

Process for the Biological Removal of Fe (II) from Reconstituted Waters on a Support of Filter Material with Coated Jujube Seeds

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Abstract

Three beakers for removing Fe (II) in reconstituted water (doped with FeSO₄) were built and tested. Given the set operating conditions ([O₂] > 4 mg·L⁻¹, P_{atm} = 1.013 bar, T = 25°C ± 1°C and [Fe²⁺]₀ = 0.5 to 2 mg·L⁻¹), removal of iron was caused by biological and possibly physical and chemical oxidation because there is a quantity of free oxygen in the medium. The extent of each type of oxidation has not been evaluated as it specifically studies the biological degradation of iron in these beaker tests by setting the operating conditions (pH > 6.5, dissolved oxygen from 0 to 8 mg·L⁻¹, Redox Potential from 100 to 400 mV). The experimental studies focused particularly on the measurements of maximum wavelength, conversion efficiencies from Fe (II) to Fe (III), the effect of the Fe (II) concentration, the influence of pH, the action of the temperature of the prepared solutions and the effect of O₂ concentration under specified operating conditions. It noticed precipitated amounts of iron deposited at the bottom of the beakers. Thus, the low concentrations of Fe (II) detected in the influent after the biological oxidation operation could be attributed to microorganisms that consume iron as a substrate.

Keywords

Biofilm, Batch Test, Biological Oxidation, Iron Removal, Beaker, Doped Water

1. Introduction

In the case where iron is detected in drinking water intended for public consumption, at levels which exceed the drinking water potability standards by

0.3 mg·L⁻¹ (WHO), it is not advised to use this water for various reasons (da Silva Almeida et al., 2021). First, aesthetic appearance: the precipitates of Fe (III) give water a reddish color in the presence of oxygen. Second, Iron gives water a taste of metal and an unwanted odor with the development of certain microorganism in the aerated environment. Third, Fe (III) precipitates in the pipes decrease the hydraulic loads where the initial diameter is designed to transport water from one point to another. Some ferrobacteria use iron for growth under certain operating conditions. When iron bacteria die and disappear, cause odors this can and unpleasant tastes (Vayenas & Lyberatos, 2005). Iron is predominantly in Fe (III) form in surface water. Fe (II) is generally found in deep layers devoid of oxygen (O₂). The oxidation state of iron in water depends mainly on pH and redox potential (*Diagrams of the predominance of iron species and their stability in water*) (Charles, 2006). Fe (II) can be oxidized in FeCO₃, Fe(OH)₂ or Fe(OH)₃ under the influence of increasing the oxidation potential or pH of the solution (Levitt et al., 2011; McKee et al., 2016).

The degradation of ferrous iron increases mainly with the increase in oxygen content in the reaction medium, redox potential and pH and can reach 90% conversion yields to a pH of 7 and a redox of 400 mV (Oliveira et al., 2021). However, Dailianis et al. (2021); Vayenas and Lyberatos (2005) have shown in their work, the iron oxidation reaction is very slow at a pH < 6 and the reaction products may remain in aerated waters for a certain time. For biological treatment, there are many kinds of bacteria that eliminate Fe (II) dissolved in groundwater. In fact, the oxidation mechanism of *Gallionella sp*, *Leptothrix ochracea* and *Crenothrix polyspora* is done by a primary intracellular reaction by enzymatic action, on the other hand the opposite action is of catalytic type because the microorganisms can secrete filaments (Mouchet, 1992).

In the literature and in the groundwater treatment places of Senegal, we noticed that to eliminate the iron dissolved in the water deprived of oxygen, the ventilation followed by a solid-liquid separation on a sand bed is the most used chemical treatment method. Michalakos et al. (1997) have shown in their scientific work that aeration is the appropriate method for the oxidation of ferrous iron in groundwater where iron concentrations are greater than 5 mg·L⁻¹ to avoid the costs associated with chemicals and the complexity of the technology. The separation of the liquid in the solid phase is possible using sand filters. Other complementary treatments can be employed, such as the use of strong oxidants like chlorine, potassium permanganate, biological oxidation... (Michalakos et al., 1997; Tekerlekopoulou et al., 2008; Vayenas & Lyberatos, 2005).

