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Biological Adsorption for Removal of Hydrogen Sulfide from Aqueous Solution by Live Eisenia Foetida Worms

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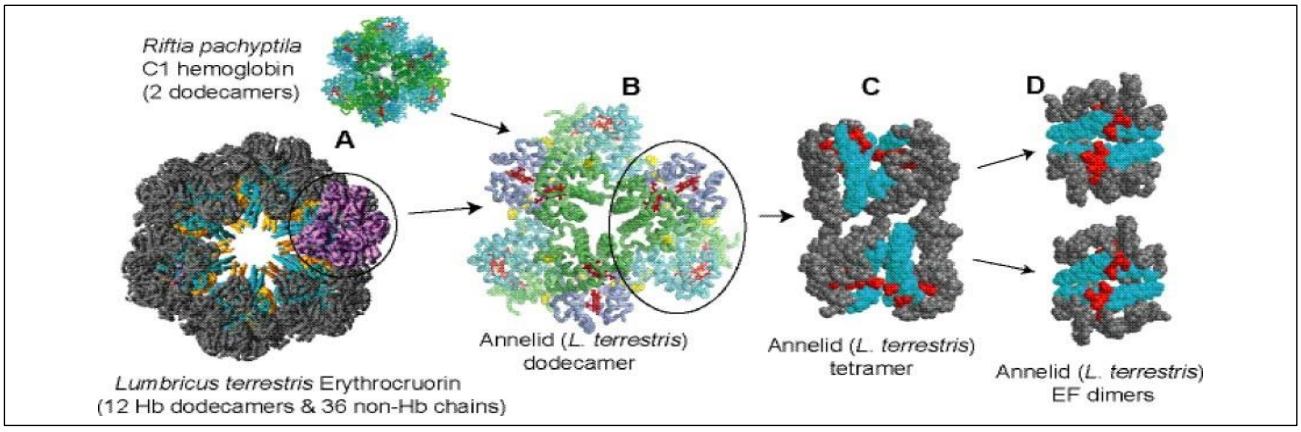
ABSTRACT

The increasing human population on earth has increased household waste and wastewater worldwide. Wastewater due to have H_2S is often toxic and has a pungent odor. To solve the problem of household waste, it is better to bury household waste and biogas production for consumption as fuel. Natural gas and biogas have H_2S. The main purpose of this research was to use Eisenia foetida worms in the H_2S removal from solution for performance in water scrubbing gas, biogas, and wastewater purification. The samples were bought from vermicomposting farms in Mashhad City. The tolerance of the worms to hydrogen sulfide was determined. In two identical cylindrical experimental treatments and control, and H_2S solution was poured. Live worms were added to the experimental treatment and the H_2S concentration was measured at every 30 minutes intervals. Adsorbed capacity and removal efficiency was calculated. In the experimental treatment adsorption capacity was 66 (mg/g) and the removal efficiency at the end of the test at 220 minutes reached 100%. The results showed that worms can adsorb and remove significant amounts of H_2S.



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1. Introduction

The increasing human population on earth has maximized household waste and wastewater worldwide. There are two most important environmental problems in countries and many countries are trying to solve these problems in a variety of ways. Likewise, there are several techniques for wastewater and household waste treatment. Contamination and wastewater water are often toxic and have a pungent odor. One of the main causes of bad odor and water toxicity is H_2S. Therefore, it is necessary to remove H_2S from wastewater water, for water purification. In addition, one of the ways to solve the problem of household waste is to the burial of household waste of which biogas is produced under anaerobic digestion conditions by bacteria [1]. Generally, biogas has 55-65% methane, and 35-45% Carbon dioxide, 0. 5-1% hydrogen sulfide and a small amount of water vapor [2]. Depending on the initial origin of the biogas producers, the concentration of H_2S varies from 80 to 10000 ppm [3]. Moreover, natural sour gas contains large amounts of acidic compounds such as carbon dioxide, hydrogen sulfide, and other sulfur compounds [4]. H_2S in gas and biogas can initially cause damage to pipes and secondly, corrosion of engine and metal parts [2]. On the third step, it is toxic for modified catalyzes and fuel cells fourth, burning H_2S leads to the exit of sulfur dioxide, which has destructive environmental effects. Therefore, gas and biogas production for consumption as fuel should be purified. For this, harmful gases such as H_2S should be removed first, so that the amount of gas energy increases [2]. The main purpose of this study was to use worms in the removal of hydrogen sulfide from gas and biogas in water. The removal of H_2S from wastewater is concerned, as well.

