Adsorption of Gold from Aqueous Systems Using Microbial Thermophilic Proteins

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Highlights:

- Modified Thermus enhanced medium as a cultivation medium for thermophilic bacterial strain to improve protein production.
- This current paper did optimization variable conditions such as temperature and initial pH for microbial protein productions.
- Adsorption isotherm for gold adsorption using microbial protein fitted with Freundlich isotherm equation.

Abstract. Precious metal such as gold can be obtained from mining. Metals in low concentrations at geothermal sites or in industrial waste are difficult to gain using the conventional mining process. Alternative approaches for recovering metals from dilute solutions have been developed, such as biosorption, i.e. adsorption using microorganisms or their derivatives. In this study, gold in an aqueous system was recovered via biosorption using proteins produced from an isolated thermophilic bacterial strain. Modified Thermus enhanced medium was used as the medium to improve protein production from the thermophilic bacterial strain. The microbial proteins showed effective conditions for Au^{3+} ion adsorption. The optimum adsorption conditions for Au ions occurred at pH 1 with an adsorption capacity of 482.0 mg/g protein. The metal ion adsorption capacity increased with increasing temperature. The adsorption isotherm was conducted at room temperature, because the Au ions could be well fitted by the Freundlich isotherm equation with q_{max} at 527.229 mg/g protein.

Keywords: *adsorption; geothermal sites; gold; microbial thermophilic; protein.*

1 Introduction

Noble metals are very valuable because of their special physical and chemical characteristics. Noble metals are not only used in jewelry, but gold for example can also be used for sheathing of electronic components, because it helps to dissipate heat and maintains the electrical properties of electrical junctions [1]. Platinum has outstanding biocompatibility combined with its good stimulant and sensing properties, ensuring its extensive use in cardiovascular applications.

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Palladium is an essential electronic component for multi-layer ceramics, capacitors, printed circuit boards, and hybrid integrated circuits [2]. Unfortunately, the implementation of such beneficial materials is hindered by the scarcity and expensive mining processes of noble metals like gold, platinum, and palladium. The main ways to obtain noble metals are mining or extraction from the earth. Noble metals can also be found in byproducts from residues, tailings, and wastewater streams of various industries, including geothermal water processes, deep seawater, industrial wastewater, and other sources with a small fraction of the noble metals [3]. Because of the special material properties of noble metals, more advanced technology is needed to obtain high purity, including zinc replacement precipitation, reverse osmosis, and electrochemical treatments [4,5]. However, the foregoing methods are either energy- and cost-intensive or cause pollution.

Biological treatment for metal recovery may be a good alternative when compared with conventional methods. Biological treatment is based on organisms that directly adsorb metal with high selectivity [6]. Biological methods such as biosorption or bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physic-chemical methods [7]. Biosorption has been proven to be quite effective in removing metal ions from a contaminated solution in a low-cost and environmentally friendly manner [8]. Various biomaterials have been examined for their biosorption properties and different types of biomass have shown levels of metal uptake high enough to warrant further research. Many microorganisms, such as bacteria, yeast and algae, can take up dissolved metals from their surroundings into their cells, which can be used for removing metal ions [7,9-11].

Biosorption occurs mainly through the binding of metal ions with the electronegative functional groups present on the cell wall or cell membrane of organisms, such as metal transport related membrane proteins [12]. Proteins can interact with metal ions; these interactions play an important role in biological systems [13-15]. Researchers have reported that pure protein from ovalbumin, lysozyme, and bovine serum albumin (BSA) can adsorb precious metals [16]. These reports suggest that proteins are good candidates for metal ion adsorbents. Proteins are complex molecules composed of a long chain of amino acids, consisting of carbon, hydrogen, oxygen, and nitrogen. Proteins show interactions with various metal ions. The functional group composition of proteins can cause specific metal-binding sites (metal-binding domains) with chelated metal ions. For example, electron-rich thiol metallothionein (MT) protein can be bonded with three kinds of divalent cations and six kinds of monovalent metal ions. MTs are widely applied for binding metal ions with proteins [17-20]. Craig *et al.* (1953) in [21] revealed strong interactions between the protein ovalbumin and chloroauric ($AuCl_3$) in buffered solution. The chemical reaction between amino

groups in ovalbumin and the $AuCl_4^-$ ion is described schematically in Figure 1. The chloroaurate ion is attracted to the positive $-NH_3^+$ centers on the surface of the protein by Coulombic forces.

