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Biosorption of Iron from Ground Water by Biomasses of Yeast (*Saccharomyces cerevisiae*)

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ELEVEN *S. cerevisiae* strains alive and dead forms were screened for biosorption and bioaccumulation of iron from prepared aqueous solution. *S. cerevisiae* strains F-707 in alive form was found to be excellent Fe^{+2} biosorbent that biosorbed 21.9 mg Fe^{+2}/ g yeast biomass. Optimization of cultural conditions revealed that optimum concentration of Fe^{+2} was 5.6 mg Fe^{+2} /l after 20 min at pH 7, agitation rate ,150 rpm and yeast biomass concentration 0.1g / l at 30ºC for maximum Fe^{+2} biosorption by *S.cerevisiae* F-707 in alive form

Keywords: Biosorption, Iron,Ground water, *Saccharomyces cerevisiae*.

In Egypt wells have been used since 3000 BC. (Sharma., 2001). Iron is mainly present in ground water in two forms: either soluble ferrous iron or insoluble ferric iron. Water containing ferrous iron is clear and colorless because iron is completely dissolved. When the water is exposed to air, it turns cloudy and a reddish brown substance begins to form. This sediment is the oxidized hydroxide iron that is not dissolved in water (Das *et al.*, 2007). Conventional methods for removing metal ions from aqueous solution such as chemical precipitation, ion exchange, electrochemical treatment, membrane technologies and adsorption on activated carbon cannot be used at large scale. This is either due to their infectiveness with low metal ion concentration or their extreme expensiveness (Wang and Chen, 2006). Biosorption and bioaccumulation belong to the group of biological methods suitable for heavy metals removal from wastewater. Big advantages of these methods are the low operating cost, minimization of the volume of chemical and/or biological sludge to be disposed of and high efficiency in detoxifying very dilute effluents (Kadukova and Vircikova, 2005). Biosorption is a rapid, metabolically passive process. The biomass usually sequesters metal through surface bonding only. Bioaccumulation is an intracellular accumulation of sorbate (Chojnacka, 2010). Bioaccumulation is a slow, metabolically active process. In this process the metals are concentrated through a combination of surface reaction, intra- and extracellular precipitation, and intra –and extracellular complexation reactions (Aksu, 1998). Ferraz *et al*. (2004) used brewery waste *S. cerevisiae* for removing silver and uranium from laboratory–prepared aqueous solutions. Parvathi and Nagendran (2008) used waste yeast *S.*

cerevisiae from beer fermentation industry for the biosorption of chromium from electroplating effluent.

Wang and Chen (2006) reported that *S. cerevisiae* has many advantages as biosorbent in metal biosorption. Firstly *S. cerevisiae* is easy to cultivate at large scale, the yeast can be easily grown using unsophisticated fermentation techniques and inexpensive growth media. Moreover, the yield of the biomass is high. Secondly, the biomass of *S. cerevisiae* can be obtained from various food and beverage industries as a by–product. Thirdly, *S. cerevisiae* is generally regarded as safe. Therefore, biosorbents made from *S. cerevisiae* can be easily accepted by the public when applied practically. Fourthly, but not the last, *S. cerevisiae* is an ideal model organism to identify the mechanism of biosorption in metal ions removal.

Material and Methods

Yeast strains

Eleven yeasts (*Saccharomyces cerevisiae*) strains were obtained from culture collection of Microbial Chemistry Dep. National Research Center, Dokki, Giza, Egypt. The yeast strains were then routinely maintained on PDA at 4ºC.

Preparation of metal ions

The stock metal ions solution was prepared by dissolving ferric chloride of analytical grade in deionized water to obtain a concentration of 1000 mg/l further dilutions were made during the course of the experiments to obtain different concentrations as desired.

Screening yeasts biomass for the biosorption of Fe^{+2} .

Fifty ml double distilled water (ddH_2O) was amended with Fe^{+2} to attain conc-entration 2.3 mg Fe^{+2}/l, then poured in 250-ml Erlenmeyer flasks inoculated with 0.1 gm from different yeast strains biomass in alive and dead forms and then incubated for 180 min. at 28ºC in shaking incubator (200 rpm). Samples were filtered using Whatman No.1 filter paper. The concentration of iron remaining in the filtrate was analysed using Atomic adsorption (thermo scientific ice 3000 series) this experiment was repeated with chosen the most strain biosorbents with decreased time to 90 min .

Under the above conditions samples were withdrawn at 10, 20, 30, 45, 90 and 180 min. intervals and analyzed for Fe^{+2} .