1.1. Mechanisms of H_2S generation in biological systems

Under acidic aerobic conditions and high moisture, the reaction of calcium carbonate with iron sulfide and oxygen leads to the production of calcium sulfate, ferric oxide, and carbon dioxide.

$$8CaCO_3 + 4FeS_2 + 8O_2 \rightarrow 8CaSO_4 + 2Fe_2O_3 + 8CO_2$$

Then, under anaerobic conditions, the reaction of sulfate with the organic matter by sulfur-reducing bacteria leads to the H_2S production [2, 3].

$$2CH_2O + SO4\ 2- \rightarrow H_2S + 2HCO_3$$

In domestic wastewater, H_2S is produced in the presence or absence of oxygen by Desulfovibrio desulfuricans bacteria and other sulfate-reducing bacteria so that the reaction of sulfate ions with organic materials leads to the production of hydrogen sulfide and carbon dioxide.

$$SO4^{2-} + \text{Organic matter} \rightarrow H_2S + CO_2$$

In places without oxygen, sulfur-reducing bacteria commonly use sulfate as an electron receptor in the anaerobic respiratory chain, providing energy and growth through this process. The mechanism of converting of sulfate into hydrogen sulfide is displayed in Fig. 1 [4-6].

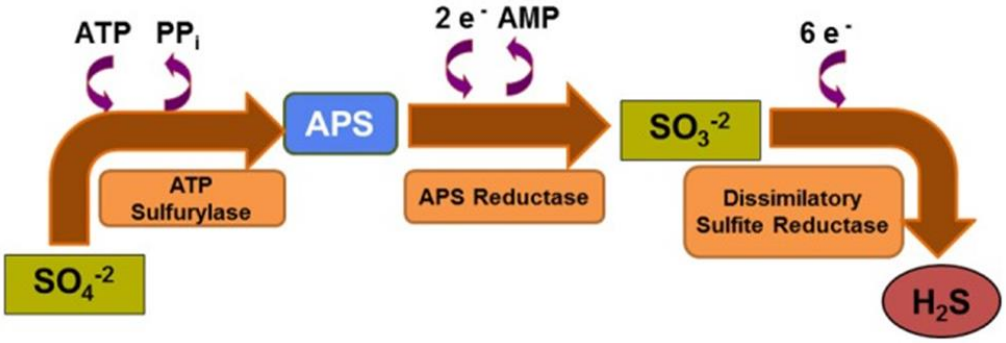

Figure 1. Microbial sulfate reduction pathway [6]

On the other hand, taurine, which is a sulfated substance, is secreted by eukaryotic cells in the body and is involved in regulating bile acid chelating, osmoregulation, and heart function, retinal growth. It is degraded by microbes Bilophila wadsworthia in intestine and a process leads to the H_2S production (Figure 2) [6, 8].

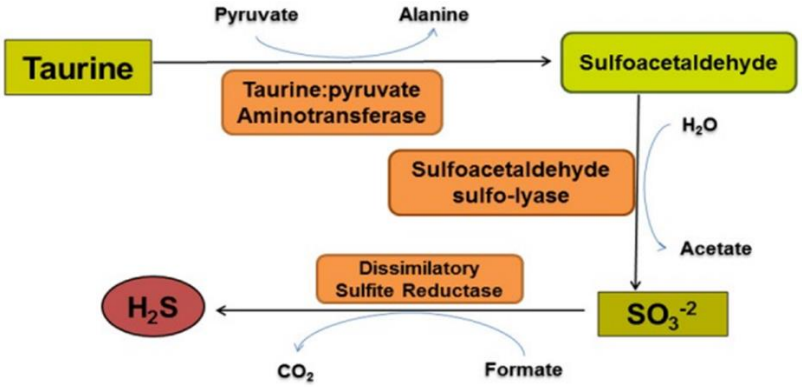

Figure 2. The taurine degradation pathway of Bilophila wadsworthia [6]

