Specific Removal of Nitrite from Lake Urmia Sediments by Biohydrogel Based on Isolated Soy Protein/Tragacanth/Mesoporous Silica Nanoparticles/Lycopene

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1. Introduction

Lake Urmia is one of the largest saltwater lakes in the world. This lake is an animal habitat called Artemia. In recent years, due to the drought conditions and the uncontrolled entry of industrial wastewater into the lake, the amount of some chemical ions and oxygen in the lake has changed dramatically. The most important pollutants in the lake are chemical organic compounds, heavy metals, nitrate, nitrite, biochemical oxygen demand (BOD), and chemical oxygen demand (COD), among which, nitrite pollutant is very important.[1,2] The emergence of nitrite ($\text{NO}_2^-$) is important for two reasons and is very problematic for human health: First, nitrite can oxidize blood hemoglobin and convert it to methemoglobin. It disrupts the oxygen supply to the body. Second, nitrite combines with some of the body’s amines and amides to produce carcinogen nitrosamines. Some studies have shown a significant relationship between the prevalence of gastric cancer and the concentration of nitrate in drinking water.[3]

Biohydrogel is made up of biological macromolecules made up of a large number of small, similar subunits that are joined together by a covalent bond to form a long chain. Biohydrogels, like synthetic hydrogels, have the ability to absorb large amounts of water. In the normal course, biohydrogel or macromolecules are intracellular compounds that enable the organism to survive in harsh environmental conditions. Biopolymer materials have been developed in various forms, so they have the capacity to be used in various industries. The biopolymer materials are reversible to the environment unlike petroleum polymers and they do not pollute the environment.[4–9] Soy protein isolate contains at least 90% protein, which is extracted from soybean meal by removing fat and carbohydrates. The use of isolated soy protein (ISP) in various industries is due to increasing the protein content of the product, high water absorption, and emulsifier properties of isolated soy protein. This product has no taste and is easily used in various industries. Isolated soy protein has a high water absorption capacity (WAC) and can act as a biohydrogel.[10] Tragacanth gum is the secretion of dried gum from several plant species. Tragacanth gum (TG) is extracted from the stem by mechanical activity. Tragacanth contains 10–15%
water, 3–4% minerals, and 3% starch. There are generally two types of active ingredients in tragacanth, one is a water-soluble substance called tragacanthin and the other is a water-insoluble solution called bassorin. Tragacanth is composed of galacturonic acid, which binds to galactose and xylose sugars. Tragacanth easily absorbs the water and forms a gel in the presence of water, and due to the adhesive properties of tragacanth, it can be used to increase the physical resistance of biohydrogels.\textsuperscript{[11,12]} Nanoparticles have been considered in various industries such as filtration, electronics, and polymer industries due to their various applications. Mesoporous silica is solid materials that have porous like honeycomb pores with hundreds of empty channels that are capable of absorbing and encapsulating relatively large amounts of bioactive molecules. Prominent features of mesoporous include high surface area, adjustable size, and good chemical and thermal stability. Scattered colloidal systems containing dispersed silica particles are used in various industries, including catalysts and biopolymers.\textsuperscript{[13]} One of the fields of application of mesoporous silica nanoparticles (MPS) is the use in medicines and cosmetics. Other uses for nanoparticles include composites, thermal insulation, and electrical insulation.\textsuperscript{[14]} Lycopene (Lyc) is a powerful antioxidant that can help human body protect against degenerative diseases. This pigment can easily react with nitrate and nitrite oxides and remove them from the environment.\textsuperscript{[15]} Levels of lycopene in the blood and adipose tissue are positively correlated with reduced risk of cancer, heart disease, and macular degeneration. This pigment can easily react with nitrate and nitrite oxides and remove them from the environment.\textsuperscript{[16]}

In this study, isolated soy protein/tragacanth modified with nanoparticles of mesoporous silica and lycopene was used to reduce BOC, COD, nitrate, and nitrite. In the first part of this study, the physicochemical and mechanical properties of ISP/TG/MPS/Lyc biohydrogel were investigated using techniques such as infrared spectroscopy and scanning electron microscopy (SEM). In the second part, the ISP/TG/MPS/Lyc biohydrogels were used to remove nitrate and nitrite from Lake Urmia sediments and the efficiency of reducing pollutants was investigated. The results showed the formation of biohydrogel with suitable physical properties, which is very effective in the specific removal of nitrite from sediments and sewage. The proposed method is very cheap and the biohydrogel is biodegradable and environmentally friendly.

