

**How to Cite:**

Qader, M. A. H. A., Al-Rawi, A. M., & Al-Ahmedy, K. K. (2022). Designing wastewater treatment plant by using biosynthesized titanium oxide nanoparticles. *International Journal of Health Sciences*, 6(S1), 1443-1457. <https://doi.org/10.53730/ijhs.v6nS1.4899>

# Designing Wastewater Treatment Plant by Using Biosynthesized Titanium Oxide Nanoparticles

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**Abstract**--In this research, a lab scale wastewater treatment plant was locally designed as a continuous culture of activated sludge and using dual water purification system and titanium oxide nanoparticles (TiO<sub>2</sub> NPs). Biosynthesis of TiO<sub>2</sub>NPs and characterization were done, then nanofilter was prepared by tightly wrapping the filter with gauze and moistened it with 1% TiO<sub>2</sub> NPs solution until saturation, a control filter was also prepared. Wastewater sample was passed through each filter. The results showed that the TiO<sub>2</sub> NPs filter was improved their quality as showed the values of physical, chemical and biological properties of wastewater which included turbidity that decreased from 561 to became 2.9 NTU, the total solid and total suspended solid were decreased from (1820, 1217 mg/l) to (421, 2 mg/l) respectively, the biological and chemical oxygen demands were decreased from (256, 410 mg/l) to became (50, 41.5 mg/l) respectively. The NO<sub>3</sub>-1, PO<sub>4</sub>-3, SO<sub>4</sub>-2 were reduced from ( 100, 8.2, 78) to (6.8, 0.9, 48) respectively. Additionally heavy metals were successfully removed and total plate count, total coliform, total fecal coliform and total fungi were highly decreased.

**Keywords**--activated sludge, wastewater treatment, wastewater, water purification.

## Introduction

Water is necessary for living on earth, but due to exponential growth of people and the rapid development of the industries population which leads to accumulation for a large amount of industrial and municipal wastewater that

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International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.

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Manuscript submitted: 18 Nov 2021, Manuscript revised: 27 Feb 2022, Accepted for publication: 15 March 2022

cause continuous deterioration of quality water resources [Corbacho *et al.*, 2018], so pollution of water represent a severe threat to environment and human health. A large amount of wastewater was discharged into rivers and may cause a large worldwide environmental crisis. Different types of treatment were performed to remove contamination from wastewater before discharged into ecosystems [Riva *et al.*, 2018].

Conventional methods such as biological treatment focusing on primary treatment which depend on physical separation of solid particles are not efficient enough to remove pollutants from contaminated wastewater [Gangaraju *et al.*, 2021], therefore, it is necessary to develop a rapid, simple, eco-friendly, effective, strict and cost effective by using sophisticated technology for sustainable development to produce safe output to the environment [Mulyanti and Susanto, 2018].

Nanotechnology is highly potent in advancing wastewater treatment via nanomaterials include nanofiltration and nanomembrane, it's a set of techniques to process of matter and obtain materials with new functionalities and enhanced features [Kyzas & Mitropoulos, 2021]. Nowadays The development of nanoscience and nanotechnological methods play a great role for wastewater purification because of highly efficient than other traditional ways. These due to their small size high surface to volume ratio, and other features [Mandeep and Shukla, 2020]. In recent years membrane were utilized by many researchers in treatment of wastewater by adding some nanoparticles [Al-Ani *et al.*, 2020]. The membrane nanofiltration technology very effective in removal of different pollutants.

Titanium dioxide ( $\text{TiO}_2$ ) is one of the most important chemically stable nanoparticles which received the most attention as photo catalyst and adsorbent in removal pollutants from wastewater [Khashan *et al.*, 2021]. These nanoparticles can be biosynthesized by some microorganisms such as bacteria as a nanofactories reducing agents for development of nontoxic ecofriendly methods to produce nanoparticles [Hamed *et al.*, 2021].

Mainly,  $\text{TiO}_2$  nanoparticles have been used as a catalyst in organic reaction and decomposition of organic wastewater. Biosynthesis of  $\text{TiO}_2$  NPs by using microorganisms (e.g. bacteria) has a great interest because of their unique properties and they have less aggregation behavior due to presence of more repulsive forces [Daham, 2021]. This research aimed to biosynthesis of  $\text{TiO}_2$  NPs by *Bacillus cereus* and study its characteristics, then designing of  $\text{TiO}_2$  nano filter to apply it in a lab scale wastewater system.