Different concentrations of Fe^{+2} including 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 and 5.6 mg Fe^{+2}/ l aqueous solution contained 0.1g /l *S. cerevisiae* F-707 biomass in alive form were incubated for 20 min. at 28ºC in shaking incubator (200 rpm).

Different concentrations of *S. cerevisiae* F-707 biomass in alive form like 0.1, 0.2, 0.3, and 0.4 g/l aqueous solution contained 5.6 mg Fe^{+2} /l were incubated for 20 min. at 28ºC in shaking incubator (200 rpm) .

The aqueous solution contained 0.1g /l of *S. cerevisiae* F-707 biomass in alive form and 5.6 mg Fe^{+2} /l, was adjusted to pH 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9 using 0.1 M HCl or 0.1 M NaOH, and left for 20 min. at 28ºC in shaking incubator (200 rpm).

Different agitation rates 100, 150, 200 and 250 rpm for 20 min. were applied to study their effects on the Fe^{+2} biosorption in solution containing 5.6 mg Fe^{+2}/l and 0.1 g alive *S. cerevisiae* F-707 biomass .

The temperature was maintained at 20, 25, 30, 35 and 40ºC and agitation rate 150 rpm were applied to study their effects on the iron biosorbstion in solution containing 5.6 mg Fe^{+2} /l and 0.1 g alive *S. cerevisiae* F-707 biomass .

Yeast pelletes were examined using Scanning electron microscope (SEM) and energy dispersive X-ray spectrometer (EDX). The yeast strain 707F was grown in distilled water without Fe^{+2} as control and grew in distilled water with Fe^{+2} contains 5.6mg/lFe^{+2}. After 20 min. incubation at 28˚C and 150 rpm, the yeast cells separated and then washed with distilled water. The washed yeast cells were smeared over the cover slip coated with poly-L-lysin for 30 min. in wet condition (Srivastava and Thakur, 2006).The specimen washed with buffer, dehydrated in a series of ethanol-water solution (30, 50, 70, and 90% ethanol, 5 min. each) and critical point dried under CO_2 atmosphere for 20 min. Mounting was done on aluminum stubs, and cells were coated with 90-J thick gold palladium coating in a polaron Sc 7640 sputter coater (VG Microtech, East Sussex, TN22, England) for 30 min. Coated cells were viewed at kV with Scanning Electron Microscopy (FEI inspect S, Holland, HBRC). Dx_4 Prime Energy Dispersive X-ray Spectrometer (EDX) was performed at different kV for confirmation of the Fe^{+2} accumulations in the yeast cells .

Statistical analysis

Statistical analysis of the obtained data was carried out for analysis of variance according to (Snedecor and Cochran. 1992) using computer Statistical programe MSTAT-C. Means were compared by the L.S.D. values at 5% level.

Results and Discussion

The data in Fig. 1 and 2) revealed that all tested S. *cerevisiae* strains in alive form were more efficient for biosorption of Fe^{+2} than the dead form .The maximum adsorption efficiency (95.34%) 21.9 mg Fe^{+2}/g yeast biomass was recorded by alive cells of *S. cerevisiae* F-707 followed by strains F-125, FC-614, F-514, F-524 which recorded 17.9, 17.7, 17.0, 16.4,15.8 mg Fe^{+2}/g biomass, respectively. So, the above five yeast strains in alive form were chosen according to their efficiencies in biosorption of Fe^{+2} for further studies. The data in Table 1. revealed that yeast strains F- 707, FC- 614, F-514 in alive form showed the highest biosorption efficiency for Fe^{+2} , as they could absorb 21.7, 19.4, 18.4 mg Fe^{+2}/g biomass respectively. *S. cerevisiae* in different forms has been studied for

different purposes of research for example, living cell/dead cell (Kapoor and Viraraghavan, 1995), intact cell/ deactivated cell, immobilized cell/free cell (Veglio and Beolchini, 1997), raw material /pretreated cell by physicochemical process, wild type/mutant cell, and flocculent /non-flocculent cell. Marques *et al*. (1999) studied engineered /non-engineered cell, also, lab culture/waste industrial cell and cells from different industries (Park *et al*., 2003).

