University of Miyazaki

Doctoral Dissertation

Studies on the adsorption of heavy metals and precious metals using biosorbent prepared from Mongolian livestock biomass

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Chapter 1
General Introduction

Environmental pollution, especially water pollution, remains a major global problem. Environmental pollution is on the rise caused by industrialization and urbanization regarding high technology development around the world. For example, developing countries face economic and environmental pollution based on the inability to solve problems properly. Environmental issues include drinking water, soil, groundwater, and air pollution. In developing countries, environmental pollution spreads because of no management of wastes and other pollution.

Human health is directly dependent on the environment. As a result, many people around the world have got sick or lost their health due to environmental pollution. Mongolia is a developing country with abundant natural resources. Mongolia is rich in mineral resources and is registered with over 8,000 mineral deposits of 80 different minerals [1.1]. The map of major mining sites in Mongolia is shown in Fig. 1-1. Research on environmental pollution is increasing due to the expansion of mining activities.

Fig. 1-1 Map of major mining sites in Mongolia [1.2]

Most of the mining resources are not refined in Mongolia and are exported to the overseas. The mining companies and Mongolian governments are not yet aware of the natural conditions at the end of the mining operations. Heavy metals are recognized as toxic pollutants around the world, according to the survey by the Environmental Action Group in the United States [1.3], more than 10 million people were found in the most polluted areas in different countries around the world. Developed countries around the world are already interfered with this problem and are suffering from health problems due to the release of various pollutants such as heavy metals from mines into the environment. For example, the Toroku mine in Japan was operated until 1962, and after the Meiji era, tin, lead, copper, zinc, etc. were produced. However, Toroku arsenic disease was proposed in 1971, emitted arsenic-containing air caused serious health problems among residents [1.4]. For this reason, Mongolia needs proper management in mining operations, waste, and other pollution problems.

In this study, heavy metal and precious metal adsorption from wastewater was evaluated using biosorbent in different conditions.

Various methods are used to reduce heavy metal pollution and eliminate environmental pollution. These include membrane filtration (nanofiltration), chemical precipitation, solvent extraction, ion exchange, and adsorption (biosorption) methods. Of these, the biosorption method using biosorbents has recently been widely applied to heavy metal adsorption studies. It differs from other methods, in that it make adsorption using environmentally friendly and low-cost materials. The following are some examples of the application of biosorbent for the adsorption of heavy metals.

In recent, biosorbent of soy protein hollow microsphere [1.5], root bark of Indian Sarsaparilla [1.6], dead biomass brown alga [1.7], yeast biomass [1.8], biochar [1.9], Salvinia plant biomass [1.10], biomass of fungal species [1.11], mango biomass [1.12] are used as an adsorbent for removing heavy metals. Sheep manure, woodchips, and compost as reactive organic materials are used for immobilization of arsenic and manganese [1.13].

Soy protein hollow microspheres have been found to make adsorption of heavy metals quite effectively, 52.94 – 254.95 mg/g for Zn(II), Cr(III), Cd(II), Cu(II), Pb(II) and Ni(II) at 70^0C [1.5].

Jin et al. studied copper adsorption by feather fiber and adsorption amount increased with the treatment of alkaline solution. The copper adsorption capacity of the feather fiber increased as a high amount of α-helix in feather fiber [1.14].

In Mongolia, 20 breeds of 32.2 million sheep produce 45.1 thousand tons of wool. Wool production plants are producing around 25 thousand tons of wool and cashmere in Mongolia, annually [1.15]. Mongolian sheep usually produce four different types of coarse wool such as down, intermediate, coarse hair, and dead hair distinguished by their characteristics. A coarse type of wool is used to produce felt. Sheep wool is classified into 4 categories (MNS 0033:2007) such as fine, semi-fine, carpet wool, and coarse wool. Mongolians produce 28% of wool as a product, export 51% to China, and 21% remain as waste. Wool fibers have a large specific surface area and are effective against removing heavy metals. Therefore, sheep wool was selected as biosorbent for heavy metal adsorption based on its availability, eco-friendly, and unique chemical structure.

In addition, a lot of keratin-based materials were used as an adsorbent for removing heavy metal ions. For example:

- copper removal by duck feather/non-woven composite [1.14];
- arsenic removal by wool [1.16];
- copper, iron, zinc, mercury, arsenic, and chromium removal by wool produced by needle punching technology [1.17];
- chromium removal by hybrid polyurethane membrane with chicken keratin [1.18];
- chromium and copper removal by electrospun nano-fibrous membranes with keratin resin [1.19];
- lead, copper, mercury, and chromium removal by chicken feather [1.20];
- chromium removal by chicken feather [1.21];
- lead removal by poultry feather fiber [1.22];
- copper, zinc, manganese, and arsenic removal by human hair fiber [1.23];
- mercury, cadmium, and copper removal by human hair treated with NaOH/Na_2S [1.24];
- gold, palladium, and platinum removal by chicken feather powder [1.25];
- zinc removal by chicken feather particle [1.26];
- copper and zinc removal by chicken feather particle treated with NaOH and dodecyl sulfate [1.27];
- lead removal by colloidal keratin solution [1.28];
- calcium, iron, magnesium, and manganese removal by keratin amino acid immobilized silica particles [1.29].

The dissertation consists of five Chapter and the framework of this study is shown in Fig. 1-2.

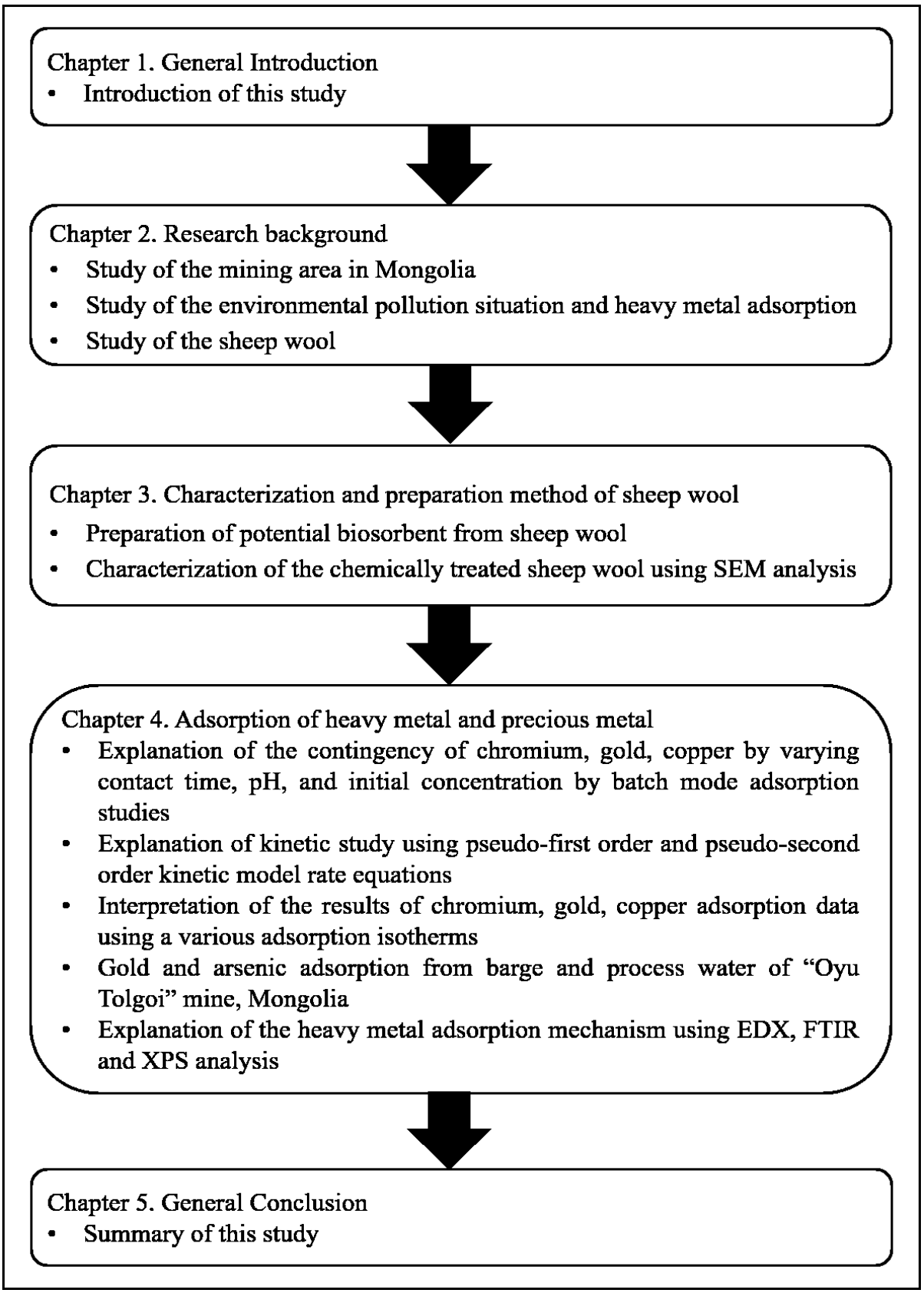

Fig. 1-2 Framework of the present study

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Chapter 2
Research background

1. Environmental pollution situation of Mongolia around the mining area

The natural environment is polluted with anthropogenic activities of mining and in recent years it remains one of the huge concerns around the world. Mongolia is one of the developing countries in the world and mining operations intensified since 1990. Mongolian Governments and miners are still not aware of the situation of the natural environment when mining is over. The world's most developed countries faced these problems already and local people suffering from poisoning by the environmental impacts of the mining industry. Nowadays heavy metal pollution emerges as mining, tailings, and wastewater [2.1]. Therefore, Mongolia needs to investigate a new removal technology and resolve the right solutions to the mining operations, mine waste, and pollution.

2. Precious metal adsorption from mining waste

Mongolia has riches by many natural resources such as gold, silver, palladium, and platinum. Although, Mongolian people are underdeveloped to extract precious metals completely from the mining area. Precious metals of gold, silver were thrown away around the mining area and there is a need to be a complete extraction method for the precious metals from the ore. The economics of Mongolia is concentrated on a few sectors that are dominated by the mining industry. The industrial sector of Mongolia is not developed well and exporting to foreign countries unprocessed raw material for a low price. Especially mining sectors need to involve new technology and method and should increase the efficient usage of the treasure of Mongolia which will improve the economic situation [2.2].

There are many kinds of biological methods exist to apply heavy metal removal from polluted areas. Biological methods include methods of biosorption [2.3], methods of using micro-organisms and bacteria [2.4], anaerobic digestion [2.5], and activated sludge methods [2.6]. One of these methods, biosorption is an eco-friendly, low-cost method, suitable for heavy metal adsorption along with other conventional methods of metal ion removal [2.7].

Some mining waste and wastewater still contain precious metals and other heavy metals of copper, nickel, silicate [2.8]. Hence, in order to make adsorption of the precious metal without the impact on co-existing ions contained in the wastewater, the adsorbent material must be high selectivity. In recent decades, applying biological methods and removing heavy

metal pollution has been paid attention including our previous study because of its high potential adsorption of heavy metal ions [2.9-2.14]. Among the biosorbent materials, there are hundreds of research bearing on the adsorption of heavy metals using sheep wool, which is widely available at low cost [2.15-2.18]. Due to the fact that sheep wool is environmentally friendly and contains a functional group of a high ability to adsorb heavy metal ions, therefore it was selected as a biosorbent material in this study.

3. Mining area in Mongolia: "Oyu Tolgoi" mining area

Mongolia is rich in underground natural resources such as coal, gold, and oil. The Oyu Tolgoi deposit is located 550 km south of the Ulaanbaatar, capital city of Mongolia, 80 km north of Mongolia-China border with a large copper and gold reserves.

The Oyu Tolgoi expected to be the third-largest copper mine in the world. The Oyu Tolgoi deposit is rich in copper, gold, silver, and molybdenum ore. Hugo-Dammet deposit is one of the world's highest copper porphyry deposits. Underground mine of Hugo North, the deepest mine of the Oyu Tolgoi deposit, will be able to extract about 80% of the total value of the highest grade deposit of the Oyu Tolgoi mining area. The underground mine will use block cave technology and this technology is cost-effective, environmentally friendly, and technologically advanced. At the initial stage of the mine operation, the ore is explored from the open pit. This means that the ore is freed and blown up to the required level and transported to the primary crusher to the retail crushing plant.

The concentrator will be used to produce copper, gold, and silver concentrate which will crush the ore into the fine powder. After that, the ore is transferred to the flotation tank, water, chemical, and air mixtures. The gas bubbles in the bombs will mask themselves with copper-containing minerals and sprout on the surface and into the outer tank of the bomb. The concentrated liquid contains about 27% of the copper. The liquid concentrate will be thickened, dried, packaged, and processed with mineral separation [2.19].

3.1 A history of the "Oyu Tolgoi" 1957-2017

The world's copper consumption is high and its perspective is to grow to 25.5 million tonnes in 2020. Over 100 years of exploitation, the Oyu Tolgoi is one of the world's top five copper-gold mines. The Oyu Tolgoi copper-gold mining area map is shown in Fig. 2-1.

Fig. 2-1 Oyu Tolgoi copper-gold mining area [2.20]

3.2 Mining and water

The southern part of Mongolia is the south Gobi region, has low water resources, low precipitation, and semi-desert climate. Water is the most important to the mining operations in this area. Hydrogeologist team discovered 3 groundwater aquifers Zagiin Hundii, Gunii hooloi, and Galbiin Gobi in 2003-2004. The Gunii Hooloi aquifer is just 35 km away from the Oyu Tolgoi project area and has water reserves of over 6.8 billion cubic meters, 150-400 m below ground level. Therefore, this water resource is the main source of water for the Oyu Tolgoi mine. The Oyu Tolgoi mine is re-using more than 80% of the water used for the household and mine processing water.

Heavy metal adsorption studies were made in the Oyu Tolgoi mine wastewater by Mongolian zeolite in Egashira et al. study. Heavy metals of Cu, Zn, Mn was adsorbed by Clinoptilolite, Mordenite, Chabazite type of zeolites. Zeolite was determined as able to remove heavy metal from Oyu Tolgoi mine wastewater [2.21]. Copper and molybdenum concentrate of the Oyu Tolgoi mining area is shown in Table 2-1.