## **Materials and Methods**

### **Bacterial strain**

In this study *Bacillus cereus* was used for the synthesis of titanium dioxide nanoparticles ( $\text{TiO}_2$  NPs), this bacterium was previously isolated and identified from wastewater samples, then it was cultured in nutrient broth medium for 24 hrs. at 37°C in orbital shaker. By using cooling centrifuge, a broth culture was precipitated at 5000 Rpm for 20 min, then the supernatant was filtered by

Whatman filter paper No.1. 100 ml of the supernatant was mixed with 20 ml of 0.025 M  $\text{TiO}_2$  in a reaction flask and stirred for 60 min then the solution was heated at 60°C for 30 min. A visible white deposit is appear, which indicate the forming of  $\text{TiO}_2$ . Positive control was prepared by incubating the culture of the bacteria with deionized water while negative control was contained  $\text{TiO}_2$  solution only Figure (1).  $\text{TiO}_2$  NPs were centrifuged at 5000 Rpm for 30 min in order to study their characterization. Pellet nanoparticles were washing many times with 70% ethanol and deionized water and then dried at 70°C by oven.

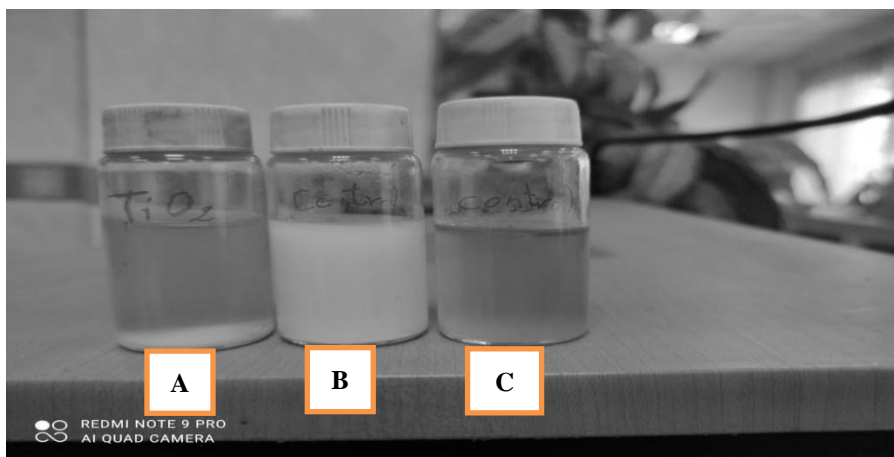


Figure 1. Biosynthesis of  $\text{TiO}_2$  NPs by using *Bacillus cereus* A:  $\text{TiO}_2$  NPs B: negative control ( $\text{TiO}_2$  solution only) C: positive control (the culture with deionized water)

### Characterization of $\text{TiO}_2$ NPs

To study the surface size, shape, morphology and distribution of  $\text{TiO}_2$  nanoparticles various instrumental analysis were used as follows:

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

Nanoparticles were confirmed by determining the absorption peak of the mixture reaction at the range (190-1100) nm using UV-light visible spectrophotometer [Shimadzu UV-(1800), 2021]. Molecules absorb light at specific wavelength reliant to chemical nature, structure and transition metal ions.

#### Scanning electron microscope (SEM) analysis

For morphological and size characterization of nanoparticles, scanning electron microscopy (SEM) was used (FEI, QUANTA-250 FEG).  $\text{TiO}_2$  NPs was examined at the University of Kofa/ faculty of Science/ Unit of Electron Microscope. Specimen were prepared by grinding or sonication of  $\text{TiO}_2$  NPs, making a colloidal suspension of  $\text{TiO}_2$  NPs, and adding a droplet of the suspension on fixing matrix. Sample dock then dried and examined, the imaging was at an accelerating voltage of 12.5-15 KV, low vacuum mode, a spot size 5 and working distances 5-10mm with different magnification powers.

**Energy dispersive spectroscopy (EDS) analysis**

Titanium nanoparticles compositional analysis was carried out with energy dispersive spectroscopy (EDS) at the faculty of Science/ University of Kofa. Point and mapping compositional analysis were examined at an accelerating voltage of 12.5-15 KV, low vacuum mode, a spot size 5 and working distances 5-10mm condition.

**Atomic force microscope (AFM) analysis**

To study the shape of surface morphology and diameter of TiO<sub>2</sub> NPs AFM was used. A sufficient amount of TiO<sub>2</sub> NPs was sent for atomic force microscopy (AFM) at Nanotechnology Research center/ University of Technology/ Baghdad. TiO<sub>2</sub> NPs samples were prepared for AFM microscopy by 5 min incubation of 10 ul TiO<sub>2</sub> NPs with 1cm<sup>2</sup> of freshly cleaved mica, modified by 20 min incubation with deposited of 100 µL of 0.01% APTES (3- amino propyl tri ethoxysilane) solution. After the mica was six times rinsed with 2 mL nanopore water and dried by compressed nitrogen, and placed in the AFM fluid cell holder for imaging.