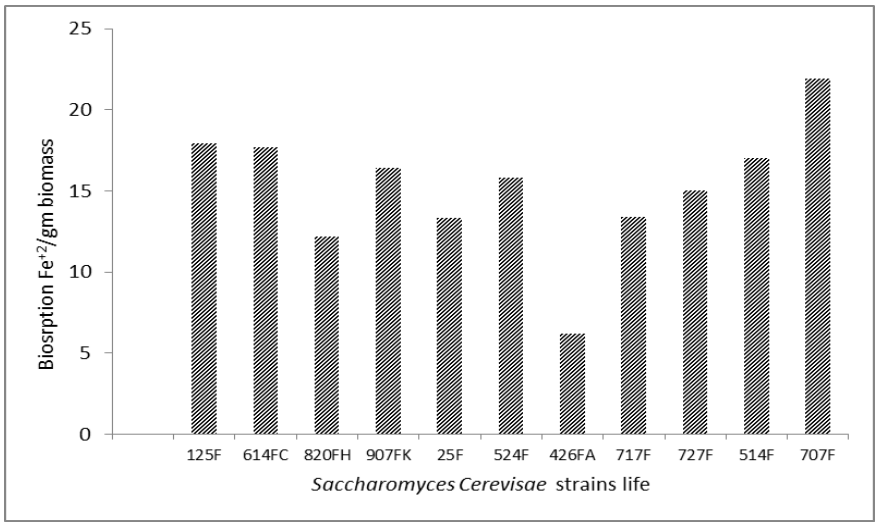

Fig. 1. Screening of some *Saccharomyces cerevisiae* strains for Fe^{+2} biosorption in alive form.

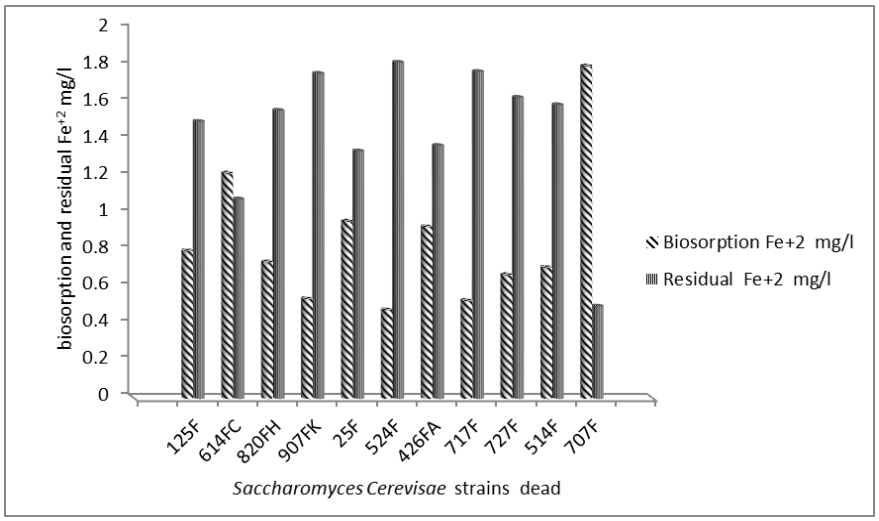

Fig. 2. Screening of some *Saccharomyces cerevisiae* strains for Fe^{+2} biosorption in dead form.

TABLE 1. Screening of some selected *Saccharomyces cerevisiae* strains for Fe^{+2} biosorption after 90 min.

Strain	Biosorption Fe^{+2} (mg/l)	Biosorption efficiency (%)	ResidualFe^{+2} (mg/l)	Residual Fe^{+2}(%)	Biosorption Fe^{+2} (mg Fe^{+2}/g biomass)
125 F-life	1.76^{d}	80	0.54^{b}	20	17.6
614 FC-life	1.94^{b}	88.18	0.36^{d}	11.81	19.4
907 FK-life	1.14^{e}	51.81	1.16^{a}	24.09	11.4
514 F-life	1.84^{c}	83.63	0.46^{c}	16.36	18.4
707 F-life	2.17^{a}	98.63	0.13^{e}	1.36	21.7

The different letters indicate significant difference at $P > 0.05$.
F, FC and FK means *Saccharomyces cerevisae* strains.

A number of references have proved that *S. cerevisiae* can remove toxic metals, recover precious metals and clean radionuclides from aqueous solutions to various extents. Schott and Gardner (1997) reported the recovery of light metals, such as aluminum by *S. cerevisiae*. Brady *et al*. (1994f) proved that the yeast cells of *S. cerevisiae* treated with hot alkali were capable of accumulating a wide range of heavy metal cations (Fe^{+3}, Cu^{+2}, Cr^{+3}, Hg^{+2}, Pb^{+2}, Cd^{+2}, Co^{+2}, Ag^{+}, Ni^{+2}, and Fe^{+2}. Heavy metals such as Pb, Au, Co, Cu, Fe and their respective cations can be completely removed from water and other aqueous environments by *S.cerevisiae* (Dhankhar *et al*., 2011, Simmons *et al*., 1995, Wang and Chen, 2006). Park *et al*. (2003) compared two strains of *S. cerevisiae* for the biosorption of cadmium. A yeast strain (ATCC834) which is used for the production of l-phenyl acetylcarbinol (lPAC) and another strain, ATCC24858 for ethanol production . They found that the thicker mannan layer and the larger specific surface layer seemed to benefit a larger cadmium uptake capacity for the strain *S. cerevisiae* ATCC834. Francisco *et al*. (2002) and Ksheminska *et al.* (2003) studied the diversity of Cr^{+6} resistant and Cr^{+6} sensitive bacteria to chromium in contaminated activated sludge. They reported that the mechanism of chromium resistance and reduction might differ in microbial community from group to group or from strain to strain within the same species.

