

## Biosynthesis of Iron Oxide Nanoparticles and Using it to Support the Efficiency of Wastewater Filtration System

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p-ISSN: 1608-9391

e-ISSN: 2664-2786

### Article information

Received: 8/8/2022

Accepted: 7/10/2022

DOI: 10.33899/rjs.2023.177285

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### **ABSTRACT**

Nanoparticles biosynthesis has gained great importance as an active eco-friendly method with economic benefit which overcame on other chemical and physical methods.

This research involved green biosynthesis of iron oxide nanoparticles (IONPs) using *Escherichia coli* (*E.coli*) isolated from wastewater in Mosul city. Characterization of nanoparticles was performed by using many techniques which included UV-Vis Spectroscopy, Scanning Electron Microscope (SEM), Atomic Force Microscope (AFM), X-Ray Diffraction (XRD) and Fourier Transforms Spectroscopy (FTIR).

Designing of a locally lab scale wastewater treatment plant was done by using IONPs adding to a dual water purification system, after tightly wrapping the filter with (1%) of IONPs solution up to saturation. Moreover, control filter was used. Sample of wastewater was passed through these filters to detect its effect on wastewater quality, the results showed that NPs filters improved physical, chemical and biological properties of wastewater including total plate count, coliform, fecal coliform and total fungi.

**Keywords:** IONPs, Biosynthesis, Wastewater treatment plant.

## INTRODUCTION

Pollution of wastewater possess a growing threat to humanity and aquatic life around the world. Water resources contributed the disposal of wastewater by self- purification to get rid of a large amount of organic waste (Alazaiza *et al.*, 2022). Because of increasing population of people and the rapid development of industries that leads to accumulation a huge amount of industrial and municipal wastewater which may cause a continuous deterioration in quality of water resources (Vaamonde, 2022).

Many conventional methods were used for wastewater treatment which included physical, chemical and biological treatment which were mostly limited and has a high economic cost, so they need to find quick and modern solutions by replacing traditional methods with update and effective methods before introducing to water bodies (Starkl *et al.*, 2022).

Recently, the world witnessed a new scientific revolution, which is the technology of petites (nanotechnology). It is a huge qualitative leap that has taken control of many different areas of life (Sagadevan *et al.*, 2022).

As wastewater treatment is a promising technology in the current century, there was an urgent need to devise a new way to solve the water pollution problem with high efficiency and then increase the provision of safe water for use by reducing pollutants to a minimum or exploring the possibility of removing them (Mojiri and Bashir, 2022).

Nowadays in line with the purposeful scientific revolution that the world is witnessing in nanotechnology and their applications in biological fields with a focus on the role of microorganisms especially bacteria as an important factor that effectively contributes to green synthesis of nanoparticles and to maintain the sustainability of a clean and safe environment, the idea of this study came to study IONPs that biosynthesized to support efficiency of filter system after locally isolation and identification of *E.coli* and used this nanoparticles in wastewater treatment.

## MATERIALS AND METHODS

### **Bacterial strain:**

Fifty samples were collected from wastewater in Mosul City. Isolation and identification of *E. coli* were done according to (Yeager *et al.*, 2022). The identification of bacteria was done and then confirmed by using the API system (Sari *et al.*, 2022).

### **Biosynthesis of IONPs by using *E. coli*:**

For the biosynthesis of nanoparticles, 100 ml of supernatant bacteria were mixed with 50 ml of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution (10M) using a conical flask and incubated in shaker incubator for 24-48 hrs. at 37°C. Control flask was prepared that contained supernatant only without salt. A change in color was observed. Then centrifugation for 30 min at 5000 rpm to study characterization. Nanoparticles were washed with ethanol and deionized water three times and then dried (Hassan and Mahmood, 2019).

