Zein/EDTA/chlorophyll/nano-clayBiocomposite Sorbent; Studying Physicochemical Properties and Ability to Remove Industrial Wastewater Contaminants

Farrokh Asadzadeh
Urmia University
sajad pirsa (pirsa7@gmail.com)
Urmia University https://orcid.org/0000-0002-6182-8798

Research Article

Keywords: Nano-biosorbent, Biodegradable, Pollutant reduction, Chemical treatment

Posted Date: September 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-886032/v1

Zein/EDTA/chlorophyll/nano-clay biocomposite sorbent; studying physicochemical properties and ability to remove industrial wastewater contaminants

Farrokh Asadzadeh[1] and Sajad Pirsa[2]*

[1]Department of Soil Science, Faculty of Agriculture, Urmia University, Urmia, Iran

[2]Department of Food Science and Technology, Faculty of Agriculture, Urmia University, Urmia, Iran

*Email: Pirsa7@gmail.com; S.pirsa@urmia.ac.ir

Abstract

In this study, a composite biosorbent of zein/ethylene diamine tetraacetic acid /chlorophyll/nano-clay (Ze/EDTA/Chl/Clay) was prepared. Thickness, mechanical properties (tensile strength and strength to breaking point), ability to reduce water hardness, water solubility, water absorption, antioxidant activity and moisture content of prepared biosorbent were studied. SEM, FTIR, XRD and TGA techniques were used to investigate the physicochemical properties and structure of the prepared biosorbent. Optimal biosorbent was selected using statistical methods and used to remove chemical contaminants from industrial wastewater in Urmia (Iran). For this purpose, total heavy metals (THM), total hardness, nitrate, nitrite, COD and BOD, TDS and salinity of industrial wastewater before and after biosorbent treatment were investigated. The results confirmed the homogeneous and cohesive structure of different zein adsorbents. FTIR results showed physical and electrostatic interactions between composite components. Nanoclay increased the thermal stability of the biosorbent. Chlorophyll and EDTA increased the biosorbent ability to absorb water and reduce the total hardness of the water. Clay nanoparticles increased the tensile strength of the biosorbent and chlorophyll and EDTA increased the biosorbent ductility. Under optimal wastewater treatment conditions, Ze/EDTA/Chl/Clay biosorbent was recognized as the best adsorbent. The use of ultrasound in wastewater treatment had a good effect. Under optimal conditions, 57.5% of THM and 67% of nitrate were removed from the wastewater. By comparing the ability of chlorophyll-containing biosorbents (Ze/Chl and Ze/EDTA/Chl/Clay) to remove nitrite and nitrate, it was found that these biosorbents have a very high selectivity in nitrate removal compared to nitrite.

Keywords: Nano-biosorbent, Biodegradable, Pollutant reduction, Chemical treatment

1. Introduction

Industrial wastewater is a by-product of manufacturing plants. Various factories that can produce the food, clothing, beverages, paper and all kinds of chemicals. In all these products, water is used as a consumable part of the production process. Due to the diversity of industrial plants and the high volume of industrial wastewater in the world, we need to identify appropriate methods for the management and treatment of industrial wastewater. The first step in managing industrial wastewater is to fully understand the effluent of factories [1 and 2]. Today, due to the increasing growth of industries in the world, the amount of pollution in the environment is increasing. Heavy metals and organic pollutants are two groups of important, main and persistent pollutants released in industrial wastewaters that it is necessary to achieve a cost-effective, sustainable and modern way to clean them. Heavy metals are a group of metals that have a specific gravity of more than 6 grams per cubic centimeter or an atomic mass of more than 50. Heavy metals gradually accumulate in the body. Heavy metals accumulate in human adipose tissue, muscle, bone, and joint. General muscle weakness, loss of appetite, nausea, inflammation of the mucous membranes of the eyes, nose and larynx, as well as skin lesions, reproductive problems, mental and neurological disorders and heart disease are the complications of exposure to heavy metals [3-6]. Nitrate is the most oxidizing form of nitrogen found in natural systems. Nitrate levels in surface waters are often low (zero to 18 mg/L) and lower than in groundwater. Discharge of large volumes of sewage and agricultural effluents or wastewater runoff is the most important source of nitrate entry into surface water and sometimes increase the concentration of nitrate in surface water to several times its amount in groundwater. Because nitrate is a water-soluble ion (such as sodium or chlorine in salt), it is not removed by water boiling. Complications of nitrate in humans occur as a result of its reduction by gastrointestinal bacteria and its conversion to nitrite NO_2^-. The formation of nitrite NO_2^- is important for two reasons and is very problematic for human health. Nitrite (NO_2^-) can oxidize hemoglobin in the blood and convert it to methemoglobin. This substance disrupts the oxygen supply to the body. Nitrite combines with some of the body's amines and amides to form nitrosamines, which are carcinogenic [7-9]. Biodegradable adsorbent polymers are a group of polymer networks whose chains are interconnected by cross-links and due to their ionic properties and type of interconnection, holes are formed in the structure of the polymer that cause a large amount of aqueous solutions absorption without dissolving in them. Zein, as one of the types of biodegradable polymers, is one of the main storage proteins of corn, which contains 50-45% of corn proteins. Despite its low nutritional value, due to its uniqueness in the formation of non-greasy, waterproof, and transparent polymers, it has a special place in the manufacture of synthetic biopolymers and biodegradable films. Because zein has good potential in some applications, several attempts have been made to provide a low-cost commercial process for zein production [10-16]. Chlorophyll is a powerful antioxidant that can help human body against degenerative diseases. This is done by neutralizing free radicals in the body. Chlorophyll can help prevent DNA damage in cells as well as better cell function. High levels of chlorophyll in the blood and adipose tissue are positively correlated with a reduced risk of cancer, heart disease and macular degeneration. This pigment can easily react with nitrate and nitrite oxidants and remove them from the environment [17-19]. Ethylene diamine tetraacetic acid (EDTA) is a chemical used for industrial and pharmaceutical purposes. This solid is a colorless, water-soluble amino carboxylic acid. The conjugate of this acid is ethylene diamine tetra acetate. This material is widely used to dissolve lime. The usefulness of this material is due to its role as a six-toothed ligand and a chelating agent, which in fact has enabled it to separate metal ions. Metal ions remain in solution after binding to EDTA and forming a complex, but their reactivity decreases. In industry, EDTA is mainly used to separate metal ions in aqueous solutions [20 and 21]. Nanoclays are unique materials that are used as additives to make nanocomposites and significantly improve the properties of polymeric materials. Under the high resolution electron microscope, it is observed that the nanoclays consist of small, non-regular clay plates about 100 nm thick. The most common nano-clay is Montmorillonite (from the smectite family). Nanoclays have a specific surface area of about 750 m^2/g. They are often reinforced with fillers to improve the mechanical properties of polymeric materials. Despite the extensive research that has been done in recent years on the interaction of soil and contaminants, no significant research has been done to increase the retention capacity of nanoparticles. Also, the evaluation of previous researches indicates that in the researches done so far, no special attention has been paid to the special capabilities of nanoclay in absorption and retention of metal contaminants [22-24].

In this study, after studying and determining the amount of heavy metals, organic compounds, nitrates and nitrites in industrial wastewater, the composite biosorbent of Ze/EDTA/Chl/Clay was used to remove industrial wastewater contaminants. In the first part of this study, the physicochemical and mechanical properties of synthesized composite biosorbents were investigated using techniques such as infrared spectroscopy, electron scanning microscopy and X-ray spectroscopy. In the second part, the synthesized biocomposite for removing pollutants from industrial wastewater was investigated and the efficiency of reducing pollutants was investigated.

2. Materials and methods

2.1. Materials

Corn zein (with more than 90% protein) was prepared with the code Z3625 from Sigma Company. Ethylene diamine tetraacetic acid with 95% purity was purchased from Famco Industrial Group (Iran, Tehran). Clay nanoparticles (with particle size of 30 to 80 nm) were prepared from Urum Aydan Sanat Company (Iran, Urmia). Chlorophyll solution (20%, with green appearance) was purchased from the Nanogilozak Company (Tehran, Iran). DPPH (2, 2-diphenyl-1-picrylhydrazyl) and other chemical compounds used in chemical tests were obtained from Merck Co. (Germany).

2.2. Preparation of Ze/EDTA/Chl/Clay biosorbent

The following method was used to prepare the biosorbent of Ze/EDTA/Chl/Clay: First, 10 g of corn zein in 100 ml of alcohol (70%) by a magnetic stirrer (RS3001, MLW, Germany) for 20 Minutes were solved with 2000 rpm. The pH of the solution was then adjusted to 9, using a NaOH 0.1 N solution. Then glycerol (30% by weight of dry matter) was added to the solution and dissolved in the solution for 20 minutes at 2000 rpm. Then, according to the statistical design of Table 1, ethylene diamine tetraacetic acid powder was added to the solution and dissolved for 1 hour by mechanical stirrer (BH8, Iran) at 1500 rpm at 70 °C/min. In the next step, according to Table 1, the chlorophyll solution was added to the above solution and the chlorophyll solution was dispersed for 1 hour by a mechanical stirrer at 1000 rpm. Finally, clay nanoparticles with the values reported in Table 1 were added to the solution and dispersed in the solution. The final solution was stirred for 1 hour by a mechanical stirrer at 1500 rpm and a uniform green solution was prepared. 20 ml of the prepared viscose solution was poured into glass plates with a diameter of 10 cm and dried at room temperature for 36 hours. Composite biosorbents prepared in the dark were stored in special zipped bags at room temperature until the experiments were performed (Fig. 1).

2.3. Investigation of biosorbent properties

2.3.1. Biosorbent thickness

A digital micrometer (Model 25A-3109) with an accuracy of 0.01 mm was used to measure the thickness of the biosorbents. The thickness of sheet biosorbents was measured at 10 different points and their mean value was calculated.

2.3.2. Mechanical properties of biosorbents

Mechanical properties of biosorbents using the texture analyzer device (Sweden, Model TVT 300 Xp) were performed. To perform this test, biosorbents were first conditioned at a relative humidity of 55% saturated calcium nitrite for 24 hours. The biosorbents were then cut to dimensions of 1×10 cm^2. The prepared biosorbents were placed between the two jaws of the device (at a distance of 5 cm from each other) and stretched at a speed of 50 mm per minute. Tensile strength factors (TS) and percentage increase in elongation at break point (EAB) were calculated by the device.

2.3.3. Solubility in water

To perform this test, biosorbent samples with dimensions of 4×4 cm were dried (at 105 °C) and their weight was measured with a digital scale (accuracy 0.001, Sartoriu, Germany) and as the initial weight (W_0) were recorded. The dried biosorbent samples were then placed at room temperature in Erlenmeyer containing 50 ml of distilled water for 24 hours. During this time, some parts of the sample dissolve in water and some remain solid. After 24 hours, the samples are filtered through pre-dried filter paper and dried again at 105 °C and again weighted (W_1). The following equation was used to calculate the solubility of the samples in water:

$$Solubility = \frac{W_0 - W_1}{W_0} \times 100 \qquad (1)$$

2.3.4. Water absorption capacity

Water absorption capacity was determined by Asadzadeh and Pirsa methods [9]. To perform this test, 2 g of biosorbent was dried in an oven at 150 °C. Then, in a 30 ml Falcon, 1 g (W_0) of the dried biosorbent sample was mixed with 9 ml of distilled water. The falcons were then centrifuged for 40 minutes (at 4,000 rpm). Finally, the solids were separated from the water and removed from the falcon. The weight of the extracted solid was measured again (W_1). The amount of water absorbed by one gram of biosorbent as the water absorption capacity was calculated by the following equation:

$$WAC\ (\%) = \frac{W_1 - W_0}{W_0} \times 100 \quad (2)$$

2.3.5. Ability to removal of total hardness of water

To measure the ability of biosorbent to remove the total hardness of water, solutions with a certain total hardness (TH0) were prepared. 2 g of biosorbent sample was placed in 20 ml of sample solution and stirred at room temperature for 1 hour using a shaker. The biosorbent was then separated from the solution using Whatman 1 filter paper and its total hardness was measured again by titration (TH1). The following equation was used to measure the ability to remove the total hardness of water:

$$THRC\ (\%) = \frac{TH_1 - TH_0}{TH_0} \times 100 \quad (3)$$

2.3.6. Measurement of antioxidant properties

The biosorbent antioxidant properties were determined by the radical scavenging method of 2DPPH using the method of Jabraili et al. (2021). To perform this test, some biosorbent (25 mg) was stirred in 5 ml of distilled water for 5 minutes.