Table 2-1 Copper and molybdenum concentrates of Oyu Tolgoi mine [2.19]

Copper concentrate content [2.19] (About 530.0 thousand tons)		Molybdenum concentrate content [2.19] (About 4.5 thousand tons)	
			
Cu	23-25%	Mo	48-50%
Mo	<0.15%	Cu	<4.0%
Fe	23-28%	SiO_2	3.5-6.0%
S	32-35%	As	<0.03%
Zn	0.4%	P	<0.02%
Pb	<0.04%	Re	500 pg/g
As	<0.30%	Se	160 pg/g
Sb	<0.08%	Te	2-3 pg/g
Ag	50-80 pg/g	Sb	<0.015%
Au	0.2-0.3 pg/g	W	<0.3%
Se	50-80 pg/g	Fe	<2.5%
Te	6.5-8.5 pg/g	S	<35%
Ni	0.006%	Ag	<20 pg/g
Bi	<0.01%	Zn	<0.04%
SiO_2	5-8%	Pb	<0.05%
Mg	0.3-1%	Bi	<0.003%
Ca	0.4-0.9%	∑moist+oil	<8%
Cd	0.001%	Oil	<4%
W_{moist}	<9.5%		

4. Sheep wool

Sheep wool is a natural fiber and being in the abundance of biological resources [2.22]. The diameter of the sheep wool varies from 12 to 47 μm relying on the kinds of sheep [2.23]. The sheep wool is comprised of carbon (49%), nitrogen (17%), oxygen (21%), hydrogen (6%),

sulfur (5%) and also trace elements (aluminum, barium, cobalt, copper, iron, manganese, molybdenum, strontium, titanium and zinc). It is unique due to the amount of sulfur in the wool (disulfide bridge) exceeds all other kinds of proteins. The chemical composition of wool is shown in Fig. 2-2. Original sheep wool usually contains mainly of wool proteins and structural lipids.

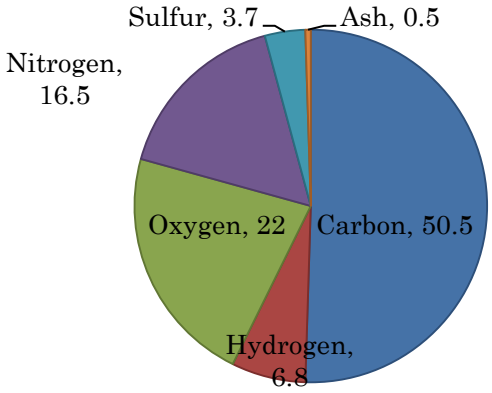


Fig. 2-2 Chemical composition of wool, wt %

Natural untreated sheep wool is made up of α-keratin and sheep wool consist of three main parts [2.24]. Other minor parts are shown in Fig. 2-3. Cortex is containing 2 kinds of cells that are ortho-cortical and para-cortical cells which have slightly different chemical composition. The cortical cell is including from spring-like structure of the smallest cortical cells and its surrounded by a high sulfur protein of a matrix. The matrix is capable to absorb water, dyestuffs, and odors.

Three main parts of sheep wool:

1. Cuticle (~ 0.5 μm thick)
2. Cortex (90% of fiber volume)
3. Medulla

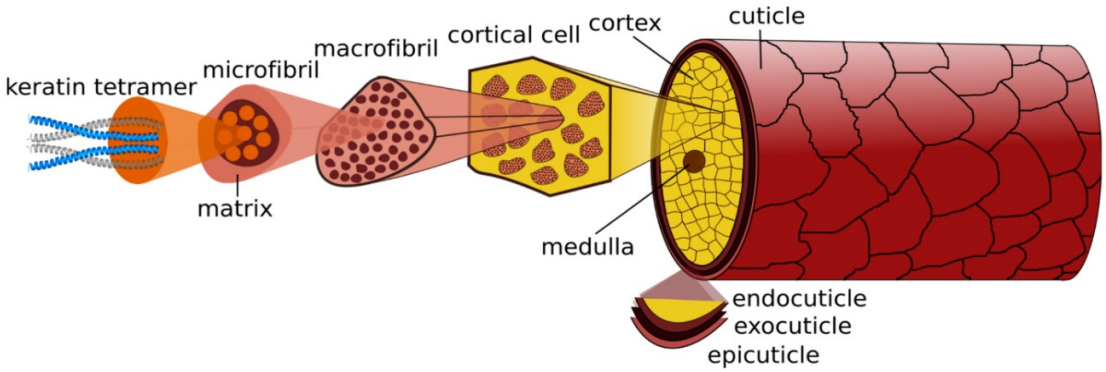


Fig. 2-3 Structure of sheep wool [2.24]

The structure of the sheep wool has many kinds of functional groups, contains amino acid residues and peptide bonds also disulfide crosslinks. The most important functional groups which sheep wool contains a set of ionizable carboxyl, hydroxyl, amino, and sulfur-containing functional groups. Sheep wool is possible to remove heavy metals from aqueous solutions even directly or after chemical treatment regarding its high content of functional groups [2.25]. These properties have gained interest in the application of wool in medical, environmental, biological, and industrial processes [2.21]. Chemical bonds in sheep wool are shown in Fig. 2-4.

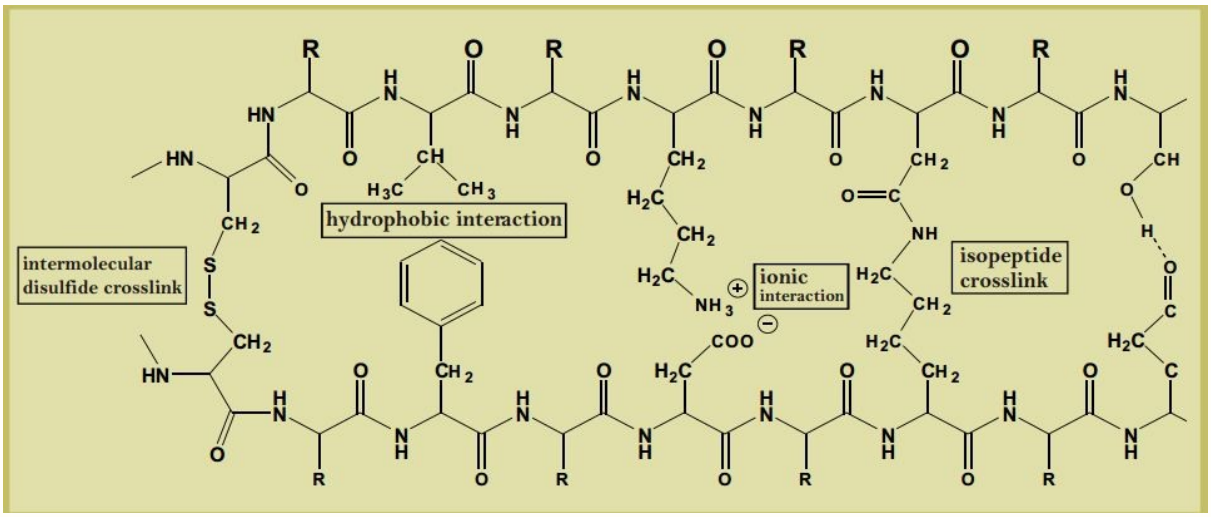


Fig. 2-4 Chemical bonds in wool [2.26]

Chemical treatment was performed to increase the adsorption content of heavy metals in sheep wool. The disulfide bond of wool is disrupted and thiol group (R-SH) is formed under the influence of chemicals, especially alkaline substances [2.27]. Sheep wool is insoluble in water it contains 90% pure keratin [2.28]. Sheep wool has lots of active functional groups

including carboxyl, hydroxyl, and others, among them thiol group can more readily make adsorption of heavy metals [2.29]. Compared to other biomaterials, sheep wool contains 18 amino acids, as shown in Table 2-2. This includes cystine, glutamic acid, serine, glycine, leucine, proline, arginine, threonine, aspartic acid, alanine, valine, tyrosine, isoleucine, phenylalanine, lysine, tryptophan, histidine, and methionine.

Sheep wool is rich in cystine content compared with other keratin-based biomaterials such as a chicken feather. Cystine has disulfide bonds which demonstrate wool incredible stability and lateral resistance against any chemical reagents [2.30]. Disulfide bond will be disrupted by alkali, bleaches, heat, sunlight, permanent set agents, non-felting agents, and moth proofing agents.

Table 2-2 Amino acid composition of sheep wool [2.31]

No.	**Component**	**Sheep wool**	
		g $100g^{-1}$	μmol g^{-1}
1	Cystine	12.02	1000
2	Glutamic	14.41	980
3	Serine	9.66	920
4	Glycine	5.25	700
5	Leucine	8.26	630
6	Proline	6.79	590
7	Arginine	9.58	550
8	Threonine	6.54	550
9	Aspartic	6.65	500
10	Alanine	4.1	460
11	Valine	5.38	460
12	Tyrosine	5.25	290
13	Isoleucine	3.41	260
14	Phenylalanine	3.8	230
15	Lysine	3.22	220
16	Tryptophan	1.43	70
17	Histidine	1.02	66
18	Methionine	0.52	39

Amino acids have amphoteric behavior it can be affected by the adsorption of metal ions. The amphoteric behavior of keratin molecules is shown in Scheme 2-1.

$$^{+}H_3N - Keratin - COOH \overset{H^+}{\leftrightarrow} \ ^{+}H_3N - Keratin - COO^- \overset{HO^-}{\longleftrightarrow} H_2N - Keratin - COO^-$$

Cationic	Zwitterionic or neutral	Anionic
pH < isoelectric point	pH = isoelectric point	pH > isoelectric point

Scheme 2-1. Amphoteric behavior of keratin molecules, key mechanism of adsorption of heavy metal ions [2.32]

Low sulfur protein and high sulfur protein play a key role in determining the super molecular structure of the wool. Proteins constitute 98% of the weight of sheep wool fiber, of which 85% protein is keratin and keratin-associated protein (KAP). Sheep wool is known to it has 73 KAP [2.33].

- Keratins
    - Acidic keratins (Type I)
    - Basic keratins (Type II)
- Keratin associated proteins (KAP)
    - High-sulfur proteins
    - Ultrahigh-sulfur proteins
    - High glycine-tyrosine proteins

Sheep wool can bind metal ion and organic compounds regarding ionizable functional groups of amino, sulfide, and carboxylate groups. To these unique abilities, the wool application is expanding in environmental, biological, and industrial processes. Usually, heavy metal ions are adsorbed to the functional group-containing sulfur and nitrogen [2.25].

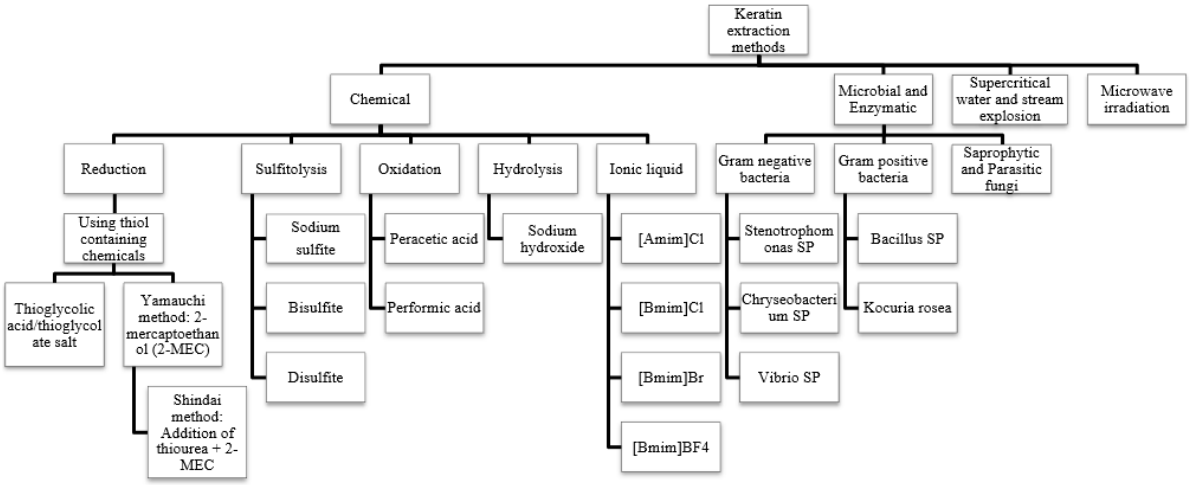
4.1 Keratin

Keratin is the main protein in human skin, hair, nails, sheep wool, horn, hoof, feathers and hair and these materials are gaily attention of many researchers cause of its great composition recently. Keratin is generally thought to be one substance, but it is a mixture of proteins and enzymes. Keratin molecule in wool contains carbon 50.65%, oxygen 18.52%, hydrogen 7.03%, nitrogen 17.7%, and sulfur 6% [2.34].

Sheep wool has a complicated structure and there have many studies were investigated for wool, in the recent 70 years [2.35]. Keratin-based materials have inherent biocompatibility, plenty in natural, biodegradable, and mechanical resistance [2.36]. Wool major structural proteins of keratin, which belong to the superfamily of intermediate filaments. Keratin and its composite of keratin interfilamentous have α- and β-; soft and hard; acidic and base keratin. Wool is appertaining α-, soft and acidic keratin type and insoluble, fiber protein. Keratin has a high amount of amino acid groups which is the capacity to bind heavy metal ions [2.37]. Other researchers studied heavy metals and toxic wastes are binding on thiol group of cystine amino acid by natural and chemically treated keratin-based materials. Though keratin-based materials, especially sheep wool has other reactive sites such as NH and OH groups [2.38]. Sheep wool was determined as the highest biosorption capacity for the keratin biomaterials compared to chicken feathers, human hair, and dog hair [2.25].

There are many ways to extract keratin from sheep wool. Chemical methods include alkali hydrolysis, ionic liquid, oxidation, reduction, and sulfitolysis, as well as microbial enzymes, microwave irradiation, supercritical water, and stream explosion methods. Each method has its advantages and disadvantages, the yield of the keratin is different. The yield of the keratin is 53% by reduction extraction, 51% by the ionic liquid, 41% by sulfitolysis, 25% by alkali hydrolysis, and 5% by oxidation method. From these methods, the alkali hydrolysis is one of the fastest and lower cost [2.39].

