SORPTION OF Au(III) BY *Saccharomyces cerevisiae* BIOMASS

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ABSTRACT

Au(III) sorption by S. cerevisiae biomass extracted from beer waste industry was investigated. Experimentally, the sorption was conducted in batch method. This research involved five steps: 1) identification the functional groups present in the S. cerevisiae biomass by infrared spectroscopic technique, 2) determination of optimum pH, 3) determination of the sorption capacity and energy, 4) determination of the sorption type by conducting desorption of sorbed Au(III) using specific eluents having different desorption capacity such as H_2O (van der Waals), KNO_3 (ion exchange), HNO_3 (hydrogen bond), and tiourea (coordination bond), 5) determination of effective eluents in Au(III) desorption by partial desorption of sorbed Au(III) using thiourea, NaCN and KI. The remaining Au(III) concentrations in filtrate were analyzed using Atomic Absorption Spectrophotometer. The results showed that: 1) Functional groups of S. cerevisiae biomass that involved in the sorption processes were hydroxyl (–OH), carboxylate ($-COO^-$) and amine ($-NH_2$), 2) maximum sorption was occurred at pH 4, equal to 98.19% of total sorption, 3) The sorption capacity of biomass was 133.33 mg/g (6.7682E-04 mol/g) and was involved sorption energy 23.03 kJ mol^{-1}, 4) Sorption type was dominated by coordination bond, 5) NaCN was effective eluent to strip Au(III) close to 100%.

Keywords: *sorption, desorption, S. cerevisiae biomass, Au(III)*

INTRODUCTION

Gold is a metal with a distinctive yellow color. By their nature, gold is used as a financial standard in many countries and is also used as jewelry, and electronics [1]. Generally, on earth, gold is found in the form of metal *(*native*)* contained in rock cracks and in the form of the mineral quartz. Traditional gold mining activities in Indonesia is characterized using simple and cheap technique i.e. exploration and exploitation techniques. The traditional technique is done by using rolling of gold and mercury as a catcher. Gold separated by roasted process until obtained the alloy of gold and silver *(*bullion*).* Waste collected in a gold processing tailings ponds, can be recycled for further gold processing. Nevertheless, the concentration of mercury contained in the final tailings discarded to the river is still large enough that cause pollution. Mercury is a toxic liquid metal and can cause serious health problems at certain exposure conditions. Mercury poisoning is marked by permanent damage to the brain, central nervous system, lungs, intestines and kidneys, even death. In addition tailings material still contains gold, silver and other metals, indicating that *recovery* is not optimal processing and tailings handling are not doing well [2]. Therefore we need an appropriate alternative gold recovery and environmentally sound.

In recent years, it has been known that certain species of microorganisms can accumulate large amounts of hazardous metals [3]. One of these microorganisms is *yeast. Yeast,* especially *S. cerevisiae* can be obtained from the wastewater results of the fermentation process the beer industry. According to Suh et al. and Vasudeva et al. [4-5] *S. cerevisiae* has a relatively high ability in sorption metal ions. Some researchers [6-9] proved *S. cerevisiae* is able to sorption Cd(II), Mn(II), Pb(II), Cr(III), and Zn(II). *S. cerevisiae* is also known to bind radio nuclides of uranium and thorium [10].

In order to solve environmental problem caused by gold mining and to increase the added value of industrial waste material of the beer fermentation process, *S. cerevisiae* sorption against ion Au(III) metal in water medium was studied.

EXPERIMENTAL SECTION

Materials

The materials used in this research were *S. cerevisiae* biomass derived from waste liquid fermentation beer industry (PT Multi Bintang) Mojosari-Mojokerto, East Java, gold metal (24 carat), hydrochloric acid, nitric acid, sodium hydroxide, potassium nitric, sodium nitric, potassium iodide and thiourea in analytical quality grade (Merck).

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Instrumentation

The instruments used in this research were Atomic Absorption Spectrophotometer GBC 932 AA, Spectrophotometer FT-IR Prestige 21, pH meters, centrifuges Centurion K 60, VXR *shaker* type, *freeze dryer,* oven, analytical balance, 100 and 200 mesh sieves, centrifuge tubes and polyethylene bottles and glass tools.

Procedure

Preparation S. cerevisiae

S. cerevisiae biomass from brewery waste was washed using distilled water and demineralized water until pH neutral, then dried by *freeze drying* method to obtain the dry *S. cerevisiae* biomass. The dry biomass of *S. cerevisiae* was sieved to get 100-200 mesh size.