**X-Ray Diffraction (XRD) analysis**

This pattern was recorded by using x-ray diffractometer (XRD, MiniFlex 600 Rigaku) with CuK radiation at 40 KeV in 2θ range of 10-80 nm. Titanium nanoparticles were sent to Nanotechnology Research center/ University of Technology/ Baghdad for X-ray diffraction (XRD) investigation. XRD is a common analytical technique that used for the study of both crystal and molecular structures, quantitative resolution of chemical species, measuring the degree of crystallinity, isomorphous substitutions, and identification the sizes of nanoparticles.

**Fourier transform infrared spectroscopy (FTIR)**

This technique was performed to identify the functional groups of nanoparticles which was recorded on (Shimadzu IR-Affinity spectrophotometer), and determines the nature and crystallization activity by collecting data of the maximum values at specific lambda from absorption and reflection spectra table (1).

Table 1  
The absorptions of common functional groups and their frequencies [Winter, 2016]

<b>IR Absorptions of Common Functional Groups</b>		
<i>Functional Group</i>	<i>Absorption Location (cm<sup>-1</sup>)</i>	<i>Absorption Intensity</i>
Alkane (C–H)	2,850–2,975	Medium to strong
Alcohol (O–H)	3,400–3,700	Strong, broad
Alkene (C=C)	1,640–1,680	Weak to medium
(C=C–H)	3,020–3,100	Medium
Alkyne (C≡C)	2,100–2,250	Medium
(C≡C–H)	3,300	Strong
Nitrile (C≡N)	2,200–2,250	Medium
Aromatics	1,650–2,000	Weak
Amines (N–H)	3,300–3,350	Medium
Carbonyls (C=O)		Strong
Aldehyde (CHO)	1,720–1,740	
Ketone (RCOR)	1,715	
Ester (RCOOR)	1,735–1,750	
Acid (RCOOH)	1,700–1,725	

### Preparation of nano filter using TiO<sub>2</sub> NPs

For prepare of nano filter, a modified filter system was prepared by using a ready fiber filter (PPF 1 Micron) as show in figure (2) which was wrapped by a gauze around it tightly and moistened very well to be saturated with a TiO<sub>2</sub> Nano solution prepared in a concentration of 1% w/v in order to optimize performance of nano filter membrane. A filter without TiO<sub>2</sub> NPs was used as a control.

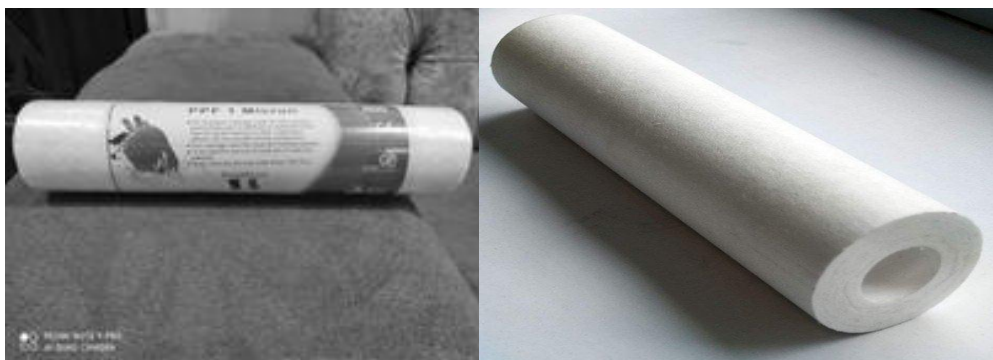


Figure 2. prepare of nano filter wrapped by gauze & 1% TiO<sub>2</sub> NPs solution (Ready Fiber Filter)

### Designing of a lab scale nanofilter wastewater treatment system

Laboratory scale wastewater treatment plant has been designed. The system was locally designed as a continuous culture of activated sludge. As explained in figure (3) it consists of a feed tank with a capacity of 100 liters with 175 ml/min discharge of wastewater to an aeration tank to insure a stay of wastewater in this tank for six hours for digestion of the organic matters. After six hours, the

wastewater passed to the sedimentation tank and stay for four hours as a detention time before reach Dual Water Purification Filter System (figure 4), which consist of three filters, the last one was a prepared from 1% TiO<sub>2</sub> NPs as mentioned in the previous paragraph.

### Wastewater sampling

Wastewater samples were collected from Al-kadraa sewage treatment station in the left side of Mosul city by using a sterile poly ethylene bottles for biological analysis and a clean plastic container, labeled properly and brought to the Laboratory for analysis. Temperature, pH and electrical conductivity (EC) were measured in the field.