In this paper, we were used the treatment method based on three batch-operated fixed bed beakers to remove dissolved Fe (II) from iron-doped synthetic waters (FeSO₄). Indeed, the beakers were filled with plastic-coated jujube nuts with an average diameter of 8.08 mm, used as filter media. The boundaries between the physical, chemical and biological removal of iron are not identified in this work. Nevertheless, the conditions of the field of biological oxidation are fixed. Experimental tests are carried on the determination of the maximum wavelength for

which the concentration is maximum, the determination of treatment yields, the study of the influence of the initial content in Fe (II), of the pH of the solution studied, temperature and concentration in O₂.

2. Material and Methods

2.1. Experimental Methodology

The kinetics of degradation of Fe (II) by isolated ferrobacteria was studied in beakers (**Figure 1**). The strains were grown in a mineral salt environment in well-defined proportions. The operation of biological degradation of Fe (II) in beakers was carried out according to the experimental parameters in particular pH, oxygen and temperature. The tests of the biological elimination of Fe (II) were carried out in water “doped” with iron at concentrations between 0.5 and 2 mg·L⁻¹, with a pH between 3.4 and 10 and a temperature ranging from 10°C to 40°C (Toyoda & Tebo, 2016).

For the measurement of the maximum wavelength, in a beaker, we put the solvent on we will do the “*optical zero*”. In another beaker we put the prepared 2 mg Fe (II) sulfate solution per litre. After determining absorbance of the solution at different wavelengths, we found that there is a wavelength at which the absorbance is maximum. Temperature and pH are continuously measured by the pH meter (multimeter). We used a DO meter HI 8043 oximeter to measure dissolved oxygen. It consists of a probe, a protective cap, an oxygen permeable teflon membrane and a cable. It also has an easy-to-read LCD screen. Dissolved oxygen is shown in mg·L⁻¹. The Fe (II) concentration is calculated by multiplying the obtained concentration by 10. Iron concentrations were evaluated according to the following relationship (Faye et al., 2019):

$$\left[\text{Fe}^{2+} \right]_{\text{total}} = \frac{\text{ABS} - 0.0577}{0.1418} \quad (1)$$

The treatment method relied on batch-operated fixed-bed beakers to remove Fe (II) from the doped water. The beakers were filled with coated jujube seeds with an average diameter of 8.08 mm, used as the filter material. For starting the



Figure 1. Batch assay on substrate of coated “jujube” seeds as medium filtering and fixation of bacteria for removal of Fe (II); 1—natural ventilation holes, 2—aluminum foil used as cover, 3—phase clear and hazy phase separation, 4—sludge.

filter, the application of the method described by (Michalakos et al., 1997; Tekerlekopoulou et al., 2013) was used. The strain was taken from a Petri dish and added to the ascending filter with 10 mg/L of Fe^{2+} in the form of $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ (Tekerlekopoulou et al., 2006). The addition of nitrogen (20 g/L of NaNO_2) and phosphorus (10 g/L of KH_2PO_4), had been made to ensure the rapid growth of bacteria. The beakers were aerated at the top of the column opening using a *MECAFER* type air compressor ($Q_G = 760 \text{ L/h}$). After a follow-up time of 10 days, the examination of the identification showed the presence of bacteria in convex form which could be of the genus *Gallionella* (*G. ferruginea*).

The column in batch mode is regularly aerated with an air compressor if the oxygen in the medium is less than $4 \text{ mg}\cdot\text{L}^{-1}$. The start-up of the biological filter was carried out 2 months to obtain maximum rates of biological oxidation of iron (Tekerlekopoulou et al., 2008). To assess the kinetics of dissolved iron depletion, plastic beakers were perforated at the bottom as a sampling point with a syringe, allowing samples to be collected based on contact time. In batch operation, a solution was added with iron concentrations in the feed between $0.5 - 2 \text{ mg}\cdot\text{L}^{-1}$ (Papadopoulos et al., 2019; Tekerlekopoulou et al., 2006). This concentration range covers concentrations found in groundwater sources in some regions of Senegal. Prepared solutions were stored at 4°C to prevent growth of bacteria in the feeding medium (Pham & Waite, 2008; Tekerlekopoulou et al., 2006). The kinetics of the oxidation of Fe (II) by isolated ferrobacteria are presented in (Figure 1).