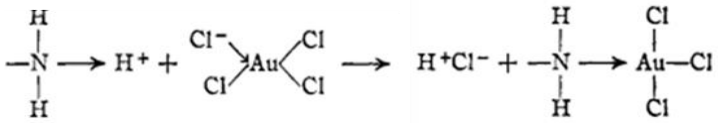

Figure 1 The reactions between protein ovalbumin and $AuCl_3$ [21].

Several researches have reported various types of proteins that are widely used as powerful biosorbents to bind metals, for example, MerP protein [12], phytochelatins [22], and metallothioneins [23]. Due to the complexity of metal-binding proteins, more than one process of binding likely takes place inside one system at the same time. Several studies have indicated a dominant role of ion exchange in metal-binding protein- mechanisms [24]. For instance, positively charged chemical groups present in biomass, such as amino groups, are capable of binding to negatively charged ions like arsenate, arsenide, chromate, or phosphate.

There are many variables that can influence metal biosorption. The most significant parameters that affect metal binding are biomass type, pH, ionic strength, and competition between metal ions. Other major factors that affect biosorption are initial metal ion concentration and biomass concentration in solution. Acidity seems to be the most important parameter in biosorption. It affects the chemistry of the metals in the solution, the activity of the functional groups in the biomass, and the competition of metallic ions [25,26]. In the experiment in this study, we used extracellular protein from thermophilic bacteria since it has heat-stable enzymes and can survive at high temperature. Most of the noble metals can be found in high-temperature aqueous systems. Thermophilic bacteria are found in hot springs, hydrothermal vent systems, sediment from volcanic islands, and deep-sea hydrothermal vents. In order to enhance the metal-binding protein, this study explored pH and temperature conditions in metal adsorption using microbial thermophilic proteins.

2 Material and Methods

2.1 Materials

Yeast extract and tryptone (BD), nitrilotriacetic acid (Alfa Aesar), $CaSO_4.2H_2O$ (Showa), $MgCl_2.6H_2O$ (Showa), 0.01 M ferric citrate (Showa) with trace element solution and phosphate buffer were used as cultivation medium. Aurum standard solution (high purity standard, USA) was used as metal for adsorption. Acetone

(Taiwan Tobacco, 95% purity) was used as protein precipitation. Bovine serum albumin (BSA Sigma) was used as protein standard.

2.2 Methods

2.3 Protein Cultivation

Meiothermus chliarophilus was cultured in modified Thermus medium [27] at 200 rpm, 55 °C, and without initial pH control. After the cultivation process, the culture broth was centrifuged at 10,000 rpm for 10 minutes. The supernatant, which contained extracellular proteins from *Meiothermus chliarophilus*, was concentrated using an Amicon 10 kDa ultrafiltration membrane. The extracellular proteins were concentrated 10-fold, washed using deionized water, and filtered with an Amicon 10 kDa ultrafiltration membrane.

2.3.1 Protein Recovery Using Different Acetone Volume Ratios

The 5 ml concentrated protein was added with 99% acetone and different protein to acetone volume ratios (1:1; 1:2; 1:3; 1:4; 1:5; 1:6; 1:7; 1:8) to get high protein recovery [28]. The mixed proteins and acetone were incubated for 30 minutes and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was removed with a pipette, being careful not to dislodge the protein pellet, leaving behind less than 100 µl of solution.

Residual acetone was removed from the pellet by air drying in an oven 30 °C for 2 hours. Five ml of deionized water was added to the dried pellet. Proteins were quantified through Bradford assay (Sigma) and measured using an Elisa OD_{595}, as shown in Figure 2 and 3. Protein recovery was calculated as the protein fraction recovered in the pellet.