Then the extract prepared from the film (2.8 ml) was added to the test tubes containing 0.2 ml of DPPH methanolic solution (1 mM). The prepared solution was kept in a room for 30 minutes. Using a spectrophotometer (Pharmacia model, USA) at 517 nm, the absorbance of the test and control samples (1 mM methanolic solution DPPH) was measured. Finally, using the following equation, the percentage of biosorbent antioxidant properties was determined [10].

$$(4)\text{Antioxidant activity } (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

$\text{Abs}_{\text{control}}$: Absorption rate of DPPH methanol sample

$\text{Abs}_{\text{sample}}$: Adsorption rate of methanol biosorbent sample

2.3.7. Scanning electron microscopy

An electron microscope (Philips, Netherlands) was used to study the surface morphology of the prepared biosorbents. To do this, the prepared biosorbent was first glued to the aluminum base with the help of silver glue. The base was then dried to a critical point and covered with gold particles. Imaging of the sample was performed with an electron microscope with a power of 20 kW at different magnifications and SEM images were recorded.

2.3.8. Fourier transform infrared spectroscopy (FTIR)

To study the chemical structure and functional groups of the biosorbents, the FT-IR spectrophotometer (model Tensor27 made by Bruker UK) was used. To do this, the biosorbent samples were completely dried and pulverized and mixed with KBr in a ratio of 1 to 100 and made into 20 mm thick tablets with a special pressure device. The prepared tablets were placed inside the tube of the device. FTIR spectra related to different samples were recorded by the device in the range of 400-4000 cm^{-1} and with a resolution of 4 cm^{-1}.

2.3.9. Thermal gravimetry analysis (TGA)

Thermal gravimetry analysis and differential thermal analysis (DTA) were used to study the thermal stability of different biosorbents. DuPont 951 TGA instrument was used to investigate the weight changes of biosorbents against temperature increase. The temperature of biosorbent samples increased from 20 to 500 °C at a rate of 20 °C/min. The temperature of the sample was kept at 500 °C for 10 minutes and finally the weight change curve was plotted in terms of temperature.

2.4. Treatment of industrial wastewater with biosorbent

Optimal biosorbents were used for chemical and physical treatment of industrial wastewater. For industrial wastewater treatment, a sample of industrial wastewater was prepared for the Urmia Industrial Town (Urmia, Iran). For treatment, 50 ml of industrial wastewater was poured into an Erlenmeyer (200 ml) and 5 g of biosorbent (the type of biosorbent was selected according to Table 2) was placed in it and according to the statistical design of Table 2 for several hours with a shaker (TM52E, Iran) was shaken. To investigate the effect of ultrasound on wastewater treatment, an ultrasound device (Sonic 6D) was used and according to the statistical design of Table 2, some samples were treated with ultrasound for 10 minutes. After treating the industrial wastewater with different biosorbents by centrifugation (Fixed 8-head centrifuge, Full digital, 4000 PRP), it was separated from the wastewater for 5 minutes. The chemical properties of wastewater, including total hardness, total heavy metals, nitrate, nitrite, BOD and COD, total dissolved solids (TDS) and salinity before and after biosorbent treatment were analyzed and the percentage of contaminant removal efficiency (PRE) was calculated using the following equation (Fig. 1).

$$PRE\ (\%) = \frac{C_0 - C_1}{C_0} \times 100 \qquad (5)$$

Where, C_0 is the concentration of contaminants before treatment and C_1 is the concentration of pollutants after treatment with biosorbent.

Fig. 1

2.4.1. Measurement of total heavy metals

Heavy metals including lead, barium, arsenic, mercury, cadmium and cream were studied as hazardous heavy metals in industrial wastewater. Metals can cause many diseases, including damage to the bones, nerves, digestive system and kidneys. Atomic absorption spectroscopy was used to measure these metals. To determine the concentration of these metals, flame atomic absorption (1200) with model (Aurora Company, Canada) and atomic absorption with graphite furnace was used.

2.4.2. Measurement of total hardness

To measure the total hardness, 50 ml of the wastewater (V_{sample}) was poured into a 100 ml Erlenmeyer flask and the pH was adjusted to 7. Then 1 ml of buffer with pH 10 was added into it. Then a few drops of Eriochrome Black T reagent were added to it and the color of the solution turned red. The solution was then titrated with EDTA 0.01 M until the color of the solution changed from red to blue. The volume of EDTA solution consumed (V_2) was recorded. This operation was performed exactly for the titration of distilled water and the volume of EDTA consumed (V_1) was recorded. Finally, the total hardness in terms of mg/L calcium carbonate was calculated from the following equation.

$$TH = \frac{(V_2 - V_1)\times 0.01\frac{mol}{lit}EDTA\times 100\frac{g}{1mol\ CaCO_3}\times 1000ml}{V_{sample}} \tag{6}$$

2.4.3. Measurement of BOD and COD

To measure BOD, BOD meter device (HACH's BOD TRAK, Austria) was used. To measure COD, samples must first be digested, as chemical digestion of samples is a prerequisite for calculating COD values. For this purpose, the DRB 200 thermal reactor of the Austrian company HACH was used. To read the COD, a spectrophotometer model DR 2800 of HACH Company (Austria) was used.

2.4.4. Measurement of TDS and salinity

To measure TDS, TDS meter (pH & TDS-METER, Crison MM 40, Spain) was used. For this purpose, the TDS meter was turned on and placed vertically and fixed inside the sample. You have to wait a few seconds for the water temperature to be adjusted by the sensors inside the device. Finally, by fixing the desired parameter number in the device, the test result was read. To measure salinity, a hand-held salinity meter model MT-128, made in China, was used.

2.4.5. Measurement of nitrite

Spectroscopic method was used to measure nitrite. To do this, 5 ml of sulfanilamide solution (0.2% W / W) and 3 ml of chloric acid solution (15% W/W) were added to 50 ml of industrial wastewater and mixed. The mixture was placed in the dark for 5 minutes at room temperature. Then 1 ml of alpha naphthyl ethylenediamine hydrochloride solution (0.1% W/W) was added and kept at room temperature for 10 minutes in a dark environment. Then the absorbance of the solution was recorded by spectrophotometer at 537 nm. Then the calibration curve of the relationship between the amount of nitrite in standard solutions and the amount of adsorption was plotted as follows and the amount of nitrite in industrial wastewater was calculated from the relationship of the calibration curve: 6 standard solutions containing alpha-naphthyl ethylenediamine hydrochloride (15% W/W), sulfanilamide (0.2% W/W), chloric acid (0.1% W/W), and different concentrations of sodium nitrite were prepared. The absorbance of the samples was recorded at 537 nm and the calibration curve was obtained.

2.4.6. Measurement of nitrate

To measure nitrate, the amount of nitrite was determined in the first step. In the second step to measure nitrate, the sample was transferred from inside the cadmium column and all nitrates were converted to nitrite. By nitrite measurement method, the total amount of nitrite in the sample and nitrite from nitrate reduction were determined. Finally, the amount of nitrate was calculated from the difference between the numbers of these two steps.

2.5. Statistical analysis

In this study, the central composite design (CCD) was used to investigate the effect of ethylenediamine tetraacetic acid, chlorophyll and nanoclay concentrations on physicochemical properties of biosorbents (Table 1-A). In the second part, to investigate the effect of biosorbent type and the effect of ultrasound on the reduction of industrial wastewater pollutants, a factorial statistical design with three replications (Table 1-B) was used. Data analysis of this section was performed at the 95 % probability level with the Design Expert-10 software. Mini-Tab-17 software and Tukey test were used to evaluate the significance of the mean results and one-way analysis of variance.

Table 1

3. Results and Discussion

Response surface statistical method was used to investigate the effect of EDTA, chlorophyll and clay nanoparticles on biosorbent physicochemical properties including thickness, mechanical properties, water solubility, water absorption, and antioxidant properties. Three-dimensional curves of the interaction of independent variables (EDTA, chlorophyll and clay nanoparticles) on biosorbent physicochemical properties were recorded with Design Expert-10 software. Mathematical models and regression coefficients between independent variables and dependent variables were calculated and the coefficient of influence of each independent variable on biosorbent physicochemical properties was investigated. Table 2 shows the mathematical models of the relationships between the independent and dependent variables.

Table 2

3.1. Investigation of thickness and mechanical properties

The thickness of polymeric biosorbents affects the mechanical properties and the ability to absorb chemicals and water. Due to the fact that polymeric biosorbents are used in harsh chemical and physical conditions, so the mechanical properties of these biosorbents are extremely important. In this study, the effect of three factors: EDTA, chlorophyll and clay nanoparticles on thickness and mechanical properties was investigated. Fig. 2 shows the three-dimensional curves of the effect of EDTA, chlorophyll and clay nanoparticles on thickness and mechanical properties (tensile strength and elongation at break).

As it turns out, chlorophyll did not have a significant effect on film thickness, but EDTA and clay increased film thickness. The effect of clay on increasing the biosorbent thickness was greater than EDTA. Due to the large amount of clay used in the preparation of biosorbent, an increase in thickness was expected in the presence of clay. Due to the dissolution of chlorophyll and EDTA in water, these materials fill the pores between the zein polymer chains and have little effect on the biosorbent thickness. The tensile strength of the biosorbent in the presence of clay increased sharply while EDTA and chlorophyll had no significant effect on the tensile strength. While the biosorbent EAB decreased in the presence of clay, chlorophyll and EDTA increased the EAB of the film. Due to the presence of mineral compounds such as aluminum, silica and magnesium as well as the presence of hydroxyl groups in the structure of clay, these compounds can cohesive the biosorbent structure and increase its tensile strength by creating hydrogen and electrostatic interactions. The same factors can also reduce the biosorbent elasticity. Due to the hydrophilic structure of chlorophyll and EDTA, these compounds can adsorb and retain more water molecules in the biosorbent structure. Due to the fact that in biopolymers, water molecules have a plasticizing role and increase the flexibility of these polymers, so the elasticity of the biosorbent in the presence of chlorophyll and EDTA has increased. Chen et al. (2014) have investigated the effect of various additives on the mechanical properties of saddle-based films, the results of which partially confirm the results of harmful research [25]. Nedi et al. (2012) investigated the mechanical and structural properties of zein film modified with clay nanoparticles. The results of the present study show a good agreement with the results of Nedi et al. [26].

Fig. 2

3.2. Investigation of solubility and water absorption capacity

Zein is soluble in alcohols such as furfuryl alcohol, tetrahydroforphoryl alcohol, glycols and aqueous alkaline solutions with a pH of 11.5 or higher. Zein is insoluble in water, anhydrous alcohols (except methanol) and acetone. The zein is rapidly denatured in solution and becomes insoluble. Due to the fact that most biosorbents are used to treat wastewater in aqueous media, so these biosorbents must have good resistance to dissolution in water and also be able to absorb water well to increase the level of contact of pollutants with biosorbents. The high contact area between the biosorbent and the wastewater increases the physical and chemical interactions between the adsorbent and the pollutants and leads to the effective removal of pollutants from the wastewater. Fig. 3 shows the three-dimensional graphs of the effect of EDTA, chlorophyll and clay nanoparticles on the solubility and the water adsorption capacity of biosorbent. As can be seen from the curves, clay has slightly reduced the biosorbent solubility, but EDTA and chlorophyll have somewhat increased the solubility. Due to the solubility of EDTA and chlorophyll, this result was expected. Although increasing the solubility of biosorbents in the presence of EDTA and chlorophyll is inappropriate, it should be noted that the gradual release of these two substances into wastewater will reduce the hardness of water, heavy metals and nitrate. On the other hand, clay has little effect on the water adsorption capacity of biosorbent, but EDTA and chlorophyll significantly increase water absorption, which is due to the hydrophilic nature of EDTA and chlorophyll. As mentioned earlier, increasing the water absorption capacity increases the interaction between biosorbent and wastewater and increases the efficiency of biosorbent in wastewater treatment. In a similar study, Asadzadeh and Pirsa (2020) investigated the physicochemical properties and the water adsorption capacity of biosorbent based on isolated soy protein/lycopene pigment, which confirms the results of the present study [9]. Zong et al. (2018) designed the biosorbent of lignin and investigated its physicomechanical properties and solubility. The results of their research are in good agreement with the present study [27].