Figure 3. Designing of a Lab scale nanofilter wastewater treatment system



Figure 4. Dual water purification system

### Analysis of samples

Samples were analyzed for physico-chemical parameters at laboratory which include: turbidity, total solid (TS), total dissolve solid (TDS), total suspended solid (TSS), hardness, biological oxygen demand (BOD), chemical oxygen demand

(COD), nitrate ( $\text{NO}_3^{-1}$ ), phosphate ( $\text{PO}_4^{-2}$ ), sulphate ( $\text{SO}_4^{-1}$ ), electron conductivity (EC) and heavy metals (Na, K, Pb, Cu, Cd, Ni). Bacteriological analysis which includes total plate count (TPC), total coliform (TC), total fecal coliform (TFC) and total fungi. All these analyses were done according to [Baird, 2017]. Samples were collected from feed tank, aeration tank and setting tank which consider before nano treatment and after treatment of  $\text{TiO}_2$  nanofiltration, then transferred as soon as possible to the laboratory for testing.

## Results and Discussion

The results showed that the  $\text{TiO}_2$  NPS were successfully biosynthesized by the formation of a visible white deposit at the bottom of the reaction flask after 25 min of heating at  $60^\circ\text{C}$  as showed in figure (5). The exact mechanism of bio formation of  $\text{TiO}_2$  NPs remains unclear [Sunkar *et al.*, 2014], while suggest that production of  $\text{TiO}_2$  NPs may be due to the negative electro kinetic potential which may easily attract the cations. The proteins in the culture supernatant could have mediated the hydrolysis of anionic complexes which result in the nanoparticles synthesis [Sunkar *et al.*, 2014]. Similar study was done by [Ibrahim *et al.*, 2019] which attened biosynthesis of  $\text{TiO}_2$  by using *Lactobacillus crispatus*, also  $\text{TiO}_2$  NPs was biosynthesized by *Staphylococcus aureus* using  $\text{Ti}(\text{OH})_2$  0.0025 M [Landage *et al.*, 2020].



Figure 5.  $\text{TiO}_2$  NPs Biosynthesis by *Bacillus cereus*

### UV visible spectroscopy analysis

UV-Vis analysis results showed that biosynthesis of  $\text{TiO}_2$  NPs by using *B. cereus* was occurred Figure (6). There are many absorption peaks but the highest were at wavelength 315.12 and 336.32 nm with the values 3.4149 and 3.2966 respectively. This represents the formation of  $\text{TiO}_2$  NPs in the reaction flask. This result was agreed with the study of [Landage *et al.*, 2020], who observed that  $\text{TiO}_2$  NPs biosynthesis by *Staph. aureus* showed UV spectra confirming absorption peak at 324 nm., while [Swathi *et al.*, 2021] suggest that UV spectra of  $\text{TiO}_2$  NPs using *Cassia fistula* at wavelength of 350 nm.