2. Experimental Section

2.1. Materials

Isolated soy protein (with more than 90% protein) was produced by Crown China. Tragacanth was as-obtained from the Medicinal Plants Store (Urmia, Iran). Mesoporous silica nanoparticles (with a particle size of 30–100 nm) were produced by Nanogilosuzak Company (Iran, Tehran). Lycopene powder (with red appearance and product code 8-65-502) was purchased from the Iran Chem Book Co (Iran, Tehran). 2,2-diphenyl-1-picrylhydrazyl (DPPH), magnesium nitrate, calcium chloride, and other chemical compounds used were purchased from Merck (Germany) and Sigma-Aldrich (USA).

2.2. Methods

2.2.1. Production of ISP/TG/MPS/Lyc Biohydrogels

To prepare biohydrogel, isolated soy protein powder (according to Table 1A) was dissolved in 100 mL of water at 70 °C by a magnetic stirrer (RS3001, MLW, Germany) at 2000 rpm. The pH of the solution was then adjusted to 10 using NaOH solution (0.1 N). Then, tragacanth powder (according to Table 1A) was added to the solution and dissolved at a temperature of 70 °C by mechanical stirrer (BH8, Iran) at a speed of 1500 rpm for 1 h. Glycerol (as plasticizer) was then added to the solution as a 40% by weight of dry matter and dissolved in the solution for 20 min under the same conditions. Then, the mesoporous silica powder (according to Table 1A) was added to the solution and dispersed by a mechanical mixer at 1500 rpm for 1 h. Finally, lycopene powder (according to Table 1A) was added to the solution and dispersed by a mechanical mixer at 1500 rpm for 1 h and a gel-like solution was formed. 25 mL of the prepared final gel (including soy protein isolate, tragacanth, silica, and lycopene) was poured into special plates with a diameter of 10 cm and dried for 48 h at room temperature (Figure 1). The dried biohydrogel was stored in zippered bags until the experiments at room temperature.

Table 1A. Prepared biohydrogels based on combined D-optimal design.

| Biophydrogel | ISP [g] | TG [g] | MPS [%] | Lyc [g] |
|--------------|---------|-------|---------|---------|
| 1            | 6       | 0     | 0       | 0       |
| 2            | 5       | 1     | 0       | 5       |
| 3            | 6       | 0     | 0       | 5       |
| 4            | 5       | 1     | 0       | 2.5     |
| 5            | 4       | 2     | 0       | 5       |
| 6            | 5.5     | 0.5   | 4       | 0       |
| 7            | 4.5     | 1.5   | 4       | 2.5     |
| 8            | 6       | 0     | 0       | 5       |
| 9            | 6       | 0     | 3       | 2.5     |
| 10           | 6       | 0     | 4       | 5       |
| 11           | 6       | 0     | 1       | 2.5     |
| 12           | 6       | 0     | 4       | 0       |
| 13           | 4       | 2     | 3       | 3.75    |
| 14           | 6       | 0     | 4       | 5       |
| 15           | 4       | 2     | 0       | 0       |
| 16           | 4       | 2     | 0       | 0       |
| 17           | 4       | 2     | 4       | 5       |
| 18           | 4       | 2     | 4       | 0       |
| 19           | 6       | 0     | 0       | 0       |
| 20           | 4       | 2     | 3       | 1.25    |
| 21           | 5       | 1     | 4       | 5       |
| 22           | 5       | 1     | 2       | 0       |
| 23           | 5       | 1     | 2       | 2.5     |
| 24           | 5.5     | 0.5   | 2       | 5       |
| 25           | 4       | 2     | 2       | 5       |
| 26           | 6       | 0     | 2       | 0       |
2.3. Biohydrogel Test

2.3.1. Solubility in Water

To determine the solubility of biohydrogel in water, biohydrogel samples (4 × 4 cm²) were dried at 105 °C and their initial weight was measured with a digital scale (Sartorius, Germany). The biohydrogel samples were then placed in containers containing 50 mL of distilled water at 25 °C for 24 h. After this time, the samples were filtered with predried filter papers and dried again at 105 °C. The solubility of biohydrogels was calculated using the following equation

\[ \text{Solubility} = \frac{W_0 - W_1}{W_0} \times 100 \]  

where \( W_0 \) is the weight of biohydrogel before the dissolution and \( W_1 \) is the weight of biohydrogel after dissolution in water.