### **Characterization of nanoparticles:**

After detecting the color shift that occurred in samples, it became necessary to complete the procedures to ensure that they fall at the nanoscale, and to verify their size and shape using various modern techniques as follows:

### **Ultra violet visible spectrophotometry optima (UV spectroscopy):**

The absorption of IONPs were measured the wavelength of mixture reaction using UV-visible spectroscopy, the absorption ranged (190-1100nm) (Al-Razy *et al.*, 2020)

### **Scanning Electron Microscope (SEM) analysis:**

The structural morphology and mean particle size of the nano composite could be characterized by a scanning electron microscope. EDX analysis was done to reveal the presence of elemental composition in the sample. The results were recorded and photographed (Majeed *et al.*, 2020).

**Atomic Force Microscope (AFM) analysis:**

Atomic Force Microscope was used to study the diameter and surface morphology of the IONPs (Akhatova *et al.*, 2022).

**X-Ray Diffraction Analysis (XRD):**

This analysis is confirming the crystal structure of nanoparticles by using X-ray diffract meter. XRD is a popular analytical technique that has been enormously used and is a very useful nondestructive characterization tool to study the physical properties crystallographic structure and chemical composition of materials as well as to determine atomic arrangement and thickness (Ali *et al.*, 2022).

**Fourier Transform Infrared Spectroscopy (FTIR):**

This technique is used to identify organic, polymeric and in some cases inorganic materials. FTIR analysis method uses infrared light to scan test samples and observe chemical property. FTIR is used to analyses functional groups in the nano composite (Fadlelmoula *et al.*, 2022).

**Processing a Lab-Scale Wastewater Treatment using a Modified Filter with biosynthesized IONPs:**

Locally, a laboratory scale system has been designed as a continuous culture using activated sludge. As shown in Fig. (1), the system consists of many tanks (feed tank, aeration tank, and setting tank), followed by a dual filter system for purification, which contains three filters the third filter was prepared using 1% IONPs. The third filter prepared from 1% IONPs by using a ready fiber filter (PPF 1 Micron) as show in Fig. (2) which moistened well to be saturated with a IONPs solution prepared in a concentration of 1%, a filter without nanoparticles was used as a control.

**Sampling of Wastewater:**

Samples were collected from Al-kadraa wastewater treatment station on the left side of Mosul city, using a labeled clean plastic container and sterile bottles for biological analysis, and brought to the laboratory for analysis as soon as possible. Field temperature, initial dissolved oxygen (DO), acidity, and electrical conductivity (EC) were measured.



**Fig. 1: A Lab scale wastewater treatment plant using modified Nanofilter**



**Fig. 2: Filter PPF-1 Micron**

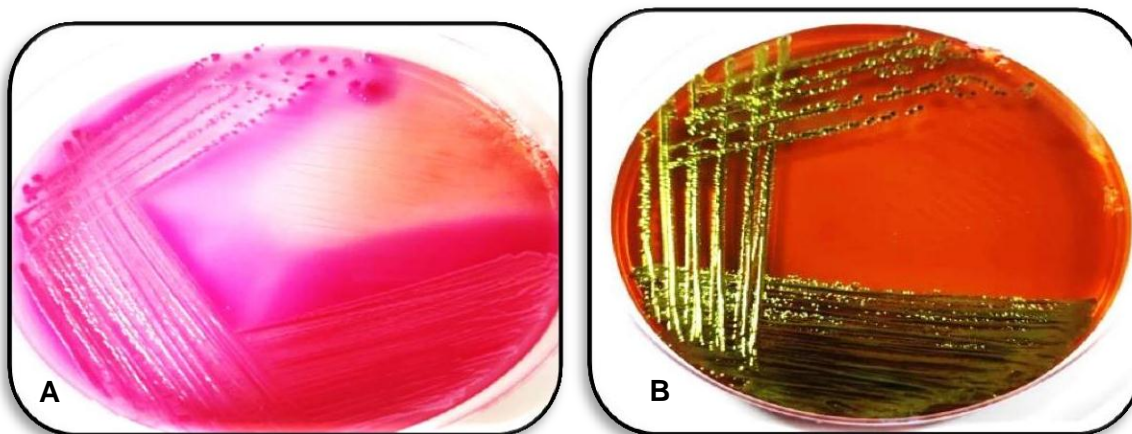
### Analysis of Samples:

