

Study of the antibacterial activity of the potassium permanganoferrate $K_3Fe_xMn_yO_8$

Amina ZAHARI¹, Amal TAZI¹, and Mohamed BLAGHEN²

¹Team of Electrochemistry and Environmental Chemistry, Laboratory of Interface, Materials and Environment, Faculty of Science, University Hassan II – Casablanca, BP5366 Maârif, Casablanca, Morocco

²Team of Environmental and Industrial Biotechnology, Laboratory of Microbiology, Biotechnology, Pharmacology and Environment, Faculty of Science, University Hassan II – Casablanca, BP5366 Maârif, Casablanca, Morocco

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ABSTRACT: Pathogenic bacteria transmitted to humans through water are, nowadays, responsible for various infectious diseases; mainly gastrointestinal. Several disposal methods for these pathogens, such as chlorination, ozonation, and UV Rays have been used and developed.

Our study consists of using a new approach to deal with resistant strains that exist in wastewater; by the application of synthetic materials, the potassium permanganoferrates $K_3Fe_xMn_yO_8$ ($1 \leq x/y \leq 4$).

Our results show the antibacterial effect of these products tested in liquid and solid medium depending on the pH on three bacterial strains: *Pseudomonas aeruginosa* (Ps.Ae), *Escherichia coli* (E.Co) et *Staphylococcus aureus* (St.Au.). In the presence of the potassium permanganoferrates, bacterial growth decreases by half in the liquid medium, with concentrations going from 0.74 mmol/l up to 0.023 mmol/l, depending on the Fe/Mn ratio and on the studied bacteria. In the solid medium, the inhibitive efficiency of $K_3Fe_xMn_yO_8$ is also demonstrated for optimized concentrations, between 0.74mmol/l and 0.023mmol/l.

The maximum inhibitions in the liquid medium are 99.4% for E.Co. and 99.9% for St.Au., are obtained with concentrations of 0.5 mmol/L of $K_3Fe_xMn_yO_8$ (Fe/Mn=4). However, with 1.2 mmol/l of permanganoferrates, the inhibition doesn't exceed 98.8% for Ps.Ae.

KEYWORDS: potassium permanganoferrates, inhibition, bacteria, wastewater.

1 INTRODUCTION

There is more and more concern over the contamination of wastewater by pathogenic and resistant bacteria, thereby being conducive to the spread of bacterial and viral diseases. This contaminated water is mainly due to fecal pollution, but also to industrial pollution. Controlling waste water, and especially sewer water, is the most important preventive measure [1].

There are several methods to reduce pathogenic bacteria, such as using chemical product (antibiotics [1],...), physical processes (γ and UV radiation) [2] or physico-chemical techniques (membrane process,...) [3,4].

However, bacterial resistance towards antibiotics has occurred rapidly after the application of the latter for the treatment of many infectious diseases. This resistance is a major factor complicating bacterial infections' therapy, and especially the spread of multi-resistant strains.

Recent studies have shown the disinfectant effect of the ferrates VI [5] K_2FeO_4 on the total bacterial community of secondary effluents, which can reach 99.9% of despondency of indigenous bacteria.

Our purpose is to present a new approach to deal with the resistant strains present in wastewater by applying synthetic molecules such as potassium permanganoferrates $K_3Fe_xMn_yO_8$ ($1 \leq x/y \leq 4$).

Potassium permanganoferrates $K_3Fe_xMn_yO_8$ do contain many proprieties: oxidizing, coagulant-flocculating, disinfectant, ... [6,7,8,9].

These compounds are considered to be super-oxidizing, and their power is above hydrogen peroxide's ($E^\circ = 1.776$ V), chlorine's ($E^\circ = 1.35$ V) and ozone's ($E^\circ = 2.076$ V), usually used for wastewater treatment and disinfection. This character can be explained by the combination of the oxidizing powers of the ferrates VI ($E^\circ(FeO_4^{2-}/Fe^{3+})=2.20$ V) and of the permanganates (VII) ($E^\circ(MnO_4^-/MnO_2)=1.697$ V).