Identification of Functional Groups Biomass with FTIR Spectrophotometer

Biomass (1 mg) before interacting with the Au(III) and after interacting with the Au(III) were mixed with dry KBr (100 mg) in pellet form and then analyzed using a FTIR spectrophotometer.

Determination of Optimum pH

Biomass of *S. cerevisiae* (100 mg) was interacted with 25 mL solution of 50 mg/L Au(III) in various pH such as 2, 2.5, 3, 3.5, 4, 4.5 and 5. Medium pH was adjusted by adding 0.1 N NaOH or 0.1 N HCl solution [11]. Furthermore, suspension was shaken with a speed of 350 rpm for 2 h and then centrifuged 2500 rpm for 10 min. The filtrate was analyzed by AAS to determine the concentration of Au(III). Sample and blank solution were analyzed under the same condition. The amount of Au(III) sorbed was considered to be the difference between the initial and the remaining amount in the reacting solution each time a sample was analyzed.

Determination of Sorption Capacity and Energy

A 50 mg of sorbent (*S. cerevisiae* biomass) was interacted with 25 mL of solution containing the various concentrations of 5, 10, 25, 50, 75, 100, 200, 300, 400, 500, and 600 mg/L of Au(III) at optimum pH which resulted from experiment of optimum pH determination above. Suspension was shaken with a speed of 350 rpm for 2 h, and then it was centrifuged at 2500 rpm for 10 min. Filtrate was analyzed by AAS to determine the remaining concentration of Au (III). Under the same condition with sample solution, blank solution was also analyzed. The data obtained was then analyzed using Langmuir isotherm model.

Determination of Sorption Type

Biomass of *S. cerevisiae* (250 mg) was interacted with 50 mL solution of Au(III) 500 mg/L at optimum pH, and it was shaken with a speed of 350 rpm for 2 h, then stored for 1 h, and after that it was centrifuged 2500 rpm for 10 min. Filtrate was analyzed by AAS to determine the remaining concentration of Au (III).

The precipitation from sorption experiments of Au(III) was added 50 mL of water and shaken 350 rpm for 10 min, followed by centrifugation 2500 rpm for 10 min. The filtrate was analyzed by AAS to determine the remaining concentration of Au(III). Then, the precipitate was interacted with 50 mL of 1 M KNO_3 and was shaken with a speed of 350 rpm for 60 min, then was centrifuged 2500 rpm for 10 min. The filtrate was analyzed by AAS to determine the remaining concentration of Au(III) separated by KNO_3 eluent. The precipitate was shaken with a speed of 350 rpm for 30 min, then centrifuged 2500 rpm for 10 min. Filtrate was analyzed by AAS to determine the remaining concentration of Au (III). Finally, the remaining precipitate was interacted with 50 mL of 0.1 M thiourea and was shaken with a speed of 350 rpm for 10 h, and then was centrifuged 2500 rpm for 10 min. Filtrate was analyzed to determine the remaining concentration of Au (III).

Determination of Effective Eluents in Au(III) Desorption

S. cerevisiae biomass (500 mg) in 50 mL polyethylene bottles was interacted with Au(III) 500 mg/L at optimum pH, and was shaken with the speed of 350 rpm for 2 h, then was centrifuged 2500 rpm for 10 min. The filtrate was analyzed by AAS to determine the concentration of Au(III) that was remained after sorption. The precipitate obtained was washed with demineralized water and dried at 80 °C to get constant weight and it was stored in dessicator overnight, which was subsequently used for desorption experiments. The precipitate was added with 25 mL of H_2O, and then it was shaken with a speed of 350 rpm for 10 min, and centrifuged 2500 rpm for 10 min. The filtrate was analyzed by AAS to determine the remaining concentration of Au(III) that was separated by H_2O. Precipitate was added with 50 mL of 0.1 M thiourea. After that, it was shaken with a speed of 350 rpm for 18 h, and centrifuged 2500 rpm for 10 min. The filtrate was analyzed by AAS to determine the concentration of Au(III), that was separated by the first thiourea eluent. These steps were repeated two times using 0.1 M thiourea eluent. To compare the effectiveness of eluent of Au(III) desorption, the procedure was also done using 0.1 M NaCN and 0.1 M KI eluents.