Sample analysis was done according to (Quispe *et al.*, 2022) which included: turbidity, total solid (TS), total dissolved solid (TDS), total suspended solid (TSS), hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate ( $\text{NO}_3^{-1}$ ), phosphate ( $\text{PO}_4^{-2}$ ), sulfate ( $\text{SO}_4^{-1}$ ), and analysis of some heavy metals (Na, K, Pb, Cu, Cd, Ni). Biological analysis included total plate count (TPC), total coliform (TC), total fecal coliform (TFC) and fungi analysis.

### RESULTS AND DISCUSSION

*E. coli* was isolated from wastewater and showed most cultural features growth on MacConkey's agar and EMB media as shown in Fig. (3).

The bacteria fermented lactose sugar and grew on MacConkey's agar with pink color while seem with green metallic shine on EMB which agree with (Willey *et al.*, 2020).



**Fig. 3: Growth of *E. coli* on different media**

**A: MacConkey agar B: EMB agar**

Identification of bacteria as showed in Fig. (4) by using API system. The results showed that this bacterium was positive to Indole, Methyl red, Catalase, Nitrate Reductase and motility while the isolate was negative for oxidase, Vogues Proskur, Citrate and Urease. These results were agreed with (Adebowale *et al.*, 2022) and (Fuchs *et al.*, 2022).





**Fig. 4: *E. coli* identification by using API system**

The IONPS were sufficiently biosynthesized by visible observed color changing of reaction from yellow to brown after 24 hrs. of incubation, color intensity was increased after 48 hrs. Fig. (5). This is color change in the color could be caused by the excitation of surface Plasmon vibrations on IONPs, which is a diacritical feature of NPs (Hashim, 2018). The vibration increased depend on the existence of dipole when magnetic field is joined with visible range to collective oscillation of conduction of electrons. The changing in color is very important and play a good role in detection of NPs forming by appearance of characteristic peak during time progresses in conjugation absorbance and give a signature of surface Plasmon resonance of IONPs (Hassan and Mahmood, 2019).

The result was agreed with (Sulman *et al.*, 2016) who studied the biosynthesis of  $\text{Fe}_2\text{O}_3$  NPs by using *Lactobacillus rhamnosus* and they observed a visible color change in flask of mixture reaction from yellow to black as a first indication of nanoparticles production.



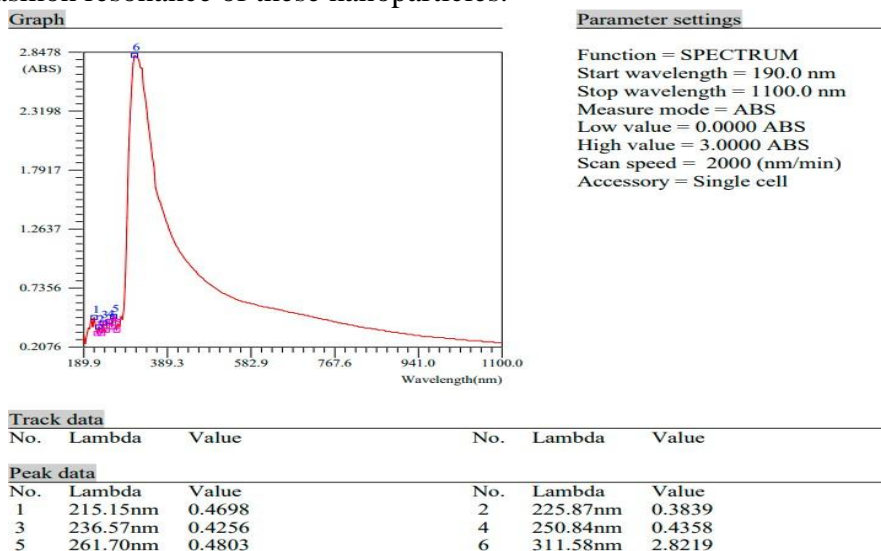
**Fig. 5: Biosynthesis of IONPs using *E. coli***  
**A: Positive result      B: control negative**