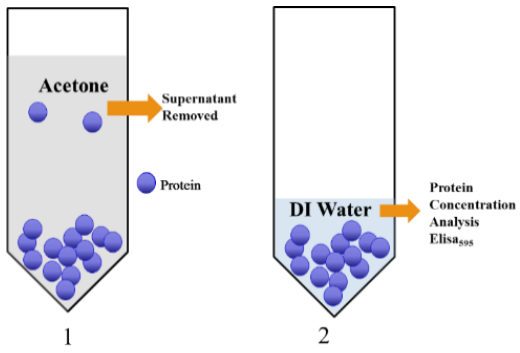

Figure 2 Protein recovery using acetone precipitation. The mixed protein and acetone (1) after centrifugation and (2) after the removal of acetone. DI was added for ELISA OD_{595} analysis.

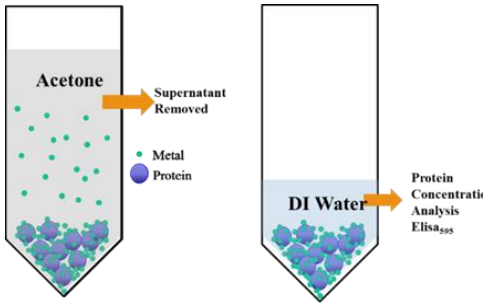

Figure 3 Protein recovery measurement. The mixed metal-binding proteins and acetone after centrifugation (a). After removal of acetone, deionized water was added for protein concentration measurement using an Elisa OD_{595} (b). Protein precipitation was conducted using acetone.

2.3.2 Metal Adsorption Condition

Fifty mg/L of 2.5 ml protein and 100 mg/L of 2.5 ml aurum metal solution were mixed in a tube and incubated for 60 minutes at room temperature. As the control variable, 10 mg/L of 2.5 ml of each metal was added with 2.5 ml of deionized water and incubated for 60 minutes. The control variable was used to know how much metal was precipitated in the acetone precipitation process. The mixed solutions were added with pure acetone with a protein to acetone volume ratio of 1:6 and then incubated for 30 minutes. The samples were centrifuged for 10 minutes at 10,000 rpm, after which the pellet was collected. This was then dried in an oven at 30 °C for 2 hours. The samples were added with 5 ml of 5 M nitric acid (HNO_3) for ICP measurement to determine the protein binding with metal as shown in Figure 4.

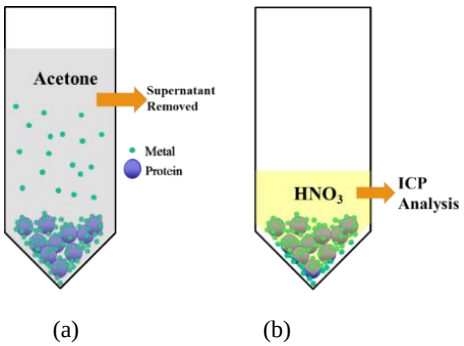

Figure 4 Metal-binding performance analysis methods using acetone precipitation. The mixed metal-binding protein and acetone after centrifugation (a). After removal of acetone, nitric acid was added for ICP measurement to determine the protein binding with metal (b).

The metal used in this study was Gold (Au). In the experiment, metal adsorption was conducted with initial pH controlled at pH 1, pH 2, pH 3, pH 4, pH 5, pH 6, pH 7 and pH 8, and with temperature varied at 25 °C, 50 °C, and 100 °C .

The adsorption capacity (q) of the metal with the protein was calculated using the following equation:

$$q\left(\frac{mg\ metal}{g\ protein}\right) = \frac{metal\ adsorption\ in\ protein\ \left(\frac{mg}{L}\right) - metal\ and\ DI\ water\ as\ control\ variable\ \left(\frac{mg}{L}\right)}{protein\ recovery\ as\ biosorbent\ \left(\frac{g}{L}\right)}$$

2.3.3 Determination of Biosorption Equilibrium

The optimum conditions in terms of pH and temperature were applied in the next procedure with different initial metal concentrations to get the value of adsorption equilibrium. 2.5 ml of 50 mg/L purified proteins were added to 2.5 ml of different initial metal concentrations (10-200 mg/L) of pure gold. The pH values were adjusted to pH 1 since this condition was optimal the for metal-binding protein. The adsorption experiments were conducted at 50 °C and mixed well with vortex, followed by incubation for 60 minutes to get the equilibrium activity. The rest of the experimental conditions and procedures for analyzing the metal adsorption measurement, protein recovery and control variables were identical to those described above.