Calculation of Sorption, Desorption, Sorption Capacity and Energy

In sorption experiments of Au(III), the metal content that remain in solution was measured by Atomic Absorption Spectrophotometer (AAS). The concentration of Au(III) that was sorbed by sorbent at the equilibrium state is calculated using equation (1) [12]:

$$q=\frac{(C_0-C_f)\cdot V}{W} \qquad (1)$$

Where q is the concentration of Au (III) that is sorbed by sorbent at the equilibrium state (mg/g), C_o and C_f is the initial and final Au(III) concentrations (mg/L), V is the volume of solution (L) and W is the weight of sorbent (g). Langmuir isotherm sorption model is chosen to determine the sorption of Au(III), that expressed by the formula (2) [13]:

$$\frac{C}{n}=\frac{1}{n_mK}+\frac{C}{n_m} \qquad (2)$$

Where n is Au(III) ion sorbed per g biomass (sorbent) at equilibrium, C is the equilibrium concentration Au(III) in solution (mg/L), n_m is Langmuir's sorption capacity. The linear equation that generated by plotting the value C/n againts the value of C can be used to determine the equilibrium constant (K) and the sorption capacity (n_m). The value of them can be determined from intercept ($1/n_mK$) and slope ($1/n_m$). Total energy of sorption per mol can be determined from the equilibrium constant value with the equation $E = -\Delta G° = RT \ln K$

Concentration of Au(III) that measured after desorption is concentration of Au (III) releasing by the eluent. Desorption data of Au(III) were analyzed and calculated by equation 3 [14]:

$$\%\text{ Desorption efficiency } = \frac{\text{released gold (mg)}}{\text{initialy sorbed gold (mg)}} \text{x}100 \qquad (3)$$

RESULT AND DISCUSSION

Determination of Functional Groups in *S. cerevisiae* Biomass

Infrared spectra *S. cerevisiae* biomass before and after interacting with Au(III) were presented in Fig. 1a and 1b. The spectra of *S. cerevisiae* biomass before interacting with the Au(III) was identified the functional groups. The absorption band at 3402.43 cm^{-1} indicates stretching of –OH in carboxylic group, which is supported by the absorption at 1033.85 cm^{-1}. It shows stretching CO- in carboxylic, this absorption can also be identified as an amine –C-N [15]. The stretching vibration of –NH bands can also showed at 3402.43 cm^{-1}. Absorption at 1635.64 cm^{-1} shows the stretching vibration of C=O groups which supported by the existence of peptide-NH group as a bending vibration at 1527.62 cm^{-1}. An asymmetric -COO stretching vibration also showed at peak area 1635.64 cm^{-1} which is supported by the symmetry stretching vibration at 1396.46 cm^{-1}. The -OH bending vibration of carboxylic acids are also identified at 1396.46 cm^{-1} [16]. Thus the biomass *of S. cerevisiae* has a functional group of -OH,-COOH and $-NH_2$.

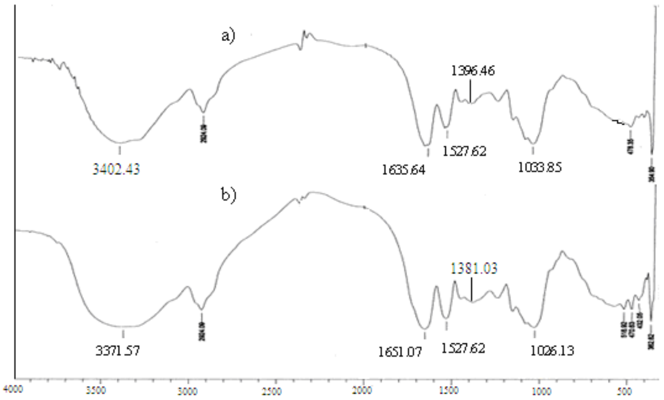

Fig 1. Infrared spectra *of S. cerevisiae* biomass before (a) and after (b) interacting with Au(III)

According to Sastroamidjojo [15], if the mass of atoms that bond to carbon atoms are greater, the vibration frequency will decrease. That statement can be explained that when a metal ion binds to ones functional group biomass *S. cerevisiae*, there will be a decreasing in wave numbers. The spectra of *S. cerevisiae* biomass after interacting with the Au(III) (1b) showed a decreasing wave number, i.e the wave number 3402.43 cm^{-1} to 3371.57 cm^{-1}, and 1033.85 cm^{-1} to 1028.13 cm^{-1}, 1396.46 to 1381.03 cm^{-1}, respectively identified as the stretching –OH, -NH, and -CO. From the identification above, it can be predicted that the functional groups that have a role in the process of sorption of Au(III) are -OH, -COOH and $-NH_2$. Based on the theory of Hard Soft Acid Base (HSAB) from Pearson, functional group-OH and-COOH are include as acid harder relatively than -NH group (base softer) preferred to interact with Au(III) ion.