2.3.2. Humidity Content

Biohydrogel samples were placed on glass plates that had already reached a fixed weight and were weighed (\( W_i \)). It was then dried in an oven at 105 °C for 24 h. The sample with the plate was removed from the oven after this time and weighed again after cooling in the desiccator (\( W_j \)). The amount of biohydrogel moisture was calculated by the following equation

\[ \text{Moisture} = \frac{W_1 - W_i}{W_i} \times 100 \]  

2.3.3. WAC

To investigate the water absorption capacity of biohydrogel, 2 g biohydrogel was dried at 150 °C inside the oven. Then, 1 g (\( W_0 \)) of the biohydrogel sample was mixed with 9 mL of distilled water in a 30 mL falcon for 30 s. The falcons were then kept at room temperature for 2 h, after which they were centrifuged at 3000 rpm for 30 min. The supernatant separated from the sediment was removed from the falcon, and its weight was measured (\( W_1 \)). Water absorption capacity was calculated as the amount of water absorbed by 1 g

\[ \text{WVC} = \frac{W_1 - W_0}{W_0} \times 100 \]  

2.3.4. Antioxidant Activity

DPPH radical scavenging activity (RSA) was performed using Pirouzifard et al.’s method. To do this, 25 mg of biohydrogel was gently stirred in 3 mL of distilled water for 5 min. Then, 2.8 mL of the film extract was added to the test tubes containing 0.2 mL of 1 × 10⁻³ M DPPH methanol solution and kept in the room for 30 min. The absorption rate of the test and control tubes at 517 nm was measured by a spectrophotometer (Pharmacia, USA). The degree of discoloration of DPPH solution indicates the power of free radical scavenging by the corresponding antioxidant. Finally, using the following formula, the percentage of free radical scavenging activity was determined

\[ \text{Radical scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \]  

where \( \text{Abs}_{\text{control}} \) is DPPH methanol absorbance and \( \text{Abs}_{\text{sample}} \) is the adsorption rate of biohydrogel methanolic sample at a wavelength of 517 nm.

2.3.5. Mechanical Properties

The mechanical properties of biohydrogel were performed by Instron universal testing instrument (Sweden, Model TVT 300 Xp). After being conditioned (for 24 h at a relative humidity of 55% (saturated calcium nitrite)), the biohydrogels were cut (1 × 10 cm in size). The biohydrogel was put between two jaws of the instrument such that the jaws moved with 5 mm min⁻¹ space. The elongation at breaking point (EB) was calculated by the device and the tensile strength factor was obtained by the following equation

\[ \text{TS} = \frac{F}{w \times d} \]  

where TS is the tensile strength in terms of MPa, \( F \) is the strength in Newton, \( d \) is the thickness, and \( w \) is the width of the biohydrogel (m).

2.3.6. Scanning Electron Microscopy

The microstructures of biohydrogels were studied with the electron microscope (Philips, the Netherlands). To do this, the dried biohydrogels (at 105 °C) were glued to the aluminum base with silver glue. The bases were then dried until the critical point and covered with gold for 5 min. Imaging of the samples was performed by scanning electron microscopy in different magnifications with a power of 20 kV.

2.3.7. Fourier-Transform Infrared Spectroscopy (FTIR)

The FT-IR Spectrophotometer (Tensor27, Bruker UK) was used to demonstrate the link between the chemical polymer groups
of the polymer matrix and the added nanoparticles. Pirsa et al.’s method was used to study FTIR of biohydrogel,[20] Tablet containing film (1 cm diameter and 20 μm thickness) was prepared by compressing the mixture of KBr powder and biohydrogel powder. Tablets containing the biohydrogel were placed inside the cell of the device. The infrared spectrum was recorded in the range of 400–4000 cm⁻¹ and with a resolution of 4 cm⁻¹.

2.3.8. Thermal Gravimetry Analysis (TGA)

TGA was used to study the thermal stability of isolated biodegradable soy protein and its various composites. The DuPont 951 TGA instrument was used to study the weight changes of the polymer matrix and the added nanoparticles. Pirsa et al.’s method was used to study FTIR of biohydrogel.