The adsorption equilibrium data were analyzed by the Langmuir equations in Eq. (1).

$$q = \frac{q_{max} b C_{eq}}{1 + b C_{eq}} \quad (1)$$

where q is milligrams of metal accumulated per gram of biosorbent material; C_{eq} is the residual metal concentration in solution; q_{max} is the maximum specific metal ion uptake per unit weight of biosorbent to form a complete monolayer on the surface bound at high C_{eq}; b is the Langmuir constant related to the adsorption energy; q_{max} represents a practical limited adsorption capacity when the surface is fully covered by metal ions.

The Freundlich isotherm is represented by Eq. (2) as follow:

$$q = K_F C_{eq}^{\frac{1}{n}} \quad (2)$$

where C_{eq} is the equilibrium concentration (mg/L); q is the amount adsorbed (mg/g); K_f is the Freundlich constant related to the adsorption capacity of the adsorbent; and n is the Freundlich exponent related to the adsorption intensity.

3 Results and Discussion

3.1 Protein-Acetone Precipitation.

Several studies have been done on protein precipitation using an organic solvent such as acetone. Crowel, *et al.* in [28] used acetone with a protein to acetone volume ratio of 1:4 to precipitate several types of proteins, such as lysozyme, bovine serum albumin, carbonic anhydrase, and myoglobin, obtaining a protein recovery of 8%, 12%, 95%, 97%, respectively. The conditions for acetone precipitation were optimized by conducting different protein and acetone volume ratios to enhance the protein recovery efficiency. At a protein-to-acetone volume ratio of 1:1, the protein recovery obtained was only 64.8%, while it increased to 81.6% at a protein-to-acetone volume ratio of 1:6. When the ratio was further increased to 1:8 and 1:10, the protein recovery was similar, at 82.6% and 83.7%, respectively. This suggests that a protein to acetone volume ratio of 1:6 is most suitable for protein recovery. (See Figure 5)

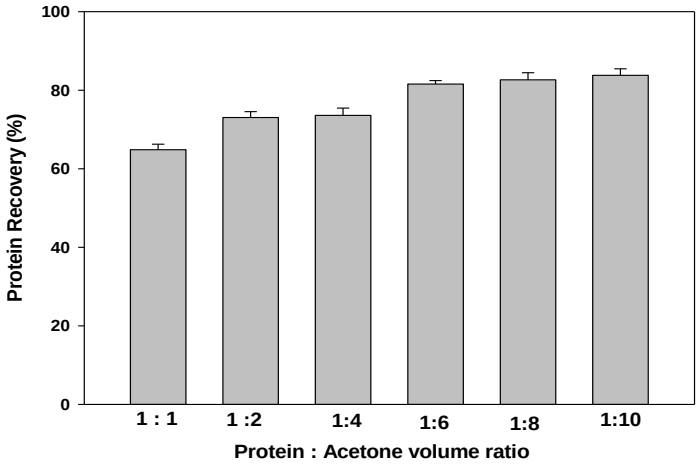

Figure 5 Effect of protein-to-acetone ratio on the recovery of protein produced from the thermophilic strain.

3.2 Metal Biosorption Conditions

Metal binding proteins are known to express in various cellular compartments to promote metal removal. In this section, we examine microbial proteins from a

thermophilic strain as biosorbent to bind with noble metal. The purpose was to identify the optimal conditions for metal-binding proteins to enhance aurum biosorption.