In this work, we aim to study the performance of these synthetic molecules in the inhibition of growth of three bacterial strains: *Pseudomonas aeruginosa*, *Escherichia coli* et *Staphylococcus aureus*, without them having any side effect on living beings and on the environment.

2 MATERIALS AND METHODS

2.1 STUDIED STRAINS

The study has been carried out on three baseline bacterial strains:

Pseudomonas aeruginosa ATCC 27853 (Ps.Ae.), *Escherichia coli* ATCC 25922 (E. Co.) and *Staphylococcus aureus* ATCC 25923 (Sta.Au.).

2.2 DISINFECTING REAGENT: $K_3Fe_xMn_yO_8$

The permanganoferrates $K_3Fe_xMn_yO_8$ ($x / y = 1, 2, 3$ and 4) are synthetized by using the dry method at ambient temperature [6,10].

2.3 DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION MIC

2.3.1 MIC IN THE LIQUID MEDIUM: MICL

The method consists of preparing a series of tubes containing 5ml of nutrient broth (5g/l of gelatine, 3 g/l cattle meat, pH = 7), to which we add the potassium permanganoferrates with concentrations between 0.014 mmol/l and 3.5mmol/l, according to a geometric progression due to 2. After that, we add 50 μ l of bacterial inoculum diluted 100 times from a 24-hour-old culturing.

The prepared tubes' galleries for molar ratios being $x/y = 1, 2, 3$ and 4 will be incubated at 37°C during 18h[11].

2.3.2 MIC IN THE SOLID MEDIUM: MICS

The used method consists of preparing a series of Erlenmeyer flasks containing 20ml of Muller-Hinton agar, to which we added amounts of permanganoferrates VI going from 0.014mmol/L to 3.5mmol/L, following a half-cascade evolution.

After homogeization, the culturing media are aseptically transferred into Petri dishes. The bacterial inoculum, diluted 1000 times from a 24-hour-old culturing, is sown on the surface. The cultures are then incubated at 37°C during 18hours.

2.4 PH MEASUREMENT

The pH measurement has been done with the help of a multi-parameter analyzer Type CONSORT - (C561–C562).

2.5 THE OPTICAL DENSITY MEASUREMENT

The optical density measurement was made by the spectrophotometer UV-1800 Serial No. A11454703649, Shimadzu (Japan).

In order to monitor the effect of permanganoferrates on bacterial growth, we have proceeded as follows:

From a 24-hours pregrowth phase of different bacterial strains diluted 100 times, are seeded into Erlenmeyer flasks containing nutrient broth with or without potassium permanganoferrate which concentration is half of the MICL's one, and are then incubated at 37°C.

Note: All the experiments have been carried out performed three times

3 RESULTS AND DISCUSSION

3.1 DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION OF POTASSIUM PERMANGANOFERRATES

3.1.1 IN LIQUID MEDIUM: MICL

The permanganoferrates MIC's VI $K_3Fe_xMn_yO_8$ obtained in a liquid medium for the different molar ratios x/y are summarized in Table 1.

Table 1. MICL in a liquid medium for the three bacterial strains *Ps.Ae.*, *E. Co.* et *St.Au.*

		MICL of $K_3Fe_xMn_yO_8$ in mmol/L			
		$x/y=1$	$x/y=2$	$x/y=3$	$x/y=4$
Bacterial strain	<i>Ps. Ae.</i>	1.485	0.743	0.372	0.372
	<i>E.Co.</i>	0.743	0.372	0.186	0.046
	<i>St.Au.</i>	0.186	0.186	0.093	0.046

According to these results (Table 1), we have found that the molar ratio 3 and 4 have revealed an inhibition for the three bacterial strains with MICL below molar ratios 1 and 2.

The inhibitory effect of $K_3Fe_xMn_yO_8$ increases with the rate of iron VI. For example, for *St.Au.*, the value of CMIL goes from 0.186 mmol/L for $x/y = 1$ and 2 ratios to 0.046 mmol/L for the ratio 4. On the other hand, for *E. Co.*, the CMIL of the ratio 4 is 16.2 times weaker than the one of the ratio 1.