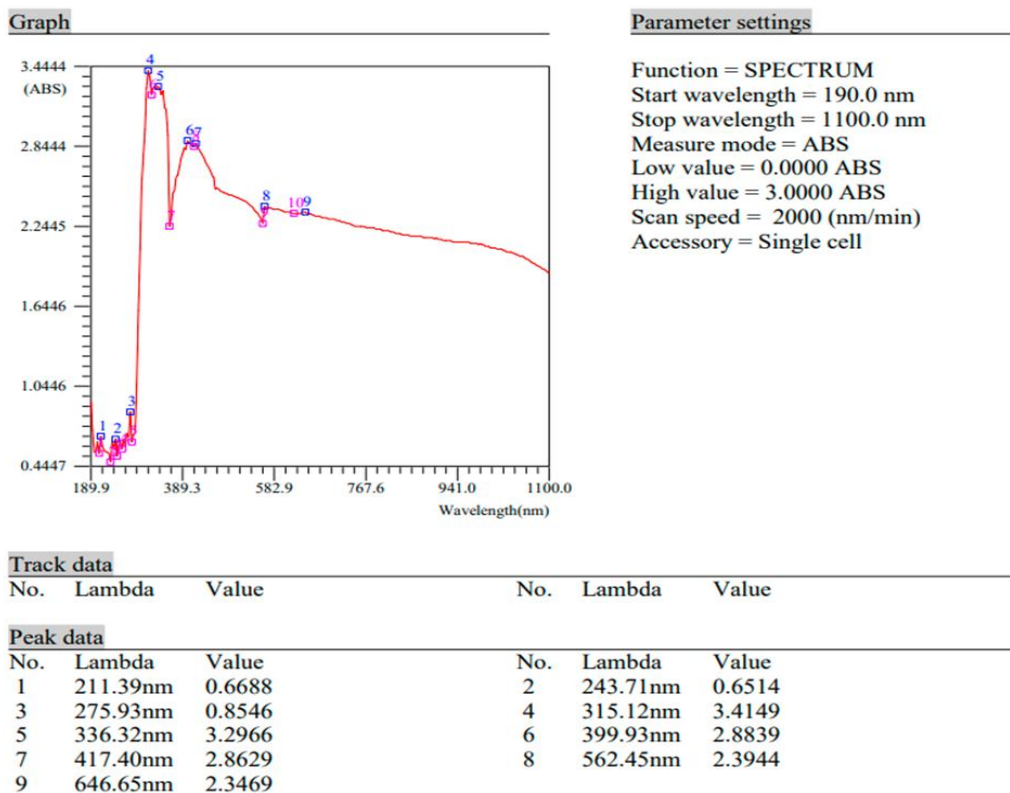


Figure 6. UV\_ visible spectrum analysis of TiO<sub>2</sub> NPs biosynthesis from *B. cereus*  
SEM and EDS analysis

Analysis of SEM revealed that TiO<sub>2</sub> NPs appeared in spherical shapes and agglomerated in crystallite rods as in figure (7). TiO<sub>2</sub> NPs was distributed uniformly with combined a smooth and rough surface. This agrees with other studies reported by [Ibrahim *et al.*, 2019] who suggested that TiO<sub>2</sub> NPs were spherical or oval in shape by using *Lactobacillus* spp., also similar result was showed by [Landage *et al.*, 2020] who used *Staph. aureus* for biosynthesis of TiO<sub>2</sub> NPs.

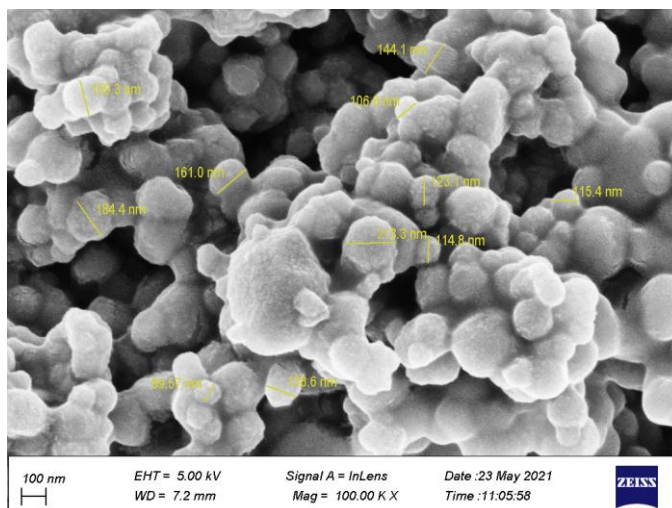


Figure 7. SEM analysis of  $\text{TiO}_2$  NPs fabricated by *Bacillus aureus*

The EDS results confirm the presence of  $\text{TiO}_2$  NPs in the suspension. The spectrum analysis revealed the appearance of signals in the titanium and oxygen regions. (Figure 8). Similar results were obtained by Elisa *et al.*, [Elisa *et al.*, 2020] and Swathi *et al.*, [Swathi *et al.*, 2021] who mention that the biosynthesis of  $\text{TiO}_2$  NPs showed elemental compositions identified as titanium and oxygen molecules.

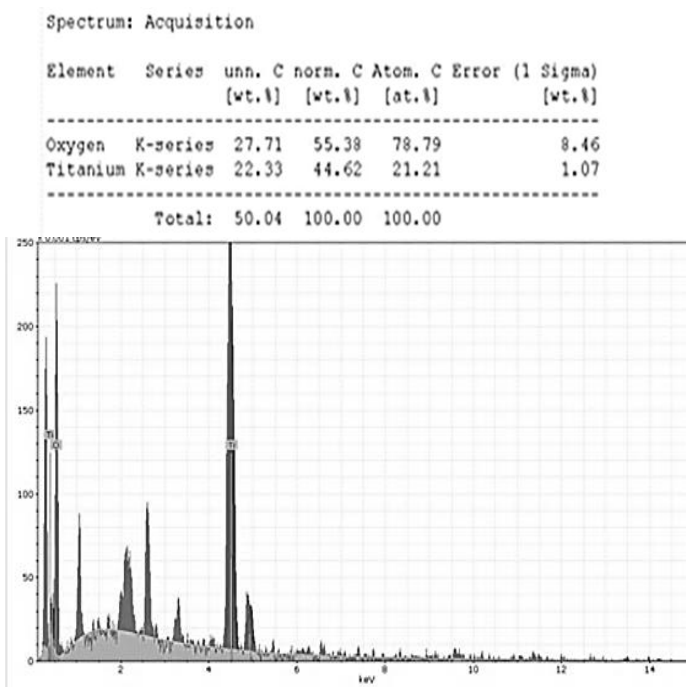


Figure 8. EDS point analysis spectrum showing availability of  $\text{TiO}_2$  NPs