In order to get the optimum initial pH condition, a biosorption experiment was conducted with 2.5 ml of 100 mg/L metal and 2.5 mg of 50 mg/L initial protein. As shown in Figure 6, the biosorption of aurum in the protein was optimal at low pH conditions. The lower the pH condition, the higher the aurum content in the protein after precipitation. The initial pH was adjusted using sodium oxide (NaOH) and hydrogen chloride (HCl), which affects the metal adsorption. The aurum concentration in the protein after acetone precipitation at pH 1 was 24.7 mg/L and 4.8 mg/L in water as control variable. The aurum content in water after acetone precipitation can be explained by metal and sodium hydroxide reacting and forming metal hydroxide. In water as control variable, pH did not lead to precipitation of aurum since metal precipitation from pH 1 to pH 8 had almost the same value.

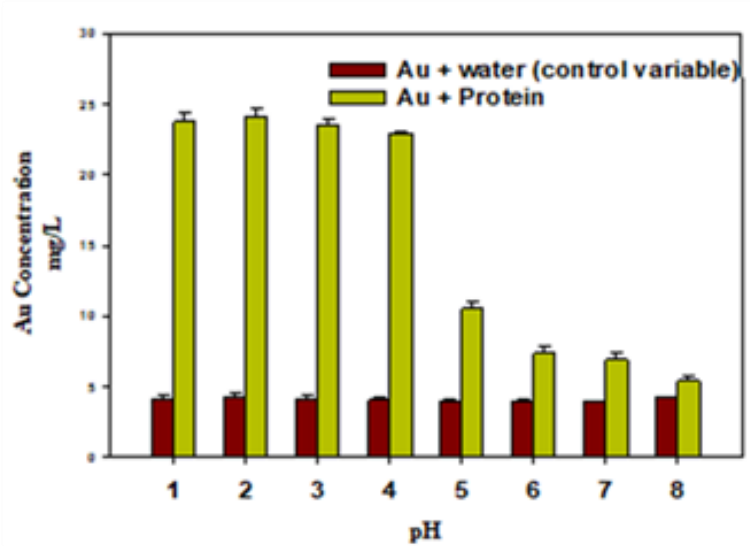

Figure 6 Effect of pH on biosorption of aurum ions by microbial proteins.

As can be seen from Figure 7, the solution pH had a significant effect on the adsorption capacity. For aurum biosorption, the data indicated that the adsorption capacity slightly changed between pH 1.0 and 4.0. Later it declined drastically for aurum ions and the optimum pH was at pH 2. The explanation for this is that at low pH, when the solution is controlled by HCl, the amount of chloride in the solution is high enough to favor the formation of chloro-anionic species that will be adsorbed into the amino groups of proteins [29]. Moreover, the protonation of

an amino group from protein-induced and electrostatic attraction of anionic metal complexes increases the number of available binding sites for precious metal ion uptake. In acidic solutions, platinum and aurum are usually present in solution in their most stable form, i.e. Pt (IV) and Au (III). They can form stable complexes, especially with amino group chelation sites of the protein, due to their soft acid characteristic [30].

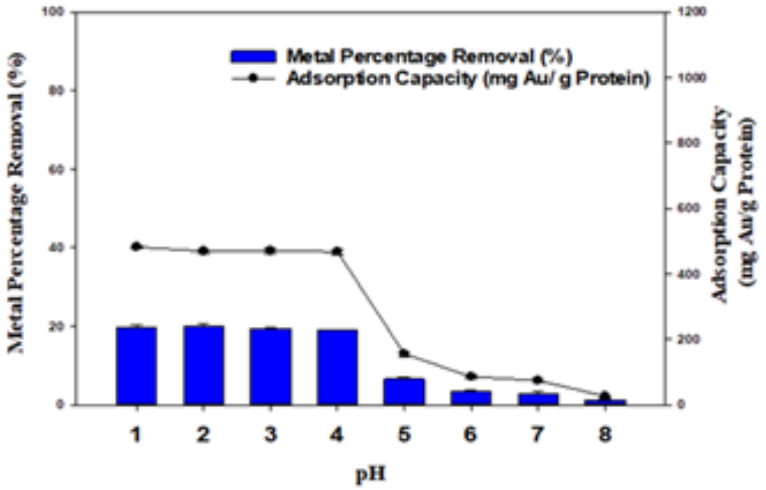

Figure 7 Percentage removal and adsorption capacity of aurum by microbial protein at different initial pH values.