Alkali hydrolysis method is cheap and fast, most proteins are dissolved or changed their structure after treatment. Recently, other methods have been used for extraction of keratin, such as uses L-cysteine, ionic liquid, enzymatic method, and microwave irradiation method. It is possible to order the yield of the keratin, L-cysteine (72%) > Microwave irradiation (60%) > Enzymatic (50%) > IL method (40%).



Scheme 2-2. Keratin extraction methods [2.40].

Keratin extraction was made by sulfites, enzyme, thiols, L-cysteine, and ionic liquids from wool. L-Cysteine extraction of keratin was carried out with 72% of keratin yield at 72^0C for 5 hours and determined 62% of the disulfide bond is dissolved. The extracted keratin contains 60 wt% of low sulfur keratin and 26 wt% of high sulfur keratin [2.41].

$$R-S-S-R+2^{-}SCH_2CH(NH_2)COO^{-} \leftrightarrow 2RS^{-}+^{-}OOC(NH_2)CHCH_2S-SCH_2CH(NH_2)COO^{-}$$

The pure keratin membrane and the fiber are prepared by a doctor-blade casting process and wet spinning method. The separation of the keratin from its dissolved solution is made by adjusting solution pH to 4 which is its isoelectric point. The pure keratin is fragile, the strength of the keratin is increased by mixtures of other substances [2.42].

The extracted keratin is mixed with polyethylene and prepared synthetic natural membranes used for the separation process. The temperature of the treatment is very important and it's possible to affect keratin yield [2.43].

The keratin extracted from sheep wool is rich in cystine amino acids. The keratin is not soluble in water or organic solvents, or diluted acids and alkaline due to its strong disulfide bonds. In other words, the keratin is a highly stable, low-soluble protein. Chemically treated chicken feathers have a low surface area, the adsorption capacity of the metal ion is much higher than a natural chicken feather because it's hydrophobic characteristic [2.44].

Keratin is extracted by using the ionic liquid from the chicken feather. Sodium sulfite also used for the extraction process and chicken feather solubility; the yield of keratin increased by the increase of an amount of sodium sulfite, but the optical amount is determined as 10 wt%

of sodium sulfite. As disulfide bonds broke, there is no difference was observed for increase the amount of sodium sulfite. The ionic liquid is highly expensive, but its reuse is possible. The following reaction occurs when sodium sulfite is used for keratin extraction.

$$RSSR' + SO_3^{2-} \rightarrow RSSO_3^- + R'S^-$$

The yield of the obtained keratin was quite high (75.1%). The ionic liquid is non-volatility and thermal stability, imidazole was used for this study as an ionic liquid and it is relatively cheap [2.45].

4.2 Sheep wool in Mongolia

Mongolia is continuing animal husbandry since ancient times, sheep are accounting for 45.5% of the total livestock head that is 70.9 million in 2019. Sheep wool is a renewable huge resource and 20 breeds of 32.2 million head of sheep produced 45.1 thousand tons of wool in Mongolia. It used as many purposes in the world, which textile raw material of clothing cashmere, felt making, and insulation materials. Felt is used as insulation materials in “Ger” (traditional house of Mongolia), which were produced by traditional methods of the paddle and rolling methods, using sheep hair. Waste wool is abundantly available in Mongolia and about 10% of low-grade short wool is discarded from the textile industry and animal husbandry.

Therefore, biosorbent of sheep wool is possible to prepare at low cost regarding a large amount of waste sheep wool and after utilization of wool is not toxic to the environment, also it can be composted for soil. Sheep wool is naturally biodegradable and minimizes the landfill spaces when the waste is buried. Sheep wool would biodegrade in a week to 1 year regarding the environmental situation and nitrogen content of the sheep wool [2.46].

5. Aims and Objectives

The purpose of this study is to use low cost, high adsorption effectivity natural biomass, to remove heavy metals from polluted water, and to extract precious metals from water even its low amount. The natural biomass was treated simple procedures of chemical treatment which were considered beneficial to industrial scale biosorption.

Many heavy metal adsorption studies using keratin based materials have been performed, but the adsorption mechanism of heavy metals has not been studied in detail. Therefore, this study main task is to understand a comprehensive heavy metal adsorption mechanism on the

adsorbent material of Mongolian sheep wool. It would be a stimulus to determine which chemical treatment is the most effective for which heavy metal adsorption.

Herein, efficient usage of waste sheep wool of Mongolia is considered. The adsorption of heavy metal and precious metal from aqueous solution was investigated by using waste sheep wool which derived from domestic animals based on its high availability in the countryside area of Mongolia.

Furthermore, to prove the effectiveness of this method works in actual condition, the method was applied to the barge and process water of the Oyu Tolgoi mine, Mongolia. The research background and plan are shown below.

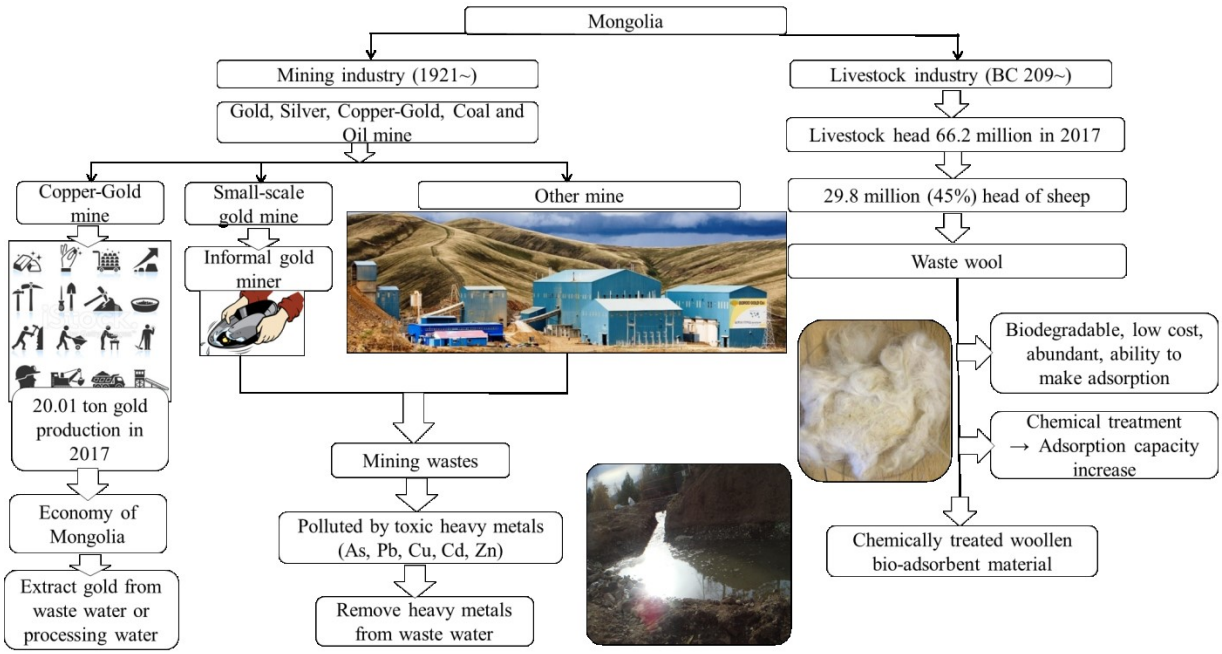


Fig. 2-5 Research background and plan

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Chapter 3
Characterization and preparation method of sheep wool

1. Introduction

Sheep wool is chemically treated with different ways and its comparative studies were continuing. There are few methods are used for converting biomaterials into better biosorbents. For example, thermal drying, grinding, pretreatment, enhancement of binding groups, elimination of inhibiting groups, graft polymerization, culture optimization, and genetic engineering.

Sheep wool core amino acids were destroyed by chemical treatment of hydrolysis, acid, alkali, and enzymatic digestion. Characterization of sheep wool is changed due to harsh chemical treatment concentration. Peptide linkages don't hydrolyze by some treatment and this allows the characterization of treated wool keratin will looks like natural sheep wool keratin after chemical treatment. Sheep wool keratin is used for mainly medical technology and industrial technology those are based on the complex fundamental structure of wool [3.1].

Usually, wool surfaces modification made by many kinds of chemical treatments that attach branched molecules and functional chemical agents to the surface of fiber [3.2].

1.1 Reduction of the wool

Reduction agents as thioglycolic acid, potassium cyanide, sodium sulfide, and sodium sulfite used for the reduction of a disulfide bond in keratin. Sheep wool is stabilized by reducing agents as the result that disulfide bonds are disrupted and thiol groups reacted with alkylating agents to form a modified woolen product.

Chemical reactions on disulfide bonds are shown here [3.3]:

1. $R-S-S-R+2HS-CH_2COOH \rightarrow 2R-SH+[S-CH_2COOH]_2$
2. $R-S-S-R+HCN \rightarrow R-SH+R-S-CN$
3. $R-S-S-R+H_2S \rightarrow R-SH+H_2S_2$
4. $R-S-S-R+H_2SO_3 \rightarrow R-SH+R-S-S-O_3H$

Sheep wool is sensitive to alkali. In alkaline solution, the alkali binds to the wool proteins with swelling and simultaneous degradation. Even at low concentrations, the reversible binding is accompanied by fiber damage. The alkali binds to the wool so firmly that it cannot be completely removed by washing with water alone. First, the alkali salts of the wool proteins must be decomposed by reaction with stronger acids. Wool containing alkali residues

undergoes decomposition on heating in a stream of dry air, the most important degradation reactions being the decomposition of cysteine and cystine.

1.2 Oxidation of the wool

Acid treatment will differ with sheep wool by its condition of reaction such as temperature, pH, and time of reaction. Wool is stable in acid solutions comparing with the treatment of alkaline. Disulfide bonds of the wool are cleaved by oxidation of wool and sulfonic acid will form [3.4].

$$Wool - SS - Wool \xrightarrow{HCO_3H+H_2O} 2Wool - SO_3H$$

Disulfide Sulfonic acid

Chemical reactions on disulfide bonds by hydrogen peroxide oxidation of sheep wool are shown below (Redox state of cystine):

$$Wool - SS - Wool \leftrightarrow 2Wool - SH$$

Disulfide Thiol (cys)

$$2Wool - SH \xrightarrow{H_2O_2} 2Wool - SOH$$

Thiol (cys) Sulfenic acid

$$2Wool - SOH \xrightarrow{H_2O_2} 2Wool - SO_2H$$

Sulfenic acid Sulfinic acid

$$2Wool - SO_2H \xrightarrow{H_2O_2} 2Wool - SO_3H$$

Sulfinic acid Sulfonic acid

1.3 Alkaline hydrolysis of the wool

Wool is completing from amino acid chains, basically settled on the disulfide cross-linkages of cystine. The disulfide bond is a basic part of the wool modification of alkaline, it affects to change the location of disulfide bonds and rearrange of keratin structure also physical-chemical characteristics of wool.

Sheep wool hydrolysis made by sodium hydroxide. Sheep wool is unstable in alkaline solution which simultaneous degradation occurs to sheep wool protein by alkali binding. Sheep wool fiber will damage even a low concentration of alkaline solution [3.5].

The reason for choosing sodium hydroxide is that it is cheaper than potassium hydroxide. The sodium hydroxide solution is considered to be either 0.1 to 1.0 M, since the pH of the solution is 12.5, 13.0, and the pH ≥ 13 is preferable to break the disulfide bond. The temperature of the solution is assumed to be up to 100^0C which solution boiling temperature, it is ideal of breaking the disulfide bond and further, the thiol group is highly formed. When the alkaline treatment of sheep wool, heat is exposed to react by a surface of the sheep wool and there is a disadvantage of uniform distribution of heat [3.6].

2. Materials and Methods

2.1 Chemicals

Analytical grade chemicals used in this work. A 1000 ppm of chromium, gold, and copper standard solutions were purchased from Wako Pure Chemical Corporation. Sodium hydroxide and hydrochloric acid were alternatively used for solution pH adjustment for the biosorption experiment. Sodium sulfide, sodium bisulfite, and sodium borohydride were purchased from Wako Pure Chemical Corporation and used for the chemical treatment of sheep wool.

2.2 Chemical treatment of sheep wool

The sheep wool sample was washed with detergent and dried at room temperature. Chemical treatment is made using sodium hydroxide (NaOH), sodium sulfide (Na_2S), sodium bisulfite ($NaHSO_3$), and sodium borohydride ($NaBH_4$) solution by simple methods in this study. The chemical treatment process of sheep wool is shown in Fig. 3-1.