On the other hand, the results of the *Ps.Au.* CMIL are much higher than the ones determined for the two other bacteria, showing the stiff resistance of this bacterium.

3.1.2 IN A SOLID MEDIUM MICs

We summarize, in Table 2, the results of $K_3Fe_xMn_yO_8$ MICs determined in the solid medium depending on the molar ratio x/y for the three bacterial strains we study.

MICs in the solid medium for the three bacterial strains *Ps.Ae.*, *E. Co.* et *St.Au.*

		MICL of $K_3Fe_xMn_yO_8$ in mmol/L			
		$x/y=1$	$x/y=2$	$x/y=3$	$x/y=4$
Bacterial strain	<i>Ps. Ae.</i>	0.743	0.743	0.372	0.186
	<i>E.Co.</i>	0.372	0.186	0.186	0.046
	<i>St.Au.</i>	0.186	0.093	0.093	0.023

For all three bacteria, comparing the Minimum Inhibitory Concentrations in the solid and the liquid media allows us to conclude that the inhibitory effect of the potassium permanganoferrate is much higher in the solid medium, except for the $x/y = 3$ ratio in which the MICs are identical to the ones found in the liquid medium for the three bacteria. Oxidation can be more influenced by the solid medium than by the liquid medium. This can be explained by the stability of the potassium permanganoferrates in the solid medium. However, in the aqueous solution, we might experience a reduction of the iron VI into the Iron (II).

In order to extend our study, we have followed the evolution of the bacterial growth of these strains in the presence of potassium permanganoferrates injected into the medium, in order to compare it with the one of their normal growth.

3.2 MONITORING BACTERIAL STRAINS GROWTH IN THE PRESENCE OF PERMANGANOFERRATE VI

The evolution of the optical density of these bacterial suspensions has been followed through time at 600 nm. The results are illustrated in Figure 1.