At higher pH values, the decreased sorption capacity may be explained by the presence of less absorbable Au (III) species because of the lower availability of chloride anions. Also, at higher pH, the solubility of metal complexes decreases, leading to precipitation, which may complicate the sorption process. Higher pH also leads to precipitation of some metals, which is not favorable for their adsorption [31].

Figure 8 shows that higher temperatures resulted in higher adsorbent capacity. When the temperature was raised from 25 °C to 100 °C, the adsorption capacity increased from 450.250 to 486.457 mg Au/g protein.

In Figure 9, the removal percentage had similar results under different temperature conditions of the adsorption process. The effect of temperature on the metal uptake capacity hardly changed over the tested temperature range of 25 to 100 °C. Temperature difference had no significant effect on the adsorption process. Energy-independent mechanisms are less sensitive to temperature since the metal removal depends on physicochemical processes [32].

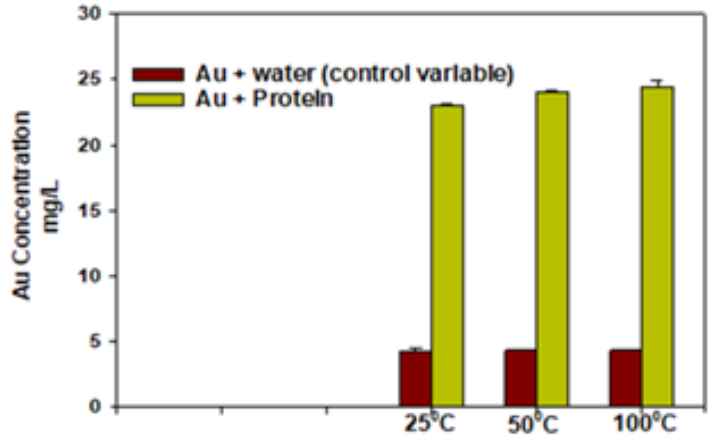

Figure 8 Effect of temperature on biosorption of aurum ions (b) by microbial protein.

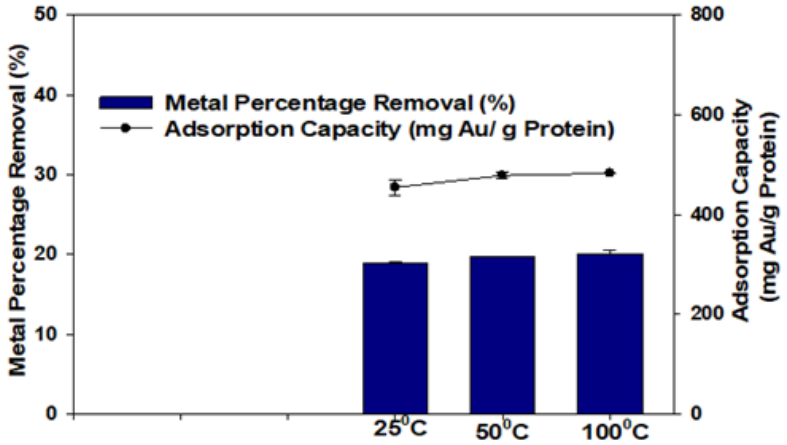

Figure 9 Percentage removal and adsorption capacity of ion aurum metals by microbial protein at different temperatures.

The adsorption isotherm and adsorption equilibrium data were analyzed using the Langmuir and Freundlich equations. The quality of adsorption by microbial thermophilic protein is determined by the amount of metal that it can attract and retain in immobilized form. An adsorption isotherm was used to evaluate sorption performance. The uptake increases with an increase in concentration and reaches saturation at higher concentrations [33]. Adsorption modeling can be performed using empirical modeling, which can predict the experimental outcome.