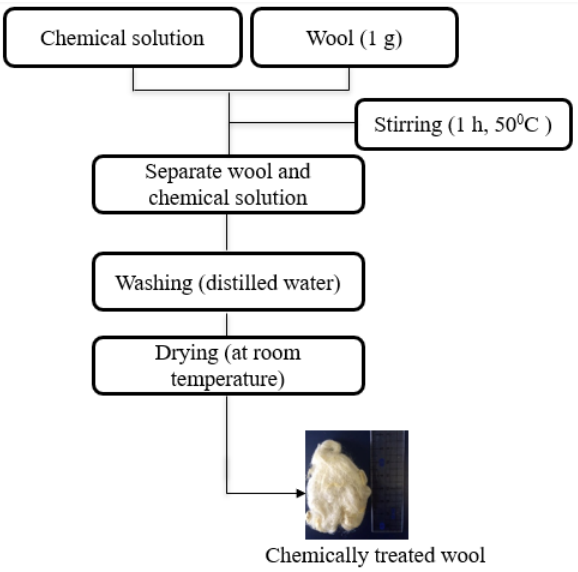

Fig. 3-1 Chemical treatment of sheep wool

Chemical treatment is made to enhance the uptake of metal ions and chemical reactions shown below. Sulfhydryl group formed after each chemical treatment.

$$R - S - S - R + OH^- \rightarrow R - SH + R - SO^- \quad [3.7]$$

$$R - S - S - R + 2H_2S \rightarrow 2R - SH + H_2S_2 \quad [3.8]$$

$$R - CH_2S - SCH_2 - R + NaHSO_3 \rightarrow R - CH_2SH + NaOSO_2SCH_2 - R \quad [3.9]$$

$$R - S - S - R \xrightarrow{NaBH_4} 2R - SH \quad [3.10]$$

Moreover, the effect of the concentration of sodium sulfide carried out at concentration of 0.002 to 0.1 M for the sheep wool chemical treatment. Sheep wool and sodium sulfide solution were prepared and mixed for 0.5 h at 50^0C temperature which is shown in Fig. 3-2. After treatment, sheep wool is washed with distilled water and dried at room temperature. The sheep wool weight loss after chemical treatment was determined by using below an equation.

$$Weight\ loss = \frac{m_b - m_a}{m_b} \cdot 100\% \quad (3.1)$$

m_b – mass of wool before treatment, [g]

m_a – mass of wool after treatment, [g]

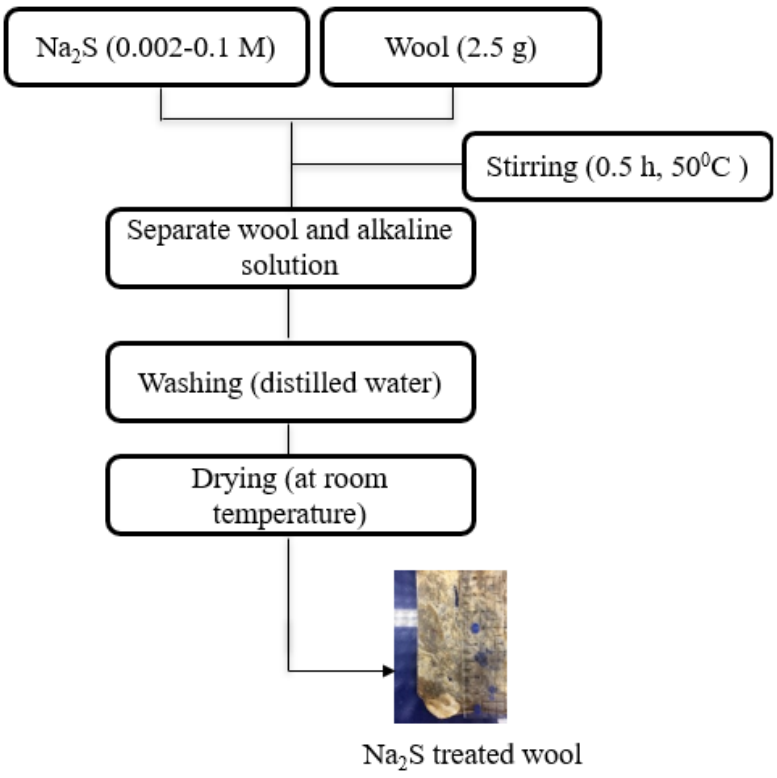

Fig. 3-2 Sodium sulfide treatment of sheep wool

3. Results and Discussion

3.1 Characterization of sheep wool and chemically treated sheep wool samples

The sheep wool was chemically treated with sodium hydroxide, sodium sulfide, sodium bisulfite, and sodium borohydride solution at 1.0 M. The weight loss of the sheep wool by before and after chemical treatment is shown in Table 3-1.

Table 3-1 Sheep wool weight loss by the chemical treatment

No.	Sample ID	Concentration of chemicals	Weight loss, %
1.	SW	none	none
2.	NaOH treated SW	1.0 M	14.01
3.	Na_2S treated SW	1.0 M	75.33
4.	$NaBH_4$ treated SW	1.0 M	0.51
5.	$NaHSO_3$ treated SW	1.0 M	2.35

*SW: Sheep wool

The sodium sulfide treatment of sheep wool was faster than other chemical treatments, which is explained by the color of the solution changed to yellow 20 minutes after the start of the experiment. Some part of the sheep wool was dissolved by the treatment of sodium hydroxide. For sodium bisulfite, color change of the solution began to be observed in 35 minutes, while for sodium borohydride, the color of the solution has not changed during the experiment. The degradation of the sheep wool was defined as the following order; Na_2S > NaOH > $NaBH_4$ > $NaHSO_3$.

Additionally, the effect of sodium sulfide concentration carried out for the sheep wool. Depending on the concentration of sodium sulfide, the properties of sheep wool changed dramatically, and at high concentrations (0.05-0.1 M) the sheep wool lost its fibrous form and turned into a sheet. However, at low concentrations (0.002-0.02 M), sheep wool retains its fibrous structure.

Table 3-2 Sodium sulfide treatment of sheep wool at various concentrations

No.	Sample ID	Concentration of Na_2S treatment	Weight loss, %
1.	SW	none	none
2.	SW-I	0.1 M	68.29
3.	SW-II	0.075 M	53.25
4.	SW-III	0.05 M	44.24
5.	SW-IV	0.02 M	1.51
6.	SW-V	0.005 M	0.95
7.	SW-VI	0.002 M	0.46

Sheep wool is not resistant to strong sodium sulfide solutions, the breakdown of sheep wool protein occurs. The decomposition of sheep wool occurs as a result of the colorless of the solution during chemical treatment, which turns to yellow [3.4]. The mass loss of sheep wool was greater with increasing concentration of sodium sulfide solutions. The sheep wool loss is shown in Table 3-2 and Fig. 3-3.

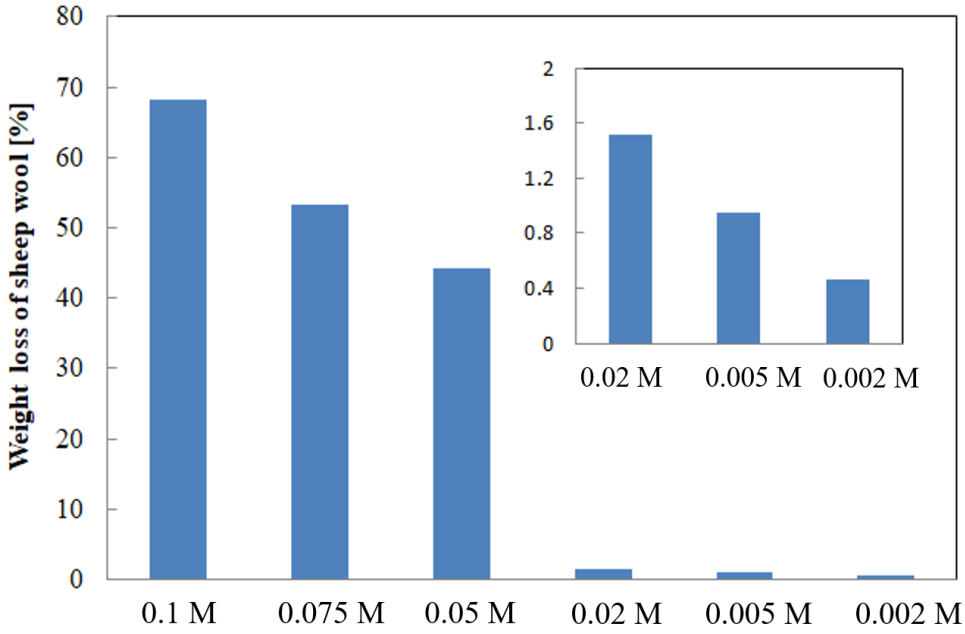

Fig. 3-3 Sheep weight loss by the sodium sulfide treatment

During the treatment of sodium sulfide in sheep wool, the color of the solution changed into yellow in high concentrations of sodium sulfide treatment, similar to the results of Ki et al. study. This indicates that the wool decomposes during chemical treatment and the disulfide

bond is disrupted. The properties of wool during chemical treatment are shown in Fig. 3-4.

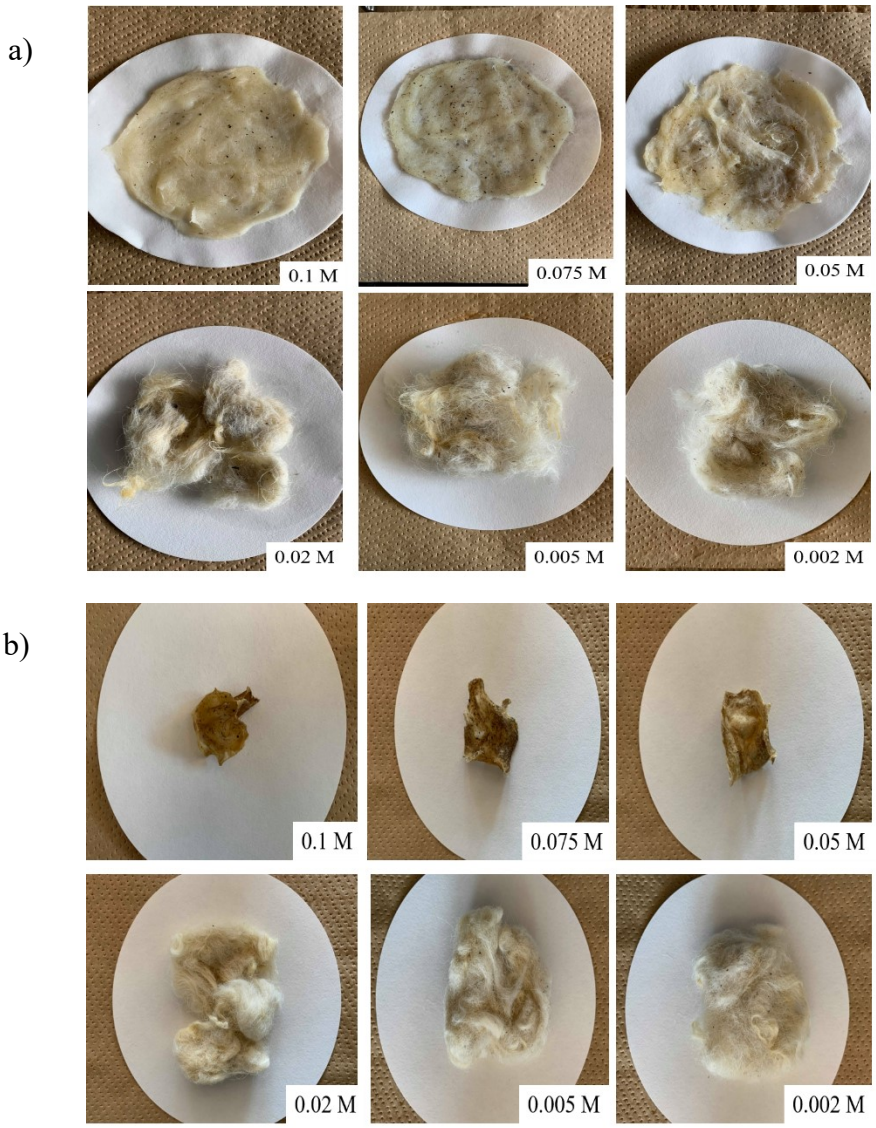

Fig. 3-4 Appearances of sheep wool treated at various sodium sulfide concentrations. a. wet, b. after drying

3.2 Morphology analysis of sheep wool

SEM analysis carried out for sheep wool and chemically treated sheep wool samples to understand the morphology of adsorbent which plays an important role in the biosorption. The results are presented in Figs. 3-5 and 3-6.

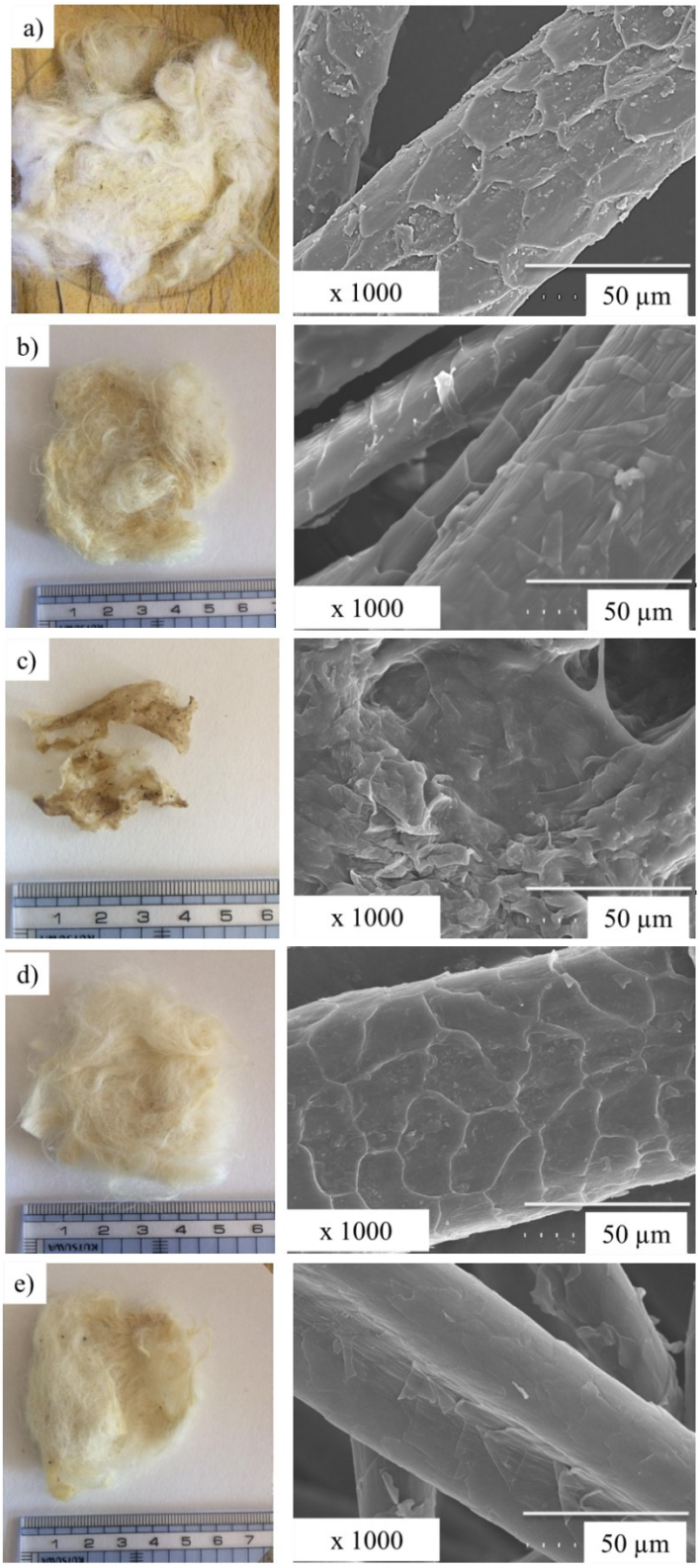

Fig. 3-5 SEM images of chemically treated sheep wools a) sheep wool, b) sodium hydroxide, c) sodium sulfide, d) sodium bisulfite, e) sodium borohydride treated sheep wool

Sodium hydroxide treatment of sheep wool has been shown to dissolve the exocuticle, the outermost part of the cuticle, that is expected to improve its contact with heavy metal ions.