Fig. 3

3.3. Investigation of total hardness reduction capacity and antioxidant properties

One of the most important factors in wastewater is the presence of water hardness and oxidizing agents that have a great impact on the health of the facility and the environment. Due to the presence of EDTA in the biosorbent structure, this biosorbent will have a high ability to remove the total hardness of wastewater and also due to the antioxidant properties of chlorophyll, the designed biosorbent will be able to reduce oxidizing agents in wastewater. Fig. 4 shows the three-dimensional diagrams of the effect of EDTA, chlorophyll and clay nanoparticles on the total hardness reduction capacity and antioxidant properties. The results show that clay and chlorophyll do not have a significant effect on the ability to remove the total hardness of wastewater, while EDTA has greatly increased the ability to remove the hardness of total wastewater. Because EDTA easily complexes with total hardness factors (calcium and magnesium), the biosorbent containing EDTA can effectively treat total hardness factors (calcium and magnesium) in wastewater. Also, due to the fact that the clay and chlorophyll do not have a specific physical and chemical interaction with total hardness factors (calcium and magnesium), they do not have much effect on it. By examining the antioxidant properties curves, it was found that all three factors of EDTA, chlorophyll and clay nanoparticles are effective on the antioxidant properties of the adsorbent. Chlorophyll has good carbon-carbon double bonds with good antioxidant properties, which increases the antioxidant properties of chlorophyll-containing biosorbents. Regarding the effect of clay on its antioxidant properties, it can be said that clay contains large amounts of mineral metals that these metals can increase the antioxidant properties of biosorbents by electrostatic adsorption or physical adsorption of DPPH free radicals. Increased antioxidant properties in the presence of EDTA may also be related to physical interactions between free radicals and EDTA. Singh et al. (2012) used EDTA-modified Chitosan film to reduce heavy metals, the results of which confirm the results of the present study [28]. Lanfer-Marquez et al. (2005) investigated and confirmed the antioxidant properties of chlorophyll and its derivatives [29].

Fig. 4

3.4. Study of SEM, FTIR and TGA

Fig. 5 shows the SEM images, the FTIR spectra, the TGA and DTA spectra of the adsorbent and its composites. The results of SEM images show that pure zein adsorbent has a smooth and uniform surface with cracks in some parts. With the addition of EDTA and chlorophyll, the surface gaps on the zein biosorbent are filled and a more uniform surface is created. In zein biodegradation modified with clay nanoparticles, the presence of clay particles in the dimensions of 40 to 100 nm at the biosorbent surface is easily detectable. In this film, there are no gaps in the biosorbent of pure saddle, which indicates that the addition of clay particles to the biosorbent structure has caused more coherence of its structure, which confirms the results of mechanical properties. The presence of clay nanoparticles in Ze/EDTA/Chl/Clay is not easily detectable, which is probably due to the fact that these nanoparticles are surrounded by chlorophyll and EDTA pigments. It can also be said that the lowest surface gap is related to Ze/EDTA/Chl/Clay biosorbent, which indicates that clay, chlorophyll and EDTA had a synergistic effect in filling the biosorbent surface gaps. Luís et al. (2019) have investigated the surface morphology of biodegradable films based on zein, the results of which confirm the results of the present study [30].

By studying the FTIR spectra, electrostatic and physical interactions between the components of the zein composite were investigated. In the pure zein biosorbent FTIR spectrum, the peak at 3300 cm^{-1} indicates tensile vibrations related to OH and NH. The two peaks at 2875 and 2955 correspond to the CH tensile vibrations in the $R\text{-}CH_2\text{-}CH_3$ and CH_3 groups. The peak at 1650 corresponds to the tensile vibrations C=C for the $R\text{-}CH{=}CH_2$ groups. Peak at 1530 is related to off-plane vibrations of the NH group. The 1450 peak corresponds to C-C vibrations in aromatic rings. Peak at 1170 is related to the vibrations of the $R\text{-}NH_2$ and $R_2\text{-}NH$ groups. By comparing the spectrum of pure zein with its various composites, it is clear that all peaks related to pure zein can be seen in its composites, but these peaks have shifted to different wave numbers, which indicates the creation of electrostatic interactions between zein, chlorophyll, EDTA and clay nanoparticles. In the zein spectrum containing EDTA, a new peak was created in 1710, which confirms the presence of EDTA in the composite structure. This peak is related to the C=O tensile vibrations in EDTA. In chlorophyll-containing zein spectra, the peak intensity of the 3250 region is also significantly reduced, probably due to the fact that different OH and NH groups in the zein and chlorophyll have interacted by hydrogen bonds and affect NH and OH vibrations. In the spectra of composites containing nanoclay, two peaks have been created in 550 and 950 wave numbers, which indicate the presence of clay nanoparticles in the composite structure. Chen et al. (2014) examined the FTIR spectrum of zein based composites. The peaks that appeared and the wave numbers recorded in this study are consistent with the reported results [31].

The effect of chlorophyll, nanoclay and EDTA on biosorbent thermal stability was investigated by studying TGA and DTA spectra. The study of the spectrum of pure zein and its composites showed that in all biosorbents weight loss is observed in two areas that the weight loss of the first stage occurs in the temperature range of 80 to 150 °C, which is related to the evaporation of volatile compounds in the structure of biosorbents as well as moisture in the structure of biosorbents. The second weight loss occurs in the temperature range of 250 to 350 ° C, which is related to the destruction of the overall biosorbent structure. By comparing the different biosorbent spectra, it was found that the addition of EDTA and chlorophyll reduced the degradation temperature of the second stage, so these materials weakened the thermal stability of the biosorbent. Since these biosorbents are used for wastewater treatment at low temperatures, the reduction of thermal stability is not important given the enormous benefits of EDTA and chlorophyll in biosorbents. On the other hand, the addition of clay nanoparticles has increased the thermal stability so that in the biosorbent containing EDTA, chlorophyll and nanoclay, the thermal stability of the biosorbent was higher than that of pure zein. Due to the presence of metal elements in the nanoclay structure and the possibility of chelation between metals and EDTA, these structures can also increase the thermal stability of the biosorbent. Pereira et al. (2019) investigated the antimicrobial and thermal properties of zein composites. The TGA spectra of zein composites are in good agreement with the results of present research [32].

Fig. 5

3.5. Removal of pollutants from industrial wastewater with biosorbent

3.5.1. Reduction of COD, BOD, TDS and salinity

BOD of wastewater is the amount of biological oxygen demand of wastewater. The aerobic microorganisms in the effluents use the oxygen in the wastewater to decompose the organic matter, and finally biological treatment occurs. The amount of oxygen needed to be consumed by all microorganisms in wastewater is called BOD. Based on a general relationship, it can be determined that the higher the BOD, the higher the organic matter in the wastewater, and finally it can be said that the effluent has lower quality. Other factors for measuring the quality of chemical and industrial wastewater include chemical oxygen demand (COD). The amount of oxygen needed to oxidize the chemicals in the wastewater is called chemical oxygen demand or COD. Like BOD, this factor indicates the quality of the effluent, and the higher the amount, the greater the presence of chemical contaminants. Total dissolved solid (TDS) or the sum of all water-soluble organic and inorganic substances are one of the most important factors in standardizing the quality of drinking water. The large amount of suspended solids (organic and inorganic substances) in water leads to a decrease in water quality. Excessive presence of these substances in water will have an adverse effect on its quality and will change its smell, taste and color. According to international standards, the unit of measurement of TDS is ppm or milligrams per liter. It should be noted that the standard TDS in drinking water should be in the range of 20 to 90 ppm and increasing or decreasing the amount of the above range will cause several problems for human health. Wastewater salinity is the amount of water-soluble salts. These salts are composed of compounds such as sodium chloride, magnesium sulfate, potassium nitrate and baking soda, which dissolve in water. Fig. 6 shows the three-dimensional curves of the effect of biosorbent type and ultrasound on BOD, COD, TDS and salinity of industrial wastewater. In all four parameters studied, ultrasound had a positive effect of 15 to 30% on the efficiency of all types of biosorbents in reducing pollutants. Ultrasonic causes very small gaps in the biosorbent polymer chains and by accelerating the movement of the wastewater molecule, it increases the contact surface of the biosorbent and the wastewater, and thus can increase the efficiency of wastewater treatment. Comparing the effect of biosorbents on the efficiency of industrial wastewater treatment from the perspective of BOD, COD, TDS and salinity, it was found that clay did not have a significant effect on reducing BOD, COD, TDS and salinity, but EDTA and chlorophyll had a significant effect. The effect of EDTA was greater than chlorophyll. Chlorophyll can have a positive effect on reducing BOD, COD, and TDS by inactivating chemical oxidizing agents (due to the antioxidant nature of chlorophyll). EDTA can also form cyclic complexes with many metals to remove them from the environment, reducing TDS and salinity. In addition, the circular structures formed are a good environment for trapping chemical and biological compounds that can lead to a reduction in BOD and COD.

In a similar study, Pirsa and Asadzadeh (2020) synthesized magnetic biosorbents and investigated its effect on the reduction of BOD and COD, the results of which confirm the results of the present study [33]. Mishra et al. (2021) have used biodegradable hydrogels to reduce salinity and TDS of wastewater, the results of which are in good agreement with the results of the present study [34].

Fig. 6

3.5.2. Reduction of total heavy metals, total hardness, nitrite and nitrate

Fig. 7 shows the three-dimensional columnar curves of the effect of biosorbent type and ultrasound on total heavy metals, total hardness, nitrite and nitrate. In the study of all 4 parameters, the use of ultrasonic in wastewater treatment has increased the treatment efficiency by 15 to 25%, so the use of ultrasonic has a good effect on biosorbent efficiency. Examination of the curves of total heavy metals and total hardness of wastewater shows that biosorbents containing EDTA have a great impact on the removal efficiency of total heavy metals and total hardness of wastewater. Due to the ability to form a complex between EDTA and magnesium, calcium and heavy metals, the presence of EDTA in the biosorbent structure has greatly increased the ability of the biosorbent to remove a variety of metals. It can be said that Ze/EDTA and Ze/EDTA/Chl/Clay biosorbents have specifically reduced the total heavy metals and total water hardness. Examination of wastewater nitrite and nitrate curves shows that chlorophyll-containing biosorbents have a high ability to remove these two hazardous chemical compounds from wastewater. While pure zein adsorbents and chlorophyll-free biosorbents have little ability to reduce nitrite and nitrate. By comparing the ability of chlorophyll-containing biosorbents (Ze/Chl and Ze/EDTA/Chl/Clay) to remove nitrite and nitrate, it was found that these biosorbents have a very high selectivity in nitrate removal compared to nitrite. In other words, Ze/Chl and Ze/EDTA/Chl/Clay biosorbents can specifically remove nitrate from industrial wastewater. Given that nitrate is an oxidizing chemical, and chlorophyll is a pigment that can react with oxidizing compounds and destroy the chemical structure of chlorophyll, it can be said that chlorophyll purifies nitrate from wastewater by reducing nitrate. Fig. 8 shows the chemical structure of the two types of chlorophyll as well as the possible mechanism of reaction of chlorophyll with sodium nitrate. Oxidation of chlorophyll with nitrate causes the destruction and opening of the annular part of the chlorophyll, which this degradation structure is likely to change the color of chlorophyll from green to yellow.

Chavoshizadeh et al. (2020) investigated the effect of nitric acid and sodium nitrate in acidic environment on the color of chlorophyll-containing gluten film. They reported that chlorophyll-containing films had a high ability to absorb and reduce nitrate. The results of Chavoshizadeh et al., fully confirm the results of the present study [18]. Zhao et al. (2015) used the beta-cyclodextrin-EDTA complex to remove heavy metals and cationic pigments from wastewater, the results of which are in good agreement with the results of the present study [35].

Fig. 7

Fig. 8

3.5.3. Optimization of purification conditions using numerical optimization ramps

Numerical optimization ramps method was used to obtain the best biosorbent and most suitable wastewater treatment conditions. The optimization module searches for a combination of factor levels that simultaneously satisfy the criteria placed on each of the responses and factors. To include a response in the optimization criteria, it must have a model fit through analysis or supplied via an equation only a simulation. Factors are automatically included “in range”. Numerical optimization uses the models to search the factor space for the best trade-offs to achieve multiple goals. Graphical optimization uses the models to show the volume where acceptable response outcomes can be found. Fig. 9 shows the numerical optimization ramps. Ramps are a graphical view of each optimal solution. Optimal factor settings are shown with red points. Optimal response prediction values are displayed in blue. Responses with models, but no goals are shown with gray. The Factors Tool is used to control which solution is shown in the graph area. Stepping through the list of solutions is a good way to explore how the factor settings effect the response. According to the results obtained from the study of optimal conditions, the Ze/EDTA/Chl/Clay biosorbent was the best adsorbent, which has the highest efficiency in removing all wastewater pollutants. Due to the fact that this biosorbent has both chlorophyll, which has the ability to remove nitrate and nitrite and reduce BOD and COD, and EDTA, which can reduce the total hardness and total heavy metals. The use of ultrasound has a good effect on wastewater treatment. Under optimal conditions, THM 57.5%, TH 52.5%, COD 61.1%, BOD 43%, TDS 73.7%, salinity 52.8%, nitrite 25% and nitrate s 67% were removed from industrial wastewater by Ze/EDTA/Chl/Clay biosorbent. Gbashi et al. (2019) have used the Numerical Optimization method to obtain the optimal degradation conditions for mycotoxins in the food industry, the results of which partially confirm the results of recent research [36].

