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IRON REMOVAL FROM GROUNDWATER USING LEPTOTHRIX DISCOPHORA

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ABSTRACT

In the present study one strain of iron oxidizing bacteria was isolated from soil and water enriched sample with FeSO4.7H2O and identified as Leptothrix discophora (L. discophora). This bacterium was used to remove iron from groundwater sample. The optimum temperatures for L. discophora growth were 45 and 35 ℃ at pH of 3 and 5, respectively. Bioremoval of ferrous iron by immobilized cells of L. discophora was investigated in repeated batch culture and continuous operation. Effects of dilution rate and initial concentrations of Fe^{+2} on bioremoval were also investigated. During repeated batch culture, the immobilized-cells were stable and showed high constant iron-oxidizing activities. Our results showed that the immobilized cells of L. discophora removed iron of the concentration of 1ppm from groundwater at natural condition in 25 min.

Key words : *Ferrous iron oxidation; Iron bioremoval; Immobilization; FeOB; Leptothrix discophora; Groundwater.*

INTRODUCTION

Iron is a common trace element in soils and groundwater and it is the fourth most abundant mineral in the earth's crust. The bulk iron content of soils is typically in the range of 0.5% to 5% (by volume), and it is dependent upon the source rocks from which the soil was derived, transport mechanisms, and overall geochemical history. Iron occurs naturally in water in soluble form as ferrous iron (Fe^{+2}) or non-soluble form as ferric iron (Fe^{+3}) (Prince *et al.,* 2003; Vreeburg, 2007). Iron presence at elevated levels can cause aesthetic problems on ornamental plants, buildings and structures, and its accumulation on irrigation equipment can lead to clogged emitters (Smith *et al.,* 1997; McNeill and Edwards, 2001 and UNDP, 2010).

In groundwater, Fe (II) are being removed by either physico-chemically or biologically based-methods. The biological treatments are more advantages than conventional physico-chemical treatments because no use of chemicals, have higher filtration rates, the possibility of using direct filtration and lower operation and maintenance costs (Mouchet, 1992). Conventionally, iron is removed from groundwater by the processes of aeration and rapid filtration (Salvato, 1992). Different mechanisms may contribute to the iron removal in filters; flock filtration, adsorptive iron removal

and biological iron removal. This mechanism is dominant depends on the groundwater quality and the process conditions (Hatva, 1989; Mouchet, 1992; Søgaard *et al.,* 2000). Nowadays biological processes to remove Fe are widely used in Europe, and there are some plants are also used in this treatment in the United States and Canada (Mouchet, 1995; Gage and Williams, 2001).

However, iron removals by biological processes are based on different stages of biofiltration where beds are colonized by Fe oxidizing bacteria. In nature, iron oxidizing bacteria (IOB) is found in widespread. It is prevalent in groundwater, swamps, ponds, in the hypolimnion of lakes, in sediments, soils, wells and water-distribution systems. In the latter they can cause significant clogging problems due to biofilm formation (Ghiorse, 1984 a;b). These bacteria which are present in raw water can multiply in sand filters under appropriate conditions and is able to oxidize divalent ions Fe^{+2} and precipitate them under their oxided forms Fe^{+3}.

Iron fixing bacteria are belonging to the filamentous genera such as *Gallionella* spp., *Leptothrix* and *Sphaerotilus* and less from the rod type, such as Psendomonas and Enterobacter. They react with soluble iron, Fe^{+2}, through an oxidation process that changes the iron to an insoluble form, Fe^{+3} (Siering and Ghiorse; 1996, Czekalla *et al.*, 1985 ; Mouchet 1992). Moreover, The Fe/Mn-oxidizing bacteria are known for their potential to form extracellular Fe- or Mn-encrusted structures in aquatic environments (Ghiorse, 1984 a;b , Spring, 2006, Hashimoto, *et al.,* 2007, Miot, *et al.,* 2009, Sakai, *et al.,* 2010, Suzuki, *et al.,* 2011). The genus *Leptothrix* forms microtubular sheaths that are distinct in morphology from the twisted stalks produced by another Fe-oxidizing genus, *Gallionella.*