As shown in Table 2. the equilibrium adsorption capacity increased as the contact time increased. The biosorption of Fe^{+2} by yeast biomass was rapid for first 20 min. and equilibrium was nearly reached after 45 min. Results revealed that the highest removal efficiency for Fe^{+2} by yeast biomass was occurred after 20 min. then the removal efficiency occurred slowly. This trend emphasizes that

sorption times have an important effect on recovery efficiency, which decreases with increase biosorbent contact time with metal ions solution. Metal accumulation inside the cell may resulted from bioaccumulation, slow metabolic dependent removal mechanism, or by simple metal diffusion (Gaad, 1990).

TABLE 2. Screening of 707 F yeast strain for Fe^{+2} biosorption after different time intervals.

Time (min.)	Biosorption Fe^{+2} (mg/l)	Residual Fe^{+2} (mg/l)	Biosorption Fe^{+2}(mg Fe^{+2}/g biomass)
10	1.8^{b}	0.5^{a}	18
20	2.16^{a}	0.14^{b}	21.6
30	2.16^{a}	0.14^{b}	21.6
45	2.16^{a}	0.14^{b}	21.6
90	2.12^{ab}	0.18^{b}	21.2
180	2^{ab}	0.3^{ab}	20

The different letters indicate significant difference at P>0.05.

Biosorption kinetics with an initial rapid metal uptake followed by slow uptake was observed, this kinetic model has been accepted for various biosorbents such as bacteria and fungi (yeast) under similar operation conditions (Tavares *et al*., 1995). In this connection Goyal *et al*. (2003) reported that the uptake of metal ions by microorganisms in batch systems has been shown to occur in two stages: an initial rapid stage (passive uptake), followed by much slower process (active uptake). The first stage is physical adsorption or ion exchange at the surface of the biomass, which is biosorption. The biosorption equilibrium occurs at the end of rapid physical adsorption stage (first-stage). Adsorption isotherm equation is frequently used to represent this equilibrium. The same behavior was observed by Han *et al*. (2006) for equilibrium time of Zn (II) and Cu (II).

Figures (3a, 3b, 3c and 3d) showed that removal efficiency increased with increasing the metal ions concentration. On the contrary biosiorption was decreased with increase dose of biomass.The best removal occured at 0.1g yeast. This agrees with Cojocaru *et al*. (2009) who found that increase in density of the negative charge on the cell surface, causing proton removal from solution, thereby decreasing biosorption capacity of Cu (II) because of competitively adsorption of protons. A similar observation was reported by Ferraz and Teixeirain (1999) who suggested an increase of electrostatic interaction at high biomass concentration inhibited metal biosorption. When the biomass concentration is low, metal ions in the solution would not only be adsorbed to the surface of the biomass, but also enter into intracellular part through facilitating the concentration gradient of metal ions (Wang, 2002). It should be mentioned that the cadmium ions adsorption capacity decreased with increase of biosorbent dosage Vasudevan *et al.* (2002). Their theory is similar to that advanced by Zou *et al*. (2006) to account for the cell

surface remaining unsaturated at higher biosorbent dosage. Uslu and Tanyol (2006) found that the initial adsorption rates of Cu (II) decreased with increased biosorbent concentration.