1.2. H_2S removal from water

Hydrogen sulfide is a toxic gas with corrosive effects [4, 7, 11-13]. There are several techniques for removing hydrogen sulfide from the water. These techniques include aeration, zonation, ion exchange, reverse osmosis, and biological and chemical treatment [3, 14, 15]. Removal of H_2S by biological treatment includes biofilters, bio trickling, and bio scrubbing towers. The basis of these methods is microbiology [16-18]. One of the methods of H_2S biological removal from gas is the injection of gas into the activated sludge tank. In this case, H_2S dissolved in sludge water is decomposed by microorganisms [9]. One of the efficient techniques for removing contaminants from water sources is the adsorption process [19]. Adsorption has a two-phase solid-liquid system. During this process, the material separates from the liquid phase and enters the solid phase [12]. Chemolithotrophic bacteria derive their energy from H_2S oxidation to thiosulfate or sulfate and sulfur [13, 20]. These heterotrophic bacteria oxidize hydrogen sulfide by sulfide: quinone oxidoreductase (SQR) and persulfide dioxygenase (PDO) enzymes [13, 21, 22]. Various bacteria are further used to remove H_2S under aerobic and anaerobic conditions [13, 23].

1.3. H_2S removal from gas

The H_2S separation in the oil and gas industry has significant economic and environmental resources [3]. There are several technologies for H_2S removal from biogas. In general, H_2S removal methods based on the type of removal action are divided into two groups as physicochemical and biotechnology methods. Although physical, and chemical methods are traditional, they are still used frequently. Biotechnology methods have been considered in the past two decades and many experiences in this field are indicated that biotechnology methods are more efficient. On the other hand, these methods do not produce the secondary flow of materials that need to be re-cleaned. However, standardizing biological removal methods requires basic and applied research. Using a combination of these methods is developing [2]. The selection and preparation of adsorbents are very important in implementing an adsorption method [3].

1.4. H_2S removal from gas by water scrubbing technique

One method of separating H_2S from gas is the water scrubbing technique. In this technique, compressed biogas is imported from the bottom and compressed water is imported from the top

of the scrubber tower and inside the scrubber, there is a column of packed material [24-27]. This increases the contact surface for absorption [26, 27]. This technique is based on the formation of gaseous hydrates. One of the new methods of gas separation is the formation of gaseous hydrates. Hydrated gas crystals are made from host gas molecules and water [27, 28]. The general formula of gas hydrates is MnH_2O, which M indicates the number of gas molecules by studying the structure of the water molecule, the researchers found that hydrogen bonds stabilize the structure of water by forming a ring of the form [27, 29]. The kinetics of gas hydrate formation has three stages. These three steps include dissolution, nucleation, and growth. The initial step is to dissolve the gas in the liquid (water) phase. This step continues until the liquid is saturated. To reach the nucleation stage, the liquid should reach supersaturating. In this state, a large amount of gas in the liquid phase is dissolved more than normal under the same conditions of temperature and pressure. The growth phase begins when the hydrate gas clusters are large enough to form a single nucleus [27, 30]. In other words, these three stages include the penetration of gas into the water surface, surface migration, and the formation of primary nuclei, and the growth stage of crystals [9, 27].

1.5. Stretcher of Annelid hemoglobin for H_2S removal

Researchers discovered that organisms living in areas rich in H_2S have certain mechanisms to neutralize the toxic effects of H_2S. These mechanisms include oxidizing enzymes or amino acids banded to sulfide [31]. Research has shown that hemoglobin in the blood of these organisms can be connected to sulfide [32]. This hemoglobin is an extracellular protein and exists in three groups of worms which are Annelids, Pogonophorans, and Vestimentiferans [31]. Studies of morphological, genetic, and embryological have indicated that these three groups are closely related [33]. Most of the information about hemoglobin of these three groups is obtained from the study of earthworm Lumbricus terrestris hemoglobin. The results of these studies are assigned to all groups [34]. It is a large giant extracellular hemoglobin with a reported molecular weight between 3000 to 4000 kDa [33, 35, 36]. Vermicompost Eisenia foetida and earthworm lumbricus terrestris are from the group of enlides [31, 32, 34]. The structure of giant extracellular hemoglobin in these worms is composed of two decamers. Each decamer includes a trimmer, each unit has a trimmer with a tetramer, and each unit of a tetramer has two dimmers, and each unit of a dimmer has four chains. In total, one decammer has 144 globin chains (Figure 3) and 36 linker peptides [34, 36-38].