### **Characterization of NPs:**

#### **UV-visible spectroscopy:**

The result of UV analysis shows that IONPs biosynthesis by *E. coli* was occurred as showed in Fig. (6). Maximum peak at 311.58 nm with value 2.8219 was observed, this peak appeared in combination of the absorbance through time.

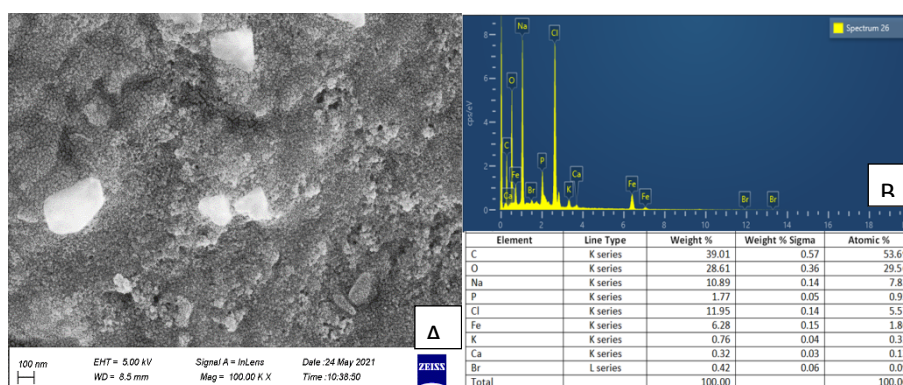
This result was agreed with (Majeed *et al.*, 2020) who biosynthesized Fe<sub>2</sub>O<sub>3</sub> NPs using *Proteus vulgaris* and the UV showed maximum absorption peak at 310 nm. All these peaks indicate a guide of a plasmon resonance of these nanoparticles.



**Fig. 6: UV visible spectrum analysis of IONPs Scanning Electron Microscope**

The examination by SEM showed the morphology of NPs which appeared to get agglomeration and are semi smooth cubical Fig. (7).