The study involved the examination of experimental conditions such as pH, temperature, contact time and iron concentration on the removal of Fe^{+2} from aqueous solutions by free and immobilized cells iron bacteria isolated. Moreover, we studied the feasibility of using the specific carrier for immobilization of *L. discophora*, and established a procedure for *L. discophora* immobilization on a laboratory scale, that is simple, fast, and easily reproducible which can be adapted to the industrial scale.

MATERIALS AND METHODS

Isolation and growth conditions of bacteria

Iron oxidizing bacteria (IOB) was isolated in the laboratory from soil and water enriched with iron ($FeSO_4.7H_2O$) as described by Smith (1992). The medium used for isolation consisted of: $(NH_4)2SO_4$ 3.0 g/L, K2HPO4 0.5 g/L, KCL 0.1 g/L, $Ca(NO_3)_2$ 0.01 g/L, $MgSO_4.7H_2O$ 0.5 g/L, H_2SO_4 (10N) 1.0ml, $FeSO_4.7H_2O$ solution300ml. These cultures were allowed to grow at 30 ℃ with shaking at 150 rpm and the organisms were tentatively identified according to Krieg (1984) and Holt *et al.* (1994). The exact identification of bacteria was carried out according to (Van Veen *et al.* (1978); Czekalla *et al.* (1985) ; and Spring,(2002).

Sampling and analytical methods

The ferrous solution was prepared from

$FeSo_4.7H_2O$: One mg/L (ppm) aqueous solution (stock solution) with de-ionized water in 1% HNO_3 solution and the stock solution was diluted with de-ionized water to obtain the working standard solutions (Oyedeji and Osinfade, 2010).

Raw and treated groundwater and the effluent of each process were analyzed after incubation time. The following parameters were measured: temperature, pH (pH-meter HACH sension1), growth turbidity (Spectrophotometer HACH DR 4000). Ferrous iron was determined by the phenanthroline method (Spectrophotometer HACH DR 4000) as described by Smith (1992).

Slides prepared for IOB were examined with a binocular microscope (Olympus BX100). Keys given in the ASTM Standard Test Method for Iron Bacteria (ASTM, 1997), Standared Methods (APHA, 1995), Methods for the Examination of Water and Associated Materials (Environmental Agency, 1998).

Optimization of temperature and initial pH for bacterial growth

The optimum temperature for growth of isolated bacterium was obtained by its growth in liquid medium at different temperatures (25, 35, 45 ℃) and optimum pH for bacterial growth was determined at pH values ranging from 2.0 to 8.0 (Lee and William, 1985).

Immobilization of IOB

The purified IOB suspension was mixed with sodium alginate solution (2%) 1:1 ratio. The IOB-alginate mixture was added dropwise into calcium chloride (0.2 M) solution with continuous shaking at 4 ℃. As soon as the drop of IOB-alginate solution mixed with $CaCl_2$ solution, Na^+ ions of Na-alginate were replaced by the Ca^{+2} ions of $CaCl_2$ solution, which finally formed Ca-alginate beads. The beads thus formed were washed 3-4 times with deionized water and finally used for further studies (Fraser and Bickerstaff, 1997).

Ca-alginate beads inoculated in water sample contain 1ppm of Fe^{+2} at different pH (3, 5, 7, 7.5 and 8) and temperature (25, 35 and 45 ℃), 5 ml were taken at 5 min interval, to determine iron concentration, and also placed in groundwater sample to test the ability for removing iron (Kim *et al.,* 2005).