Determination of Optimum pH

The result of sorption of Au(III) by *S. cerevisiae* biomass at pH 2, 2.5, 3, 3.5, 4, 4.5, and 5 were presented in Table 1. Variation of pH were done at pH 2 to 5 (acid pH). At pH above 6 Au (III) had begun to precipitate as Au $(OH)_3$ [11]. Visualization sorption of Au(III) at various pH was presented in Fig. 2. It showed that sorption Au (III) on the *S. cerevisiae* biomass was relatively not much different. *S. cerevisiae* biomass can sorp of Au(III) in quite high concentration at pH range 2–5 that was equal to 10.68–12.27 mg/g. Gardea-Torresdey et al. [17] reported that Au (III) sorption through by *Malone alfalfa* biomass is almost independent

Table 1. Sorption 50 mg/L Au(III) by 100 mg *S. cerevisiae* biomass on various medium pH

pH system	Au(III)$_{initial}$ (mg/L)	Au(III)$_{eq}$ (mg/L)	Au(III) sorbed (mg/L)	**n** sorps Au(III) (mg/g)	% Au(III) sorbed
2	50	1.6429	48.3570	12.0893	96.7141
2.5	50	1.5532	48.4468	12.1117	96.8937
3	50	1.2450	48.7550	12.1887	97.5100
3.5	50	1.2008	48.7992	12.1998	97.5984
4	**50**	**0.9061**	**49.0938**	**12.2735**	**98.1878**
4.5	50	1.1308	48.8693	12.2173	97.7385
5	50	7.2758	42.7242	10.6811	85.4483

Table 2. Sorption 25 mL of Au(III) in various concentrations at pH 4 by 50 mg *S. cerevisiae* biomass (29 ± 1 °C)

Au(III) initial (mg/L)	Au(III)eq (mg/L)	Au(III) sorbed (mg/L)	Au(III) sorbed (mg/g)	Au(III) sorbed (mol/L)	Au(III) sorbed (mol/g)	Au(III)eq/n (g/L)
0	0	0	0	0	0	0
5	0.5311	4.4689	2.2344	2.696E-06	1.1342E-05	0.2377
10	0.6507	9.3493	4.6747	3.303E-06	2.3729E-05	0.1392
25	0.6866	24.3134	12.1567	3.485E-06	6.1709E-05	0.0565
50	4.7131	45.2869	22.6434	2.392E-05	1.1494E-04	0.2081
75	6.9617	680383	34.0191	3.534E-05	1.7269E-04	0.2046
100	10.1473	89.8527	44.9263	5.151E-05	2.2805E-04	0.2259
200	32.7198	167.2802	83.6401	1.6609E-04	4.2457E-04	0.3912
300	90.7463	209.2537	104.6268	4.6064E-04	5.3110E-04	0.8673
400	164.0344	237.0740	117.9828	8.3266E-04	5.9890E-04	1.3903
500	250.5879	249.4121	124.7061	1.2720E-03	6.3303E-04	2.0094
600	346.1395	253.8605	126.9302	1.7571E-03	6.4432E-04	2.7270

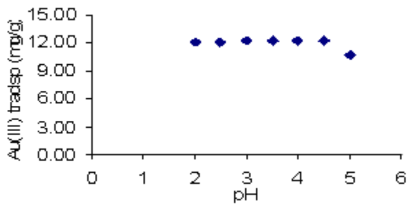

Fig 2. Sorption of Au(III) by *S. cerevisiae* biomass on the various medium pH

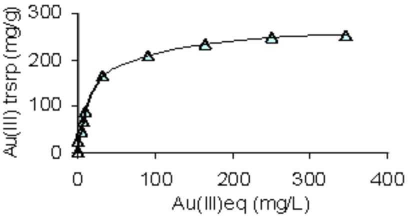

Fig 3. Curve of Langmuir Isotherm Sorption of *S. cerevisiae* biomass on the Au(III) at pH 4

on pH. The sorption of Au (III) by *Malone alfalfa* biomass at pH range 2.0 to 6.0 is high. Unlike common sorption of metal cations that highly depend on pH, $[AuCl_4]^-$ can be sorbed at pH 2 to 5.5, because Au(III) is as *square planar* anions [18].