2.2. Physical Characteristics of Coated Jujube Seeds

The determination of the physical characteristics of coated jujube seeds obeys the procedure described in my scientific work on: “Comparative study of two fillings (nuts of monkey bread and jujube) in a packed column: hydrodynamics and transfer of matter (Mamadou et al., 2018). Figure 2 shows the jujube seeds before and after the coating operation.

The physical parameters of the coated jujube seeds are shown in following Table 1.



Figure 2. (a) Jujube seeds before the coating operation; (b) Jujube seeds after the coating operation.

Table 1. Characterization of physical parameters of coated “Jujube” Seeds.

Materials	Apparent volumetric mass (ρ_{app}) in $\text{g}\cdot\text{mL}^{-1}$	Actual volumetric mass (ρ_r) in $\text{g}\cdot\text{mL}^{-1}$	Porosity (ϵ)	Average particle diameter (dp) in mm	Specific surface in m^{-1}
Jujube seeds before	0.585	1.139	0.437	8.15	576.62
Jujube seeds after	0.597	1.304	0.423	8.08	548.13

3. Results and Discussions

3.1. Measurement of the Maximum Wavelength

To measure the amount of biomass in the three (3) beakers over time, we first determine the maximum wavelength from the release of a small amount of wash sludge in distilled water. The preparation of the solutions to be assayed follows the same protocol for the determination of Fe (II) by ortho-phenanthroline (Faye et al., 2019). Indeed, the principle will be to take the prepared solution and pass the monochromatic light at different wavelength, then build a graph of the absorbance as a function of the wavelength. The variation curve of the optical densities as a function of the wavelengths is described in **Figure 3**.

The peak of the graph (**Figure 3**) shows the maximum values of $\lambda_{max} = 530 \text{ nm}$ and $O.D_{max} = 0.676$. In the following experiments, we will set ourselves to the wavelength $\lambda_{max} = 530 \text{ nm}$ (adjust the device: UV-visible spectrophotometry) such that the absorbance (light absorbed) is maximal so that we will have a greater accuracy of optical density values.

3.2. Effect of Temperature

Oxidation rates of Fe (II) depend strongly on the temperature of the reaction medium. Indeed, different temperatures have been set, from 10°C to 60°C to $\text{pH} = 9.6$, with the addition of airflow (generated by the *MECAFER* type compressor) at each temperature as it has been demonstrated in literature that dissolved oxygen affects the Fe (II) oxidation rate to Fe (III). The optimum temperature found for Fe (II) oxidation was 40°C (**Figure 4**).

Over a temperature range of 10°C to 40°C , the oxidation rate increases. This phenomenon leads to the activation of the biological reaction in this temperature range. This evolution could be modeled by the Arrhenius equation in this region (Štembal et al., 2005):

$$\left(\frac{k}{k_{ref}} \right)_T = Ae^{-E_a/RT} \quad (2)$$

where k is a magnitude of the evolution of the microbial system, E_a activation energy, A is a constant and T temperature.

From 40°C , the biological oxidation rate of Fe (II) decreases. This decrease is explained by the thermal deactivation of the enzymatic system. Correlation equations could describe the evolution of oxidation rates as a function of temperature (Štembal et al., 2005). However, experimental data were insufficient to estimate

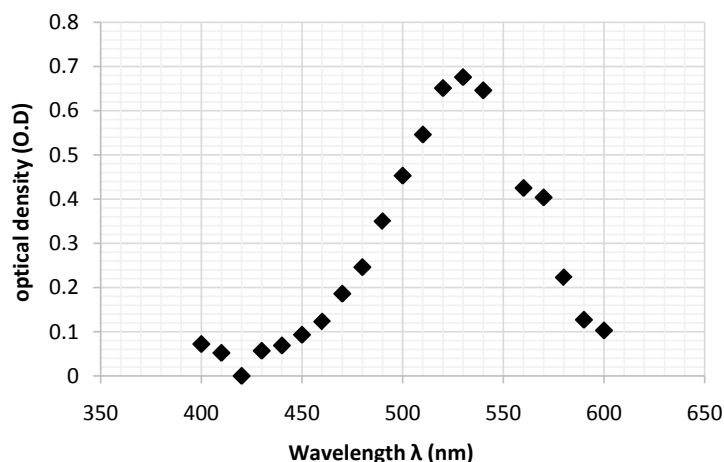


Figure 3. Curves of variation of optical densities as a function of wavelengths.