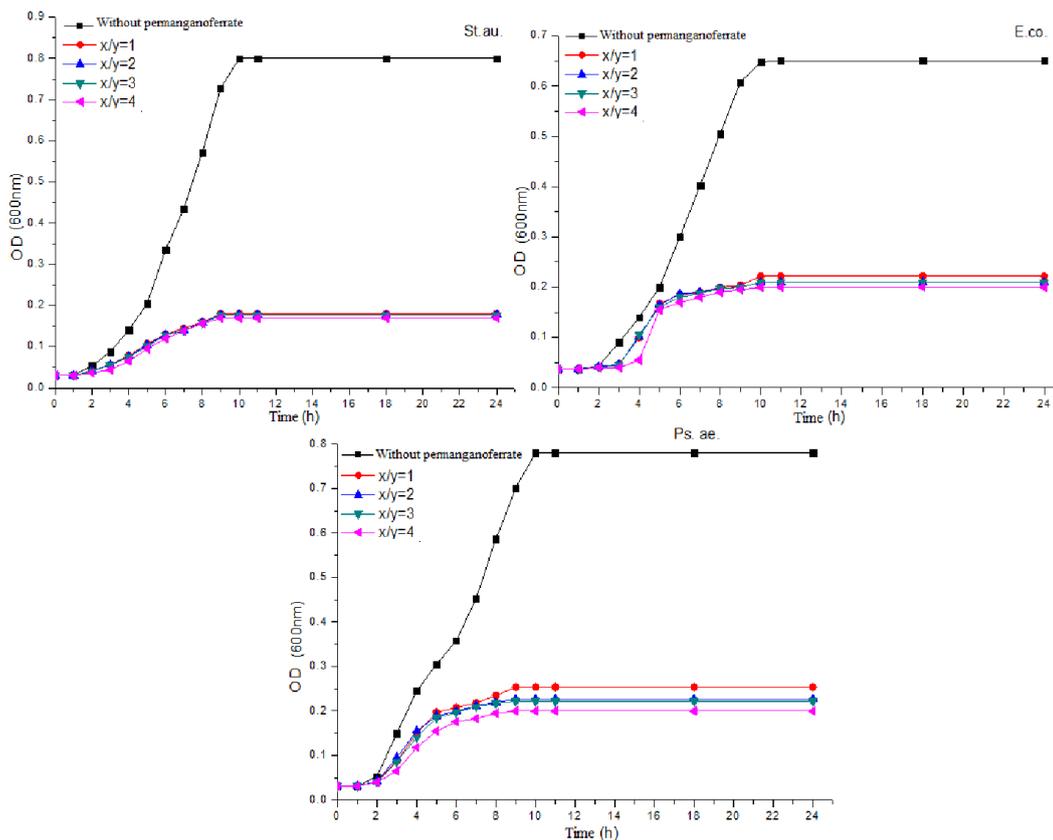


Fig.1. The effect of $K_3Fe_xMn_yO_8$ on bacterial growth (*Ps.Ae.*, *E. Co.* and *Sta.Au.*) [$K_3Fe_xMn_yO_8$]=M1CL/2

In the presence of potassium permanganoferrates VI for various molar ratios and for half of the M1CL, the general curve profile doesn't change in a longer latency period.

The inhibitory effect of the potassium permanganoferrates is demonstrated by a decrease in growth rate for the three bacterial strains, going over 50% of inhibition (63% for *Ps.Ae.*) after 6 hours.

The results shown in Figure 2 demonstrate that when the concentration of permanganoferrate increases, the bacterial growth decreases, regardless of the strain and of the chosen molar ratio.

We can see that 99.4 % is reached for *E.Co.*, and that 99.9 % is reached for *St.Au.*, with concentrations being 0.5 mmol/l which molar ratio is Fe/Mn= 4. The inhibition for *Ps.Ae.* is around 99 % and has been reached with a concentration of 1.2 mmol/l of permanganoferrate .

The inhibitive effect of these materials can be explained by their high oxidizing power, which is most pronounced for molar ratios 3 and 4, in which the amount of Iron (VI) is high.