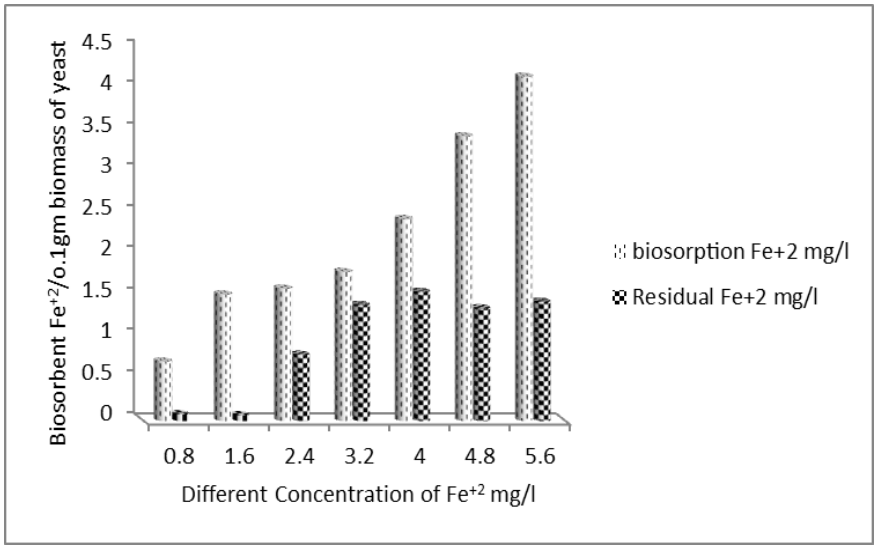

Fig. (3a). Estimation of the biosorbed and residual Fe^{+2} using different concentrations in the culture filtrates of strain 707F.

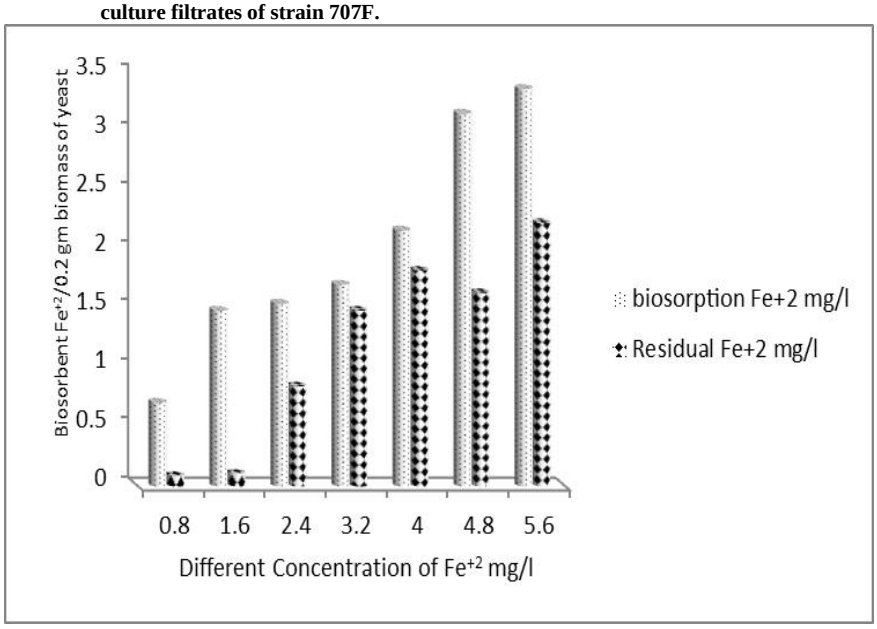

Fig. (3b). Estimation of the biosorbed and residual Fe^{+2} using different concentrations in the culture filtrates of strain 707F.

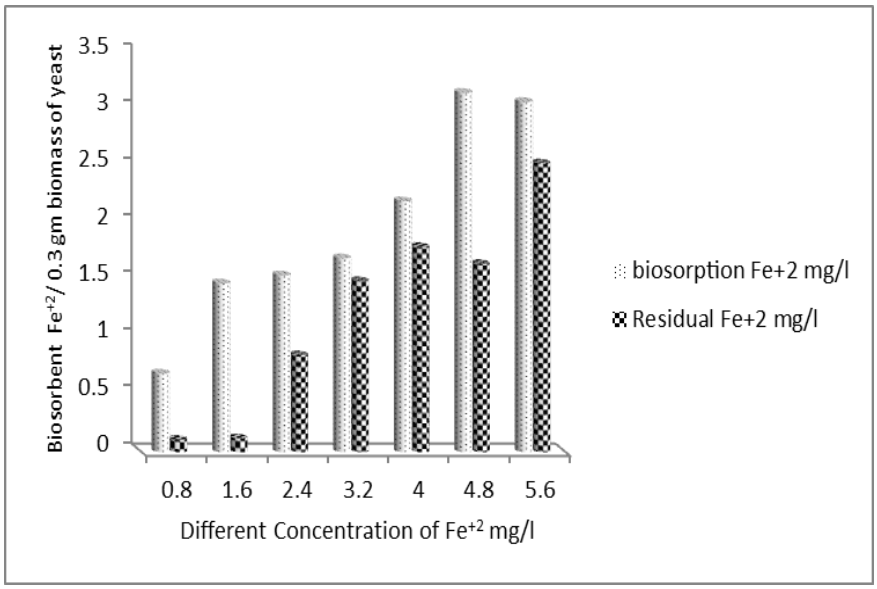

Fig. (3c). Estimation of the biosorbed and residual Fe^{+2} using different concentrations in the culture filtrates of strain 707F.