Sorption studies at pH 2–5, giving the maximum sorption of Au(III) by *S. cerevisiae* biomass equal to 12.27 mg/g (98.19%) at pH 4. The same result is reported by Peirano et Al. [19] on the sorption of Au(III) using a biopolymer chitosan *cross-linked* with glutaraldehyde. Sorption of Au(III) at pH 3–5 was big enough and has reached maximum sorption at pH 4. Niu and Volesky [20] reported that the optimum pH of sorption $[AuCN_4]^-$ by the chitin material is at pH 3.5. Nakajima [11] reported that maximum sorption of Au(III) by *P. maltophilia* occurred at pH 3.

Sorption of Au(III) at pH 4 cause the active sites of *S. cerevisiae* biomass are become protonated and had positive charged. On the other hand, the metal cation Au(III) become to auric acid species such as $AuCl_4^-$, $AuCl_{3,}$ or $Au(OH)Cl_3^-$. At concentration of Cl^- approaching 10^{-4} M, the species of auric acid fully interact with the protonated active sites, such as $R\text{-}NH_3^+$ [19]. Sorption of Au (III) decreased to 85.45% at pH 5 because the number of active sites *S. cerevisiae* biomass that are protonated decreased, and of Au(III) ions are more exist in the form of $AuCl_{3,}$ or $Au(OH)Cl_3^-$, $Au(OH)_2Cl_2^-$, $Au(OH)_3Cl^-$.

Determination of Sorption Capacity and Energy

The sorption data on variation of Au(III) concentration was shown in Table 2 and illustrated graphically in Fig. 3. The application of Langmuir isotherm sorption model with the mathematical expression in eq. 2 obtained parameters such as sorption capacity (n_m) equal to 133.33 mg/g (6.7682 E-04 mol/g), sorption equilibrium constant (K)

Table 3. Sorption of Au (III) by 250 mg *of S. cerevisiae* biomass before Desorption

Au(III) initial (mg/L)	Au(III) eq (mg/L)	Au(III) sorbed (mg/L)	mean	Au(III) sorbed (%)
500	3.732	496.268	496.001	99.200
500	4.133	495.867		
500	4.133	495.867		

Table 4. Desorption Percentage of Au(III) on the *S. cerevisiae* biomass in Various Eluents by sequential

Eluent	Au(III)$_{desorbed}$ (%)
H_2O	0.287
KNO_3	0.194
HNO_3	0.123
Tiourea	22.256
Total	22.860

Table 5. Desorption of Au (III) by 0.1 M thiourea, 0.1 M NaCN and 0.1 M KI eluent

Eluents	Fraction	Au(III) desorbed (%)	desorption Total (%)
0.1 M Tiourea	Washed water	0.1620	84.6074
	H_20	0.1617	
	Tiourea	37.8509	
	Tiourea	30.9599	
	Tiourea	15.4729	
0.1 M NaCN	Washed water	0.1620	95.8781
	H_20	0.1617	
	NaCN	72.8692	
	NaCN	21.2788	
	NaCN	1.4064	
0.1 M KI	Washed water	0.1620	9.7230
	H_20	0.1617	
	KI	2.7169	
	KI	3.2905	
	KI	3.3919	

equal to 9.6254 E+03 $(mol/g)^{-1}$, and the energy of sorption (E) equal to 23.03 kJ/mol. Based on the results of this research, energy involved in the sorption of Au(III) by biomass of *S. cerevisiae* was in the range of 10–40 kJ/mol, indicating physical energy [21].

Sorption of Au(III) by *S. cerevisiae* biomass is the causal effect of the changing solution characteristic from brown color to permanent purple color (as higher concentration of metal as darker purple color). Greene et al. [22] reported that the color of micro algae changed to purple *of Cassius Purple* of wavelength 530 nm when sorption of metals from solution hydrochloro auric Au(III). This is because Au(III) reduced to Au(I) and gradually became Au(0) inside the cell biomass.