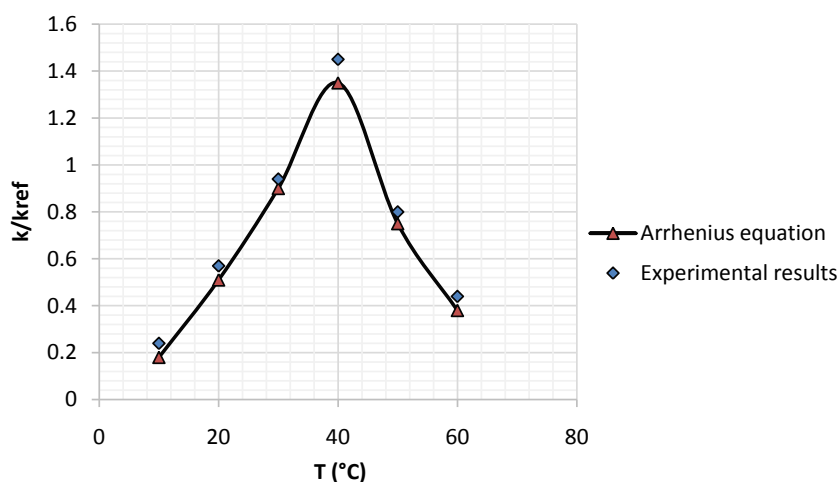


Figure 4. Effect of temperature on the oxidation rate of iron in the batch bioreactor (beaker 3) at pH = 9.6, $[O_2] = 6.5 \text{ mg}\cdot\text{L}^{-1}$, $P_{\text{atm}} = 1.013 \text{ bar}$ and $[Fe^{2+}]_0 = 2 \text{ mg}\cdot\text{L}^{-1}$.

treatment efficiencies at elevated temperatures. Hence the importance of proceeding with pilot tests to find the limit temperature of thermal deactivation of biological oxidation in our pilot column.

3.3. Effect of pH

The influence of pH on iron degradation was studied by varying this quantity by intervals of 0.5, from 7 to 10.5, while maintaining the other parameters constant (**Figure 5**) (Hesslein, 2005).

In these experiments, the maximum value of the oxidation rate of Fe (II) was observed at pH 9.5 (**Figure 5**). This value is close to the value obtained during tests in tubes for the elimination of iron present in water reconstituted by ferro-bacteria isolated on a Petri dish (9.8) (Faye et al., 2019). We have tried to reproduce the works of whose optimum pH was 7.3. The results of our experiments are not very far from those described by these two previous authors. The results of (Brouwers et al., 2000) are similar to those of (Adams & Ghiorse, 1987).

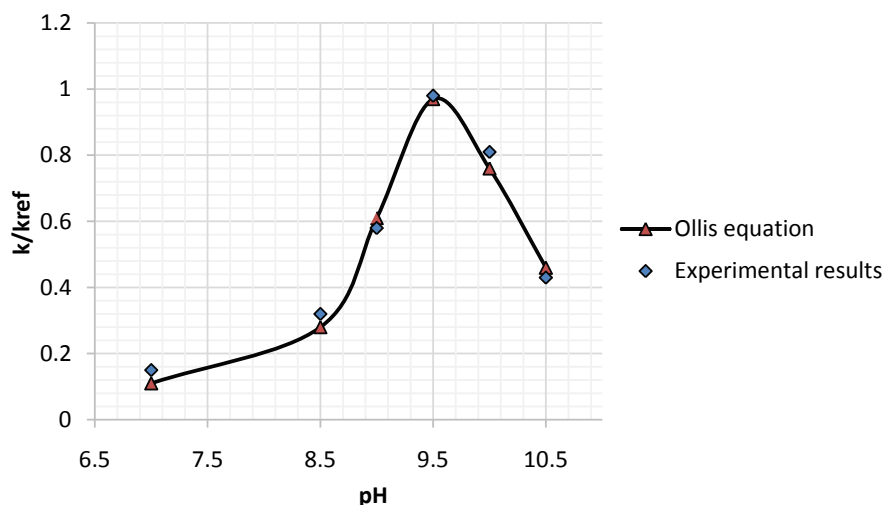


Figure 5. Influence of pH on the oxidation rate of Fe (II) in the batch bioreactor (beaker 3) at $T = 25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $[\text{O}_2] = 6.5 \text{ mg}\cdot\text{L}^{-1}$ and $[\text{Fe}^{2+}]_0 = 2 \text{ mg}\cdot\text{L}^{-1}$.