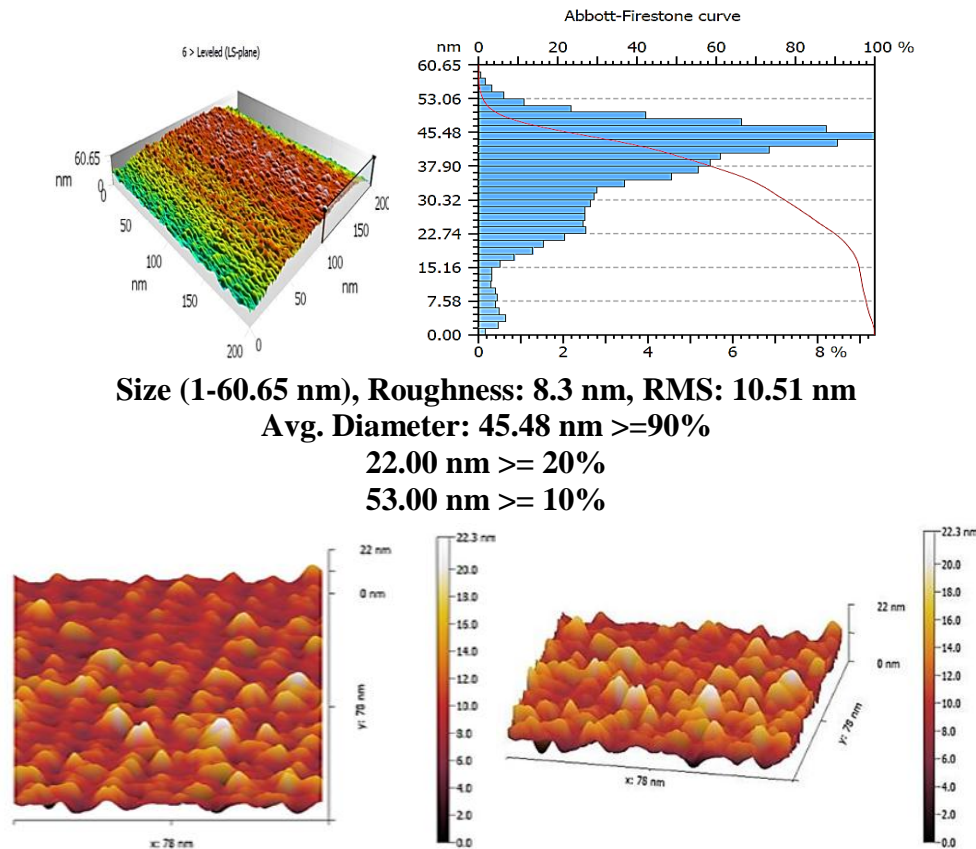
This result was agreed with (Subhashini *et al.*, 2018) who used the extracts of red algae *Gracilaria edulis* for biosynthesis of Fe<sub>2</sub>O<sub>3</sub> NPs and found the formation of cube shape. This may due to a narrow electron beam, micrographs of SEM yield a three-dimensional characteristic for understanding the surface structure of the sample have a great depth of field. Building blocks of various bioactive reducing agents, moreover the reason for agglomeration of the nanoparticles may be due to H-bonding presented in bioactive molecules (Ustun *et al.*, 2021).



**Fig. 7: SEM and EDS analysis of IONPs fabricated by using E. coli bacteria**

#### Atomic Force Microscope Analysis (AFM):

The cross section of IONPs was multiform in shape and the particle size was about 39.87 nm. As shown in Fig. (8).

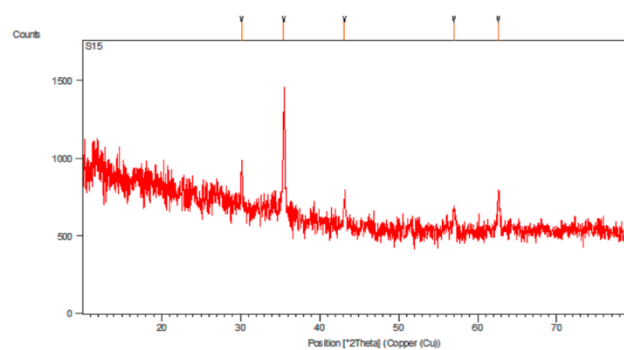


**Fig. 8: Analysis of AFM of IONPs synthesized by *E. coli***

The result was agreed with Hasan and Mahmood (2019), who used the same bacterium (*E. coli*) for biosynthesis of  $\text{Fe}_2\text{O}_3$  NPs, their analysis of AFM showed that the shape of these nanoparticles was seemed to be spherical, but the size was approximately was about 67 nm.

### X-Ray Diffraction Analysis (XRD):

Pattern of XRD extracellular biosynthesized of IONPs show up more than one peaks with  $2\theta$  Theta 2Q values at  $2\theta = 38.88, 40.12$  and  $45.91$  corresponding to the lattice planes indexes 110, 113 and 024 Bragg's reflection respectively as showed in Fig. (9). Depending on face centered multiform iron structure, results lattice planes were referring indexed to the average size of IONPs fabricated JCPDS data with file number (04-0783). Calculation by Debye-Scherrer equation.