Fig. 9

4. Conclusion

Corn zein polymer adsorbent was prepared and modified using chlorophyll, EDTA and clay nanoparticles. 5 different types of biosorbents were used for industrial wastewater treatment. The results confirmed the homogeneous and cohesive structure of different zein adsorbents. FTIR results showed physical and electrostatic interactions between composite biosorbent components. Nanoclay increased the thermal stability of the biosorbent. Chlorophyll and EDTA caused the biosorbent ability to absorb more water and reduce the total hardness of water. Clay nanoparticles increased the tensile strength of the biosorbent and chlorophyll and EDTA increased the biosorbent elongation. Ze/EDTA/Chl/Clay biosorbent was recognized as the best adsorbent under optimal wastewater treatment conditions. The use of ultrasound in wastewater treatment had a good effect. Under optimal conditions, THM 57.5%, TH 52.5%, COD 61.1%, BOD 43%, TDS 73.7%, salinity

52.8%, nitrite 25% and nitrate 67% were removed from the industrial wastewater. By comparing the ability of chlorophyll-containing biosorbents (Ze/Chl and Ze/EDTA/Chl/Clay) to remove nitrite and nitrate, it was found that these biosorbents have a very high selectivity in nitrate removal compared to nitrite. Finally, it can be claimed that the biosorbents Ze/EDTA/Chl/Clay specifically remove nitrate from industrial wastewater.

References

[1] Patwardhan, A.D., 2017. Industrial wastewater treatment. PHI Learning Pvt. Ltd..

[2] Orhon, D., Babuna, F.G. and Karahan, O., 2009. Industrial wastewater treatment by activated sludge. IWA Publishing.

[3] Gunatilake, S.K., 2015. Methods of removing heavy metals from industrial wastewater. Methods, 1(1), p.14.

[4] Hu, J., Chen, G. and Lo, I.M., 2006. Selective removal of heavy metals from industrial wastewater using maghemite nanoparticle: performance and mechanisms. Journal of environmental engineering, 132(7), pp.709-715.

[5] Mohod, C.V. and Dhote, J., 2013. Review of heavy metals in drinking water and their effect on human health. International Journal of Innovative Research in Science, Engineering and Technology, 2(7), pp.2992-2996.

[6] Mostafavi, S., Rezaverdinejad, V. and Pirsa, S., 2019. Design and fabrication of nanocomposite-based polyurethane filter for improving municipal waste water quality and removing organic pollutants. Adsorption Science & Technology, 37(1-2), pp.95-112.

[7] Ma, L., Hu, L., Feng, X. and Wang, S., 2018. Nitrate and nitrite in health and disease. Aging and disease, 9(5), p.938.

[8] Taziki, M., Ahmadzadeh, H., Murry, M.A. and Lyon, S.R., 2015. Nitrate and nitrite removal from wastewater using algae. Current Biotechnology, 4(4), pp.426-440.

[9] Asadzadeh, F. and Pirsa, S., 2020. Specific removal of nitrite from Lake Urmia sediments by biohydrogel based on isolated soy protein/tragacanth/mesoporous silica nanoparticles/lycopene. Global Challenges, 4(12), p.2000061.

[10] Jabraili, A., Pirsa, S., Pirouzifard, M.K. and Amiri, S., 2021. Biodegradable nanocomposite film based on gluten/silica/calcium chloride: physicochemical properties and bioactive compounds extraction capacity. Journal of Polymers and the Environment, pp.1-15.

[11] Kumar, A., Wang, X., Nune, K.C. and Misra, R.D.K., 2017. Biodegradable hydrogel-based biomaterials with high absorbent properties for non-adherent wound dressing. International wound journal, 14(6), pp.1076-1087.

[12] Kunioka, M., 2004. Biodegradable water absorbent synthesized from bacterial poly (amino acid) s. Macromolecular bioscience, 4(3), pp.324-329.

[13] Pirsa, S., Mohtarami, F. and Kalantari, S., 2020. Preparation of biodegradable composite starch/tragacanth gum/nanoclay film and study of its physicochemical and mechanical properties. Chemical Review and Letters, 3(3), pp.98-103.

[14] Mohammadi, B., Pirsa, S. and Alizadeh, M., 2019. Preparing chitosan–polyaniline nanocomposite film and examining its mechanical, electrical, and antimicrobial properties. Polymers and Polymer Composites, 27(8), pp.507-517.

[15] Pirsa, S., Farshchi, E. and Roufegarinejad, L., 2020. Antioxidant/antimicrobial film based on carboxymethyl cellulose/gelatin/TiO 2–Ag nano-composite. Journal of Polymers and the Environment, 28(12), pp.3154-3163.

[16] Pirsa, S., 2020. Biodegradable film based on pectin/Nano-clay/methylene blue: Structural and physical properties and sensing ability for measurement of vitamin C. International Journal of Biological Macromolecules, 163, pp.666-675.

[17] Ferruzzi, M.G., Böhm, V., Courtney, P.D. and Schwartz, S.J., 2002. Antioxidant and antimutagenic activity of dietary chlorophyll derivatives determined by radical scavenging and bacterial reverse mutagenesis assays. Journal of food Science, 67(7), pp.2589-2595.

[18] Chavoshizadeh, S., Pirsa, S. and Mohtarami, F., 2020. Sesame oil oxidation control by active and smart packaging system using wheat gluten/chlorophyll film to increase shelf life and detecting expiration date. European Journal of Lipid Science and Technology, 122(3), p.1900385.

[19] Sharifi, K.A. and Pirsa, S., 2021. Biodegradable film of black mulberry pulp pectin/chlorophyll of black mulberry leaf encapsulated with carboxymethylcellulose/silica nanoparticles: Investigation of physicochemical and antimicrobial properties. Materials Chemistry and Physics, p.124580.

[20] Zhang, W., Huang, H., Tan, F., Wang, H. and Qiu, R., 2010. Influence of EDTA washing on the species and mobility of heavy metals residual in soils. Journal of hazardous materials, 173(1-3), pp.369-376.

[21] Hanay, O., Hasar, H. and Kocer, N.N., 2009. Effect of EDTA as washing solution on removing of heavy metals from sewage sludge by electrokinetic. Journal of hazardous materials, 169(1-3), pp.703-710.

[22] Ait-Mouheb, N., Schillings, J., Al-Muhammad, J., Bendoula, R., Tomas, S., Amielh, M. and Anselmet, F., 2019. Impact of hydrodynamics on clay particle

deposition and biofilm development in a labyrinth-channel dripper. Irrigation Science, 37(1), pp.1-10.

[23] Jiang, Y., Khan, A., Huang, H., Tian, Y., Yu, X., Xu, Q., Mou, L., Lv, J., Zhang, P., Liu, P. and Deng, L., 2019. Using nano-attapulgite clay compounded hydrophilic urethane foams (AT/HUFs) as biofilm support enhances oil-refinery wastewater treatment in a biofilm membrane bioreactor. Science of the Total Environment, 646, pp.606-617.

[24] Asdagh, A. and Pirsa, S., 2020. Bacterial and oxidative control of local butter with smart/active film based on pectin/nanoclay/Carum copticum essential oils/β-carotene. International Journal of Biological Macromolecules, 165, pp.156-168.

[25] Chen, Y., Ye, R. and Liu, J., 2014. Effects of different concentrations of ethanol and isopropanol on physicochemical properties of zein-based films. *Industrial Crops and Products*, *53*, pp.140-147.

[26] Nedi, I., Di Maio, E. and Iannace, S., 2012. The role of protein–plasticizer–clay interactions on processing and properties of thermoplastic zein bionanocomposites. Journal of Applied Polymer Science, 125(S2), pp.E314-E323.

[27] Zong, E., Huang, G., Liu, X., Lei, W., Jiang, S., Ma, Z., Wang, J. and Song, P., 2018. A lignin-based nano-adsorbent for superfast and highly selective removal of phosphate. Journal of Materials Chemistry A, 6(21), pp.9971-9983.

[28] Singh, K., Suri, R., Tiwary, A.K. and Rana, V., 2012. Chitosan films: crosslinking with EDTA modifies physicochemical and mechanical

properties. Journal of Materials Science: Materials in Medicine, 23(3), pp.687-695.

[29] Lanfer-Marquez, U.M., Barros, R.M. and Sinnecker, P., 2005. Antioxidant activity of chlorophylls and their derivatives. Food Research International, 38(8-9), pp.885-891.

[30] Luís, Â., Domingues, F. and Ramos, A., 2019. Production of hydrophobic zein-based films bioinspired by the lotus leaf surface: characterization and bioactive properties. *Microorganisms*, *7*(8), p.267.

[31] Chen, Y., Ye, R. and Liu, J., 2014. Effects of different concentrations of ethanol and isopropanol on physicochemical properties of zein-based films. Industrial Crops and Products, 53, pp.140-147.

[32] Pereira, L.A.S., Pagnossa, J.P., Miranda, K.W.E., Medeiros, E.S., Piccoli, R.H. and Oliveira, J.E.D., 2019. Antimicrobial zein coatings plasticized with garlic and thyme essential oils. Brazilian Journal of Food Technology, 22.

[33] Pirsa, S., Asadzadeh, F. and Sani, I.K., 2020. Synthesis of Magnetic Gluten/Pectin/Fe 3 O 4 Nano-hydrogel and Its Use to Reduce Environmental Pollutants from Lake Urmia Sediments. Journal of Inorganic and Organometallic Polymers and Materials, 30(8), pp.3188-3198.

[34] Mishra, A., Nath, A., Pande, P.P. and Shankar, R., 2021. Treatment of gray wastewater and heavy metal removal from aqueous medium using hydrogels

based on novel crosslinkers. Journal of Applied Polymer Science, 138(16), p.50242.

[35] Zhao, F., Repo, E., Yin, D., Meng, Y., Jafari, S. and Sillanpää, M., 2015. EDTA-cross-linked β-cyclodextrin: an environmentally friendly bifunctional adsorbent for simultaneous adsorption of metals and cationic dyes. Environmental Science & Technology, 49(17), pp.10570-10580.

[36] Gbashi, S., Madala, N.E., De Saeger, S., De Boevre, M. and Njobeh, P.B., 2019. Numerical optimization of temperature-time degradation of multiple mycotoxins. Food and Chemical Toxicology, 125, pp.289-304.

Figure legends:

Fig.1. Preparation of biosorbent and treatment of industrial wastewater with prepared biosorbent

Fig. 2. Three-dimensional curves of the effect of EDTA, chlorophyll and clay nanoparticles on the thickness and mechanical properties of biosorbents

Fig. 3. Three-dimensional diagrams of the effect of EDTA, chlorophyll and clay nanoparticles on solubility and water absorption capacity

Fig. 4. Three-dimensional diagrams of the effect of EDTA, chlorophyll and clay nanoparticles on the total hardness reduction capacity and antioxidant properties

Fig. 5. SEM (A) images, FTIR spectra (B), TGA and DTA (C) spectra of zein biosorbent and its composites

Fig. 6. Three-dimensional columnar curves of the effect of biosorbent type and ultrasound on BOD, COD, TDS and salinity of industrial wastewater

Fig.7. Three-dimensional columnar curves of the effect of biosorbent type and ultrasound on total heavy metals, total hardness, nitrite and nitrate

Fig. 8. Chemical structure of two types of chlorophyll and possible mechanism of reaction of chlorophyll with sodium nitrate

Fig. 9. Numerical optimization ramps and the obtained optimum condition

Table 1- A. Biosorbents prepared using central composite statistical design

Run	A: EDTA (g)	B: Chl (μl)	C: Clay (%)
1	2	0	1
2	0	200	0
3	4	100	1
4	2	100	1
5	0	200	2
6	0	0	2
7	4	200	2
8	2	100	1
9	2	100	1
10	2	100	1
11	4	0	0
12	4	0	2
13	2	100	1
14	4	200	0
15	0	100	1
16	2	100	0
17	0	0	0
18	2	100	2
19	2	200	1
20	2	100	1

Table 1-B. List of tests used to remove pollutants from industrial wastewater

Run	Bio-absorbent type	Ultrasound

1	Pure Ze	N-Ul*
2	Ze/EDTA/Chl/Clay	N-Ul
3	Ze/Chl	N-Ul
4	Ze/EDTA/Chl/Clay	Ul
5	Ze/Chl	Ul
6	Ze/EDTA	Ul
7	Ze/Clay	N-Ul
8	Pure Ze	Ul
9	Ze/EDTA	N-Ul
10	Ze/Clay	Ul