Also, the strength of sheep wool decreases after sodium hydroxide treatment (Fig. 3-5b). The mass loss of wool after sodium sulfide treatment is very high compared to other treatments. After treatment (Fig. 3-5c), the wool cuticle and cortical cells are completely dissolved, and the appearance is no longer fibrous. No changes were found in the sheep wool surface or cuticle after sodium bisulfite treatment (Fig. 3-5d). However, after the treatment of sodium borohydride as shown in Fig. 3-5e, the surface of the sheep wool became smooth and all the cuticles were dissolved.

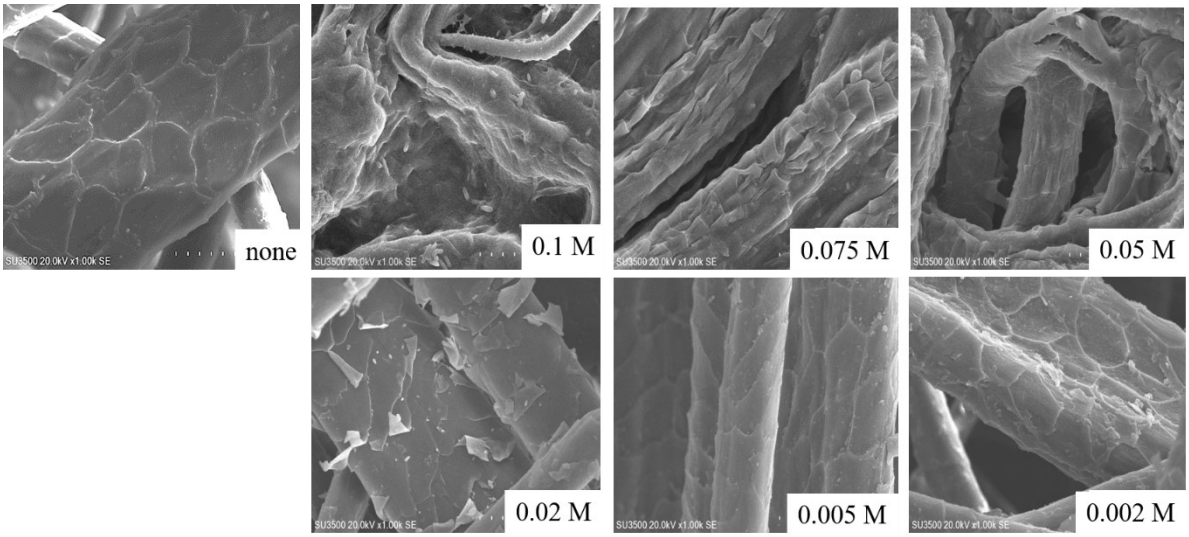

Fig. 3-6 SEM images of sodium sulfide treated sheep wools at various concentrations

After the treatment of high concentration (0.05 M to 0.1 M) sodium sulfide, it can be seen that the cuticle on the surface of sheep wool is completely dissolved. For Na_2S (0.02 M) treated sheep wool, some parts of the cuticle appears to be dissolved. Na_2S (0.005 M) and Na_2S (0.002 M) treated sheep wool are similar to untreated wool with no changes on the wool surface because of the low concentration. This shows that the concentration of the chemical being treated is directly related to the characteristic of the sheep wool and the adsorption capacity of heavy metals.

4. Summary

In this study, biosorbent material of Mongolian sheep wool was treated by different chemicals. Sodium hydroxide, sodium sulfide, sodium bisulfite, and sodium borohydride were used for chemical treatment. The highest sheep wool loss was observed in sodium sulfide treated wool while the lowest sheep wool loss was determined in sodium bisulfite.

The properties of sheep wool treated with sodium sulfide have changed the most during chemical treatment. Therefore, sheep wool was treated again using sodium sulfide solutions at different concentrations of 0.002 M to 0.1 M. The sodium sulfide concentrations of 0.02 M and 0.05 M were selected as adsorbent for further adsorption study because of their different characteristics.

5. References

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Chapter 4
Adsorption of heavy metal and precious metal

1. Introduction

Keratin material makes both physical and chemical adsorptions. Physical adsorption is the adsorption will be made on the surface or porous particle of the keratin material. Here, chemical adsorption will be made on the chemical functional groups, peptide bond, and the active site of the amino acid residue of the keratin protein.

Disulfide bonds of keratin disrupted by chemical treatment of sheep wool and form thiol group, further mercaptides will form metal ion complexation between thiol group and metal ions. The increase in the adsorption amount of the metal ion depends on the potential binding site for both sheep wool and substance.

Recently keratin-based materials are attracting the attention of the researcher due to its low cost and biodegradable properties. Heavy metals of copper, chromium, cadmium, cobalt, mercury, aluminum, nickel, zinc, gold, silver, and lead were adsorbed using biosorbent of sheep wool from wastewater [4.1]. Keratin-based materials were used as an adsorbent for adsorbing precious metals from acidic aqueous solutions, for example, gold, silver and mercury adsorption by sheep wool [4.2], silver, palladium and platinum adsorption by chicken feather powder [4.3], gold nanoparticles adsorption by wool powder [4.4] and gold cyanide adsorption by chicken feathers [4.5].

The R – COOH, R – NH_2, and R – SO_3H residues were extracted from the sheep wool and also studied how they interact with the two valence metals ion. There have been numerous experiments and studies made on the practice of interaction between wool and heavy metal ions. In recent years, heavy metal adsorption has been studied by the use of chemically treated sheep wool material. Kadokura et al. (1982) analyzed the possibility of the adsorption of two valence metals such as Hg^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, and Zn^{2+} by gel particles prepared from sheep wool keratin [4.6]. The heavy metals ion adsorption studies have investigated adsorption capacity and adsorption mechanisms but there is no result about the characterization of the formed complex. Summary of heavy metal removal studies made by keratin-based adsorbents is shown in Table 4-1.

Table 4-1 Heavy metal removal studies using keratin-based adsorbents

No.	Type of adsorbent	Metal	q_{max} (mmol/g)	Reference
1	Hair (human, hog, cattle and tannery hair)	Hg(II)	-	[4.7]
2	Human hair (natural)	Cu(II)	$0.551x10^{-3}$	[4.8]
		Mn(II)	$0.020x10^{-3}$	
		Zn(II)	$0.153x10^{-3}$	
		As(III)	$0.134x10^{-5}$	
3	Treated human hair	Hg(II)	3.33	[4.9]
		Hg(I)	2.00	
		Ag(I)	1.32	
		Cu(II)	0.463	
		Cu(I)	0.337	
		Pb(II)	0.436	
		Cr(III)	0.021	
		Cr(VI)	0.233	
		Cd(II)	0.592	
		Ni(II)	0.176	
4	Wool (natural)	Ni(II) Cu(II) Zn(II) Cd(II) Hg(II) Pb(II)	-	[4.10]
5	Chicken feather powder	Au(II)	0.305-0.812	[4.3]
6	Treated wool	Ag(I)	1.78	[4.11]
7	Wool	Cr(VI)	0.663	[4.12]
8	Human hair (natural)	Cu(II)	0.087	[4.13]
		Zn(II)	0.131	
9	Treated wool	Cu(II) Co(II)	-	[4.14]
10	Wool in native Reduced wool Oxidized wool	Cu(II) Co(II) Ni(II) Zn(II) Cr(VI)	-	[4.15]

11	Wool	Cu(II)	0.165	[4.16]
12	Wool	Au	-	[4.17]
13	Nanofibrous membrane of wool/silk	Cu(II)	0.045	[4.18]
14	Human hair (natural)	Fe(II) Pb(II) Zn(II)	$0.551x10^{-3}$ $0.020x10^{-3}$ $0.153x10^{-3}$	[4.19]
15	Wool grafted PIAH chelating fibers	Cu(II) Hg(II) Ni(II)	0.769 1.740 1.240	[4.20]
16	Wool fiber[a] Wool powder[b]	Co(II) Cu(II) Cd(II)	0.0039[a], 0.0077[b], 0.0097[a], 0.0091[b], 0.0093[a], 0.0086[b]	[4.21]
17	Wool powder	Co(II)	0.911	[4.22]
18	Keratin(wool)/PA6 blend nanofibers	Cu(II)	1.63	[4.23]
19	Keratin (wool) nanofiber membrane	Cu(II) Ni(II) Co(II)	0.173 - -	[4.24]
20	Wool powders	Zn (II) Cr (VI)	-	[4.25]
21	Keratin (wool) colloidal solution	Pb(II)	0.209	[4.26]
22	Duck feather treated with NaOH (0.1mol/L)	Cu(II)	0.822	[4.27]
23	Perm-lotion-treated human hair	Pb(II) Cu(II)	0.110 0.141	[4.28]
24	Wool powder	Cu(II)	0.763	[4.29]
25	Natural and electron irradiated sheep wool	Cu(II)	0.236	[4.30]

2. Materials and methods

Heavy metals adsorption of sheep wool studies were carried out using batch adsorption experiments. Adsorption rate decreases or increases due to external effects when

conducting biosorption. The adsorption values of heavy metals are affected by many factors, such as solution temperature, concentration, pH, absorbent size and dosage, mixing speed, and the effect of the co-existing ions [4.31].

Sheep wool was treated with special chemicals and added about 5-10 mg each to 15 ml of heavy metal solution. The prepared solution was soaked in a water bath (Model No. T-N22S, Thomas Co.ltd) for 48 hours, a temperature of 303 K at 50 rpm. After adsorption, the sheep wool, heavy metal solution were separated and solution filtered using 0.22 μm RephiQuik Syringe Filter (RJP1322NH, RephiLe Bioscience, Ltd). The inductively coupled plasma atomic emission spectrometer (Shimadzu ICPS-8100, Japan) was used to determine the heavy metal content of the solution.

The quantity of heavy metal adsorption is calculated according to the following equation:

$$q_e = \frac{(C_0 - C_e) \cdot V}{m} \qquad (4.1)$$

Equation (4.2) shows the calculation method of the percentage of heavy metal adsorption:

$$Adsorption, \% = \frac{C_0 - C_e}{C_0} \cdot 100 \qquad (4.2)$$

q_e – amount of metal ions adsorbed at equilibrium per weight of biosorbent, [mmol/ g]

C_0, C_e – metal ion concentration of initial and after biosorption, [mmol/l]

V – volume of the solution, [l]

m – amount of adsorbent, [g]

For the kinetic studies, 5-10 mg of sheep wool was agitated separately in 15 ml of chromium, gold, and copper aqueous solution for 30 to 2880 minutes. At the end of the adsorption period, the sheep wool was separated from the heavy metal solution and the metal content of the solution was determined by ICP.

2.1 Effect of the initial aqueous solution pH

The pH of the aqueous solution is one of the most important chemical parameters affecting the biosorption process [4.32]. The pH of the solution influences both the metal aqueous speciation and the chemical functional groups of the biosorbents surface speciation.

2.2 Effect of the contact time

To improve the understanding of the biosorption process, a variety of kinetic models need to be tested on experimental results. Also, modeling gives valuable information about

adsorption mechanisms. The pseudo-first order and pseudo-second order [4.33, 4.34] models are used in this study and kinetic models are determined by Equations of (4.3) and (4.4).

$$log(q_e - q_t) = logq_e - \frac{k_1}{2.303}t \qquad (4.3)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (4.4)$$

q_t – amount of metal ions adsorbed at time per weight of biosorbent, [mmol/ g]

q_e – amount of metal ions adsorbed at equilibriums per weight of biosorbent, [mmol/ g]

t – time, [min]

k_1 – pseudo-first order kinetic rate constant, [min^{-1}]

k_2 – pseudo-second order kinetic rate constant, [g/mmol min]

2.3 Effect of the initial metal concentration

The relationship between the concentration of heavy metal solutions and adsorbed metal ion can explain by the biosorption isotherm. From this, it is possible to clarify the adsorption mechanism between biosorbent and heavy metal by adsorption capacity of sheep wool. In this study, the Langmuir, the Freundlich isotherm models were used and the adsorption capacity was calculated by using below equation [4.35].

$$q_e = \frac{q_{max} K C_e}{1 + K C_e} \qquad (4.5)$$

Equation (4.6) shown the linearized form of the Langmuir isotherm model:

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \frac{1}{K \cdot q_{max}} \cdot \frac{1}{C_e} \qquad (4.6)$$

q_{max} – maximum biosorption capacity, [mmol/g]

C_e – heavy metal concentration at equilibrium, [mmol/L]

q_e –equilibrium biosorption capacity [mmol/g],

K – Langmuir biosorption constant, [L mg^{-1}]

The equation of the Freundlich isotherm model and its linearized form are shown below.

$$q_e = K_F C_e^{\frac{1}{n}} \qquad (4.7)$$

$$logq_e = logK_F + \frac{1}{n} logC_e \qquad (4.8)$$

C_e – heavy metal concentration at equilibrium, [mmol/L]

q_e –equilibrium biosorption capacity [mmol/g],

K_F and n – Freundlich constant

3. Results and Discussion

3.1 Effect of pH to chemically treated sheep wool

The sheep wool and chemically treated sheep wool was evaluated as an adsorbent material for the adsorption of Cr(III), Cr(VI), Au(III) and Cu(II) at different pH.

3.1.1 Adsorption behavior of Cr(III) and Cr(VI)

The pH of the solution affects both Cr(III) and Cr(VI) adsorption, the state of the metal ions are determined as different. The effect of the initial pH on the adsorption capacity was studied in the range of pH 1.0 to 7.0 for Cr(III) and Cr(VI) solution. Chromium uptake is strongly different from Cr(III) and Cr(VI) depending on the pH of the solution. As shown in Fig. 4-1, adsorption amount of Cr(III) increased as increasing pH. The highest adsorption occurred at pH 5.0-6.9 for sheep wool and sodium sulfide treated sheep wool.