Much research work in the biofiltration sector has shown that the biological oxidation yields of Fe (II) are strongly influenced by pH. Indeed, the effect of pH in the column can be modeled by the equation of (Zhang et al., 2002) under the same operating conditions:

$$\left[\frac{k}{k_{ref}} \right]_{\text{pH}} = \frac{k_{\text{pH}}}{1 + [\text{H}^+]/K_1 + K_2/[\text{H}^+]} \quad (3)$$

k is the oxidation constant of Fe (II), $k_{ref} = k$ at $\text{pH} = 9.5$; $[\text{O}_2] = 6.5 \text{ mg}\cdot\text{L}^{-1}$; $T = 25^{\circ}\text{C}$; $K_1 = 3.05 \times 10^{-8}$; $K_2 = 2.46 \times 10^{-8}$, $k_{\text{pH}} = 2.82$.

Thus, the increase in the oxidation conversion rate of Fe (II) to Fe (III) between a pH of 7.0 to 9.5 (Figure 5) is caused by the enzymatic activity of microorganisms which is pH dependent. Above $\text{pH} = 9.5$, the concentration of measured Fe (II) ions decreases with increasing pH due to the likely inhibition of bacteria. Although a decrease in Fe (II) could be explained by the decrease in yields at high pH (Teien et al., 2008), it is believed that this finding is unlikely since there is no formation of precipitate in the column. The absence of Fe (III) suggests that there is an imbalance with O_2 or that the precipitates formed as FeCO_3 , $\text{Fe}(\text{OH})_2$ or $\text{Fe}(\text{OH})_3$ were supersaturated and that, therefore, Fe (II) remained under its control dissolved or reduced form. Additionally, if a precipitate is present, it would have been retained on the outer walls of the jujube nuts. Thus, the decrease in Fe (II) conversion yields above $\text{pH} = 9.5$ is most likely the result of the deactivation of the biological reaction, hence the inhibition of the bacterial population.

3.4. Effect of O_2 Concentration

The influence of oxygen (O_2) on iron degradation was studied at different concentrations of dissolved oxygen, keeping the other parameters constant (Figure 6) (Zhang et al., 2002).

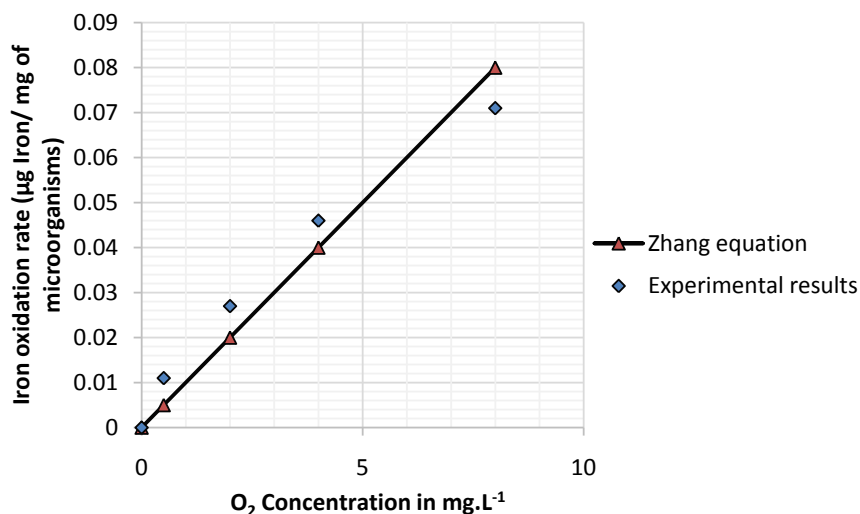


Figure 6. Effect of dissolved oxygen concentration on iron oxidation rate in the bioreactor (beaker 2) at $T = 25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $[\text{O}_2] = 6.5 \text{ mg}\cdot\text{L}^{-1}$, $\text{pH} = 9.5$, $[\text{Fe}^{2+}] = 1 \text{ mg}\cdot\text{L}^{-1}$ and redox potential = 300 mV.