RESULTS

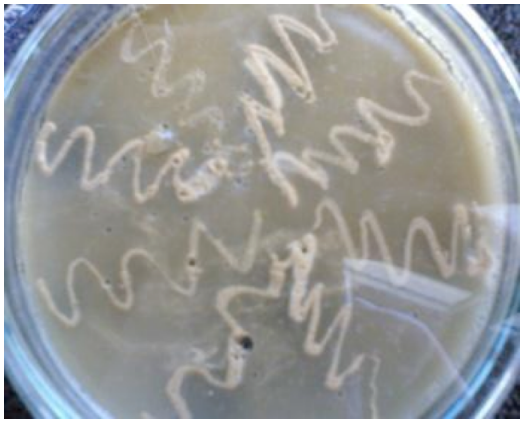
Isolation of iron bacteria:

Iron bacteria were isolated from different samples of soil and water polluted with iron on the selective medium. The isolated bacteria (Plate 1a) from all samples were of the same type. The purified bacteria were examined under light microscope as seen in Plate (1b).

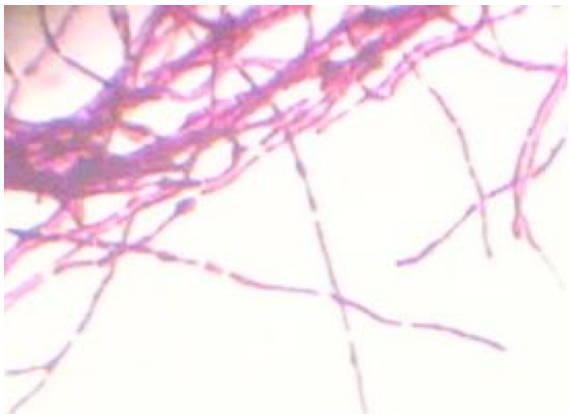
The purified bacterial isolate was grown on the selective medium containing different concentrations of iron and the best concentration of iron for growth was 10g/l (Table 1).

Table (1) : The effect of $FeSO_4.7H_2O$ concentration on bacterial growth.

$FeSO_4.7H_2O$ (g L^{-1})	Bacterial Growth
5	few
10	high
15	few
20	few
25	negative
30	negative



(a) Different isolates of FeOB on solid selective medium.



(b) *Leptothrix discophora* under light microscopy (Gram stain)

Plate (1) : Iron bacteria.

Identification of iron bacteria:

The bacterial isolate formed flocculent and filamentous growth smooth colony. Microscopically, cells are smaller than those of the other species of *Leptothrix*. They may occur in narrow sheaths or be free-swimming; free cells are motile by a thin polar flagellum.

On glucose-peptone agar, the trichomes are thin and the colonies are small, often no more than 0.1-0.3 mm in diameter, with smooth edges. Increased supply of nutrients such as glucose, peptone, methionine, purine bases, vitamin B12, biotin and thiamine hardly improve growth. When Mn^{+2} is supplied to this agar medium, the black-brown colonies will be somewhat larger (0.5-2 mm) and, sometimes ,filamentous .If widely spaced, they often are surrounded by a dark brown halo of pinpoint granules or by a diffuse light brown halo of oxidized manganese (Holt *et al.,* 1994).

Sheath formation asserted via dilute crystal staining solution (Siering and Ghiorse, 1996) that the isolate is Gram-negative and chemoorganotrph. Glucose and peptone were the optimum carbon and energy sourc-

es,respectively (Krieg, 1984). The species of genus *Leptothrix* was identified using either classical bacteriological tests and automated identification system as a *discophora* . Finally, the iron bioremoval isolate was identified as *Leptothrix discophora*.

Effect of environmental factors on Leptothrix discophora growth:

The measured growth of IOB at different temperatures (25, 35 and 45 ℃) and pH values (3 and 5)after 3 days of incubation are illustrated in Figure (1a,b). At pH 3 increasing temperature increased the rate of growth, whereas at pH 5 the optimum temperature for growth was 35 ℃. Generally the growth curve began to decline after the sixth day of incubation.