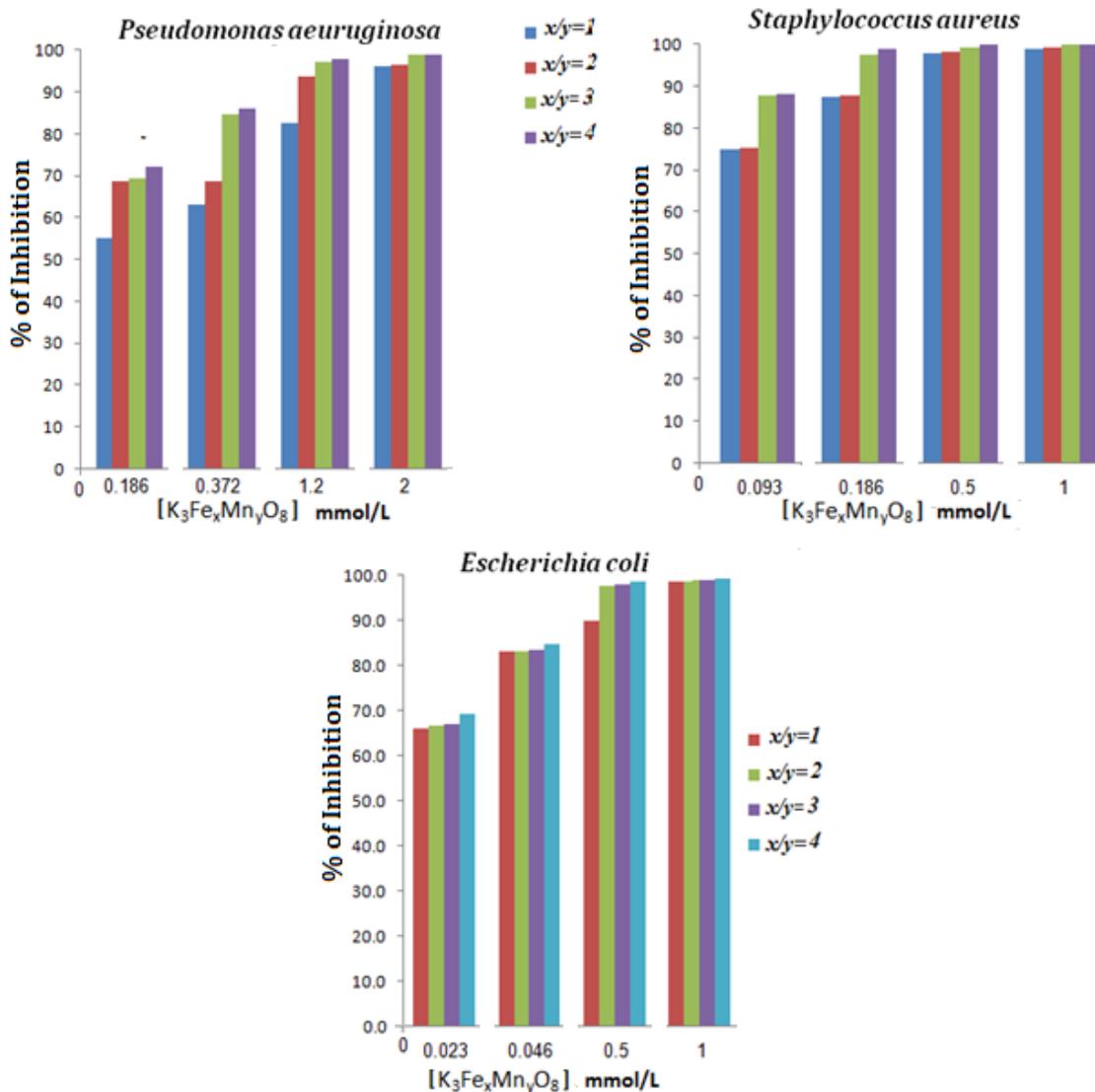


Fig. 2. % of growth inhibition of the three bacterial strains according to the different concentrations [K₃Fe_xMn_yO₈] (1≤x/y≤4)

3.3 THE EFFECT OF THE PH ON BACTERIAL GROWTH

According to Figures 3 and 4, the incubation of the three bacteria for different pH shows that the latter remains practically constant, except for a pH value below 8, for which we notice a rise in the pH value of the medium after 10 hours, and then reaches its maximum after 18 hours of incubation.

After 10 hours, we note a rise amounting to +1 in the pH 5 and 7, followed by a stability with a bacterial growth rate of almost 100%, whatever bacteria is used.

When the pH=8, we notice that the two bacterial strains, E.Co. and St.Au, show a steady pace which is almost identical with bacterial growth rates of respectively 62.87% and 50%.

However, for the Ps.Ae., we notice a change in pH of ±1, which stabilizes after 18 hours of incubation with a bacterial growth rate of 85.64%.

The results are shown in Figures 3 and 4.