The modeling of the oxidation yields of Fe (II) shows a proportionality with the oxygen (O_2) contents, which shows that all the O_2 concentrations considered to be higher than the exact concentration required for the biofilter would cause the oxygen saturation of the bed (Duesterberg et al., 2008; Zhang et al., 2002). Experimental difficulties have been encountered with accurate control of O_2 for oxygen concentrations greater than about $8 \text{ mg}\cdot\text{L}^{-1}$. The equation of (Zhang et al., 2002) was used to model the influence of dissolved oxygen on the oxidation rate of Fe (II):

$$\left(\frac{k}{k_{ref}} \right)_{\text{O}_2} = k_{\text{O}_2} [\text{O}_2] \quad (4)$$

$[\text{O}_2]$ is the oxygen concentration = $8.05 \text{ mg}\cdot\text{L}^{-1}$ [oxygen saturation medium at $8.05 \text{ mg}\cdot\text{L}^{-1}$ for an air temperature of 25°C and an ionic strength of $0.05 \text{ mg}\cdot\text{L}^{-1}$].

3.5. Measurement of Oxidation Rates of Fe (II)

For a wavelength $\lambda_{\text{max}} = 530 \text{ nm}$, measurements of the development of microorganisms were made. Within a few days after sowing, a fixed biofilm had developed on the media of the coated jujube seeds. Figure 7 below shows the evolution of microbial cells in the median filter (Zhu et al., 2010).

After a follow-up period of 39 days, the biofilm reaches the permanent phase, the biological oxidation of Fe (II) to Fe (III) becomes incomplete. However, if Fe (II) is added to the column during this period, the Fe (II) conversion efficiencies are the same as the biofilters that received Fe (II) at 2 months of operation. Thus, the oxidation kinetics of the Fe (II) was reasonably constant during the first 10 days of the stationary phase, compared with the results of (Mouchet, 1992) which indicated that the highest oxidative activity of *Gallionella* genus by Fe (II) was obtained in early stationary phase cultures after at least 2 months of culture.

These batch tests will predict in subsequent experiments, on a column in continuous mode, which addition of the Fe (II) could be done in the first 10 days of the stationary phase of growth.

3.6. Effect of Fe (II) Concentration

The biological oxidation of iron was to follow the enzymatic kinetics of Michaelis-Merten, with the corresponding velocity law:

$$\frac{d\text{Fe(II)}}{[X]dt} = -\frac{k[\text{Fe(II)}]}{K_s + [\text{Fe(II)}]} \quad (5)$$

This modeling of bacterial growth is very close to the Monod model which is the oldest. The latter is an empirical model, which accounts for the exponential

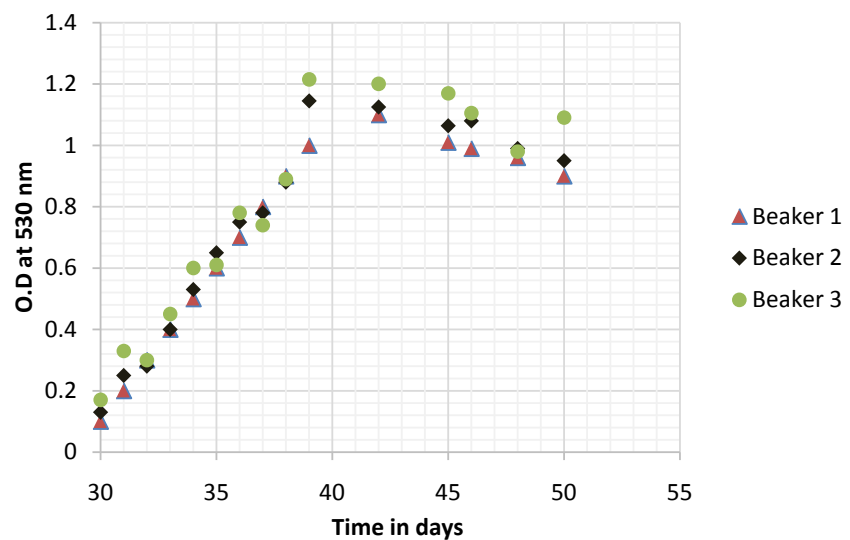


Figure 7. Growth curve of microorganisms as a function of time. The cultures were cultured at laboratory temperature and without pH control in the containers (beaker 1) at $T = 25^\circ\text{C} \pm 1^\circ\text{C}$, $[\text{O}_2] = 6.5 \text{ mg}\cdot\text{L}^{-1}$, $\text{pH} = 9.5$ and $[\text{Fe}^{2+}] = 1 \text{ mg}\cdot\text{L}^{-1}$.