2.4. Treatment of Lake Sediments with Biohydrogels

In this section, the optimal biohydrogels were used to reduce the pollution of Lake Urmia sediments. To treat lake sediments with biohydrogel and to evaluate the efficiency of pollution reduction, sediments of different parts of the lake were sampled. 20 g of lake sediments were dispersed in 100 mL of distilled water and stirred with a mixer for 1 h. The water was then separated from the solids by passing through a strainer. Separated water was poured into the beaker and 5 g of biohydrogel was placed inside it and it was shaken for several hours (according to the statistical design of Table 1C) with a balloon shaker (TM52E, Iran). After treatment of the sediment solution with biohydrogel, the biohydrogel was separated from the solution (Figure 1). The chemical properties of the sediment solution (BOD, COD, nitrate, and nitrite) were analyzed before and after treatment with biohydrogel and the pollutant removal efficiency (PRE) was calculated using the following equation

\[
\text{PRE} \% = \left(\frac{C_0 - C_1}{C_0}\right) \times 100
\]

where \(C_0\) is the pollutant concentration before treatment and \(C_1\) is the pollutant concentration after treatment with biohydrogel.

2.4.1. Nitrite Determination

Spectroscopic methods were used to measure nitrite. For this purpose, 50 mL of sediment solution was mixed with 5 mL of sulfanilamide solution (0.2% w/w) and 3 mL of hydrochloric acid solution (15% w/w) and kept at room temperature and dark condition for 5 min. Then, 1 mL of α-naphthyl ethylene diamine hydrochloride solution (0.1% w/w) was added to it and kept at room temperature and dark condition for 10 min. The solution absorbance was recorded at a wavelength of 537 nm. To obtain the amount of nitrite, the calibration curve of the relationship between the amount of nitrite in standard solutions and the absorbance values was plotted as follows, and from the calibration curve relationship, the amount of nitrite in the sediment solution was calculated: 6 standard solutions including sulfanilamide (0.2% w/w), hydrochloric acid (0.1% w/w), alpha naphthyl ethylene diamine hydrochloride (15% w/w), and various concentrations of sodium nitrite were prepared. The absorption rate of the samples was recorded at 537 nm and the calibration curve was obtained.[21]

2.4.2. Nitrate Determination

To measure nitrate, the amount of nitrite was determined in the first step. In the second step, for nitrate measurement, the sample was transferred from inside the cadmium column and the resulting solution, which is actually the sum of the nitrite in the sample and the nitrite from the nitrate reduction, was determined. Finally, the amount of nitrate was calculated based on the difference between the numbers of these two steps.

2.4.3. BOD and COD Determination

BOD meters (BD 600) and COD meters (AL100) were used to measure BOD and COD. The BOD meter can measure the BOD of solution samples in the range of 0–4000 mg L⁻¹. The COD meter can measure the COD of solution samples in the range of 0–15 000 mg L⁻¹.

2.5. Statistical Analysis

Statistical analysis of this study was performed in three parts. In the first part, combined D-optimal design was used to investigate the effect of isolated soy protein: tragacanth gum ratio, silica mesoporous nanoparticles concentration, and lycopene concentration on physical and mechanical properties of biohydrogel (Table 1A). Data analysis of this section was performed at the 95% probability level with the Design-Expert-10 software. In the second part, completely random design was used to investigate the effect of isolated soy protein: tragacanth gum ratio, silica mesoporous nanoparticles concentration, and lycopene concentration on structural, morphological, and thermal properties of biohydrogel (Table 1B). In the third part, a factorial design was used to investigate the effect of biohydrogel type and biodegradable treatment time with the sediment solution (Table 1C).

In this section, the experiments were performed in three repetitions and the confidence level was considered to be 95%. Minin-tab-17 software and Tukey test were used to analyze one-way variance and check the significance of the difference in results.

| Table 1B. Optimum biohydrogels for treatment of Lake Urmia sediment treatment. |
|-----------------|-----|-----|-----|-----|
| Biohydrogel     | ISP | TG  | MPS | Lyc |
| BH1             | 6   | 0   | 0   | 0   |
| BH2             | 6   | 0   | 0   | 5   |
| BH3             | 6   | 0   | 4   | 0   |
| BH4             | 4   | 2   | 0   | 0   |
| BH5             | 4   | 2   | 4   | 5   |

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### 3. Results and Discussions

The statistical response surface method was used to investigate the effect of isolated soy protein: tragacanth gum ratio, silica mesoporous nanoparticles concentration, and lycopene concentration on solubility, moisture content, water absorption capacity, and antioxidant properties. This method is a set of mathematical methods that determines the relationship between one or more response variables to several independent variables. Mathematical relationships, mathematical models, and regression coefficients between the generated responses and the independent variables were calculated and reported in Table 2.