*Ul; using ultrasound, N-Ul; Not-using ultrasound

Table 2. Mathematical models and regression coefficients between independent and dependent variables

Response	Equation	R^2	$AdjR^2$
Thickness (μm)	=0.3495+0.066*A+0.013*B+0.102*C	0.915	0.910
TS (MPa)	=12.33-1.9*A-1.2*B+3.55*C+0.75*AB-0.5*AC+0.25*BC-0.09*A^2+0.40*B^2-2.34*C^2	0.878	0.861
EAB (%)	=58.9+18.9*A+10.7* B-7.7*C	0.817	0.751
Solubility (%)	=22.21+4.6*A+6.5*B-0.9*C-0.62*AB+0.12*AC-0.125*BC+0.45*A^2-1.04 B^2-1.04*C^2	0.965	0.931
WAC (%)	=67.75+19.9*A+9.9*B-2.1*C-0.62*AB+0.37*AC+0.37*BC	0.898	0.894
THRC (%)	=51.3+40.1*A-0.6*B-1.6*C	0.793	0.882
Antioxidant activity (%)	=61.1+0.7*A+7.4*B-8*C-5.75*AB+11.25*AC+3.5*BC	0.983	0.972

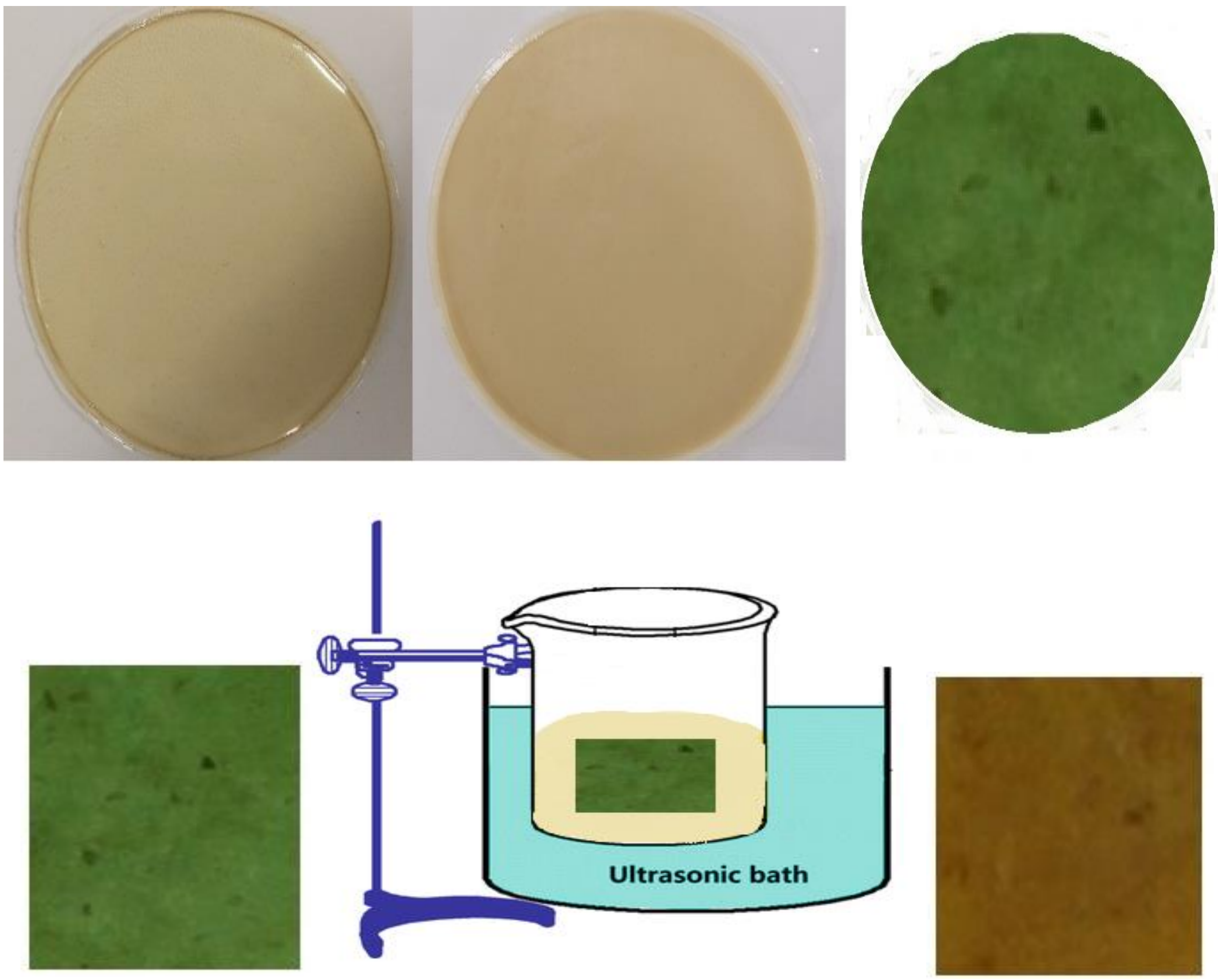


Fig. 1

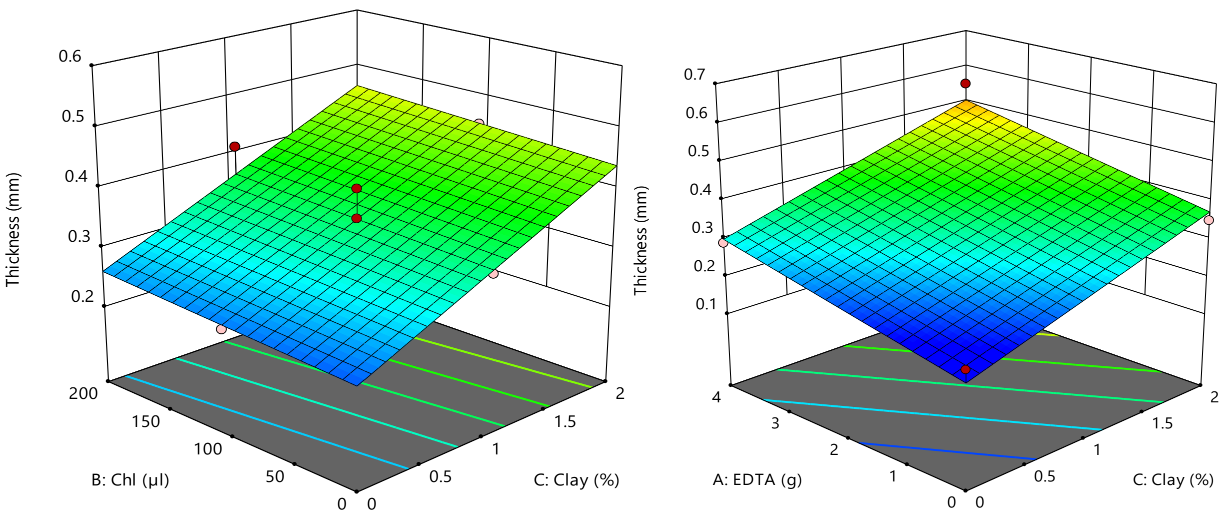

0.6
0.5
0.4
0.3
0.2
Thickness (mm)
200
150
100
50
0
B: Chl (µl)
0
0.5
1
1.5
2
C: Clay (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Thickness (mm)
4
3
2
1
0
A: EDTA (g)
0
0.5
1
1.5
2
C: Clay (%)

Fig. 2

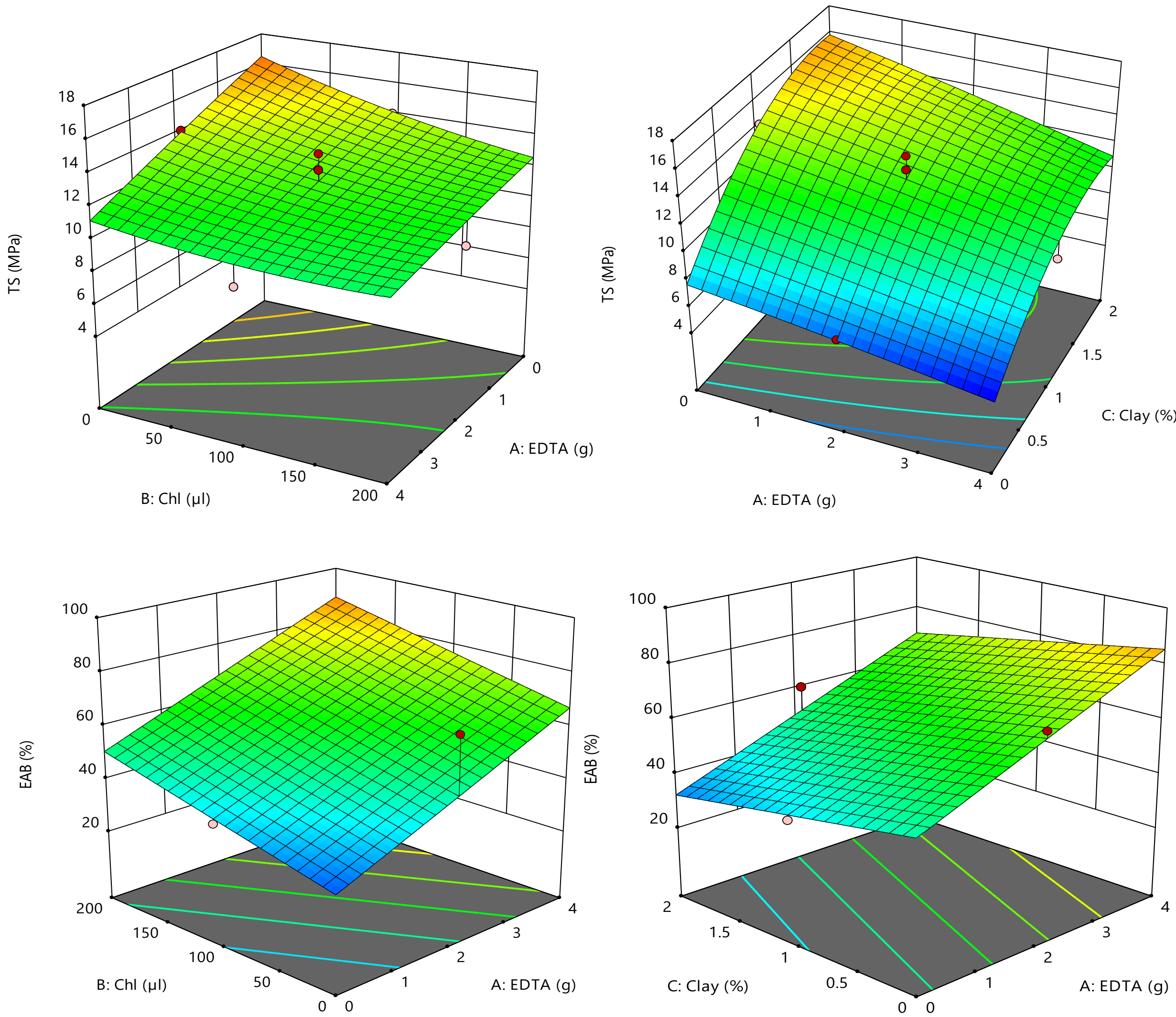

TS (MPa)
B: Chl (µl)
A: EDTA (g)
TS (MPa)
A: EDTA (g)
C: Clay (%)
EAB (%)
B: Chl (µl)
A: EDTA (g)
EAB (%)
C: Clay (%)
A: EDTA (g)