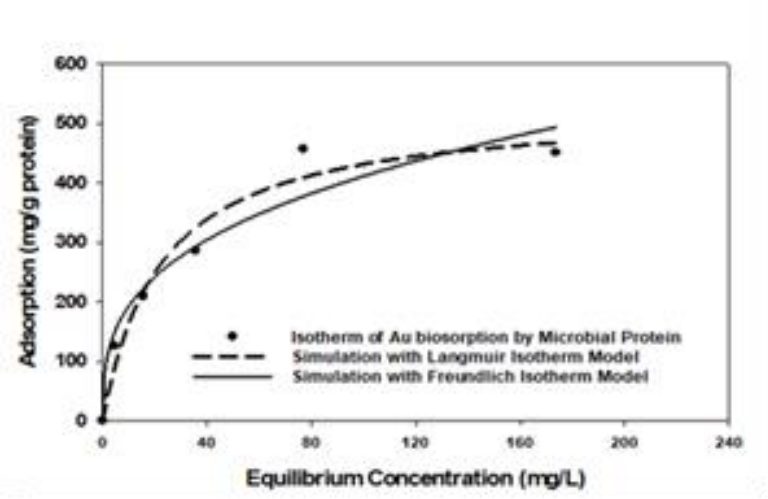

Figure 10 Isotherm of aurum biosorption by thermophilic protein strain.

A conventional adsorption isotherm model, the Langmuir isotherm equation, can be used to describe the adsorption process in the monolayer. The modification of the Langmuir isotherm that can be applied for multilayer adsorption is called the Brunauer–Emmett–Teller (BET) isotherm. BET's assumptions, such as surface uniformity, distribution of sites and negligible influence of the occupied site on the neighboring site, are related to the Langmuir monolayer adsorption [34]. The Langmuir model uses Q_{max} as a constant to represent the maximum uptake by the adsorbent.

The Freundlich isotherm assumes that through the increase of the adsorbate concentration, the adsorption will increase [35,36]. The n value (adsorption intensity) of the Freundlich equation is 3.039. If n = 1, the adsorption is linear; if n < 1, the adsorption is a chemical process; if n > 1, the adsorption is a physical process. The adsorption process is favorable when n > 1 since it indicates that the process is spontaneous [37]. From the regression coefficient (R^2) in Table 1, the adsorption data were fitted with both the Langmuir and the Freundlich isotherm model.

The R^2 values from the Langmuir isotherm model were slightly higher than from the Freundlich isotherm, which indicates that Langmuir adsorption is the best model for metal ion adsorption with R up to 0.9835 in the case of Au (III) for microbial thermophilic protein. Langmuir adsorption isotherm parameters can be used to predict the affinity between the metal and the adsorbent using a dimensionless constant called the equilibrium parameter (R_L) based on the following equation:

$$R_L = \frac{1}{(1 + bC_i)} \quad (3)$$

where b is the Langmuir constant and Ci is the initial concentration. The R_L value in the Au (III) adsorption to the microbial thermophilic protein was found to be 0.008 to 0.649. The value of R_L indicates the Langmuir isotherm type to be irreversible ($R_L = 0$), linear ($R_L = 1$), unfavorable ($R_L > 1$), or favorable ($0 < R_L < 1$) [38].

Table 1 Estimated parameters from experimental simulation data with Langmuir isotherms and Freundlich isotherms.

		Au
Langmuir Model	q_{max} (mg/ g protein)	527.299
	Kd (mg/l)	22.184
	R^2	0.9835
Freundlich Model	K_f	90.4236
	1/n	0.329
	R^2	0.9723

Note: The initial aurum concentrations ranged from 10 to 200 mg/l. The adsorption was conducted under an initial pH of 1 for Au.

4 Conclusions

Metal binding proteins produced from a thermophilic strain were utilized as biosorbents for binding with noble metal (aurum). The results showed that the microbial proteins can adsorb the aurum efficiently. For aurum, the optimum adsorption occurred at pH 1 with an adsorption capacity of 482.0 mg/g protein. The metal adsorption capacity increased with an increase in temperature. The metal adsorption capacity reached 483.2 mg Au/mg protein at temperature 100 °C. The adsorption isotherms for Au were fitted with the Langmuir equation.

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