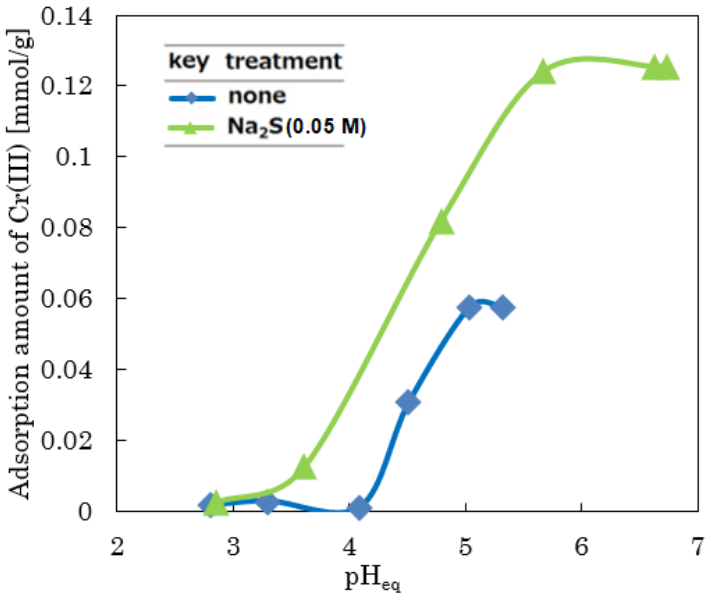


Fig. 4-1 Effect of pH on adsorption of Cr(III)

(Experimental condition: V: 15cm^3, C_{ini}: 0.1 mmol/l, pH_{ini}=2.5-5.0)

Cr(VI) adsorption has occurred at low pH, there is no adsorption observed at high pH, especially at pH 5.8-8.5. The level of biosorption was increased from 0.96 to 9.62 mmol/l. However, increasing the pH to >6.0 decreased uptake (which was about 0.23 mmol/g at pH 12.0) was determined the same as our study [4.36].

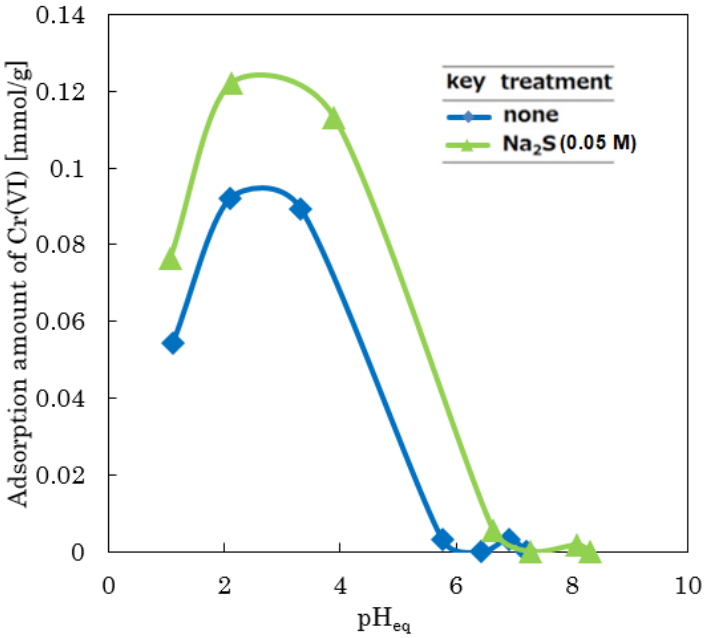


Fig. 4-2 Effect of pH on adsorption of Cr(VI)

(Experimental condition: V: 15cm^3, C_{ini}: 0.1 mmol/l, pH_{ini}=1.0-7.0)

3.1.2 Adsorption behavior of Au(III) and Cu(II)

The effect of the initial pH on the biosorption capacity was studied in the range of pH 2.9 to 6.8 at an initial gold and copper solution concentration of 0.05 mmol/l and 0.16 mmol/l, respectively. In both wool and chemically treated wool systems, the metal uptake is strongly dependent on the pH value of the aqueous solution (Figs. 4-3, 4-4).

Equilibrium pH was measured for 2.9 to 5.9 after the amount of adsorption for gold and it was reduced by increasing pH. A high adsorption amount of gold is determined as 1.9 to 4.5 of equivalent pH in sodium sulfide treated wool and chemically treated wool adsorbed two times higher amount of gold than untreated sheep wool.

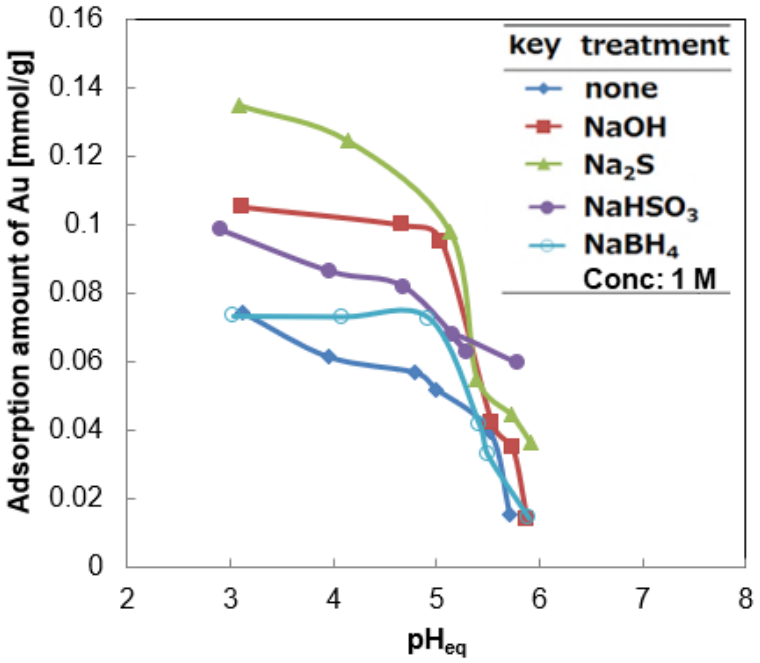


Fig. 4-3 Effect of pH on adsorption of Au(III)

(Experimental condition: V: 15cm^3, C_{ini}: 0.05 mmol/l, pH$_{ini}$=2.9-6.8)

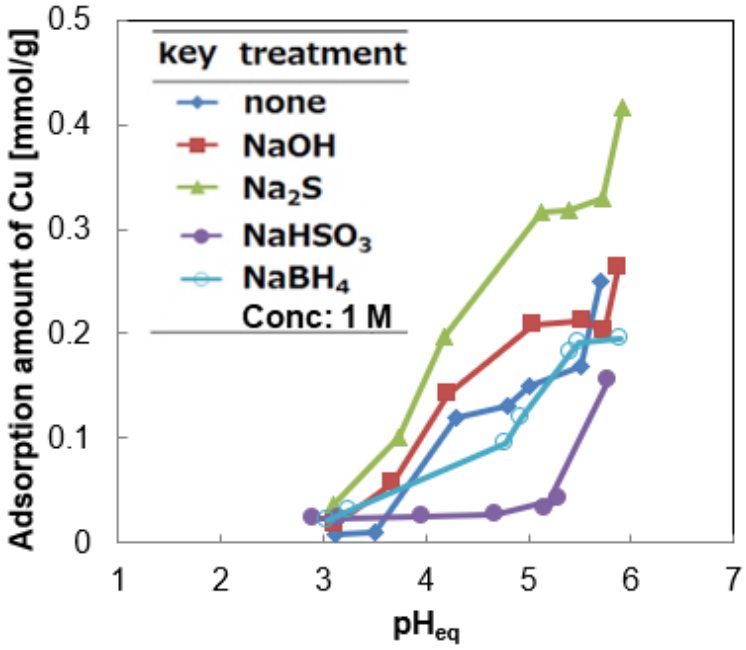


Fig. 4-4 Effect of pH on adsorption of Cu(II)

(Experimental condition: V: 15cm^3, C_{ini}: 0.16 mmol/l, pH$_{ini}$=2.9-6.8)

There was a competition between the Cu^{2+} ion and H^+ protons for the metal active sites in the biosorbent at low pH. Besides, the carboxylic groups retain photons and amino groups get protonated at lower pH. Thereby, it would reduce the probability of any positively charged

ions binding. The copper adsorption amount sharply increased at pH 5.1 to 6.0 for all the biosorbents regarding protons decrease. In sodium sulfide treated sheep wool, the amount of copper increased up to 0.42 mmol/g.

3.2 Biosorption kinetics modeling

The sheep wool and chemically treated sheep wool was evaluated as an adsorbent material for the adsorption of Cr(III), Cr(VI), Au(III) and Cu(II) at different time.

3.2.1 Adsorption behavior of Cr(III) and Cr(VI)

The contact time is one of the key parameters for the successful practical application of biosorbent. The biosorption values of Cr(III) are determined different for sheep wool and sodium sulfide treated sheep wool. After sodium sulfide treatment, the biosorption amount increased 4-5 times. The equilibrium was reached in 12 hours for both, sheep wool and sodium sulfide treated wool which is shown in Fig. 4-5.

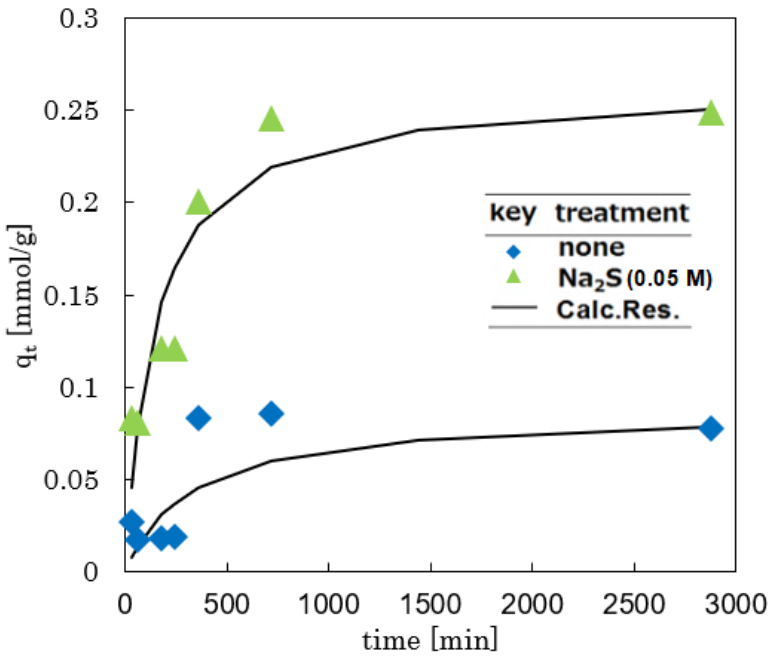


Fig. 4-5 Time course of Cr(III) adsorption by sodium sulfide treated sheep wool (Experimental condition: V: 15cm^3, C_{ini}: 0.19 mmol/l, pH_{ini}=5.0)

The biosorption of the Cr(VI) into sheep wool and sodium sulfide treated sheep wool at the different contact time, is shown in Fig 4-6. From the results, there is no difference observed for adsorption amount after sodium sulfide treatment of the sheep wool. Sheep wool

and sodium sulfide treated sheep wool, both show the same biosorption capacities and reaches the equilibrium in 6 hours.

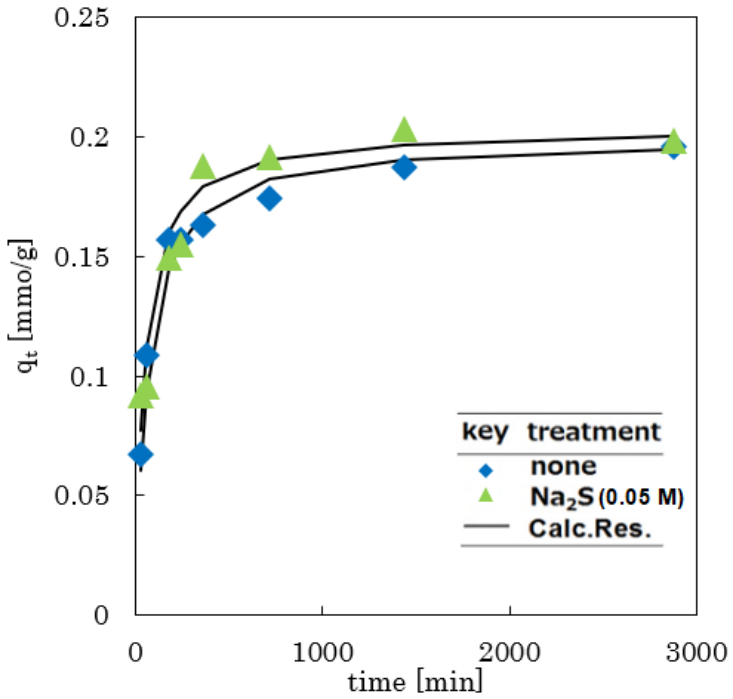
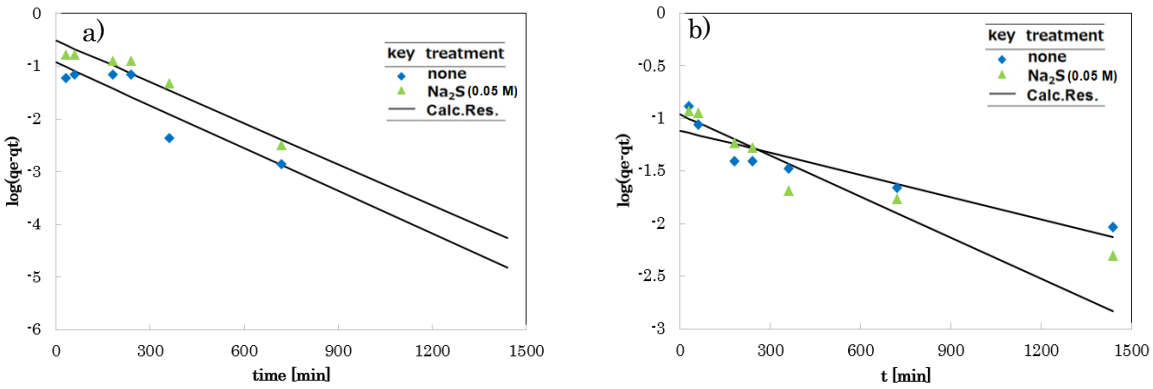


Fig. 4-6 Time course of Cr(VI) adsorption by sodium sulfide treated sheep wool (Experimental condition: V: 15cm^3, C_{ini}: 0.19 mmol/l, pH_{ini}=2.0)

Adsorption kinetics depends on the metal ions, sheep wool interaction, and adsorption conditions. The plots of $log(q_e-q_t)$ vs t for the pseudo-first order model, t/qt vs t for the pseudo-second order models are shown in Fig. 4-7. Kinetic parameters calculated from experimental data for the adsorption processes and values are given in Table 4-2.