Many researchers have reported hexagonal bilayer disk structures for this form (Figure 4) [24, 37, 39].

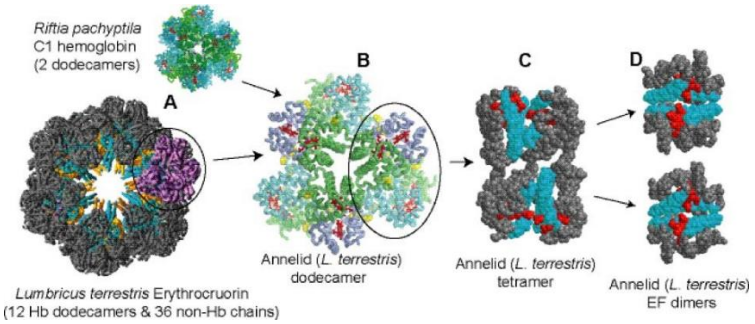

Figure 3. Hierarchical subunit arrangement in gain extracellular hemoglobin of annelid [36]

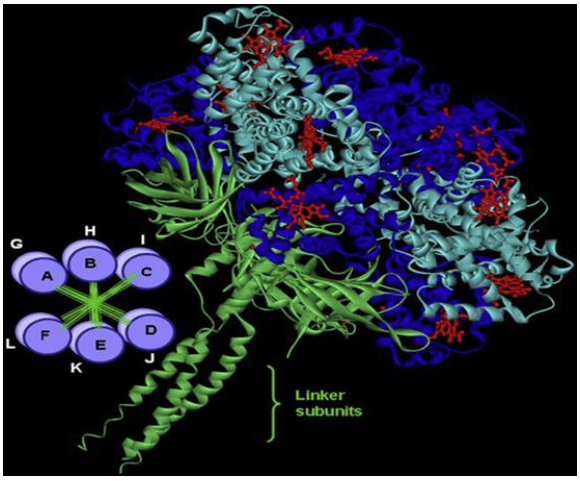

Figure 4. Hexagonal bilayer disk structures for gain extracellular hemoglobin [37]

In new research, gain extracellular hemoglobin in annelids is called erythrocruorin [36, 39]. This hemoglobin in the body of these organisms can perform two functions simultaneously. These two functions include oxygen and hydrogen sulfide transfer [33, 40]. Dimensions of vermicompost Eisenia foetida worm hemoglobin have been measured by TEM images and reported several times (Table 1) [41].

Table 1. Dimensions of vermicompost Eisenia foetida worm hemoglobin [41].

Species	High (nm)	Distance between two parallel sides(nm)	Staining	References
Eisenia foetida	17.5	26	uranyl acetate	Frossard, 1982
Eisenia foetida	16.5	26	phosphotungstate,	Frossard, 1982
Eisenia foetida	14	25	phosphotungstate,	Ochiai and Enoki, 1981

There are also two different positions of hemoglobin in the body of the worm. These two positions include the vascular blood and coelomic fluid. These two types of hemoglobin can bind to O_2 and H_2S and transfer them to the symbiont [33, 42]. In worms, sulfide is supplied to symbiotic bacteria by hemoglobin [33]. Worms Oligobrachia mashikoi and Riftia pachyptila, which have no mouth, are nourished by internal symbiotic bacteria that live in their trophosome [33]. An intestinal study of vermicompost Eiseina fetida revealed that it contained 21 Symbiotic bacteria[43]. Materials such as oxygen, hydrogen sulfide, carbon dioxide, and food are absorbed by the worm from the environment and delivered to the symbiotic bacterium through a vascular system in an organ called the trophosome [3, 42]. Chemolithoautotrophic bacterial are symbionts [42, 44] that derive their energy from sulfide oxidation [44].