### AFM analysis

Atomic force microscope image of TiO<sub>2</sub> NPs showed the data of three dimension (figure 9). It measures the height of NPs, length and width, in addition to other physical properties (morphology and surface textile) which appear to be smooth. The image displays that the photochemical are capped on the surface of the nanoparticle, therefore, it gives us the best understanding of topography and roughness of nanoparticle [Santhoshkumar *et al.*, 2017]. The strong crystalline nature can be showed in the form of diagonal formations with ridges as show in figure (9). AFM analysis revealed that the TiO<sub>2</sub> NPs was in the size of 7.101 nm.

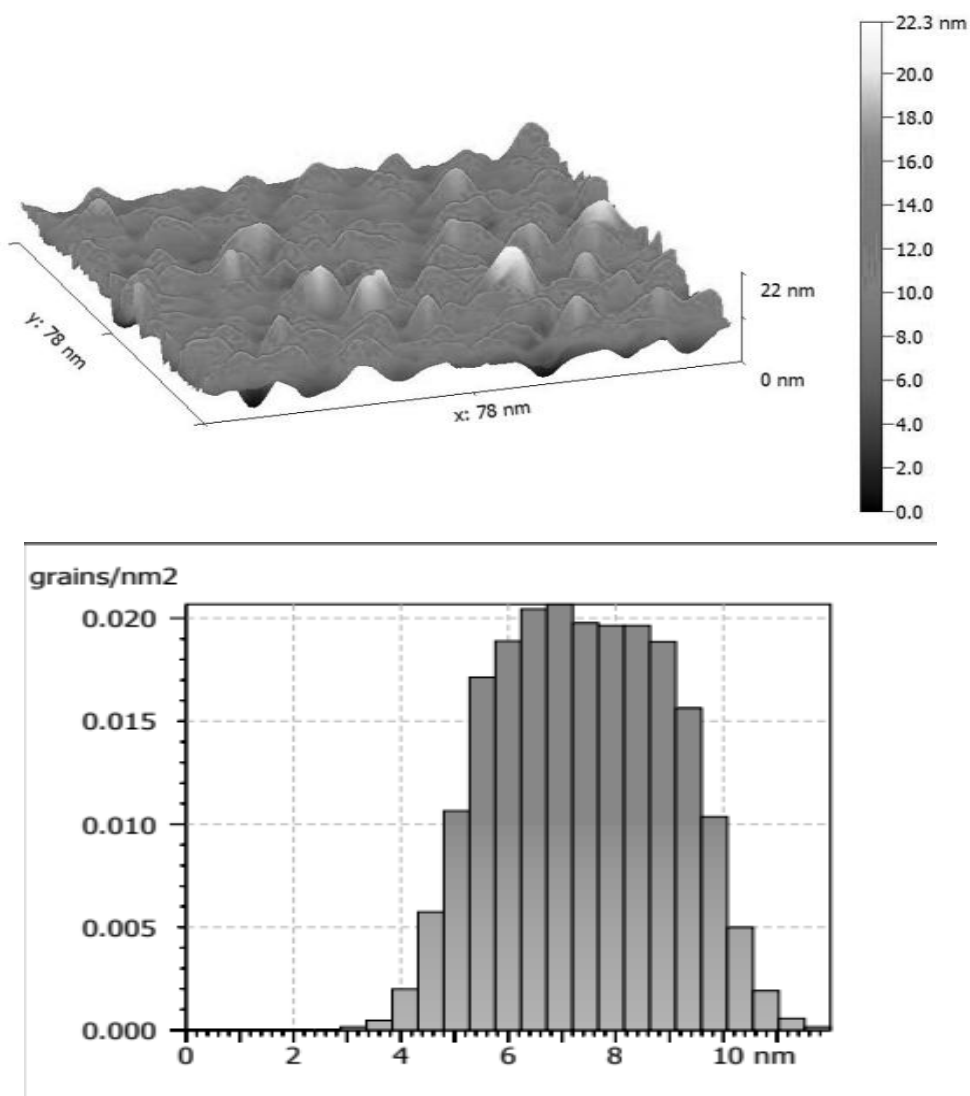


Figure 9. AFM analysis of TiO<sub>2</sub> NPs fabricated by *Bacillus cereus*  
 A: 3D characterization B: granularity and cumulation distribution chart.