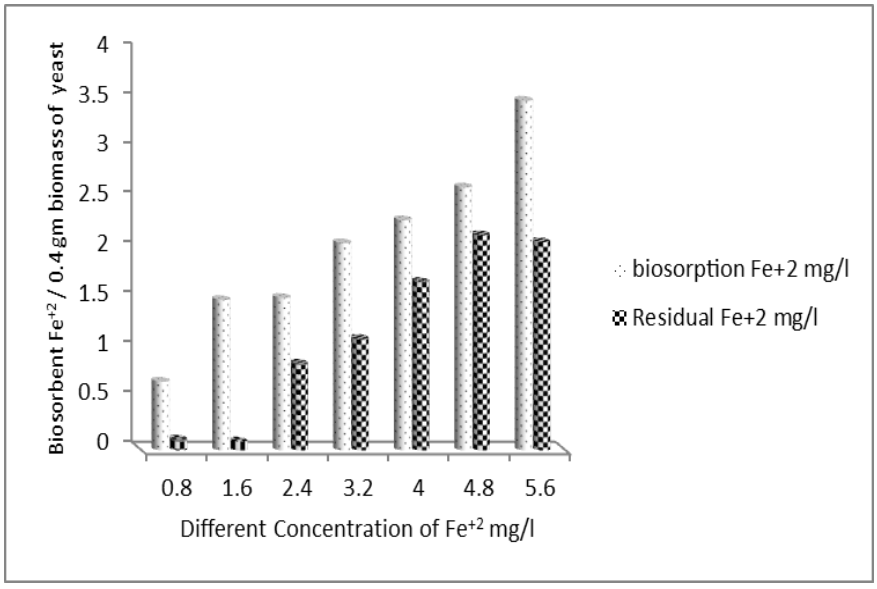

Fig. (3d). Estimation of the biosorbed and residual Fe^{+2} using different concentrations in the culture filtrates of strain 707F.

The charge of the adsorbate and adsorbent often depends on the pH. The adsorption Fe^{+2} as a function of pH were measured. As shown in Table 3. there were increases in biosorption uptake equilibrium with increasing pH from 4.5 to 9 for Fe^{+2} ions. Similar results were detected by Volesky (1990) who found that the optimal pH value is 5–9 for Cu (II) biosorption by *S. cerevisiae*. Also, Mapolelo and Torto (2004) proved that the optimal pH values are greater than 5 for $Cu^{+2}$and Zn^{+2}. Generally, an increase of pH causes deprotonation of metal ions binding sites exposed by cellular surface. However, a decrease of pH causes competition between protons and positively charged metal ions. However these rules concern only cations. Since biosorption is reversible process, decreasing pH would result in deprotonation. This property is used in regeneration of biosorbents (Naja and Volesky, 2010).

TABLE 3. Effect of different pH values on Fe^{+2} uptake by strain 707 F after 20 min.

pH	Biosorption Fe^{+2} (mg/l)	Residual Fe^{+2} (mg/l)	Biosorption Fe^{+2} (mg Fe^{+2}/g biomass)
4.5	5.21^{bc}	0.39^{cd}	52.1
5	5.32^{ab}	0.28^{de}	53.2
5.5	4.8^{e}	0.8^{a}	48
6	5.06^{d}	0.54^{b}	50.6
6.5	5.11^{cd}	0.49^{bc}	51.1
7	5.35^{a}	0.25^{e}	53.5
7.5	5.25^{ab}	0.35^{de}	52.5
8	5.31^{ab}	0.29^{de}	53.1
8.5	5.28^{ab}	0.32^{de}	52.8
9	5.37^{ab}	0.23^{de}	53.7

The different letters indicate significant difference at P>0.05

Removal of Fe^{+2} increases with the agitation time as shown in Table 4. even until 150 rpm the removal increases slightly but it is lower when the agitation speed increased to 200 and 250 rpm. Accumulation starts to predominate after some time of the *Saccharomyces cerevisiae* growth around 150 rpm of agitation .These results agreement with Handan *et al*. (2002). Biosorption of chromium (VI) on to cone biomass of *Pinus sylvestris* was studied with variation in agitation speed. The biosorption of Cr(VI) was increased when the maximum chromium biosorption occurred at 150 rpm agitation.

TABLE 4. Effect of agitation rate on Fe^{+2} uptakes by strain 707 F after 20 min.