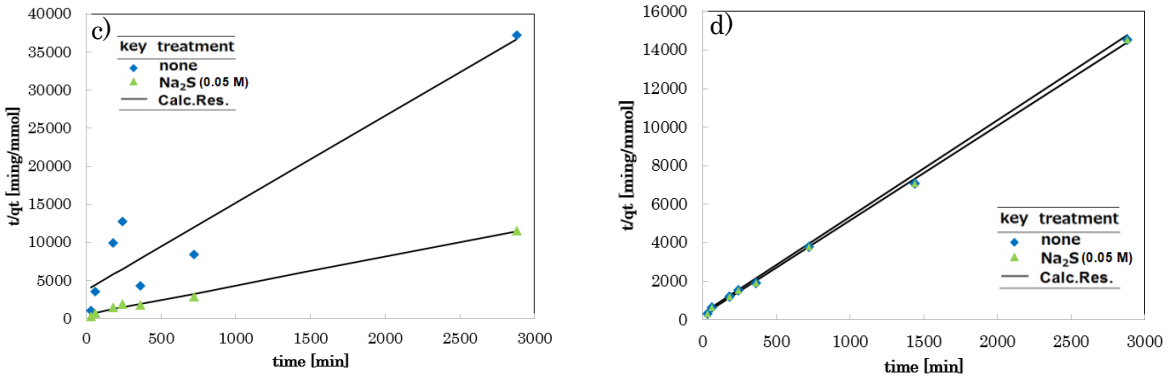


Fig. 4-7 a: Pseudo-first order kinetics of Cr(III); b: Pseudo-first order kinetics of Cr(VI) onto wool and sodium sulfide treated wools; c: Pseudo-second order kinetics of Cr(III); d: Pseudo-second order kinetics of Cr(VI) onto wool and sodium sulfide treated wools

In general, pseudo-first order model does not fit well with the results adsorption study. It will be fitted by pseudo-first order model when biosorption was fast in the initial rapid step. The kinetic parameters and correlation coefficients of the pseudo-second order kinetic model are greater compared with the pseudo-first order model in this study. Additionally, the calculated q_e values agree very well with the experimental data for this study. It indicates that chromium was adsorbed on sheep wool by chemisorption regarding the formation of chemical bonds between biosorbent and metal ion.

Table 4-2 Kinetic parameters obtained from experimental data of chromium adsorption

Chemical treatment	Pseudo-first order model			Pseudo-second order model		
	k_1 (1/min)	q_e (mmol/g)	R^2	k_2 (g/mg min)	q_e (mmol/g)	R^2
Cr(III)						
none	0.0062	0.1169	0.8282	0.0336	0.0930	0.8977
Na_2S (0.05 M)	0.0059	0.3057	0.9362	0.0243	0.2412	0.9936
Cr(VI)						
none	0.0016	0.0758	0.8421	0.0732	0.1994	0.9995
Na_2S (0.05 M)	0.0029	0.1084	0.8451	0.0999	0.2035	0.9993

3.2.2 Adsorption behavior of Au(III) and Cu(II) by chemically treated sheep wool

Kinetic models are used to clarify the mechanism of the adsorption process of an adsorbent. The time course of Au(III) and Cu(II) results are shown in Figs. 4-8, 4-9.

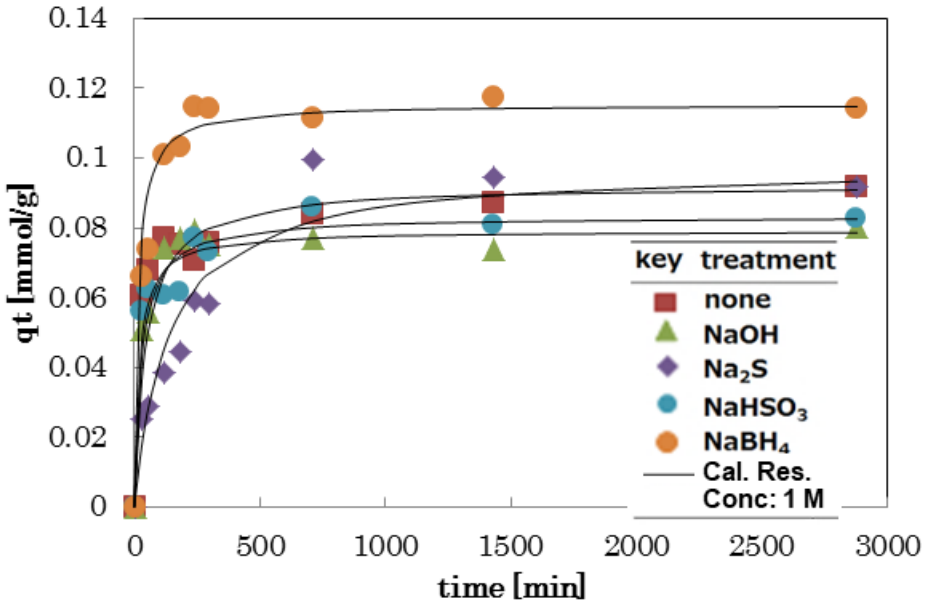


Fig. 4-8 Time course of Au(III) adsorption by chemically treated sheep wool

(Experimental condition: V: 15cm^3, C_{ini}: 0.05 mmol/l, pH$_{ini}$=2.0)

The sheep wool and chemically treated sheep wool samples were determined as possible to make adsorption of gold from aqueous solutions. The rate of gold adsorption was increased by time increase and reached equilibrium within 3 hours of except sodium sulfide treated sheep wool samples. In 48 hours of contact, gold adsorption rates reached 91% and higher in wool and chemically treated wool samples.

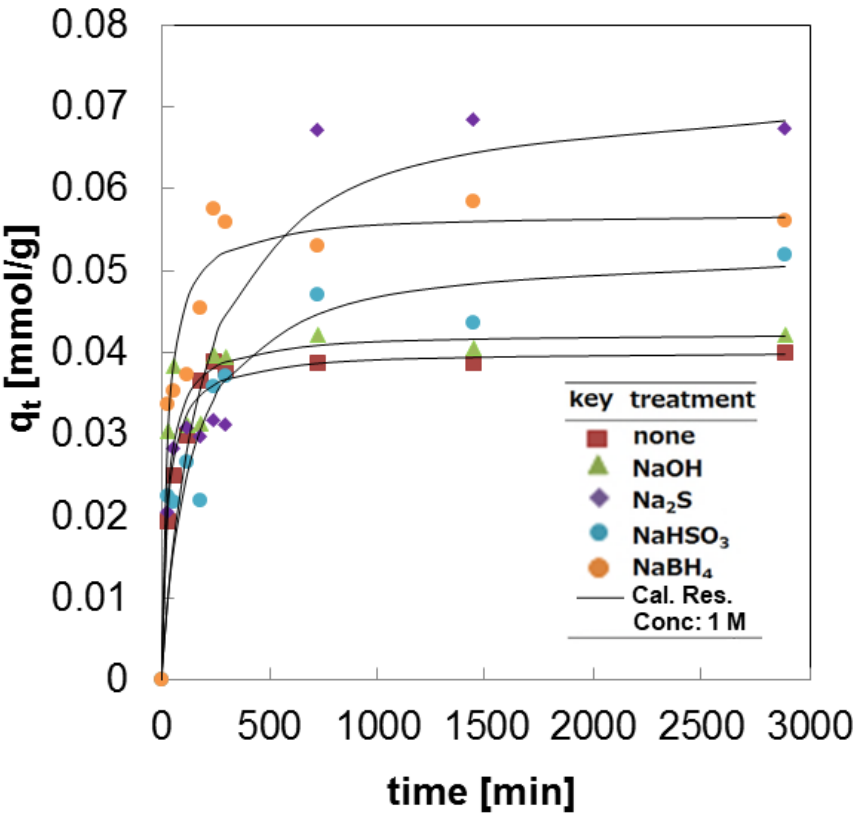


Fig. 4-9 Time course of Cu(II) adsorption by chemically treated sheep wool

(Experimental condition: V: 15cm^3, C_{ini}: 0.16 mmol/l, pH$_{ini}$=2.0)

The pseudo-second order models of Au(III) and Cu(II) adsorbed chemically treated sheep wool are shown in Fig. 4-10. The pseudo-second order kinetic constant, other parameters were determined and summarized in Table 4-3.

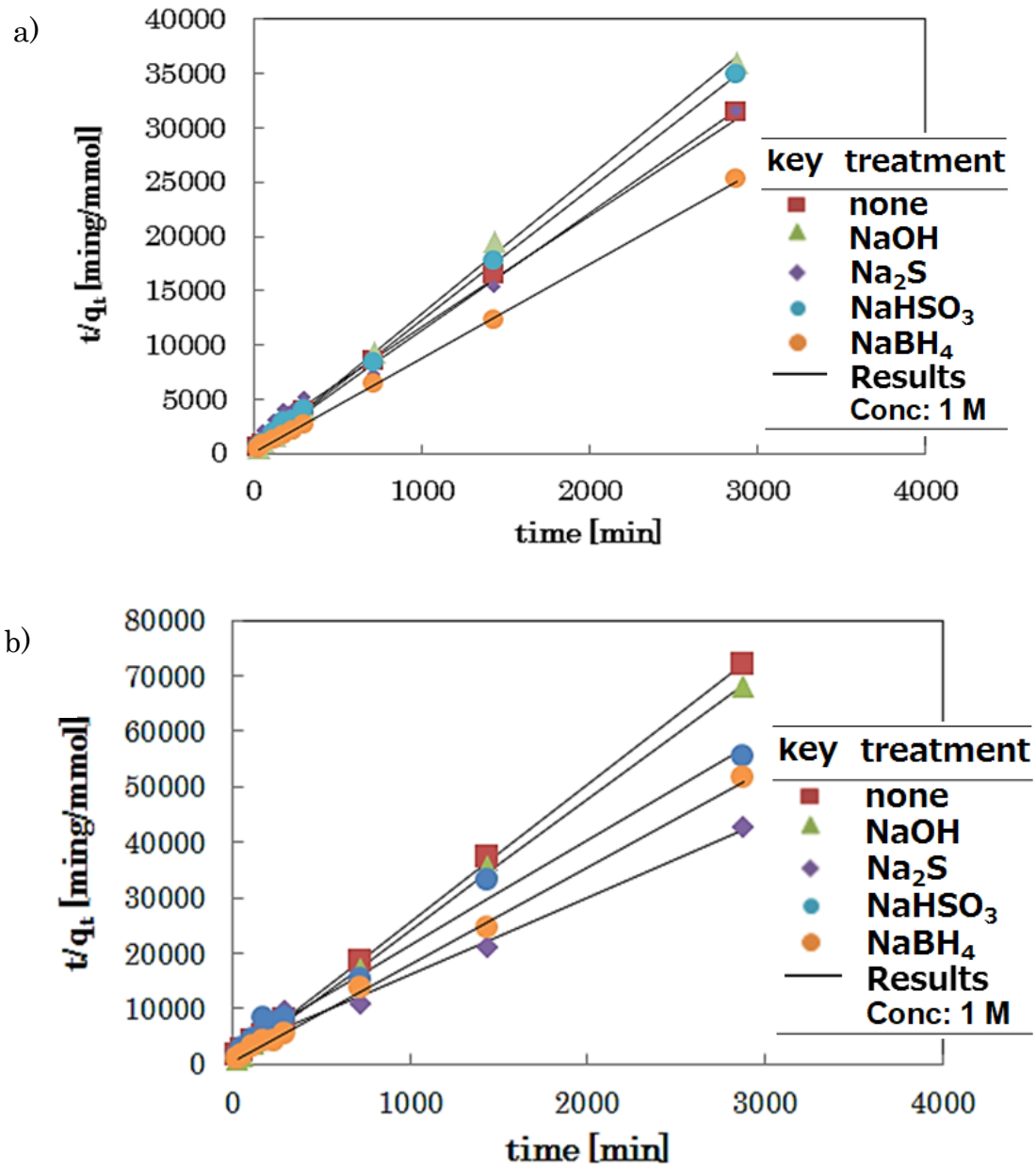


Fig. 4-10 a: Pseudo-second order kinetics of Au(III); b: Pseudo-second order kinetics Cu(II) onto wool and chemically treated wools

Pseudo-second order kinetic models well fit the experimental data. This suggests that heavy metals may have been adsorbed on the surface of the sheep wool by chemisorption. In this adsorption system, chemical bonds formed between the heavy metal ion and sheep wool [4.37].

Table 4-3 Kinetic parameters obtained from experimental data of Au(III) and Cu(II) adsorption

Metal	Chemical treatment (Conc: 1.0 M)	Pseudo-second order model		
		k_2 (g/mg min)	q_e (mmol/g)	R^2
Au(III)	none	0.2318	0.0923	0.9991
	NaOH	0.6243	0.0792	0.9985
	Na_2S	0.0740	0.0978	0.9931
	$NaHSO_3$	0.4186	0.0833	0.9993
	$NaBH_4$	0.5669	0.1154	0.9996
Cu(II)	none	0.8867	0.0402	0.9997
	NaOH	0.8626	0.0425	0.9994
	Na_2S	0.0729	0.0728	0.9842
	$NaHSO_3$	0.1439	0.0528	0.9912
	$NaBH_4$	0.6434	0.0571	0.9988

3.2.3 Adsorption behavior of Au(III) and Cu(II) by sodium sulfide treated sheep wool

The plots of t/q_t vs. time of each Na_2S treated sheep wool for the adsorption studies of Au(III) and Cu(II) shown in Figs. 4-11, 4-12, respectively.