### XRD analysis

X-ray diffraction pattern of TiO<sub>2</sub> NPs synthesized by *B. cereus* as showed in figure (10), indicates a high purity, small size, and crystallinity of sample. The intense peaks at  $2\theta = (25.3, 28.1, 32.4, 45.2)$  corresponding to around the lattice planes 101, 103, 021 and 112 respectively, that indexed as anatase phase of TiO<sub>2</sub>. This result was agreed with [Swathi *et al.*, 2021] and [Ibrahim *et al.*, 2019] who indicated that nano particle structure was correspond to anatase crystalline.

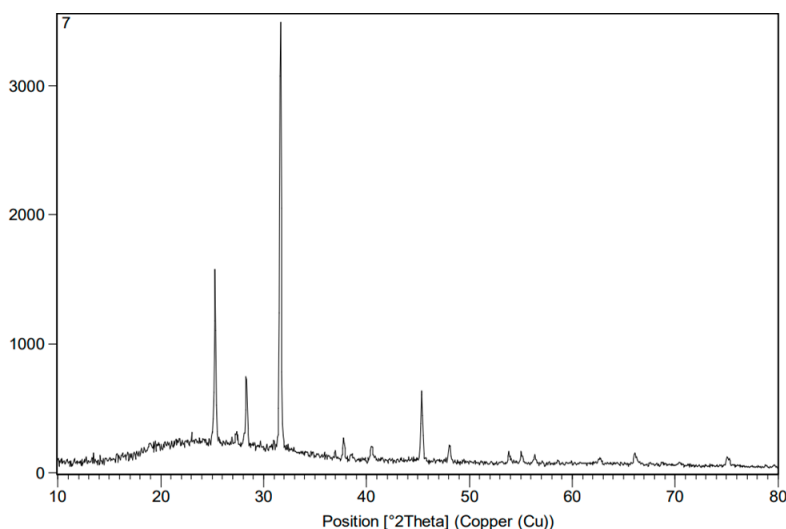


Figure 10. XRD analysis of TiO<sub>2</sub> NPs

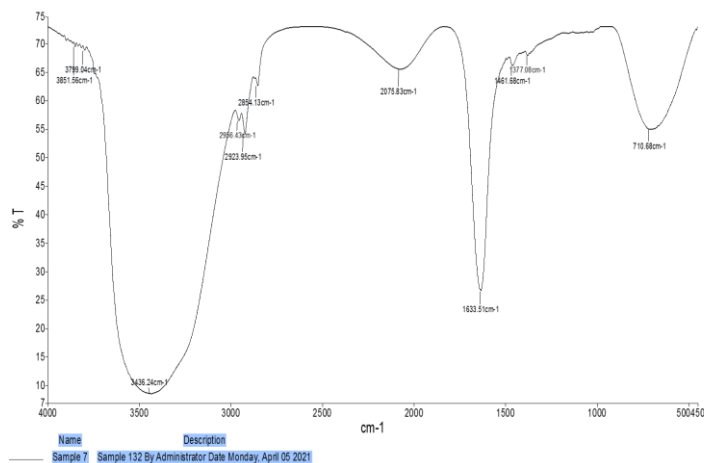
### FTIR analysis

As showed in the table (2) there are (11) different peaks which represents the presence of a broad band and many functional groups. The peak of 3851.56 cm<sup>-1</sup> corresponds to the phenol group. Peak at 3799.04 corresponds to O-H group, while alcoholic group at the peak of 3436.24 cm<sup>-1</sup>. At 4,5,6,7 peaks the results showed the presence of many bonds of protein and peptide with nanoparticles which produced by the bacteria, while amino groups and C=C groups were due to carbohydrate and lipids. Finally, Ti-O-Ti bond was present at the last peak. The free amine's groups which binds with nanoparticles and help in the nucleation of nanoparticles formation. Figure (11) showed the FTIR analysis results.

Table 2  
Peaks position of FTIR analysis of TiO<sub>2</sub> NPs

Peak Number	Frequency Range cm <sup>-1</sup>	Transmission T%	Functional Groups
1	3851.56	69.60	O-H stretching
2	3799.04	68.97	Amide/Phenol
3	3436.24	8.52	Bonded of amine & amide
4	2956.43	56.58	C=C/ C-H/ protein

5	2923.95	54.30	C-H
6	2854.13	62.71	N-H/ C-O
7	2075.83	65.63	Silicon compounds
8	1633.51	26.70	C=O/ Aromatic
9	1461.68	66.30	Methylene group
10	1377.08	68.04	Halogen compound
11	710.68	55.03	Aliphatic group

Figure 11. FTIR analysis of TiO<sub>2</sub> NPs

### Characterization of wastewater samples before and after treatment

The influent and effluent at Al-kadraa wastewater treatment plant were characterized as showed in table (3). Different parameters were performed. In general, the characterization of the influent wastewater reaching the plant indicates that it is comparable with the samples after treatment with nanofiltration.