Optimization of iron bioremoval:

By free cells of *Leptothrix discophora*:

In this experiment the remaining iron was measured in the media after incubation at different temperature (25, 35 and 45 ℃) and pH (3 and 5). The data clearly suggest that pH 3 was better than pH 5 at all tested temperatures and at 35 ℃ removed iron effectively than 25 and 45 ℃ (Figure (2 a,b and c).

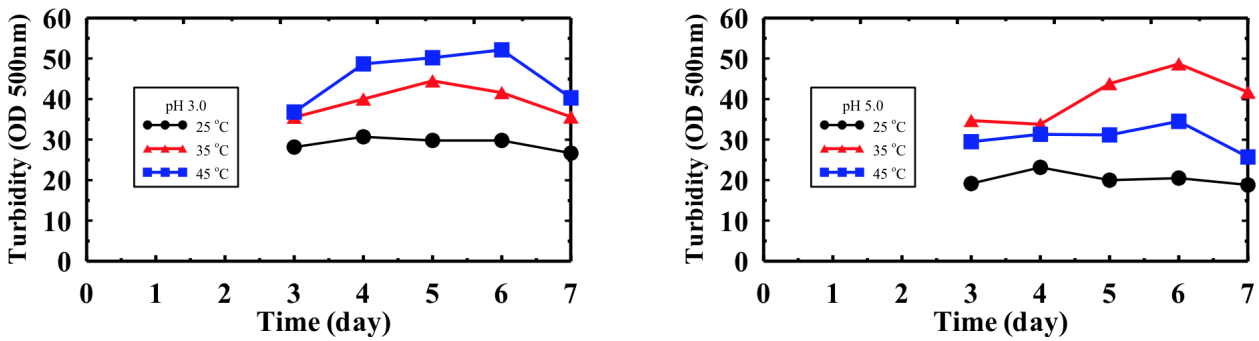
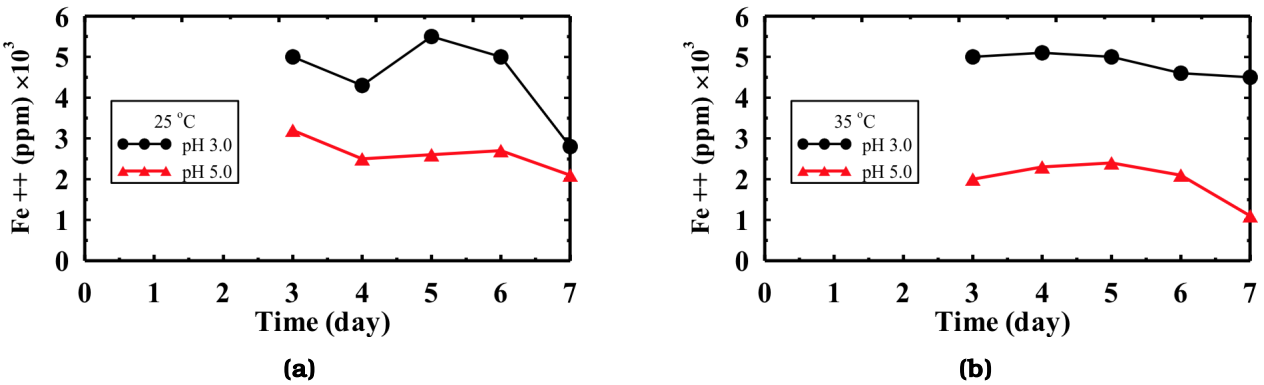
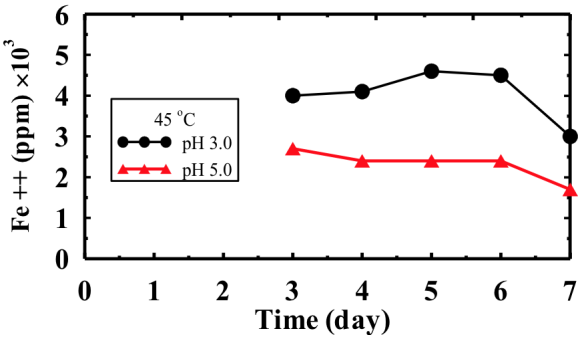


Fig. (1) : Effect of temperature on bacterial growth at pH 3.0(a) and pH 5.0 (b).