#### 3.1. Investigation of Solubility, Humidity, WAC, and RSA

Figure 2 shows the 3D curve of the effect of the isolated soy protein: tragacanth gum ratio, silica mesoporous nanoparticles concentration, and lycopene concentration on solubility, moisture content, water absorption capacity, and antioxidant properties. As can be seen from the 3D curves, the addition of tragacanth gum, MPS, and lycopene reduces the solubility of biohydrogel in water. The most effective in reducing water solubility has been MPS, followed by lycopene and tragacanth. Because lycopene pigments are insoluble in water, a reduction in solubility was expected due to increased lycopene. Mesoporous silica nanoparticles are waterproof naturally and can reduce the solubility by being placed between isolated polymer chains of soy protein and increasing its structural strength. In the structure of tragacanth, there is a substance called tragacanth (bassorin), which is insoluble in water, which reduces the solubility of biohydrogel in water. Kim et al. reviewed isolated soy protein films and their results about ISP confirm our results.[22]

The curve of moisture content and water absorption capacity shows that MPS does not have much effect on the moisture content of biohydrogel and water absorption capacity and only slightly increases the moisture content and water absorption capacity, which is probably due to the presence of cavities. In its structure, it has the ability to trap water molecules. Lycopene reduces moisture content and water absorption capacity. Due to the fact that this pigment is hydrophobic, the presence of lycopene molecules in the biohydrogel structure reduces the presence of water molecules. The presence of tragacanth gum in the biohydrogel structure has increased the moisture content and water absorption capacity. Due to the fact that tragacanth is a compound that strongly absorbs water and has a gel-like property, an increase in the percentage of moisture and water absorption capacity in the presence of tragacanth was expected. In general, the presence of MPS and tragacanth increases the percentage of moisture and water absorption capacity by creating vacant spaces in the biohydrogel structure and creating gel-like properties. Montazer et al. prepared the tragacanth-silver gum hydrogel and examined its moisture and adsorption properties, the results of which confirm the results of the present study.[23] Hosseini et al. examined the pharmacological properties and the water uptake capacity of tragacanth nanoparticles. The results of the present study are in line with the research of Hosseini et al.[24]

The curve of the antioxidant properties of biohydrogel shows that tragacanth does not have a significant effect on antioxidant properties, but MPS has greatly increased the antioxidant properties of biohydrogel. In general, silica nanoparticles have the ability to physically absorb free radicals, which is why they cause these radicals to go out. The mesoporous silica nanoparticles also have the ability to disable radicals due to their very high surface-to-volume ratios. Lycopene is a member of the carotenoid family of phytochemicals and a natural pigment that, unlike other carotenoids, is not converted to vitamin A. This pigment has a very high antioxidant property, so the antioxidant properties of biohydrogel are greatly increased in the presence of lycopene. There are many studies that have confirmed the antioxidant properties of lycopene.[25,26]
Figure 2. 3D curve of the effect of independent factors on solubility, humidity, WAC, and RSA of ISP biohydrogels.
3.2. Investigation of Mechanical Properties

Figure 3 shows the 3D curve of the effect of the isolated soy protein: tragacanth gum ratio, silica mesoporous nanoparticles concentration, and lycopene concentration on the mechanical properties. As it turns out, lycopene pigment does not have a significant effect on the tensile strength and EB of biohydrogel. Due to the fact that lycopene is not soluble in water and only physically placed in the pores of biohydrogel, it has not had a significant effect on its mechanical properties, although the lycopene has affected biohydrogel morphology. MPS has somewhat increased tensile strength and reduced its EB. Due to the porous structure of MPS and its high surface area, there is a possibility of physical interactions between isolated soy proteins and nanoparticles, which leads to a strong structure in the biohydrogel and increases the tensile strength of biohydrogel. The addition of tragacanth to biohydrogel has increased the tensile strength of biohydrogel, which is probably due to creating a strong 3D network with high crosslinks between ISP and tragacanth. This result has been confirmed by Tian et al.\cite{27} There are many studies that show that adding polysaccharides to the structure of protein films reduces their elasticity. The results of this study are well consistent with previous research.\cite{28}

