²	44	34	28	18	1
Dilution 10 ⁻²	8.4 × 10 ²	23	16	12	09	1
Dilution 10 ⁻³	6.3 × 10 ³	31	25	21	10	1
Dilution 10 ⁻⁴	3 × 10 ⁴	39	33	23	14	1

The different results obtained in coliforms (Table. 6) by agar count (24 hours of incubation at 37°C) after treatment of the water of Oued Fez by different doses of ferrate VI, confirms the dose requested to clean up this water.

4.2. Identification of pathogenic species and strains in water samples

The types of contamination indicator bacteria to be identified are:

* The total mesophilic flora (FMAT) and the total coliforms allowing to know the degree of pollution of a water.

*Total coliforms (CT) and faecal coliforms (CF) are indicators of faecal contamination making it possible to

by ferrate	ferrate	ferrate
Dilution 10 ⁻³	2.1 × 10 ⁵	1
Dilution 10 ⁻⁴	2.2 × 10 ⁵	1
Dilution 10 ⁻⁵	1.2 × 10 ⁶	1
Dilution 10 ⁻⁶	1.1 × 10 ⁶	2
Dilution 10 ⁻⁷	1.1 × 10 ⁷	1

The results found (Table. 4) by enumeration on agar after incubation at 37°C for 24 h before and after treatment of wadi Fez water with 12 mg/l of ferrate VI confirms the results obtained by measuring the optical density (Table. 5).

Table 5: Results obtained in coliforms after treatment of Oued Fès water with ferrate VI (K₂FeO₄), determined by count on agar (24h incubation at 37°C).

Treatment of different dilution by ferrate	Before addition of ferrate	After hearing 12 mg / l of ferrate
Dilution 10 ⁻¹	742 × 10 ²	1
Dilution 10 ⁻²	8.4 × 10 ²	1
Dilution 10 ⁻³	6.3 × 10 ³	1
Dilution 10 ⁻⁴	3 × 10 ⁴	1

According to the table. 5, the number of the total coliform colony obtained after treatment of the water of Oued Fez with ferrate VI (K₂FeO₄) and calculated by counting on agar (24 h of incubation at 37°C), we deduce that 12 mg / l of ferrate VI is sufficient to completely cleanse this water of total coliforms under the most favorable conditions.

assess with more or less safety or precocity, the risk of contamination by faecal matter that can carry micro-organisms pathogens. There is a difference between the presence of coliform bacteria, witnesses of faecal contamination, and the presence of pathogenic bacteria. Coliform bacteria are present in the intestines of warm-blooded animals, but they are also present in soils on plant debris, etc. Those that inhabit the intestine can be identified by their tolerance to a temperature of 44 – 45 °C. The presence of these thermotolerant coliforms is indisputable proof of contamination by fecal matter. In raw water, the number of coliforms is an indicator of the probability of the presence of pathogenic bacteria in treated water. The presence of these coliforms is an indicator of the ineffectiveness of the water sterilization method. However,

the demonstration of faecal contamination calls on only commensal bacteria of the intestine but never saprophytes.

Faced with these technical difficulties, three categories of bacteria indicating fecal contamination are used: Total coliforms, given that fecal coliforms are part of the group of total coliforms. The absence of total coliforms in drinking water is a relatively reliable indication of the absence of pollution of faecal origin. So the detection of faecal contamination is based on the count of thermotolerant coliforms and streptococci.

4.3. Bactericidal power and mechanism of action of iron VI

Ferrate (VI) is a supercharged iron compound in which the iron is in the state oxidation + 6, it is better known as iron (VI). The Ferrate is extremely powerful, can provide multiple treatments from a single application, does not create disinfection by-products, is environmentally friendly and solves the difficult processing which presents challenges that other oxidants cannot touch. Plus, Ferrate is often the least expensive and most effective treatment option.

More recent Jianping Yu and al [39] have illustrated the principle of disinfection of microorganisms and viruses by ferrate VI, in which ferrate VI kills microorganisms by oxidizing their nucleic acids so as to destroy their protein structures. This specificity confirms their bactericidal power in water treatment, because it reacts on the structure of the microorganism and leads to their destruction. On the other hand, iron hydroxide compounds obtained by reduction of ferrate VI lead to the aggregation and precipitation of microorganisms. What is absent in other usual substances.

Ferrate VI therefore has the advantages of being an alternative, powerful and safer substance for disinfection of water compared to other usual products such as hypochlorite, aluminum sulphate, ozone and sulphate. ferric because it does not produce any indigenous compounds during processing according to Jiang and al., 2006 [40]. Figure 9 presents the principle of disinfection of microorganisms by ferrate (VI).