**Fig. 9: XRD analysis of IONPs**

Usually, this analysis used to define the structural features of substance, it's important to confirm the crystalline nature and the films of phase identification IONPs. In this research, samples were put on substrate of glass. Our result of XRD was agreed with (Subhashini *et al.*, 2018) who

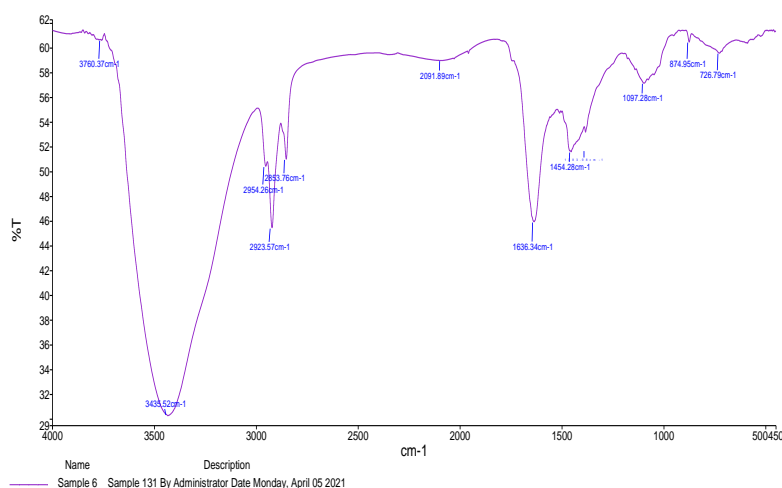
obtain a similar peak by green synthesis of IONPs by using algae with a sharp peak at 44.8. These results confirmed the multifunctional of IONPs.

### Fourier Transform Infrared Spectroscopy (FTIR):

Analysis that used for identification and detection of functional effective groups in the samples that appear as different peaks. ( Table 1) showed the common functional groups and their different frequencies. The results of IONPs showed different functional groups that are confined between (700-4000)  $\text{cm}^{-1}$ , Fig. (10). Two peaks at 3760.37  $\text{cm}^{-1}$ , 3435.52  $\text{cm}^{-1}$  correspond to the hydroxyl groups. While carboxylic groups at the peaks of 2954.26  $\text{cm}^{-1}$ , 2923.57  $\text{cm}^{-1}$  respectively. While carbonyl groups were presence at 5,6 peaks. Then the amino and C=C groups which could be due to carbohydrate and lipids. lastly, Fe-O-Fe bonds were present at the final two peaks. Proteins could bind with nanoparticles through amine groups or by crystalline residues in the proteins, which might help in the nucleation of nanoparticles formation. All these functional groups play an important role as effective metal reducing agent (Joshi *et al.*,2022).

**Table 1: The frequencies of different functional groups**

| Functional Group                           | Group Frequency ( $\text{cm}^{-1}$ ) |
|--|--------------------------------------|
| -C-H (stretch)                             | 2850-2960                            |
| =C-H (stretch)                             | 3000-3100                            |
| $\equiv$ C-H (stretch)                     | $\sim$ 3300                          |
| C=C (stretch)                              | 1620-1680                            |
| C $\equiv$ C (stretch)                     | 2100-2260                            |
| -O-H (alcohols, H-bonded, stretch)         | 3200-3600                            |
| -O-H (carboxylic acids, H-bonded, stretch) | 2500-3000                            |
| -N-H (stretch)                             | 3300-3500                            |
| -N-H (bend)                                | $\sim$ 1600                          |
| C=O (stretch)                              | 1670-1820                            |
| C $\equiv$ N (stretch)                     | 2220-2260                            |
| -S-H (stretch)                             | 2550-2600                            |
| -S-S- (stretch)                            | 470-620                              |
| Si-O-Si (stretch)                          | 1020-1095                            |
| Si-O-C (stretch)                           | 1080-1110                            |
| -N=N- (stretch)                            | 1575-1630                            |



**Fig. 10: FTIR analysis of IONPs**



Our result agreed with (Abid *et al.*, 2021) who studied FTIR analysis of IONPs produced by used peel extract of garlic and onion, also (Hassan and Mahmood, 2019) revealed the existence of many peaks during biosynthesis of IONPs by *E. coli* due to their structure which were in general absorbed from the surrounding of mesoporous structure.