Table 3  
characterization of Al-Kadraa WWTP before and after treating with TiO<sub>2</sub> nanofiltration

Parameters	Feed Tank	Aeration Tank	Setting Tank	TiO <sub>2</sub> Nanofilter	Control Filter
Temp. ° C	25	25	23	23	22
pH	9.3	9.2	9.2	8.8	9.0
Turbidity NTU	561	563	19.2	2.9	8.3
Total Solid mg/l	1820	1930	495	421	440
Total Dissolve Solid mg/l	603	621	471	419	430
Total Suspended Solid mg/l	1217	1309	24	2	10
Hardness mg/l	540	512	424	400	412
BOD mg/l	256	255	50	26	38
COD mg/l	410	406	72.6	41.5	57.4

NO <sub>3</sub> <sup>-1</sup> mg/l	100	94	11	6.8	7.2
PO <sub>4</sub> <sup>-3</sup> ppm	8.2	7.7	2.2	0.9	1.8
SO <sub>4</sub> <sup>-2</sup> ppm	78	70	62	48	52
EC Mhos/cm	815	815	812	774	788
Na ppm	28	26	22	20	22
K ppm	10	10	9	9	10
Pb ppm	0.186	0.177	0.133	0.068	0.097
Cu ppm	0.237	0.221	0.196	0.064	0.083
Cd ppm	0.211	0.203	0.097	0.027	0.055
Ni ppm	0.141	0.141	0.081	0.022	0.023
Total Plate Count CFU	31×10 <sup>-5</sup>	43×10 <sup>-5</sup>	5×10 <sup>-5</sup>	13×10 <sup>-1</sup>	17×10 <sup>-1</sup>
Total Coliform CFU	20×10 <sup>-2</sup>	22×10 <sup>-2</sup>	3×10 <sup>-2</sup>	5×10 <sup>-1</sup>	5×10 <sup>-1</sup>
Facial Coliform CFU	14×10 <sup>-2</sup>	21×10 <sup>-2</sup>	2×10 <sup>-2</sup>	Nil	Nil
Fungi CFU	12×10 <sup>-2</sup>	13×10 <sup>-2</sup>	3×10 <sup>-2</sup>	Nil	Nil

The first three columns of the previous table (feed tank, aeration tank and setting tank) represent the wastewater before treatment, while the last two one represent treated wastewater with TiO<sub>2</sub> nanofilter and without TiO<sub>2</sub> nanofilter as a control. All the parameters appeared improving the values after treatment e.g. turbidity values at the beginning (feed tank) 561 NTU decreased to 2.9 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 NO<sub>3</sub><sup>-1</sup>, PO<sub>4</sub><sup>-3</sup> and So<sub>4</sub><sup>-2</sup>. Regarding to cations and heavy metals the results showed remarkably decreased in these values. Finally the biological analysis showed that the CFU of bacteria and fungi were decreased and eliminate after treatment. The results of our study were deduced presented to found 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,that called as hybrid process for water treatment [Jayashree *et al.*, 2014].

(Ziental *et al.*, 2020), refired that the fundamental mechanism of TiO<sub>2</sub> NPs has possessed a chemical stability, high surface to volume, chemical stability, and high photo reactivity. TiO<sub>2</sub> has received more attention due to its stability under harsh conditions, improving the hydrophilic character, minimizing the fouling phenomena and improving mechanical properties of the composite membrane [Al-Ani *et al.*, 2020], and reducing agent [Hamed *et al.*, 2021].

Our 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 wastewater treatment plant situated in the municipality of Medina Sidonia (Spain) to removed contaminants from wastewater, and they suggest that NF as a tertiary treatment as a viable method for removing various pollutants. Mandeep & Shukla, [Mandeep and Shukla, 2020] suggest that NFM play a vital role in recovery of many nutrients from industrial effluents in India, also [Liu *et al.*, 2019] used gold NPs of NFM to achieve higher recovery of phosphorous from wastewater (96.1%) and heavy metals (100%). Batool *et al.*, [Batool *et al.*, 2021] suggest that incorporating of different amount of TiO<sub>2</sub> NPs with membrane may lead to increase in porous structures and hydrophilicity and this lead to remove many salts from the wastewater [Sakarkar

*et al.*, 2021]. TiO<sub>2</sub> which have unique properties (non-toxicity, low cost, high chemical stability and biocompatibility) to have great potential in engineering photocatalytic based membrane for recovery of wastewater for reuse [Tetteh *et al.*, 2021].

## Conclusion

TiO<sub>2</sub> NPs were successfully biosynthesis by using a friendly green method using *B. cereus*, 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.

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