Fig. 2

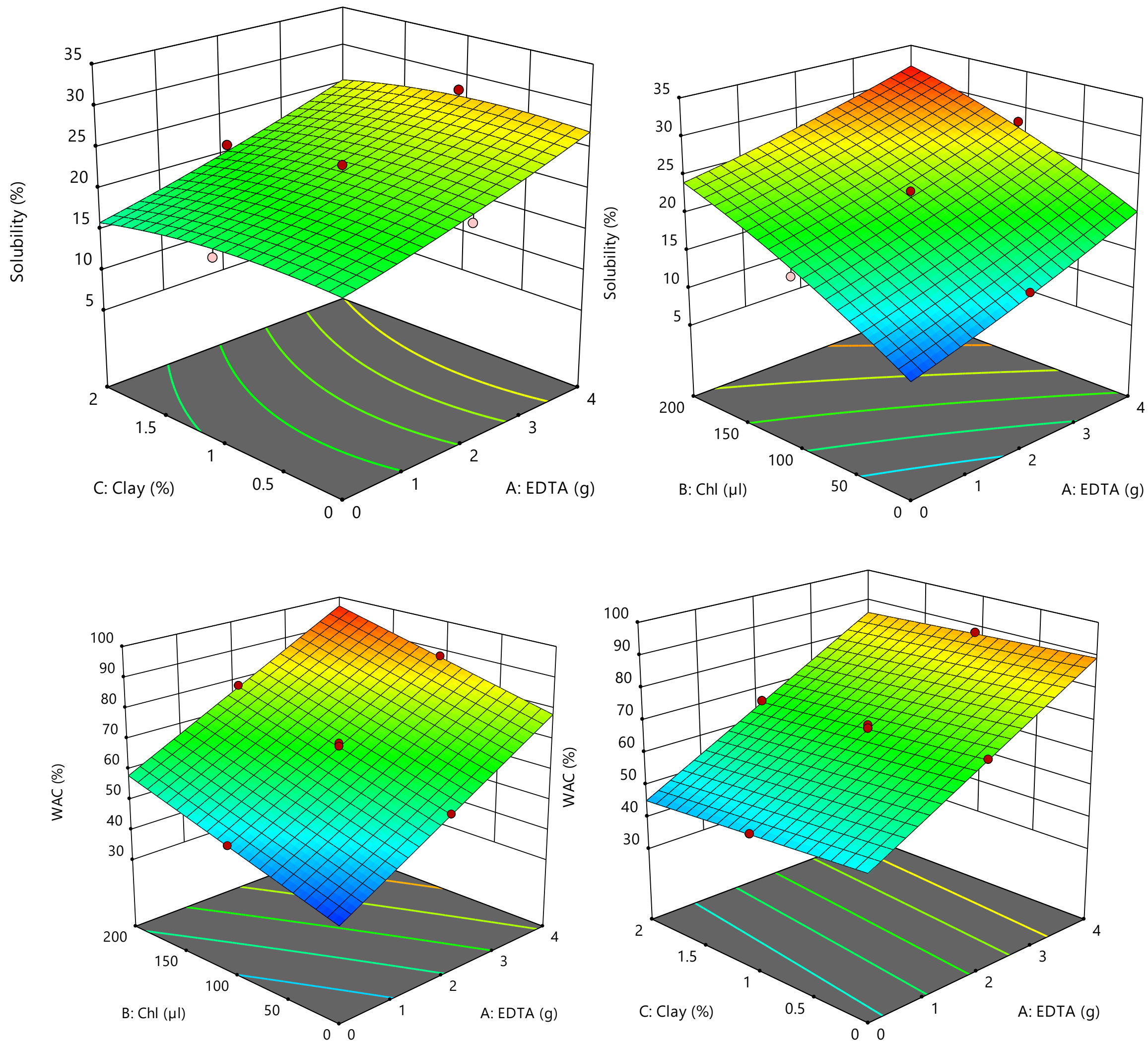
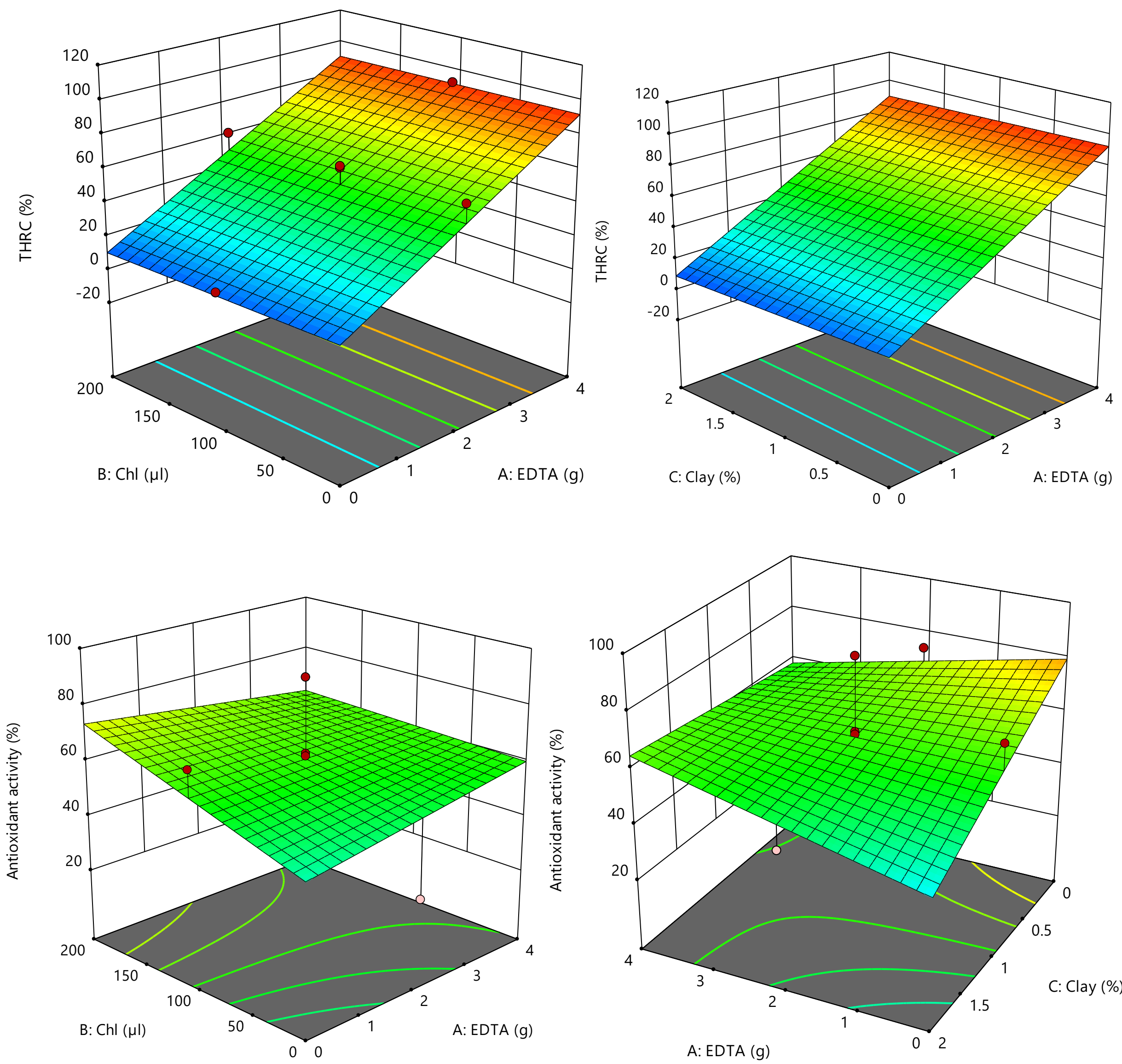

Solubility (%)
C: Clay (%)
A: EDTA (g)
Solubility (%)
B: Chl (µl)
A: EDTA (g)
WAC (%)
B: Chl (µl)
A: EDTA (g)
WAC (%)
C: Clay (%)
A: EDTA (g)

Fig. 3



THRC (%)
120
100
80
60
40
20
0
-20
B: Chl (µl)
200
150
100
50
0
A: EDTA (g)
0
1
2
3
4
C: Clay (%)
2
1.5
1
0.5
0
Antioxidant activity (%)

Fig. 4

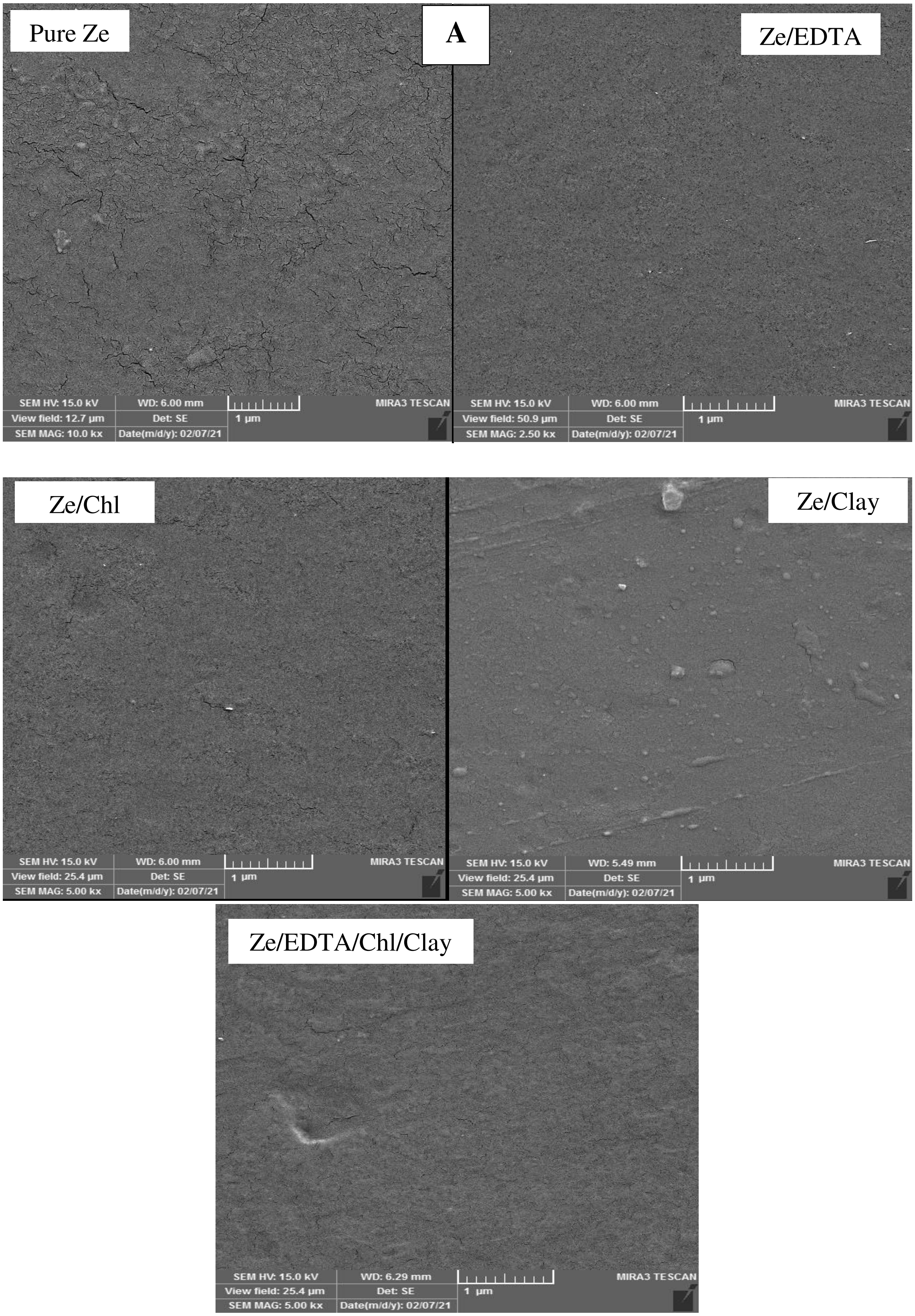
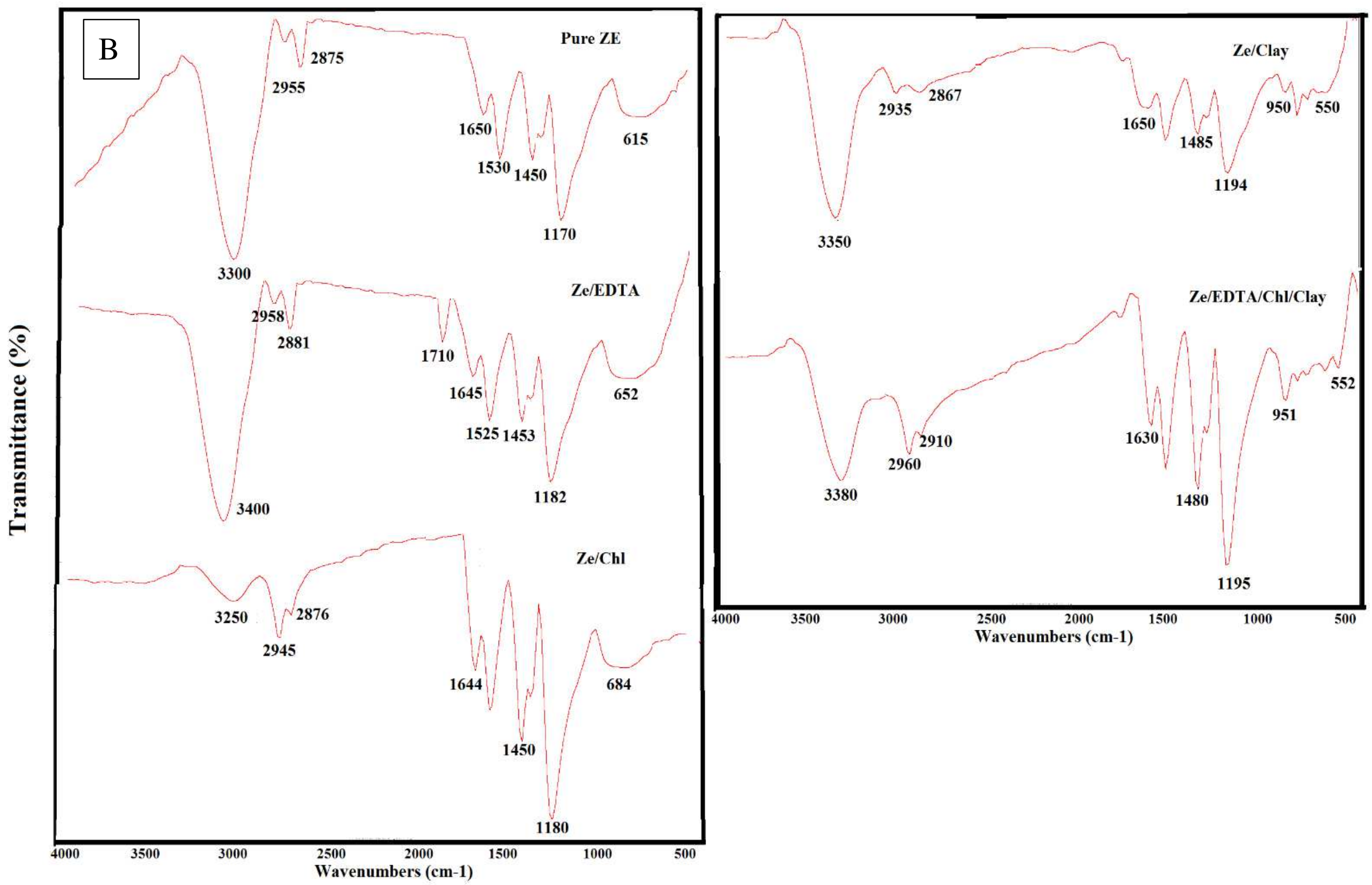