(c)

Fig. (2) : Effect of pH on bioremoval of iron at 25 ℃(a), 35 ℃ (b)and 45 ℃(c).

By immobilized cells of *Leptothrix discophora:*

The immobilized *Leptothrix discophora* before and after removal of iron as shown in Plate (2) and Figure (3).The best removal of iron was recorded when bacterial growth at pH 7.5 and 100% of iron was removed after 15 minutes. While it required 30 minutes at pH 7 and 8 to reach the same percentage. Also it was found that the 35 and 45 ℃ were better than 25 ℃ to get rid of about 100% of dissolved iron after 15 minutes Figure (4).

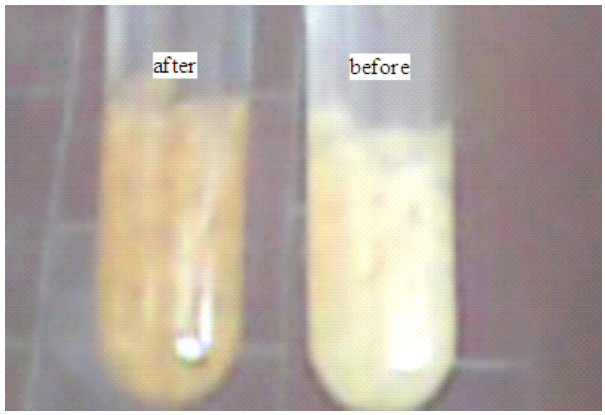


Plate (2) : *Leptothrix discophora* immobilized cells after and before removal of iron.

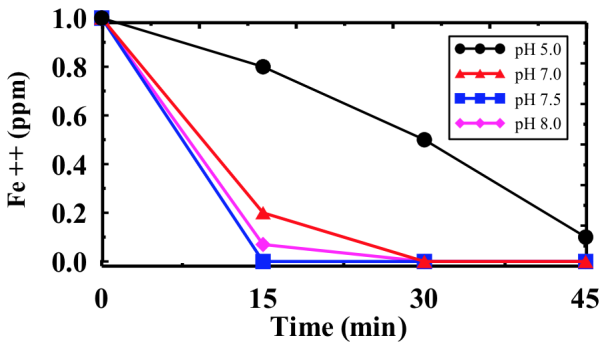


Fig. (3) : Effect of pH on bio removal of iron by immobilized cells of *Leptothrix discophora*.

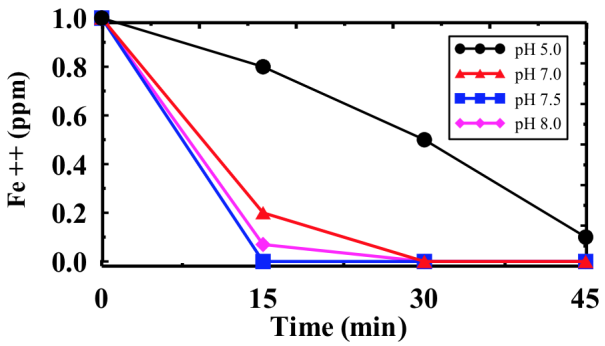


Fig. (4): Effect of temperature on iron removal by immobilized cells of *Leptothrix discophora*.

Free and immobilized Leptothrix discophora to remove iron :

The immobilized cell removed iron almost completely under our experimental conditions (Fig.5 a,b and c). Experimentally, temperature greatly affected the removal of iron , especially with the free bacteria. At 35 ℃ free bacteria efficiently removed iron from water that reached as low 0.3 ppm.