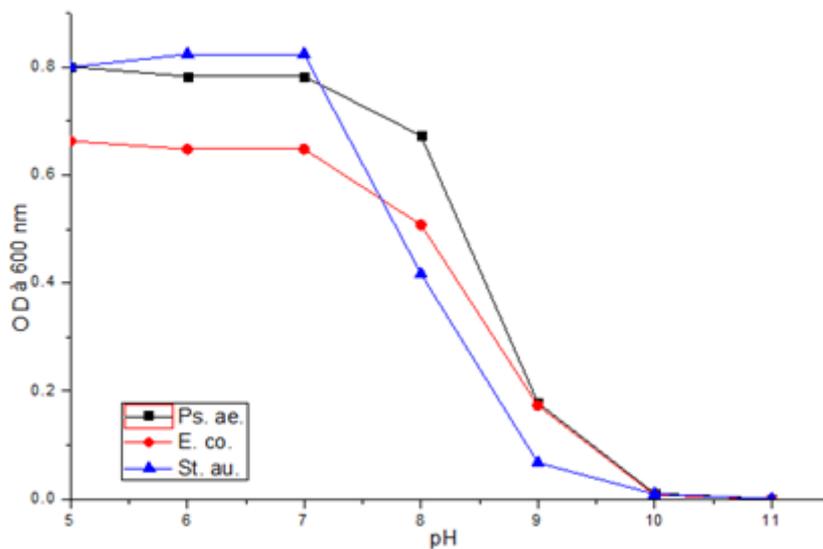


Fig. 3. Effect of the pH on bacterial growth

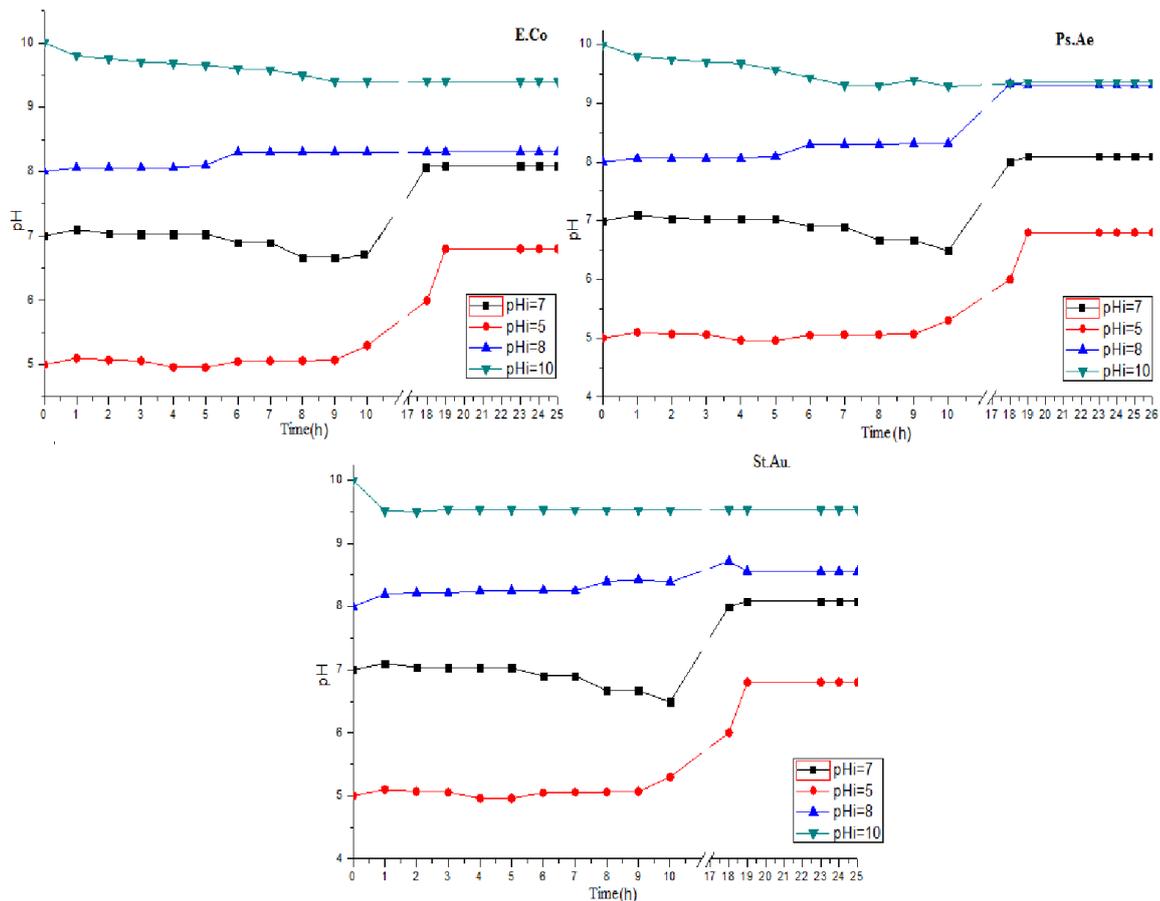


Fig. 4. Monitoring of the pH of the culture medium according to time for each bacterial strain

4 CONCLUSION

Therefore, inhibition by potassium permanganoferrates proves to be important and effective and does not generate any adverse/harmful effect on the environment and the living beings' health. Comparatively to the results obtained (3.3) with potassium permanganoferrates ($K_3Fe_xMn_yO_8$), we reach maximum inhibitions from 98.8 to 99.9%, depending on the case, whereas the pH of the medium is of the order of 8, which shows, once again, the super-oxidizing effect of these materials.

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