A
Pure Ze
SEM HV: 15.0 kV
WD: 6.00 mm
MIRA3 TESCAN
View field: 12.7 µm
Det: SE
1 µm
SEM MAG: 10.0 kx
Date(m/d/y): 02/07/21
Ze/EDTA
SEM HV: 15.0 kV
WD: 6.00 mm
MIRA3 TESCAN
View field: 50.9 µm
Det: SE
1 µm
SEM MAG: 2.50 kx
Date(m/d/y): 02/07/21
Ze/Chl
SEM HV: 15.0 kV
WD: 6.00 mm
MIRA3 TESCAN
View field: 25.4 µm
Det: SE
1 µm
SEM MAG: 5.00 kx
Date(m/d/y): 02/07/21
Ze/Clay
SEM HV: 15.0 kV
WD: 5.49 mm
MIRA3 TESCAN
View field: 25.4 µm
Det: SE
1 µm
SEM MAG: 5.00 kx
Date(m/d/y): 02/07/21
Ze/EDTA/Chl/Clay
SEM HV: 15.0 kV
WD: 6.29 mm
MIRA3 TESCAN
View field: 25.4 µm
Det: SE
1 µm
SEM MAG: 5.00 kx
Date(m/d/y): 02/07/21

Fig. 5



B
Pure ZE
2955
2875
3300
1650
1530
1450
1170
615
Ze/EDTA
2958
2881
3400
1710
1645
1525
1453
1182
652
Ze/Chl
3250
2945
2876
1644
1450
1180
684
Transmittance (%)
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)
Ze/Clay
2935
2867
3350
1650
1485
1194
950
550
Ze/EDTA/Chl/Clay
3380
2960
2910
1630
1480
1195
951
552
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

Fig. 5

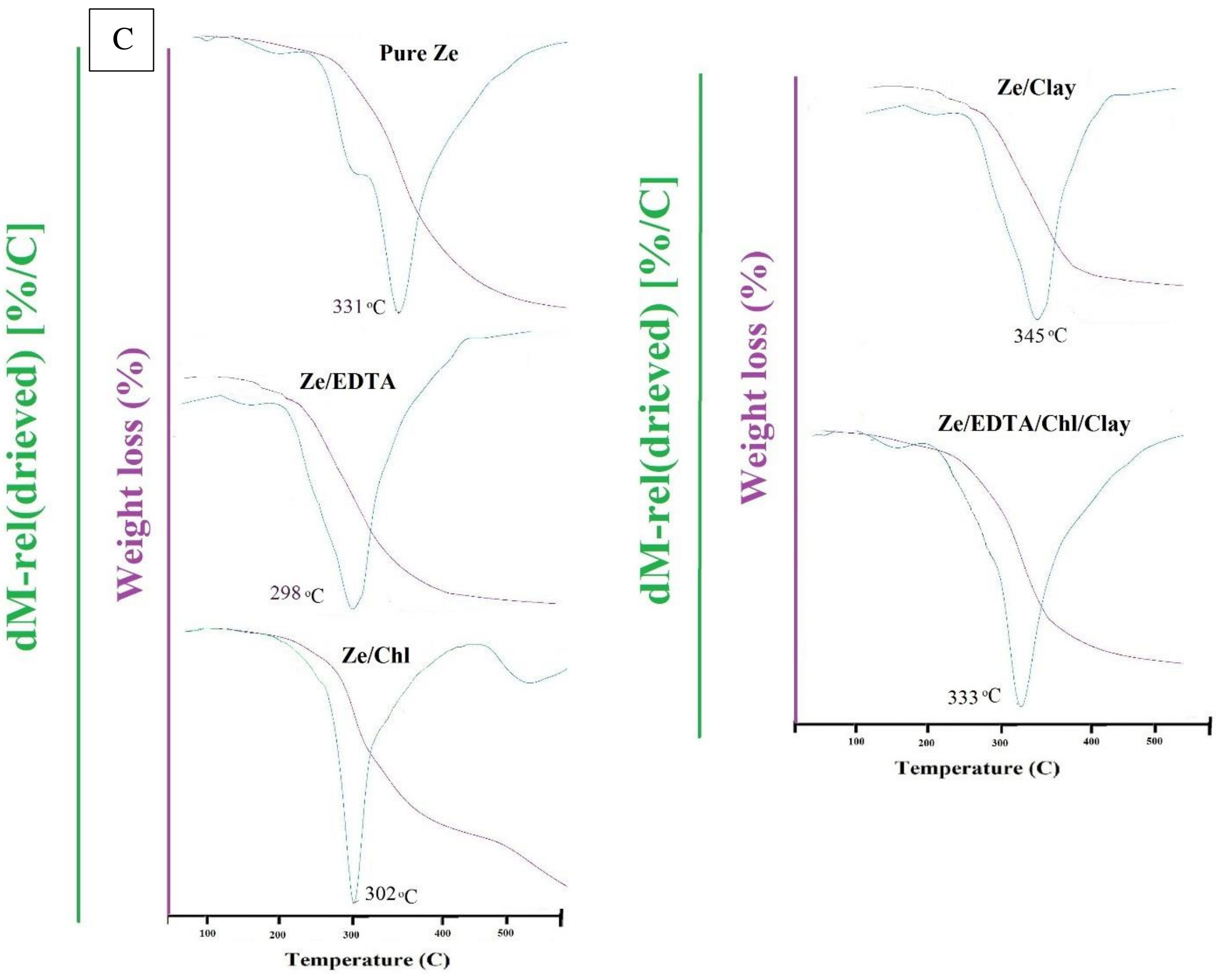
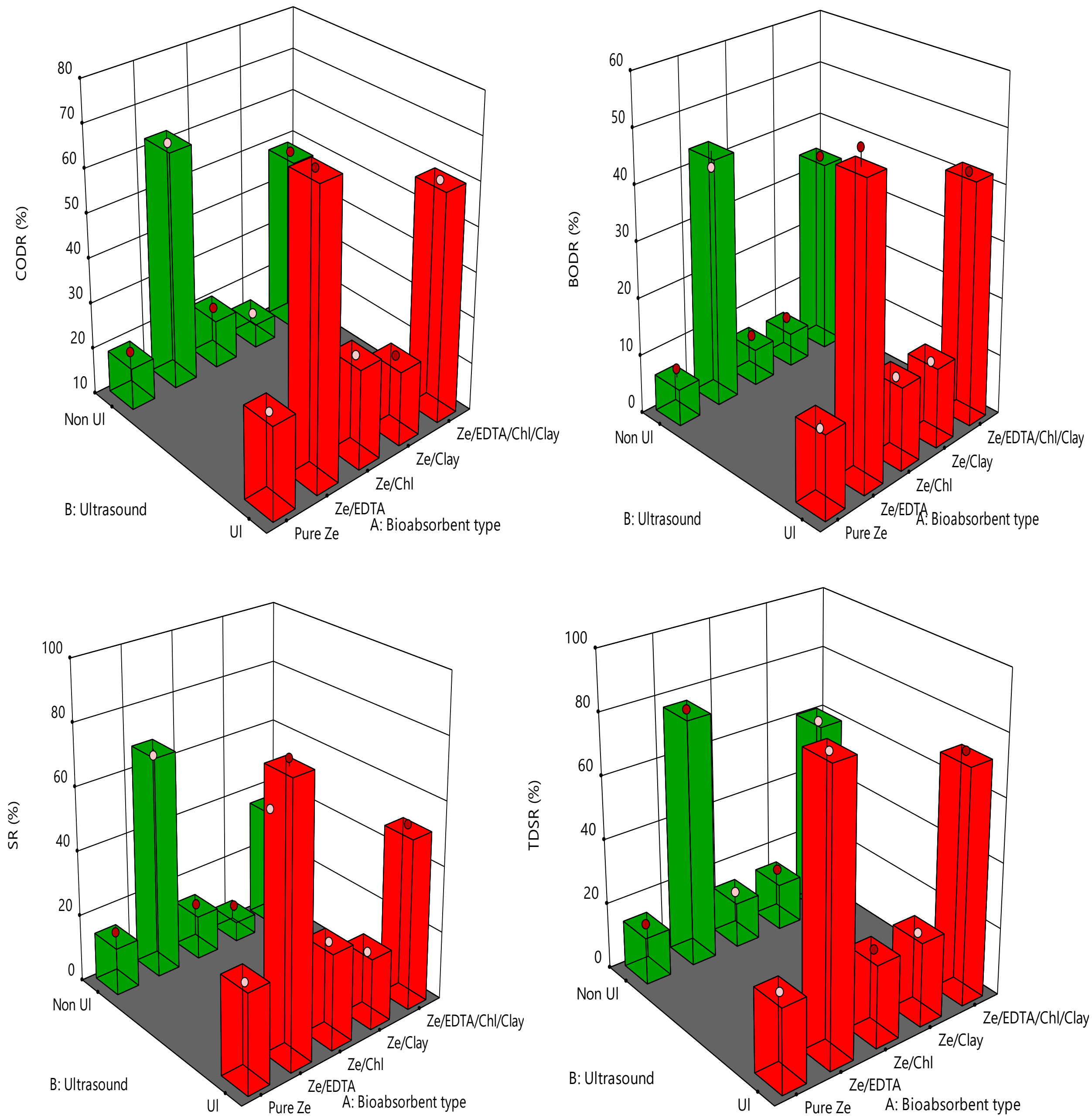

C
Pure Ze
331 °C
Ze/EDTA
298 °C
Ze/Chl
302 °C
dM-rel(drieved) [%/C]
Weight loss (%)
100
200
300
400
500
Temperature (C)
Ze/Clay
345 °C
Ze/EDTA/Chl/Clay
333 °C
dM-rel(drieved) [%/C]
Weight loss (%)
100
200
300
400
500
Temperature (C)

Fig. 5



CODR (%)
B: Ultrasound
A: Bioabsorbent type
Non Ul
Ul
Pure Ze
Ze/EDTA
Ze/Chl
Ze/Clay
Ze/EDTA/Chl/Clay
BODR (%)
SR (%)
TDSR (%)