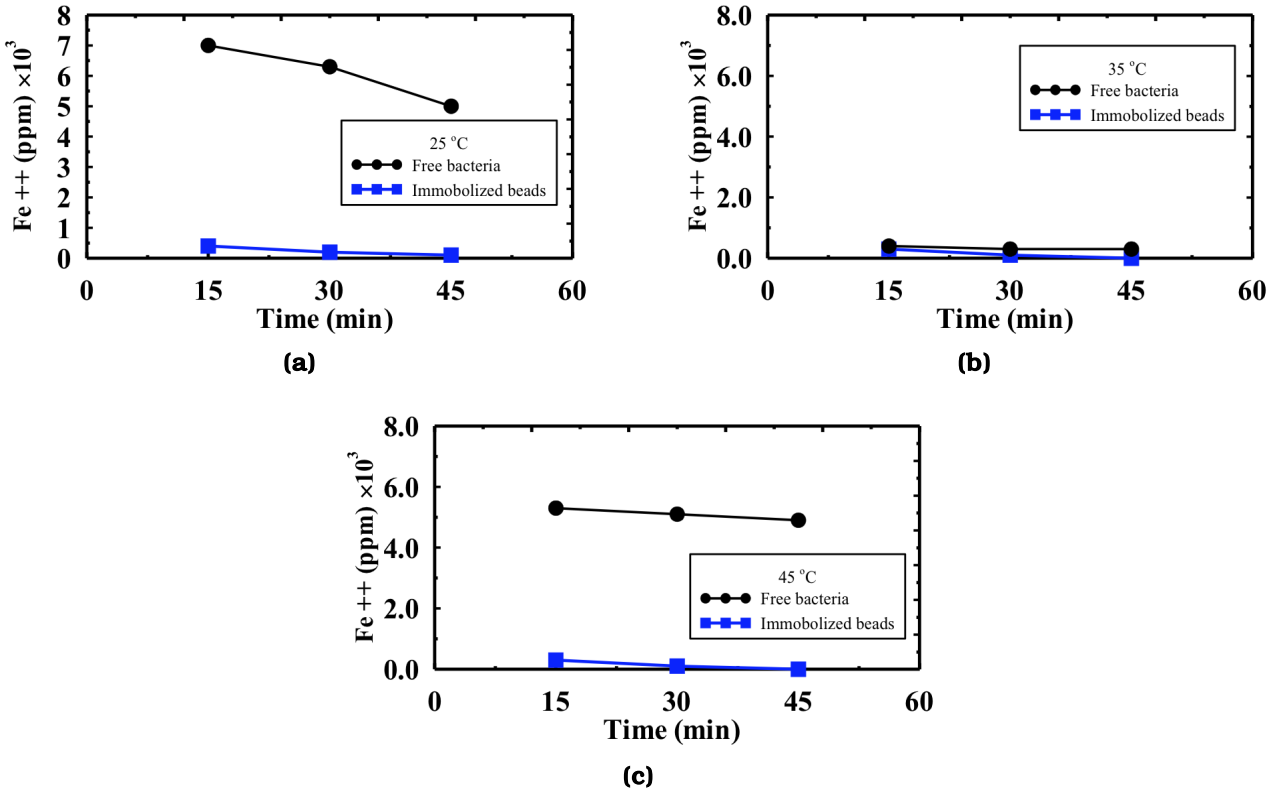


Fig. (5) : Comparison between free and immobilized cells of *Leptothrix discophora* in bioremoval of iron at 25 ℃(a), 35 ℃(b) and 45 ℃(c) .