#### **Description Samples of Wastewater before and After Treatment:**

The influent and effluent samples at Al-kadraa WWTP were featured as showed in (Table 2). Many parameters were done. Generally, characterization of the influent wastewater reaching the plant that it is comparable with samples after nanofiltration treatment.

The first three columns of the previous table (feed tank, aeration tank and setting tank) represent the wastewater before treatment, while the last two columns represent treated wastewater with and without filter as a control. All the parameters appeared to improve the values after treatment e.g., turbidity values at the beginning (feed tank) 561 NTU decreased to 5.3 NTU, regarding to total solid and total dissolve solid, the results showed that the values became slim after treatment as well as the hardness, BOD and COD, this is in addition to  $\text{NO}_3^{-1}$ ,  $\text{PO}_4^{-3}$  and  $\text{So}_4^{-2}$ . Regarding cations and heavy metals, the results showed a remarkably decreased in these values. Finally, biological analysis showed that the CFU of bacteria and fungi were decreased and eliminated after treatment. The results of our study were deduced and presented to find the potential applicability of this methodology. This laboratory scale wastewater treatment plant is a combination of many processes such as aeration, water flow, agitation and filtration. The hybrid process for water treatment demonstrates that the fundamental mechanism of IONPs possesses chemical stability, high surface to volume, and high photo reactivity. IONPs have received more attention due to their stability under harsh conditions, improving the hydrophilic character, minimizing the fouling phenomena and improving mechanical properties of the composite membrane and reducing agent (Jayashree *et al.*, 2014). These results were agreed with many other researchers who deals with preparation of nanofiltration membrane (NFM), (Corbacho *et al.*, 2019) installing NFM at the outlet of secondary decanter at a wastewater treatment plant situated in the municipality of Medina Sidonia (Spain) to removed contaminants from wastewater, and they suggested that NF as a tertiary treatment is a viable method for removing various pollutants.

**Table 2: Wastewater features before and after Nano treatment**

| Before treatment                   |                    |                    |                   | After treatment |                    |
|------------------------------------|--------------------|--------------------|-------------------|-----------------|--------------------|
| Features                           | Feed tank          | Aeration           | Setting           | IONPs filter    | Control            |
| Temp. °C                           | 25                 | 25                 | 23                | 23              | 23                 |
| PH                                 | 9.3                | 9.2                | 9.2               | 9.0             | 9.0                |
| Turbidity NTU                      | 561                | 563                | 19.2              | 5.3             | 8.1                |
| Total solid mg/l                   | 1820               | 1930               | 495               | 442             | 440                |
| Total dissolve solid mg/l          | 603                | 621                | 471               | 435             | 428                |
| Total suspended solid mg/l         | 1217               | 1309               | 24                | 7               | 12                 |
| Hardness mg/l                      | 540                | 512                | 424               | 410             | 411                |
| BOD mg/l                           | 256                | 255                | 50                | 35              | 40                 |
| COD mg/l                           | 410                | 406                | 72.6              | 45.3            | 60.2               |
| NO <sub>3</sub> <sup>-1</sup> mg/l | 100                | 94                 | 11                | 9.1             | 9.8                |
| PO <sub>4</sub> <sup>-3</sup> ppm  | 8.2                | 7.7                | 2.2               | 1.5             | 1.7                |
| SO <sub>4</sub> <sup>-2</sup> mg/l | 78                 | 70                 | 62                | 50              | 51                 |
| EC Mhos/cm                         | 815                | 815                | 812               | 780             | 785                |
| Na ppm                             | 28                 | 26                 | 22                | 21              | 21                 |
| K ppm                              | 10                 | 10                 | 9                 | 9               | 9                  |
| Pb ppm                             | 0.186              | 0.177              | 0.133             | 0.090           | 0.091              |
| Cu ppm                             | 0.237              | 0.221              | 0.196             | 0.083           | 0.091              |
| Cd ppm                             | 0.211              | 0.203              | 0.097             | 0.041           | 0.055              |
| Ni ppm                             | 0.141              | 0.141              | 0.081             | 0.043           | 0.043              |
| Total plate count CFU              | 31x10 <sup>5</sup> | 43x10 <sup>5</sup> | 5x10 <sup>5</sup> | zero            | 15x10 <sup>2</sup> |
| Total coliform CFU                 | 20x10 <sup>2</sup> | 22x10 <sup>2</sup> | 3x10 <sup>2</sup> | zero            | 15                 |
| Fecal coliform CFU                 | 14x10 <sup>2</sup> | 21x10 <sup>2</sup> | 2x10 <sup>2</sup> | zero            | 2                  |
| Fungi CFU                          | 12x10 <sup>2</sup> | 13x10 <sup>2</sup> | 3x10 <sup>2</sup> | zero            | 2                  |