1.6. Mechanisms of H_2S removal by hemoglobin Annelid in worms

Arp et al. were the first to show that sulfide binding in hemoglobin of reifitia worm is done in the site of disulfide bridges and is carried out through the exchange mechanism of thiol-disulfide which is the result of a regenerative reduced cysteine amino acid and a per sulfide group [32, 45].

$(-CH-CH_2-SS-CH_2-CH-) + H_2S \longrightarrow (-CH-CH_2-SSH) + (-CH-CH_2-SH)$

(Cysteine+per sulfide group ⟶ hydrogen sulfide+ disulfide bridges)

In their work, Suzuki et al. announced that sulfide binding to hemoglobin occurs in the position of

free cysteine. Therefore, disulfide bridges and free cysteine are the binding sites of hydrogen sulfide in hemoglobin [32, 33, 46, 47]. In this case, S-sulfohemoglobin is formed by binding sulfide to hemoglobin [33, 46]. These results were obtained by binding mercury to cysteine at the site of sulfide binding to hemoglobin [33]. In the structure of this hemoglobin, the sulfide binding position is other than the oxygen-binding position [3]. Red worms Vermicomposting has removed H_2S from experimental treatment by absorbing it. In these conditions, H_2S is absorbed by worms and in practice, H_2S concentration has been zero in experimental treatment containers. The basis of this research is relied on H_2S neutralization in worms. The main purpose of this study was to use worms in H_2S removal from gas and biogas in water. In addition, the removal of hydrogen sulfide from wastewater. This is a new research on the methods of H_2S removal with biological treatments. Similar research has not been reported to date. The authors hope that the results of this article will be used and developed to remove hydrogen sulfide.

2. Materials and Methods

2-1. Preparation of H_2S saturation solution

The hydrogen sulfide saturation solution was prepared based on the method Hughes [7]. To prepare the H_2S saturation solution, the five connected wash bottles were used. For this, they were connected via a high-quality PVC clear hosepipe with a tight connection, and the wash bottles were placed in a container which was filled up by water. By passing oxygen from inside the series of wash bottles, a leak test was performed. After ensuring no leakage, 200 mL of distilled water was poured inside the first wash bottles and in each of three wash bottles, the next 200 mL of the trapping solution was poured, as well. In the fifth wash bottle, 100 mL of distilled water was poured. In the next step, the solutions of wash bottles were deoxygenated. Thus, argon gas was passed from within the series of wash bottle solutions for 40 minutes. Followed the series of wash bottles connected to the H_2S reservoir and the H_2S saturation, the solution was prepared.

2.2. Earthworm samples

The Eisenia foetida samples were bought from vermicomposting farms in the Mashhad City of Khorasan Razavi Province in Iran. These samples were transferred inside the substrate containing cow manure under live conditions in laboratories at the Research Institute of Applied Sciences at Shahid Beheshti University in Tehran. The samples were kept in the laboratory for 24 hours for adaptation and to avoid stress conditions (Figure 5). To perform laboratory tests, some samples were selected from the cow manure substrate. The selected samples were washed with water, and then the water was discharged from the sample, and the wash samples were weighed (Figure 6).

Figure 5. The samples in cow manure

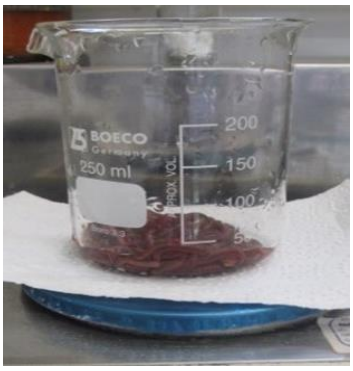

Figure 6. The samples weighed

2.3. Determination of the worm's tolerance to H_2S

Determination of the worms' tolerance to H_2S concentration was performed. For this, dilution from main stocks containing H_2S saturation solution was carried out [7, 48]. Therefore, based on the Koch method in 1883 dilution from the main solution was prepared [49]. In five series test tubes, 90 mL of deoxygenation of distilled water was poured. Then in the first test tube, the amount of 10 mL from the stock of hydrogen sulfide saturation solution was poured, and so dilution was done in the rest of the test tubes. Therefore, the concentrations of hydrogen sulfide were prepared from 0.01 to 0.00001 mol/L in test tubes. Then, 10 worms were transferred to each of test tubes and the cap was closed. The survival of worms was investigated in solution. This initial baseline measurement was done for the final evaluation.