Fig. 6

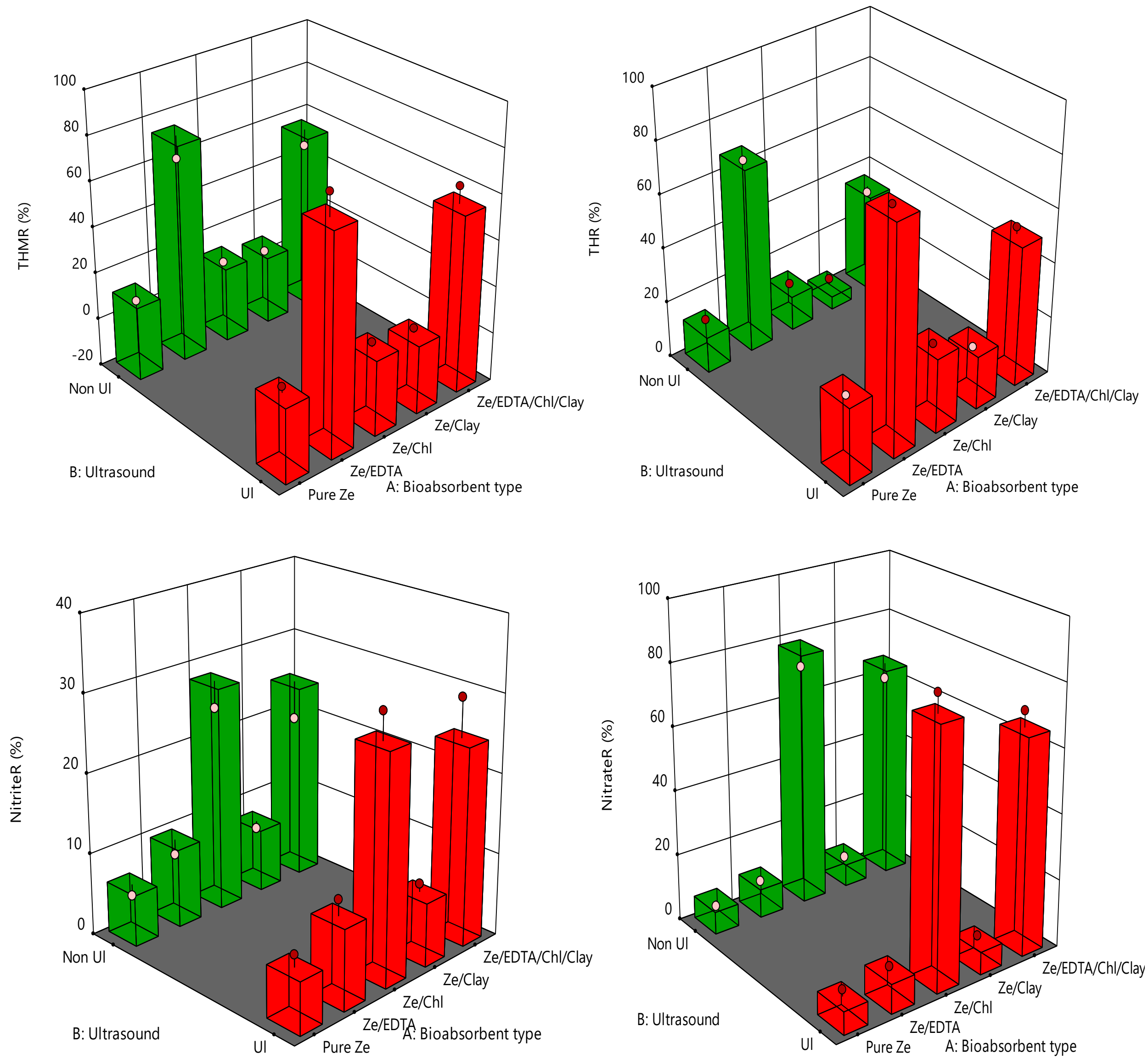
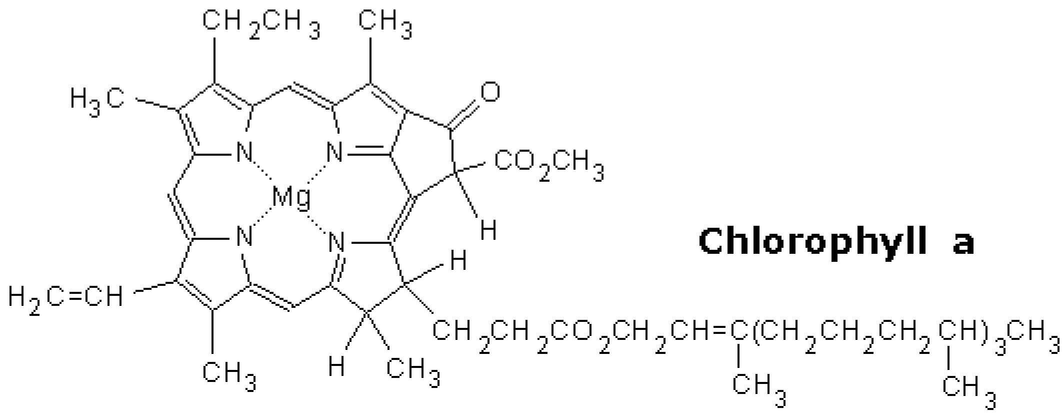
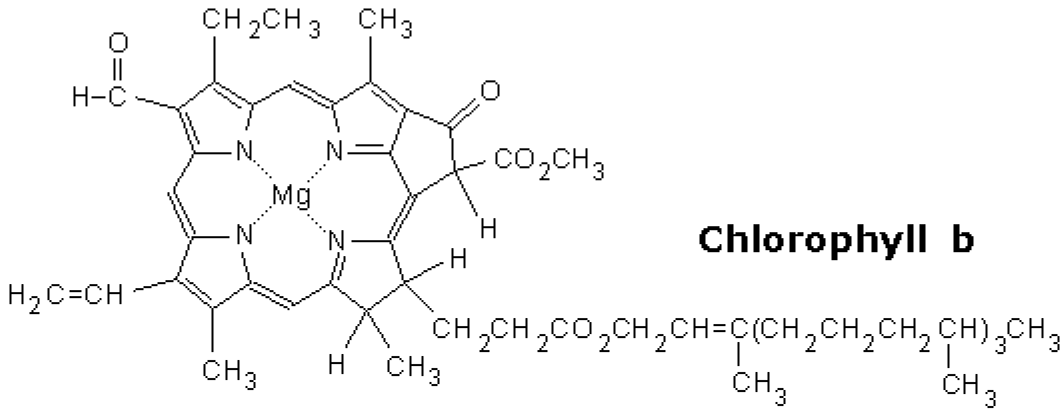

THMR (%)
100
80
60
40
20
0
-20
Non UI
UI
B: Ultrasound
Pure Ze
Ze/EDTA
Ze/Chl
Ze/Clay
Ze/EDTA/Chl/Clay
A: Bioabsorbent type
THR (%)
NitriteR (%)
NitrateR (%)

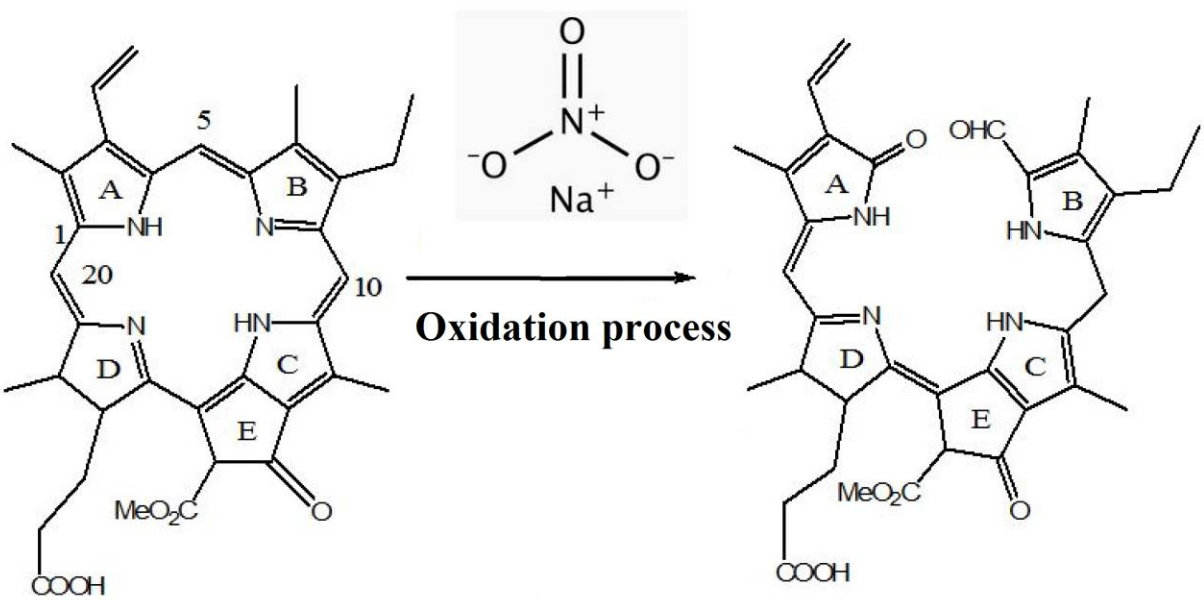
Fig. 7



Chlorophyll a



Chlorophyll b



chlorophyll

Fig. 8

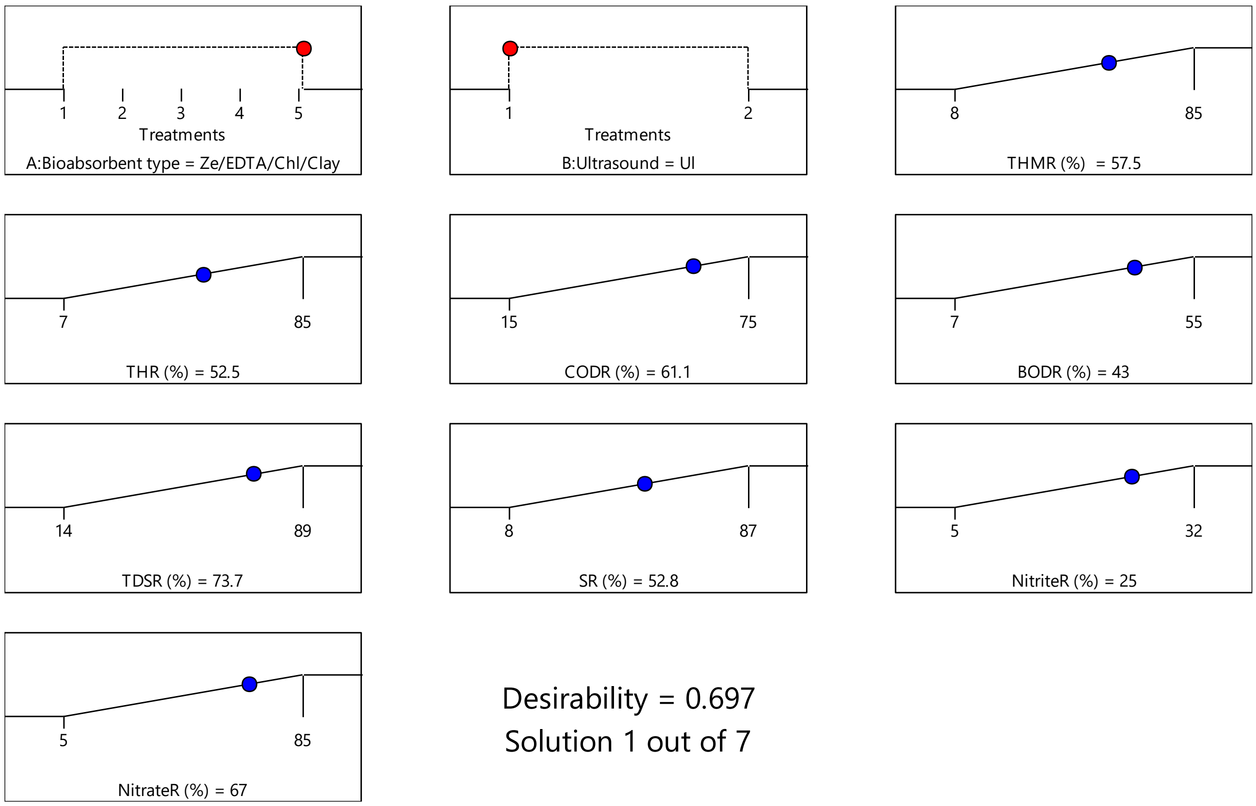

1
2
3
4
5
Treatments
A:Bioabsorbent type = Ze/EDTA/Chl/Clay
1
2
Treatments
B:Ultrasound = Ul
8
85
THMR (%) = 57.5
7
85
THR (%) = 52.5
15
75
CODR (%) = 61.1
7
55
BODR (%) = 43
14
89
TDSR (%) = 73.7
8
87
SR (%) = 52.8
5
32
NitriteR (%) = 25
5
85
NitrateR (%) = 67
Desirability = 0.697
Solution 1 out of 7

Fig. 9