Iron removal from groundwater:

The ability of immobilized cells was tested to remove iron from groundwater sample was taken from a well at Met abo khaled; Meet Ghamr, Dakahlia. The results showed that the immobilized cells removed 100% of the iron from the used groundwater after 25 min., (Fig. 6). More than 90% of iron was removed after 5 min of adding the immobilized cells and in the rest 20 min the rest of contaminated iron was removed.

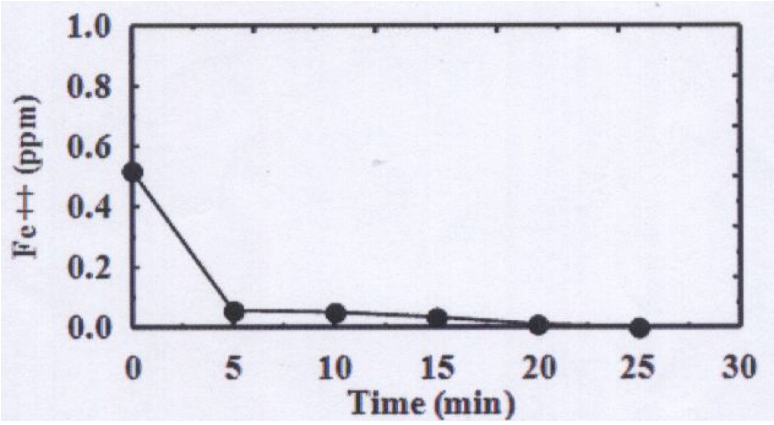


Fig. (6) : Iron bioremoval by immobilized cells of *Leptothrix discophora* from groundwater sample.

DISCUSSION

Iron is the most abundant transition element in the Earth's crust, approximately one-third of the Earth's mass is estimated to be iron. Its concentration is relatively high in most crustily rocks (lowest in limestone), which is more or less pure calcium carbonate (Farago, 1986).

The biological oxidation of ferrous iron (Fe^{+2}) by *L. discophora* is potentially a useful industrial process for the bioremoval of iron and in the regeneration of ferric iron (Fe^{+3}) as a leaching agent in oxidation processes (Longa *et al.,* 2004).

There are several factors that play substantial roles in the rate of oxidation of Fe^{+2} by *L. discophora.* These factors include Fe^{+2}/Fe^{+3} iron concentration, cell and oxygen concentrations, pH, temperature and reactor type (Daoud and Karamanev, 2006). Accordingly, studies were carried out using batch experiments for assessment of optimal environmental conditions, such as initial Fe^{+2} concentration and pH, for efficient bio-oxidation of Fe^{+2} to Fe^{+3} ions (Malhotra *et al.,* 2002).In this study, the culture growth was affected by the Fe^{2+} ion concentration.

The increase in iron concentration resulted in an increase in the culture growth until maximum value was achieved, after which a decrease in bacterial growth occurred (Table 1). Continuous oxidation was affected by the dilution rate and initial the Fe^{2+} ion concentration (Ehsan, 2008).

The oxidation of iron takes place rapidly by the bacterial cell. One species of *Leptothrix* was isolated from water enriched with iron and it reflects a specific environmental condition. Regulation of Fe metal uptake would seem reasonable in order to fulfill this need. Temperatures inside well between 30 and 35℃ was suitable for growth of *Leptothrix* species, particularly *Leptothrix* AT22. On the other hand, these bacteria and other various bacteria are able to acidify the medium, which ensures a better solubility of ferric ions and enables iron uptake (Peine, *et al.,* 2000).

L. discophora SS-1 is a gram-negative heterotrophic bacterium that has the unique

feature of producing two distinct extracellular macromolecules that catalyze the oxidation of Fe^{+2} and Mn(II) (Corstjens, *et al.,* 1992). This bacterium is recalcitrant to genetic manipulation, and less is known about the molecular mechanism of Mn(II) oxidation in this organism than about those in other Mn^{+2} oxidizing bacteria such as *Pseudomonas, Pedomicrobium*, and *Bacillus* species (Tebo, *et al.,* 2004).