Agitation rpm	Biosorption Fe^{+2} (mg/l)	Residual Fe^{+2} (mg/l)	Biosorption Fe^{+2} (mg Fe^{+2} /g biomass)
100	5.15^{b}	0.45^{c}	51.5
150	5.24^{a}	0.36^{d}	52.4
200	4.87^{d}	0.73^{a}	48.7
250	4.99^{c}	0.61^{b}	49.9

The different letters indicate significant difference at P>0.05

Temperature has an influence on the biosorption of metal ions, but to a limited extent under a certain range of temperature, which indicates that ion exchange mechanism exists in biosorption to some extent . Biosorption process is usually not operated at high temperature because it will increase the operational cost (Wang**,** 2002). In our results the different temperature from 20ºC to 40 ºC proved that the optimal temperature values are greater and don't have more effect on biosorption of iron. Brady and Duncan (1994b) found that temperature (5–40°C) had minor effect on the accumulation level of Cu^{+2}, Co^{+2} or Cd^{+2} by free cells of *S.cerevisiae* in suspension . Adsorption reactions are normally exothermic ,so biosorption capacity increases with decrease of temperature (Kapoor andViraraghavan.1997) . In the range of 15°–40°C ,the maximum equilibrium biosorption capacity for Pb(II) ,Ni(II) and Cr(VI) ions by the inactive *S.cerevisiae* was reached at temperature of 25°C.The decrease in capacity at higher temperature between 25and40°C revealed that the processes of biosorption for these metal ions by *S. cerevisiae* are exothermic.The decrease of biosorption capacity at higher temperature may be due to the damage of active binding sites in the biomass (Özer and Özer ,2003).However, (Goyal *et al* ., 2003) found that the metal biosorption of Cr(VI) by *S. cerevisiae* increases with increasing temperature in the range of 25–45°C,they Explained that higher temperature would lead to higher affinity of sites for metal or binding sites on the yeast. The energy of the system facilitates Cr(VI) attachment on the cell surface to some extent .When the temperature is too high ,there is a decrease in metal sorption due to distortion of some sites of the cell surface available for metal biosorption (Table 5).

The SEM and EDX examination of Fe^{+2} biosorption by 707 F revealed the presence of Fe^{+2} as discrete particles attached or near cell surface of yeast. The EDX confirmed that Fe^{+2} was uniformly distributed and adsorbed to the cell surfaces as 707 F. Electron microscopic observation carried out by Mullen *et al.* (1989) clarified the presence of Ag^{+2} as discrete particles at or near the cell wall of both Gram-positive and Gram-negative bacteria and the presence of silver was confirmed by energy dispersive X-ray analysis (EDX). Large particles containing gold were localized in *Sargassum natans* cells by EDX carried out in

conjunction with scanning electron microscopy (Srivastava and Thakur, 2006). Leusch *et al*. (1995), using the X-ray photoelectron spectroscopy, observed that iron was present in two oxidation states when brown seaweed *S. fluitans* was exposed to Fe^{+2}, while only Fe^{+3} was present when the biomass was exposed to ferric ions. SEM and EDX analysis of *S.fluitans* revealed that metal ions (Cr^{+6}) was uniformly distributed and adsorbed to the surface of the cells initially. Limin *et al*. (2009) mentioned that during sorption most of the lead was adsorbed on the surface of the cell. Lead adsorption by *S. cerevisiae* has mainly resulted from both ion exchange and the surface complexation as indicated by SEM-EDX analysis. Also studies revealed that chromium adsorption have marked effects on the fungal morphology. (Fig. 4a and 4b).

TABLE 5. Effect of different temperature on Fe^{+2} uptake by strain 707 F after 20 min.

Temperature ºC	Biosorption Fe^{+2} (mg/l)	Residual Fe^{+2} (mg/l)	Biosorption Fe^{+2} (mg Fe^{+2}/g biomass)
20	5.29^{b}	0.31^{b}	52.9
25	5.08^{c}	0.52^{a}	50.8
30	5.28^{a}	0.32^{c}	52.8
35	5.35^{a}	0.25^{c}	53.5
40	5.35^{a}	0.25^{c}	53.5

The different letters indicate significant difference at P>0.05
The similar letters insignificant at P>0.05