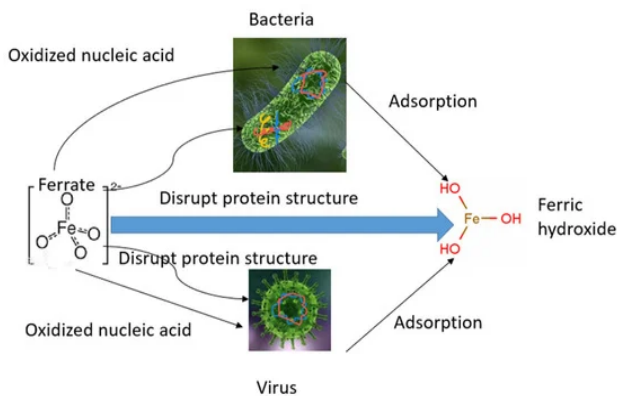


Fig. 9: Principle of ferrate (VI) disinfection [39].

5. Discussion

The required mass of iron VI to inhibit *E. coli* is 4 mg/l at pH=8 at a contact time of 20 minutes (Figure 7, Table 1, 2). This presents a great similarity with the results of the various previous studies [23], [41]. First, the inactivation of *E. coli* in 100% requires a quantity of 4 to 6 mg/l of iron VI and a contact time varies from 20 to 30 minutes under the most favorable conditions and on the other hand the disinfection efficiency of ferrate VI increases when the pH exceeds 8. This low mass of iron VI to inactivate *E. coli* (4mg/l at pH=8) for 20 minutes depends on the purity of the ferrate used and the favorable conditions provided for the inactivation of this bacterium (pH of the medium).

The necessary mass of iron VI to completely inactivate (*Proteus*, *Shigella* and *Pseudomonas* sp.) is set at 6 mg/l at pH=8 for a period of 20 minutes (figures 4, 6 and 8; table 1, 2). However, for the other bacteria tested (*Missouri Bacteria* and *Streptococcus*), requires only 5 mg/l of ferrate VI at pH = 8 for a contact time of 20 minutes (Figures 3 and 5; Table 1, 2). These results are compatible with that obtained by Murmann and Robinson [33].

Murmann and Robinson [33] also studied the inactivation of *Pseudomonas* and *Missouri Bacteria* in river water, in order to show that the amount of iron VI and the pH of the water influence this disinfection. First, the *Pseudomonas* sp was completely destroyed at a dose of 0 to 50 mg/l, on the other hand *Missouri Bacteria* in river water is between 2 and 100mg/l. The difference observed between the quantities of iron VI used in the treatment of two bacterial strains under the same operating conditions explains the resistant capacity of each bacterium.

According to our results, treatment of different bacterial strain at pH = 8 by iron VI and the results of various previous studies, we deduce that we really face an environmental problem of the water of the Fez rivers.

Sanitation of the water of Oued Fez requires a more in-depth study to limit the polluting and indigenous factors (bacteria and viruses), having a high resistant capacity to iron VI in order to reduce the quantity of ferrate VI used for the disinfection of this water and optimize the responsible factors at this dose of 12mg/l.

According to our work (Tables 4 and 5), 12 mg/l of iron VI is enough to disinfect Oud Fez water, which is compatible with that presented in the literature by Murmann and Robinson (1974) [33]. As according to previous studies, 10 mg/l of ferrate VI is only capable of inactivating faecal coliforms at 99.90% and inactivating total coliforms at 99.70% (Aubertin and al., 1996) [42], which is compatible with our results obtained during the treatment of water from the Fes wadi (table 5).

Jiang and Lloyd (2002) [26] have shown that oxidation

state VI iron eliminates micropollutants contained in wastewater, precipitates phosphate and attacks molecules with electron-rich functional groups thanks to its coagulant power, as well as he showed that ferrate VI is an incredible superoxidant for various organic compounds and produces non-toxic by-products. It also has iron VI presents an effective alternation for other disinfectants thanks to their bactericidal power to disinfect and inactivate native and chlorine-resistant microorganisms, some compounds of which can be degraded and eliminated in a few seconds to a few minutes its produce dangerous compounds and undesirable (Sharma, 2010) [41].

Previous studies (Jiang and al., 2001[44]; Jiang and Wang, 2003[45]; Lee and al., 2009[46]; Yngard and al., 2008, 2007 [47], [48]; Sharma and Sohn, 2009 [49]; Sharma and al., 2007[50]; Jain and al., 2009[51]), confirmed that the transformation of iron VI to iron III after processing renders a super coagulant for metals and radionuclides in water.

Shuchang and al (2021) [52] have well documented that pH is one of the most vital parameters that affects Fe(VI) kinetics and self-decomposition mechanism. The lowest rate of Fe(VI) self-decay occurs at alkaline pH. This confirms our results found at pH=8.

6. Conclusion

These studies present the interest of ferrate VI as a powerful bactericide, the dose of which required to inactivate E. coli is 4 mg/l; Streptococcus and Missouri Bacteria is 5 mg/l; Proteus, Shigella and Pseudomonas sp is 6 mg/l in alkaline medium at pH=8 for a contact time of only 20 minutes.

The dose required to inactivate the various native bacterial strains to more than 99.9% during the treatment of water from the Fez wadi in Fez under study is only 12 mg/l at pH= 8.

Our results confirm the bactericidal power of ferrate VI in the treatment of water as a superoxidant, coagulant, flocculant to clean up the environment, which have been demonstrated by previous studies (Sharma, 2010) [43].

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