The voltammetric microelectrode measurements provided evidence of Fe redox cycling in both the mat and puffball materials. The steep opposing gradients of Fe(II) and O_2 are analogous to those documented previously in circumneutral Fe steep and fresh-water sedimentary environments (Emerson and Revsbech, 1994; Sobolev and Roden ,2004; Druschel, *et al.,* 2008; Bruun, *et al.,* 2010), as well as in situ gradients in Fe rich microbial mats at the Loihi Seamount (Glazer and Rouxel, 2009). This study includes determination of the contribution of microbial activity to Fe^{+2} oxidation by FeOB pure cultures in water samples. Pure isolate of FeOB was identified as *L. discophora* (plate 1a, b and c). This bacteria optimized for growth in selective medium ,showed that 45 ℃ at pH 3 and 35℃ at pH 5 gave the best amount of growth at 600nm.The results which are in a good agreement with those of Iman *et al.,* (2008). The results indicated that oxidation of iron by *L. discophora* increased with time and pH at the same temperature (Figure 2). Morever; bioremoval of iron by immobilized cells of *L. discophora* depends on pH, time of treatment, initial concentration of iron and temperature The optimum conditions for bioremoval by immobilized cells of *L. discophora* are pH 7.5 at 35 ℃ for 1 ppm of iron (Fe^{2+}) in 15 minute. Therefore, we conclude that the decrease of pH and temperature caused decrease in iron oxidation in groundwater.

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الملخص العربى

ازاله الحديد من المياه الجوفيه باستخدام *Leptothrix discophora*

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المياه الجوفيه من انقي مصادر المياه الطبيعيه التي يعتمد عليها الكثير من سكان العالم، الا ان بعض مياه الابار يكون ملوثا ببعض المواد العضويه والعناصر الثقيله منها الحديد والمنجنيز.

الحديد هو رابع اكثر المعادن وفره في القشره الارضيه، ويوجد الماء في ثلاث صور مختلفه منها الحديدوز(يذوب في الماء) والحديديك (لايذوب في الماء) وفي صوره غرويه (لايمكن ازالته بالترشيح ولا بالترسيب) ,والنسبه المصرح بها من الحديد في مياه الشرب هي ٠,٣ جزء من مليون حسب منظمه الصحه العالميه. ازاله الحديد من المياه الجوفيه تعتبر ذات اهميه قصوي وذلك لحمايه البيئه وصحه الانسان خاصه في المناطق التي تستخدم فيها المياه الجوفيه للشرب .

في هذا البحث تم عزل بكتيريا الحديد من مصادر مختلفه تربه مشبعه بالمياه ومياه جوفيه) وتم تعريف سلاله واحده منها علي مستوي الجنس والنوع بطرق التعريف البكتريولوجيه علي انها : *Leptothrix discophora*

كما تمت دراسه بعض العوامل البيئيه المؤثره علي نمو ونشاط البكتريا علي اكسده الحديد ,منها ودرجه الحراره والزمن وتركيز الحديد ، فوجد ان افضل درجه حراره للاكسده هى ٣٥ درجه مئويه عند درجه حموضه تساوي ٥ وذلك مع البكتيريا الحره.

واثبتت النتائج ان استخدام البكتريا المقيده في الجينات الكالسيوم اكثر قدره من البكتريا الحره في اكسده الحديد تحت نفس الظروف البيئيه عند استخدام البكتريا المقيده مع عينه مياه جوفيه تم احضارها من بئر مياه جوفيه غير صالح للاستعمال الادمي لارتفاع نسبه الحديد به الي اكثر من ٠,٩ جزء في المليون فاثبتت كفائه عاليه علي ازاله الحديد من المياه الجوفيه الي حد كبير.

JOESE 5

IRON REMOVAL FROM GROUNDWATER USING LEPTOTHRIX DISCOPHORA

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