3.3. SEM and FTIR Tests

Figure 4 shows SEM images (panel A) and FTIR (panel B) spectra of ISP biohydrogels. As can be seen from the SEM images, pure ISP biohydrogel has a smooth, uniform, nonslit surface, while ISP/TG biohydrogel surface has a slit, which may be due to the gel properties of tragacanth, which increases the moisture content of the biohydrogel and has caused a gap at the level of the biohydrogel. In the ISP/MPS biohydrogel, small gaps are seen on the biohydrogel surface, and also the MPS nanoparticles are distributed nonuniformly in the biohydrogel. The size of these nanoparticles in some areas is between 20 and 100 nm, while in other areas, the size of the particles is larger, due to the accumulation of particles. Due to the fact that in the process of biohydrogel preparation, the particles were dispersed in the solution by mechanical methods, the accumulation of particles was expected in some areas. In ISP/Lyc biohydrogel, as it turns out, lycopene pigment has disrupted the uniform structure of biohydrogel, which may be due to the fact that this pigment does not dissolve in the water environment of biohydrogel. In the ISP/TG/MPS/Lyc biohydrogel, due to the presence of tragacanth, MPS and lycopene, as well as nonuniform distribution of MPS nanoparticles in the biohydrogel the cracks are observed in the biohydrogel structure.

In the FTIR spectrum of pure ISP biohydrogel, the peak at 3278 cm$^{-1}$ is related to the tensile vibrations of O–H and N–H. Peaks at 2850 and 2920 are related to C–H tensile vibration. Peak at 1620 is related to the C=O tensile vibration of the amide group and peak at 1540 is related to the tensile vibration of the N–H group of the amide group. Peak at 1025 is related to C–O tensile vibration and peak at 1240 is related to C–N tensile vibration. In the ISP/TG biohydrogel spectrum, all peaks
related to pure ISP are observed, which almost all of these peaks shifted to higher or lower wave numbers, indicating the interaction between ISP and tragacanth molecules. There is no new peak in the ISP/TG spectrum compared to the pure ISP spectrum, as most of the interatomic links in the ISP are also seen in tragacanth, and the corresponding peaks overlap. In the ISP/MPS spectrum, all peaks related to a specific ISP are seen, which have shifted to other wave numbers. Also, two new peaks have been created in this spectrum, which indicates the presence of silica nanoparticles in ISP/MPS biohydrogel. In this spectrum, peak at 990 is related to Si–OH vibrations and peak at 670 is related to Si–C vibration. The ISP/Lyc spectrum also shows all the peaks associated with a pure ISP that have been shifted to other wave numbers. In this spectrum, two new peaks are seen in 1710 and 1740, which are related to C=C tensile vibrations. The ISP/TG/MPS/Lyc spectrum also shows all the peaks related to a pure ISP that have been shifted to other wave numbers. Silica and lycopene peaks are also seen. The overall results of the study of FTIR spectra indicated that tragacanth, SiO₂, and lycopene affected the FTIR spectra of ISP. Han et al. examined the isolated FTIR spectrum of soy protein whose research results confirm the FTIR results of the present study.[30] Li et al. studied the FTIR spectrum of silica and its composites. The peaks related to Si–OH and Si–C in this study were confirmed by the study of Li et al.[31]

3.4. X-Ray Diffraction (XRD) and TGA Tests

Figure 5 shows the spectra of XRD (panel A) and TGA (panel B) of ISP biohydrogel and its composites. According to the XRD spectra, as seen in the pure ISP spectrum, only a very wide peak in the 2θ of 15°–22° is seen, which indicates the amorphous structure of ISP. Previous research has shown that ISP is noncrystalline.[32] As it turns out, with the addition of tragacanth, silica and lycopene, no new peaks have been created in the XRD, and the height of the broad peaks related to isolated soy protein has decreased and its width has increased, so the addition of tragacanth, silica, and lycopene to the ISP increases the amorphous structure of ISP. The lowest height is related to the ISP/TG/MPS/Lyc composite biohydrogel. Silica’s XRD peak also appears at 2θ of 20°, which overlaps with ISP peak.[33] In a similar study, the XRD spectrum of ISP was found to have a broad peak at 2θ of 20°, confirming the results of the present study.[32]