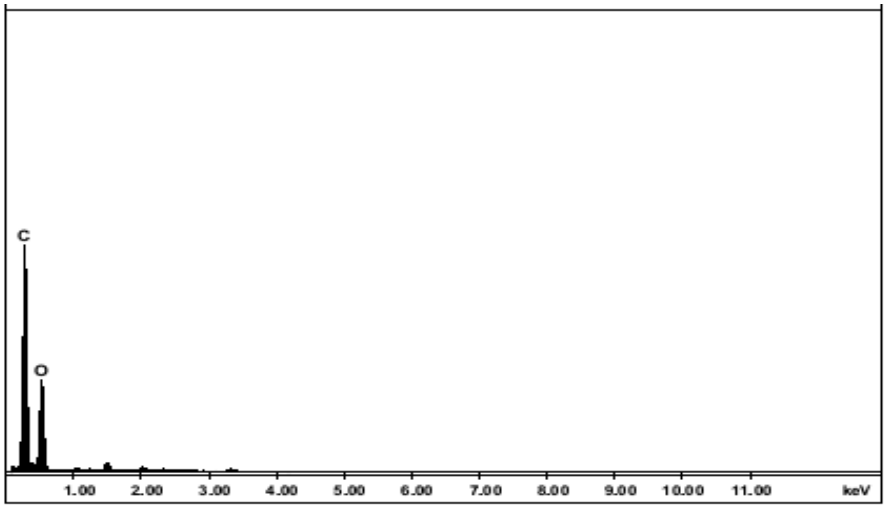

Fig. (4a) Energy dispersive x-ray (EDX) of the biosorbed Fe^{+2} by strain 707 F (control) by Scanning electron microscopy .

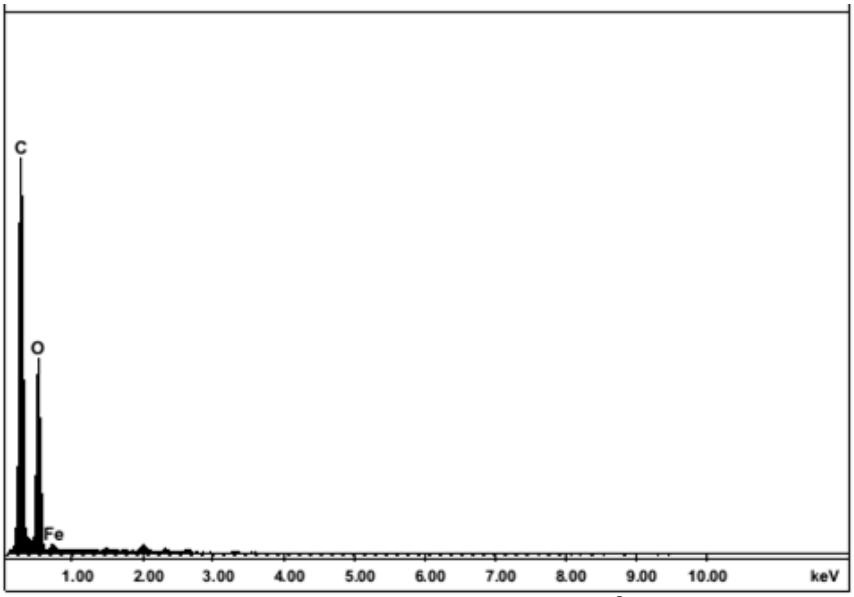

Fig. (4b) Energy dispersive x-ray (EDX) of biosorbed Fe^{+2} by strain 707 F cells treated with 5.6 mg/l Fe^{+2} by Scanning electron microscopy.

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الامتصاص الحيوى للحديد من المياه الجوفية باستخدام الكتلة الحيوية لخميرة السكارواميسيس سيرفيسيا

[١]نزيهه حسنين و[١]محمد احمد و [٢]عمرو مصطفى و [٢]مها الشافعى و[٢]مروى احمد و[٣]هند خاطر
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قد أصبح التلوث بالمعادن الثقيلة واحدة من أخطر المشاكل البيئية اليوم زفي مصر تم استخدام الآبار والمياه الجوفية كمصدر لمياه الشرب منذ زمن سحيق . يوجد الحديد أساسا في المياه الجوفية في شكلين اما ذائب او غير قابل للذوبان تم استخدام احد عشر سلاله من الخميرة فى شكل خميرة حية وميتة لفحص الامتصاص الحيوي والتراكم الحديد فى الخميره من محلول مائي اصطناعي وقد وجد ان سلالة الخميرة الحية F-707 هى الاكثر امتصاص للحديد حيث امتصت ٩و٢١ملى جرام من الحديد لكل جرام خميرة وقد تم التوصل للتركيزات المثلى لامتصاص الحديد وهى ٦و٥ ملى جرام من الحديد فى اللتر بعد٢٠ دقيقة عند عدد اهتزازات ١٥٠ ووسط متعادل ٧ ودرجة حراره ٣٠ درجه مئويه