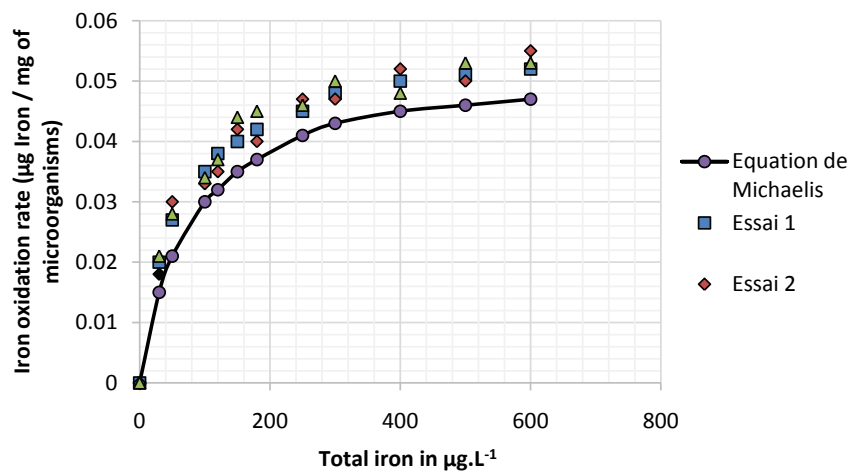


Figure 8. Fe (II) oxidation rate versus Fe (II) concentration, showing the Michaelis-Merten oxidation kinetics for Fe (II) at $T = 25^\circ\text{C} \pm 1^\circ\text{C}$, $\text{pH} = 9.5$ and $[\text{O}_2] = 6.5 \text{ mg}\cdot\text{L}^{-1}$.

phases of growth and slowdown, very close to Michaelis Merten's law relating to enzymatic reactions. K_s is the half-rate constant at half the initial concentration, k is the maximum rate constant of Fe (II) ($\mu\text{mol Fe}^{2+}/\text{mg cells. min}$), and $[X]$ is the concentration of microorganisms in $\text{mg}\cdot\text{L}^{-1}$.

The value of the constant k varies with the pH of the water, the temperature of the medium and the concentration of dissolved oxygen. The operating parameters were maintained constant $[\text{O}_2] = 6.5 \text{ mg}\cdot\text{L}^{-1}$, $T = 25^\circ\text{C}$ and $\text{pH} = 9.5$. Fe (II) conversion yields increase with increasing Fe (II) contents (**Figure 8**) (Teien et al., 2008). The analysis of the experimental data by the nonlinear regression method gives values of K_s , $17.02 \text{ mol}\cdot\text{L}^{-1}$, and k $0.082 \text{ mol}/(\text{min}\cdot\text{mg})$.

4. Conclusion

Ultimately, the main objective of this work was the biological elimination of dissolved iron in reconstituted water (doped with FeSO_4). Jujube seeds covered with a plastic layer were used as a filter medium. The main conclusions of this experimental work are mentioned below:

- 2 months is expected for the maturation and development of iron bacteria by following the optical density (O.D) of the sampled solutions;
- the natural ventilation of the column reduces the operating time of the compressor;
- the particle size of the seeds has an impact on the iron conversion yields, but this aspect is not dealt with in this part of our study;
- the biological oxidation yields of iron are improved by physical and chemical oxidation because at the beginning of the aeration operation there is a quantity of free oxygen in the reaction medium.

Finally, the batch tests on the beakers by the biological Fe (II) removal process in reconstituted water on a filter material support show that for all the parameters studied, the coated jujube seeds can be used as a filter material support for the fixed culture of bacterial populations. Thus, for proper operation of the process, the optimal parameters noted at the end of the batch tests are: temperature at 40°C , a pH at 9.5 and dissolved oxygen at 6.5 mg/L .

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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