In all TGA spectra related to ISP and its components, in the weight change curve relative to temperature, there are weight changes in two areas. The first area, which is around 90–130 °C, is due to the fact that the biohydrogels have lost water in the biohydrogel structure. In addition to releasing water molecules, this weight loss can also be related to the release of compounds with low boiling points from the hydrogel structure. The second
weight loss, which is also the main weight loss, occurs in the temperature range of 200–400 °C, which is related to the complete destruction of biohydrogel. As can be seen, with the addition of silica, tragacanth, and lycopene, the second weight loss is less happened and occurs at higher temperatures, so that the lowest weight loss is related to the ISP/TG/MPS/Lyc biohydrogel. These results show that silica, tragacanth, and lycopene increase the thermal stability of biohydrogel. It should be noted that MPS had the greatest effect on thermal stability and lycopene had the least effect. Due to the high melting point of silica, the results were expected. Regarding the fact that the thermal stability of ISP/TG is higher than ISP/Lyc, it can be noted that because tragacanth and isolated soy protein form a strong 3D polymer network, this issue increases its thermal stability. However, lycopene has had only physical interactions with the isolated soy protein. In a similar study, the thermal stability of isolated soy protein was studied by examining TGA, and the obtained results are in line with the results of the present study.

3.5. Removal of Pollutants from Urmia Lake Sediments with Biohydrogel

To investigate the effect of ISP biohydrogel and its composites on the removal of Lake Urmia sediment pollutants, the sediment solution of this lake was treated for 1, 2, and 3 h with five optimal biohydrogel (according to Table 1B) and the removal efficiency of BOD, COD, nitrate, and nitrite were examined. Table 3 shows the removal efficiency of contaminants treated...
with different biohydrogels at different times. Figure 6 also shows the column diagram of the efficiency of removal of BOD, COD, nitrate, and nitrite by five types of biohydrogel in three different treatment times.

As can be seen from the results, all five biohydrogels have the ability to reduce BOD, COD, nitrate, and nitrite in sediments. As the treatment time of the biodegradable sedimentation solution increases, the efficiency of pollutant removal increases. The lowest removal efficiencies are related to pure ISP biohydrogel. In ISP/TG biohydrogel, the removal of all contaminants has increased with the addition of tragacanth gum to the biohydrogel structure.

As the results showed, with the addition of tragacanth gum to the ISP structure, the humidity content and the WAC increased. As the biohydrogel WAC increases, the pollutants become more in contact with the biohydrogel polymer network, increasing the likelihood of physical contact and thus increasing the percentage of pollutant removal. The rate of elimination of pollutants in ISP/MPS biohydrogel also shows a relative increase compared to pure ISP. Due to the porous structure of silica nanoparticles, the placement of these particles in the isolated

| Biohydrogel type | Time [h] | BOD RE [%] | COD RE [%] | Nitrate RE [%] | Nitrite RE [%] |
|------------------|----------|------------|------------|----------------|----------------|
| ISP              | 1        | 25         | 35         | 30             | 31             |
|                  | 2        | 31         | 38         | 36             | 38             |
|                  | 3        | 39         | 43         | 42             | 43             |
| ISP/TG           | 1        | 36         | 41         | 35             | 36             |
|                  | 2        | 42         | 45         | 42             | 45             |
|                  | 3        | 47         | 48         | 47             | 47             |
| ISP/MPS          | 1        | 32         | 40         | 42             | 43             |
|                  | 2        | 35         | 44         | 46             | 48             |
|                  | 3        | 41         | 47         | 51             | 54             |
| ISP/Lyc          | 1        | 24         | 34         | 42             | 75             |
|                  | 2        | 32         | 37         | 46             | 79             |
|                  | 3        | 38         | 42         | 53             | 82             |
| ISP/TG/MPS/Lyc   | 1        | 41         | 47         | 46             | 81             |
|                  | 2        | 45         | 52         | 53             | 83             |
|                  | 3        | 49         | 57         | 56             | 87             |

Figure 6. The effect of biohydrogel type and treatment time on the removal efficiency of pollutants from lake sediments.
polymer chain of soy protein increases the internal porosity of biohydrogel, which leads to increase biohydrogel contact with pollutants and increases chemical interactions with biohydrogel, and therefore removal efficiency also increases.