2.4. Measuring containers

In order to perform the tests, two identical cylindrical containers from steel were constructed. Each container from the top has an opening and closing lid and on the bottom, the side has an outlet valve for sampling (Figure 7). One of the containers was considered an experimental treatment and the other as a control. Each container was filled with 300 mL of oxygenated distilled water. The H_2S concentration in each container was 0.0002 mol/L (equal to 7000 ppm). Then, the amount of 25 g of live Eisenia foetida worms was added to the experimental treatment container. Both containers were placed on a metal pillar base (Figure 8). Sampling was done by opening the output valve at the intervals time for the H_2S assay.

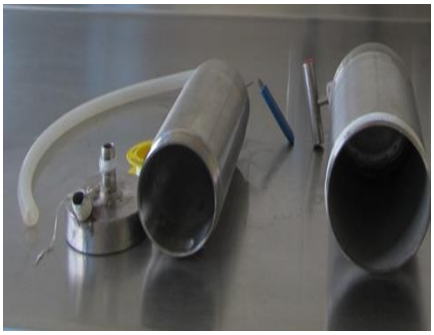

Figure 7. Two identical cylindrical containers

Figure 8. Containers placed on a metal pillar base

2.5. Assay method

Hydrogen sulfide concentration of the experimental treatment and control were measured at each 30 minutes' interval. This measurement is based on the Gunway method, on which if the H_2S solution is diluted within the deoxygenated Sodium bicarbonate buffer at pH 9.6, the concentration of anion HS- adsorption at 230 nm wavelengths will be conducted with the molar absorptive of 7200 mol^{-1} to indirectly measure the H_2S concentration in the aqueous solutions [7, 50]. This measure was done for experimental and control treatment over 220 minutes. The amount of H_2S adsorbed capacity (AC) (mg/g) was calculated by Equation 1[51].

$$AC = \frac{(c_i - c_t)}{m} V \qquad (1)$$

In which, C_i is the initial H_2S concentration (mg/g), C_t is the concentration of solution at time t, V is the volume of the solution (L), and m is the adsorbent mass (g). In this equation, the adsorbent mass in the control treatment is assumed 25 g equal to the mass of worms in the test treatment for the calculation. The H_2S removal efficiency (RE %) was calculated by Equation 2 [51].

$$RE = \frac{(c_i - c_t)}{c_i} \times 100 \qquad (2)$$

3. Results and Discussion

The concentration of H_2S saturated solution in water is 0.11 mol/l at room temperature. The results of determining worm's tolerance to H_2S indicated that in all concentrations from 34 to 341000 ppm, worms were survived after 15 hours. The results of experimental and control treatment for absorbing hydrogen sulfide from solution over 220 minutes are demonstrated (Figure 9). Based on these results, after 220 minutes, the absorbance of hydrogen sulfide in the experimental treatment reached zero, while the absorbance remains constant in the control treatment. Therefore, H_2S concentration in the solution of experimental treatment has reached zero. In practice, 7,000 ppm of the hydrogen sulfide is completely removed within 220 minutes, in other words, it was absorbed by the worms. In these conditions, the total H_2S in the solution is absorbed by worms. In addition, the H_2S concentration in the worm-containing solution in the experimental treatment was reduced to zero during the 220 minutes, while the concentration in the control treatment initially decreased slightly and then did not decrease (Figure 10). Moreover, the adsorption capacities of live Eisenia foetida worms with time for H_2S removal in the test treatment at 20 minutes 0.03(mg/g) and the end of the test at 220 minutes reached 0.07(mg/g), while removal efficiency in the control treatment at 20 minutes reached 0.007(mg/g) and remained almost constant during the test up to 220 minutes. Therefore, adsorption capacities in the test treatment have increased almost over time with a constant ratio (Figure 11). On the other hand, the removal efficiency in the test treatment at 20 minutes reached 20% and at the end of the test at 220 minutes reached 100%, while removal efficiency in the control treatment at 20 minutes reached 20% and remained almost constant during the test up to 220 minutes (Figure 12).