Determination of Sorption Type

Data from the desorption of Au (III) by *S. cerevisiae* biomass using the eluent of H_2O, 1 M KNO_3, 0.5 M HNO_3, and 0.1 M thiourea was carried out in a sequence that were presented in Table 4. The concentration of Au(III) sorbed by the *S. cerevisiae* biomass before desorbing by the four eluents was also presented in Table 3. Sequential desorption method can be applied to determine the sorption type of metal cations on a biomass [23]. The principle of this desorption model is the usage of eluents which has strength increasing in a sequence that is (1) eluent H_2O to replace Van der Waals forces, (2) electrostatic bonding or ion exchange is replaced by KNO_3, ion exchange occurs between cation K^+ of KNO_3 and the cations are bound by the active side of the previously sorbent, (3) HNO_3 used to desorp of metal cations that are bound by the force of hydrogen, (4) Desorption using thiourea eluent is to remove the metal cations that are bound by coordination bond, thiourea is a very strong chelating, forming more stable bond.

Table 4 shows the percentage of desorption of Au(III) of each eluent. Thiourea has the greatest ability in the releasing that was equal to 22.26%. Desorption with KNO_3, HNO_3 and H_2O are a very small, approximately 0.1–0.2%. Thiourea (CH_4N_2S) has two tautomeric forms (thiourea and isotiourea) and has three functional groups (amine, imine and thiols). Amine and imine functional groups are intermediate ligands/base, whereas thiols groups included to the soft base. Gold metal as well as, both ion Au(I) and Au(III) are classified into a soft acid [11]. According to the hard soft acid-base theory of Pearson, that soft acids with soft bases or strong acid with strong base will form a strong bond, as well as intermediate acid with the intermediate base [24]. The presence thiols groups on thiourea will bind well with the metal Au(III), so that thiourea has a greater ability to desorp Au (III) from its bond with the sorbent as compared with some eluent (H_2O, KNO_3 and HNO_3).

Determination of Effective Eluents in Au(III) Desorption

Au(III) is classified into a soft acid that binds strongly with the soft base (ligands) complexing agent and sulfur-containing ligands such as thiourea, merkaptoetanol, tiomalat acid, and tiogliserin [22,25]. Ramellow et al. [26] and Nakajima [11] had proved the ability of 0.1 M thiourea in releasing Au(III). In this research, researcher used eluent 0.1 M thiourea, 0.1 M NaCN and 0.1 M KI.

Table 5 shows the percentage of desorption of Au(III) of each eluent. The order of the eluent effectiveness in Au(III) desorption are NaCN > thiourea > KI. Metal Au(III) from it solution was sorbed by the *S. cerevisiae* biomass after reduced to Au (I) as in previous discussions. Elution of Au(I) using thiourea to form $Au(SCN_2H_4)_2^+$ has a formation constant (β_2) equal to 21.3, using NaCN to form $Au(CN)_2^-$ has a formation constant (β_2) equal to 33.7, and using KI to form AUI_2^- has a formation constant (β_2) equal to 23.8 [22]. Formation constant of $Au(CN)_2^-$ is the biggest, so it can desorbs Au(I) at 95.89%. Desorption using thiourea is larger than KI although the formation constants AUI_2 is larger than $Au(SCN_2H_2)_2^+$. It is because the thiourea (SCN_2H_4) can form a chelate, so it forms a more stable bond.

CONCLUSION

Based on experimental data in the study of sorption of Au(III) using the *S. cerevisiae*, it can be concluded that functional groups of *S. cerevisiae* that have a role in the process of sorption of Au(III), are -OH, -COOH and -NH_2. The maximum sorption of Au (III) occurred at pH 4 that equal to 12.27 mg/g (98.19%), sorption capacity (n_m) of *S. cerevisiae* on the Au(III) equal to 133.33 mg/g (6.7682 E-04 mol/g), sorption equilibrium constant (K) equal to 9.6254 E+03 $(mol/g)^{-1}$, and the sorption energy (E) equal to 23.03 kJ/mol. Based on sequential desorption method, it has been determined sorption type of Au(III) by *S. cerevisiae* biomass following type of complex formation (coordination bonding) because thiourea eluent has the greatest ability in the releasing of Au(III). Sequence eluent to desorption of Au(III) from the most effective were 0.1 M NaCN solution at 95.89%, followed by 0.1 M thiourea at 84.61% and 0.1 M KI solution which was sorption 9.72%.

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