In ISP/Lyc biohydrogel, the elimination of BOD and COD pollutants did not differ significantly from that of pure ISP biohydrogel, and the rate of nitrate removal increased slightly. But what is remarkable is that the rate of nitrite removal in ISP/Lyc biohydrogel has increased significantly compared to pure ISP biohydrogel. The significant increase in the nitrite removal efficiency indicates chemical interactions between lycopene pigment and nitrite. It is noteworthy that in the interaction of ISP/Lyc biohydrogel with nitrite, the red color of the biohydrogel goes to colorlessness, in other words, the higher the concentration of nitrite, the more colorless the red color of the biohydrogel. Also, nitrate has no effect on ISP/Lyc biohydrogel color.

The possible mechanism for the interaction of lycopene with nitrite is as follows: Nitrite ($\text{NO}_2^{-}$) is converted to nitrosyl cation ($\text{N}=\text{O}$) in aquatic environments, where nitrosyl may be breaking the double bonds in the lycopene structure and destroying the lycopene structure (discoloration of the lycopene pigment confirms this) and nitrosyl itself is trapped in the structure of lycopene and loses its carcinogenic power, or in other words, is eliminated from the environment. As we know, nitrate converted to nitrosyl cation can react with dialkyl amine to produce carcinogenic nitrosamines (Figure 7). The fact that lycopene does not have a significant effect on nitrate removal may be due to the fact that nitrate cannot be converted to the nitrosyl cation ($\text{N}=\text{O}$) in aquatic environments and cannot break the double bonds in the lycopene structure, so the discoloration of the lycopene does not occur. In ISP/TG/MPS/Lyc biohydrogel, due to the fact that both tragacanth and porous silica nanoparticles and lycopene pigments are present and this biohydrogel has the highest moisture and the highest WAC, this biohydrogel has the most contact with pollutants and the most removal efficiency of BOD, COD, nitrate, and nitrite. However, in ISP/TG/MPS/Lyc biohydrogel the highest removal efficiency related to nitrite contaminant removal. In other words, the target of BOD, COD, and nitrate contaminants is probably only physical, while in addition to physical removal, chemical removal also occurs in nitrate removal.

Khopde et al. examined the mechanism of reaction and trapping of NO$_3$ molecules in the polymer chain of carotenoids. The results of Khopde et al. confirm the results of the present study and the proposed mechanism.[36] Cerón-Carrasco et al. theoretically and using software calculations examined the interaction of NO$_3$ and beta-carotene radicals, their results are in line with the results of the present study.[37]

4. Conclusion

In this study, biohydrogel was prepared based on isolated soy protein/tragacanth gum/mesoporous silica nanoparticles/lycopene. Biohydrogel properties showed that this biohydrogel could absorb water 3–4 times its weight. Lycopene and silica nanoparticles reduced solubility. Tragacanth gum increases the moisture content and water absorption capacity of biohydrogel.

Figure 7. a) Molecular structure of lycopene, b) conversion of nitrite to nitrosyl, c) reaction of nitrosyl cation with lycopene, and d) nitrosyl reaction with dialkyl amine.
Biohydrogel has a completely amorphous structure that its amorphous is increased by the addition of tragacanth gum, mesoporous silica nanoparticles, and lycopene. SEM images showed that tragacanth gum, mesoporous silica nanoparticles, and lycopene make cracks in the biohydrogel structure, and the distribution of silica nanoparticles on the biohydrogel structure was confirmed. According to the FTIR results, there is only physical interaction between lycopene and isolated soy protein, and electrostatic interactions were confirmed between isolated soy protein and tragacanth gum and silica nanoparticles. Five types of biohydrogel were used to treat Lake Urmia sediment solution. The results showed that all biohydrogels have the ability to reduce BOD, COD, nitrate, and nitrite, but biohydrogels containing lycopene pigments remove much more nitrite contaminants than other contaminants. In other words, the biohydrogel containing lycopene selectively purifies nitrite from the sediment solution of Lake Urmia.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biohydrogels, lycopene, mesoporous nanoparticles, nitrite, pollutant reduction

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