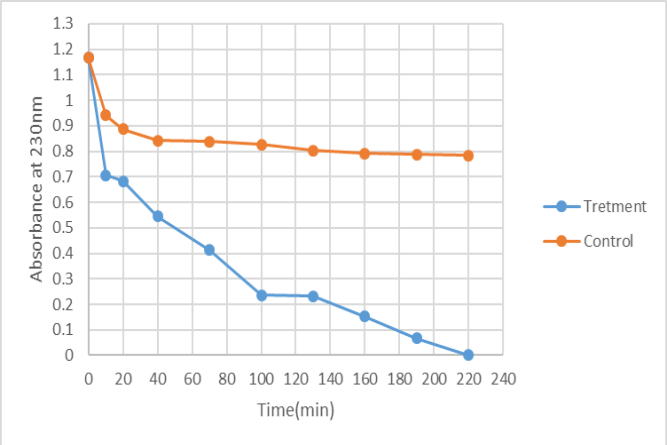

Fig. 9. Absorbance spectra for H_2S removal with time by live Eisenia foetida worms

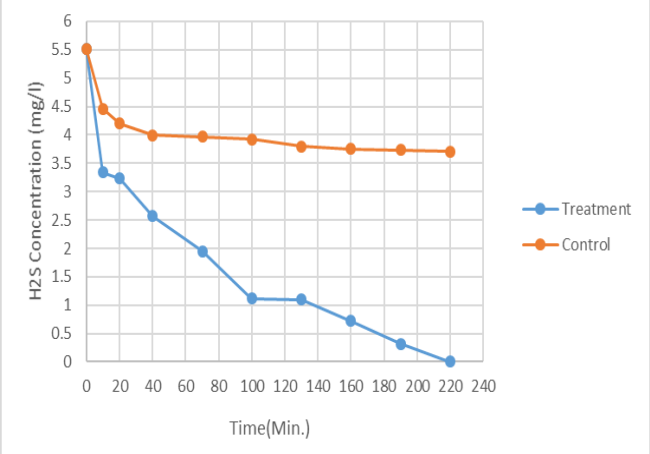

Fig. 10. Concentration spectra for H2S removal with time by live Eisenia foetida worms

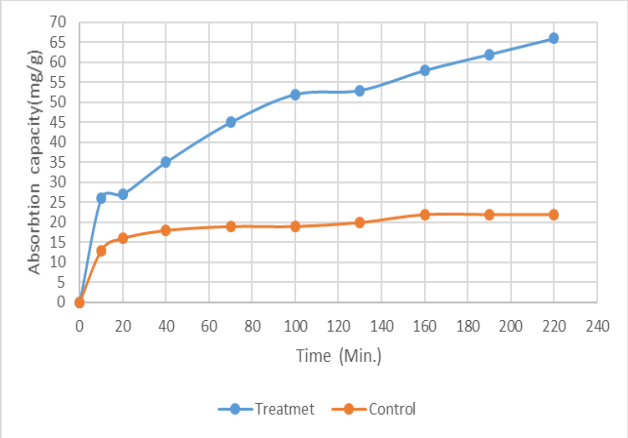

Fig. 11. Adsorption capacities of live Eisenia foetida worms with time for H_2S removal

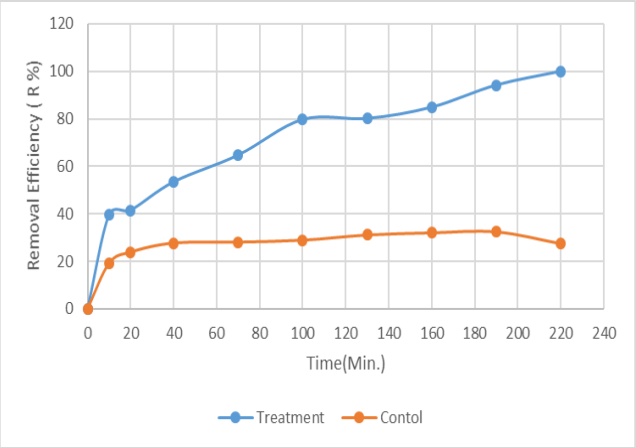

Fig. 12. The effect of contact time on H_2S removal efficiency (RE %) by live Eisenia foetida worms