## CONCLUSIONS

Iron oxide nanoparticles were successfully biosynthesis by using *E. coli* which isolated from wastewater in Mosul city. Characterization of these nanoparticles and used to prepare a new filter to utilize in designing of a lab scale nanofilter wastewater treatment system and obtain treatment wastewater with a good quality and high efficiency in all characteristics of wastewater.

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## التصنيع الحيوي لدقائق اوكسيد الحديد النانوية واستخدامها في دعم كفاءة نظام تنقية مياه الصرف الصحي

قصي كمال الدين الاحمدي

اميرة محمود الراوي

مي عبد الحافظ العلاف

رئيس جامعة الموصل

قسم علوم الحياة /كلية العلوم/جامعة الموصل

مركز بحوث البيئة /جامعة الموصل

### الملخص

يعد التصنيع الحيوي للجسيمات النانوية طريقة فعالة وصديقة للبيئة وله أهمية كبيرة وفوائد اقتصادية بحيث انها تغلبت على طرق التصنيع الكيميائية والفيزيائية.

تضمن هذا البحث التصنيع الحيوي الاخضر لدقائق اوكسيد الحديد النانوية باستخدام بكتيريا *Escherichia coli* (E.coli) المعزولة من مياه الصرف الصحي في مدينة الموصل.

تم توصيف ودراسة خصائص الجسيمات النانوية المحضرة حيويًا باستخدام عدة تقنيات تمثلت في جهاز مطياف الأشعة فوق البنفسجية (UV-vis spectroscopy)، المجهر الإلكتروني الماسح (SEM)، مجهر القوة الذرية (AFM)، جهاز تحليل حيود الأشعة السينية (X-Ray Diffraction)، وكذلك جهاز تحليل مطياف الأشعة تحت الحمراء (FTIR) Fourier Transforms Infrared Spectroscopy.

تم تصميم محطة معالجة مختبرية (Lab-scale) محلية باستخدام الدقائق النانوية التي صنعت من البكتيريا وضافتها الى نظام تصفية مزدوجة باستخدام محلول 1% من IONPs وصولاً الى حد ما فوق التشبع فضلاً عن استخدام فلتر ترشيح بدون الدقائق النانوية لنموذج سيطرة وتم دراسة تأثيرها في تحسين نوعية المياه (بإضافة جسيمات الحديد النانوية الى منظومة ترشيح مستوردة) واختبار تأثيرها على مواصفات المياه. اظهرت النتائج كفاءة الفلتر النانوي وكفاءته العالية في تحسين الخصائص الكيميائية والفيزيائية والبيولوجية لمياه الصرف الصحي والمتضمنة العدد الكلي للبكتيريا Total Plate Count والعدد الكلي لبكتيريا القولون Total Coliform وبكتيريا القولون البرازية Total Fecal Coliform والفطريات Total Fungi.

**الكلمات الدالة:** الدقائق النانوية لأوكسيد الحديد، التصنيع الحيوي، محطة معالجة مياه الصرف الصحي.