The method used in this research is biological treatment. Therefore, in this research, vermicompost worms remove hydrogen sulfide under laboratory conditions. This is the first study which was done in this regard. So, similar results are scarce for comparison with these results; however, this method has advantages compared with physical and chemical methods. These advantages include low operation cost, low energy consumption, no secondary pollution, high efficiency, and environmental friendliness [52-55]. In addition, worms are readily available, have great reproductive and production power, can remove large amounts of H_2S, and can be replaced by new worms. Moreover, they are a biological treatment plant for the purification of contaminated water from H_2S. Worms' hemoglobin erythrocruorin has special properties compared to vertebrate hemoglobin. These characteristics include that worms' hemoglobin does not have red blood cells and worms' hemoglobin oxidation rate is lower than that of mammals, and studies have shown that it can be safely transferred in mice, rats, and hamsters without any side effects [38].On the other hand, the structural feature of the Fe-heme site in vertebrates hemoglobin in the worm has been observed in Eisenia Foetida. The structure of dodecamer hemoglobin reduces cooperation and increases the affinity for oxygen [34]. Likewise, the oxygen affinity is controlled by calcium cation, while this control is done in vertebrate hemoglobin with phosphate anion [56]. Kim reported that the adsorption capacity (AC) value of chestnut bur and bark for potentially toxic elements (PTEs) in wastewater treatment was 16.18 and 9.31 mg/g, respectively [57, 58]. Aghababaei reported the use of biochar for PTEs removal from wastewater. The results revealed that the AC was 264 mg/g. On the other hand, the used Forestry wastes for removing PTEs from wastewater removal efficiency (RE) was more than 69% [58, 59]. Liu reported the use of biofilter for removing hydrogen sulfide from the sludge to treat odors. The results showed that the RE was higher than 90% [60, 61]. Almarcha and Lafita in two separate studies implemented biofilters to treat odorous emissions. The results indicate that RE was higher than 96% [60, 62, 63]. Morgan-Sagastume investigated using a biofilter with a compost media for the removal of H_2S. The results revealed that RE was close to 100% [53, 64]. Ramos developed using Thiobacillus to remove H_2S from biogas. The results showed that RE was close to 96% [55, 65]. In all the research that has been done for the H_2S removal by biological treatments, the amount of RE and AC has been almost the same as in this research. Although the results are the same when comparing methods for RE and AC, the

continuous treatment of elimination, the amount of removal per unit time, cost, how to regenerate, and reproduce biological agents are among the effective factors for the utilized method. The method of using biological to remove H_2S is cheaper than physical and chemical ones and the use of worms to remove $H_۲S$ is much more efficient and powerful than the methods of using bacteria. Worms can adsorb and remove significant amounts of H_2S. One of the most significant factors to choose an absorbent is the price [66]. The economic advantages of using biological treatment include high efficiency, lower price, less secondary pollution, and low initial capital [53, 58, 66, 67-70] which in control treatment compared with the experimental treatment, it had less absorption, since there was no reaction in the control treatment to absorb H_2S. However, continuous adsorption of H_2S in the experimental treatment resulted in a decrease in H_2S concentration in the aqueous solution. So that in the end, the H_2S concentration in the solution has reached zero and RE was 100%. Absorption of H_2S by biological methods leads to an increase in pH in the solution [13, 71]. In this research, the removal of oxygen from the test solution is considered a limiting factor, because in the presence of oxygen, sulfuric acid is produced by sulfur-oxidizing chemotrophic bacteria [13, 72].

4. Conclusion

According to the results of this study, the worms can remove H_2S dissolved in water, so purification of water contaminated with hydrogen sulfide can be done with worms. In addition, in the purification of biogas with the water scrubbing technique, we can use worms to remove H_2S dissolved in water and return the purified water to the cycle. On the other hand, this process prevents the entry of H_2S into the environment. This should be considered as a prominent issue that worms have been under oxygen deprivation conditions and in the real case, they have been forced to use H_2S.

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