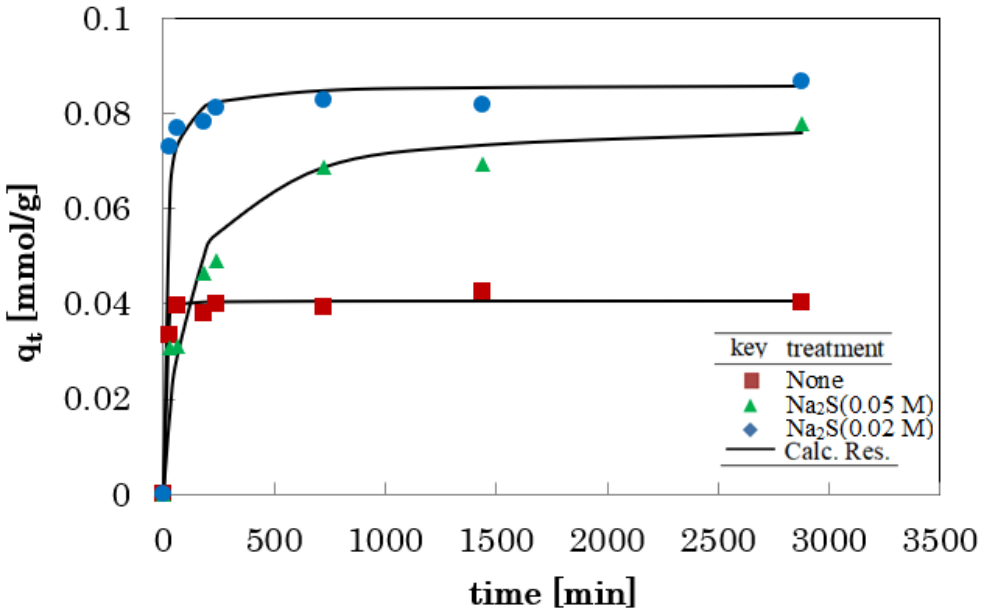


Fig. 4-11 Time course of Au(III) adsorption by chemically treated sheep wool (Experimental condition: V: 15cm^3, C_{ini}: 0.05 mmol/l, pH_{ini}=2.0)

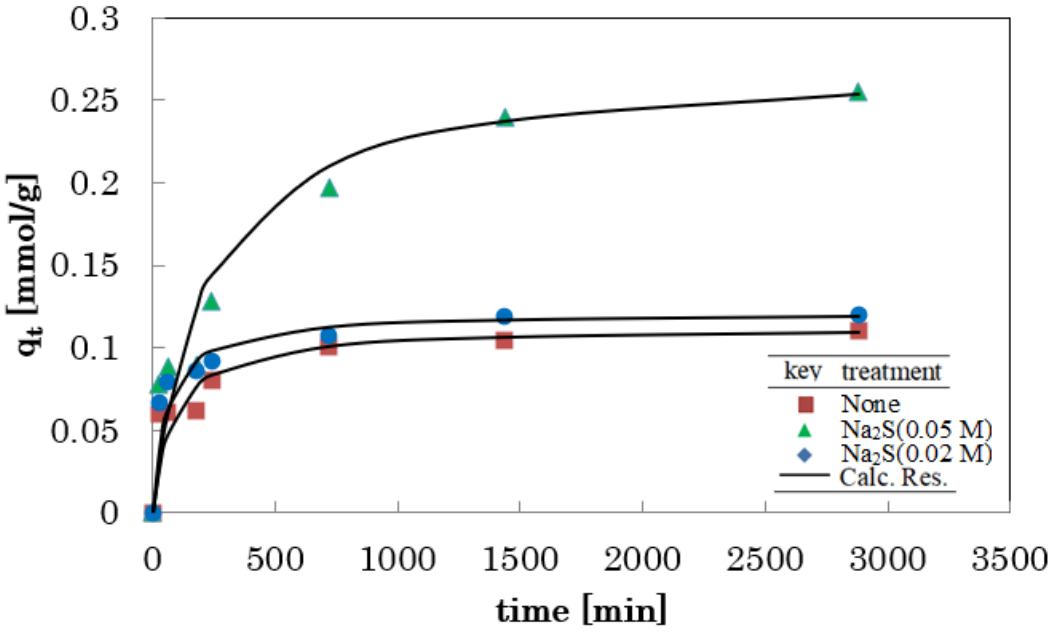
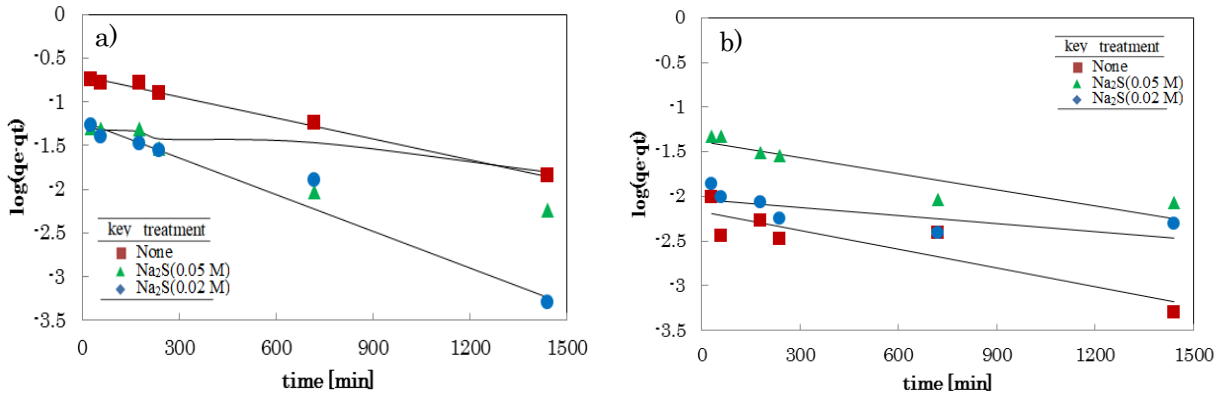


Fig. 4-12 Time course of Cu(II) adsorption by chemically treated sheep wool (Experimental condition: m: 10 mg, V: 15cm^3, C_{ini}: 0.16 mmol/l, pH$_{ini}$=4.9)

The plots of $log(q_e\text{-}q_t)$ vs. time and t/q_t vs. time of each sodium sulfide treated sheep wool for the pseudo-first order, pseudo-second order model is shown in Fig. 4-13 a-d. The pseudo-first order, pseudo-second order kinetic constant, other parameters were calculated and summarized in Table 4-4.



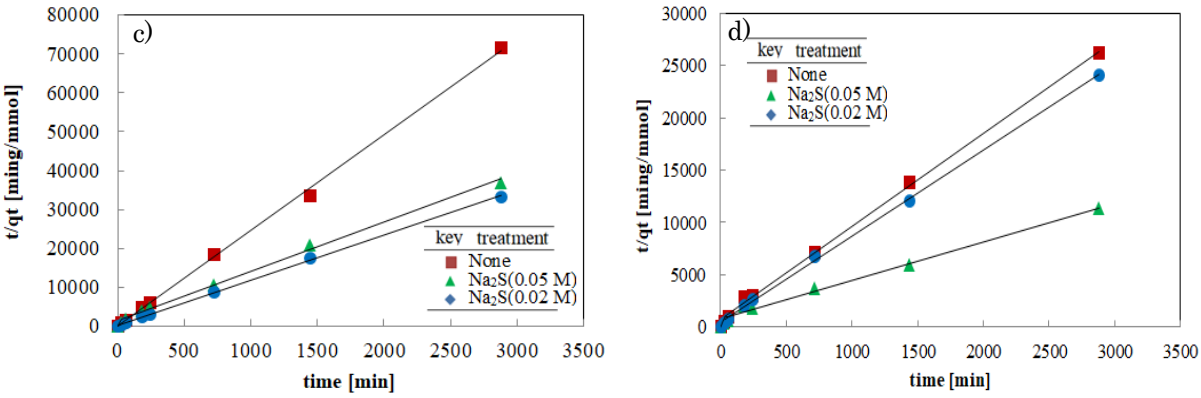


Fig. 4-13 a: Pseudo-first order kinetics of Au(III); b: Pseudo-first order kinetics of Cu(II); c: Pseudo-second order kinetics of Au(III); d: Pseudo-second order kinetics of Cu(II) onto wool and sodium sulfide treated wools

The reaction between adsorbent and heavy metals is expressed as pseudo-second order kinetic model [4.38]. The experimental data of adsorption well fitted to the pseudo-second order kinetic model compared with the pseudo-first order kinetic model. Therefore, gold and copper are adsorbed on the sodium sulfide treated sheep wool by chemisorption.

Table 4-4 Kinetic parameters obtained from experimental data of Au(III) and Cu(II) adsorption

Chemical treatment	Pseudo-first order model			Pseudo-second order model		
	k_1 (1/min)	q_e (mmol/g)	R^2	k_2 (g/mg min)	q_e (mmol/g)	R^2
Au(III)						
None	0.0016	0.0067	0.7955	0.1066	0.1126	0.9985
Na_2S (0.05 M)	0.0014	0.0411	0.8333	0.0172	0.2721	0.9913
Na_2S (0.02 M)	0.0007	0.0092	0.5119	0.1451	0.1216	0.9993
Cu(II)						
None	0.0016	0.0505	0.9158	21.265	0.0406	0.9991
Na_2S (0.05 M)	0.0018	0.1985	0.9330	0.1203	0.0786	0.9941
Na_2S (0.02 M)	0.0032	0.0604	0.9606	1.0942	0.0859	0.9991

3.3 Biosorption isotherm modeling

The sheep wool and chemically treated sheep wool was evaluated as an adsorbent material for the adsorption of Cr(III), Cr(VI), Au(III) and Cu(II) at different initial concentration.

3.3.1 Adsorption behavior of Cr(III) and Cr(VI)

The effect of the initial metal ion concentration on the adsorption of chromium was evaluated in the range from 0.019 to 1.923 mmol/l. Corresponding adsorption results are shown in Figs. 4-14 and 4-15. The adsorption amounts greatened to increasing the initial metal concentration of scale-up in the mass-transfer driving force.

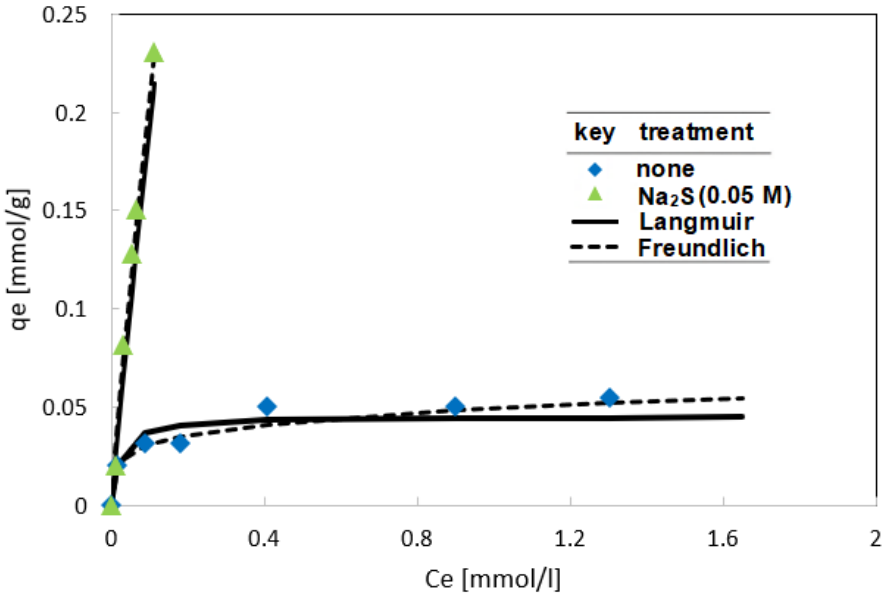


Fig. 4-14 The biosorption isotherm of Cr(III) by sheep wool
(Experimental condition: V: 15cm^3, C_{ini}:0.019-1.923 mmol/l, pH_{eq}=5.4)

Langmuir and Freundlich isotherm models were used to fit the adsorption data. All the isotherm constants and parameters calculated and summarized in Table 4-5. Thereupon experimental data of the adsorption of chromium on sheep wool, Cr(III) had good agreement with the Freundlich isotherm model more than the Langmuir adsorption isotherm.

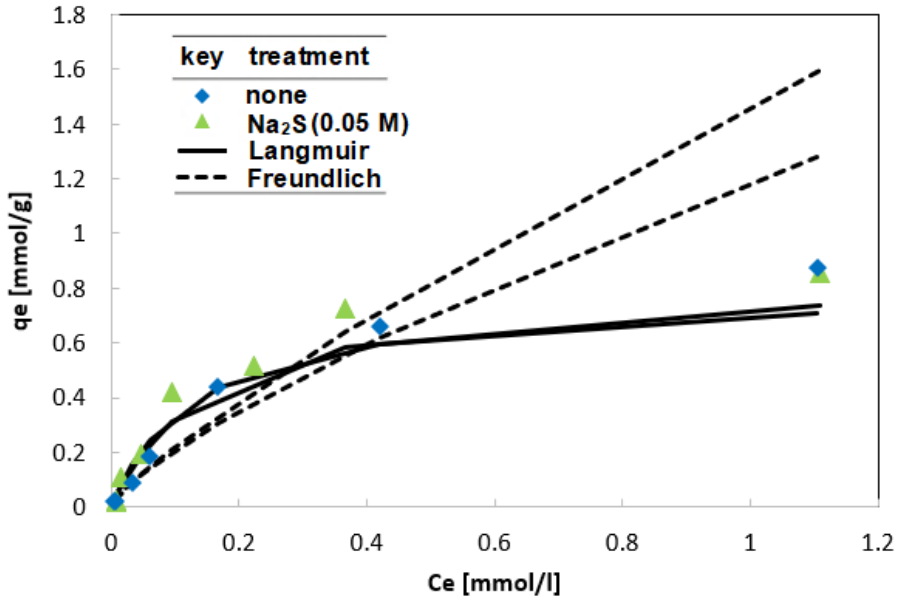


Fig. 4-15 The biosorption isotherm of Cr(VI) by sheep wool

(Experimental condition: V: 15cm^3, C_{ini}: 0.019-1.923 mmol/l, pH_{eq}=2.1)

Therefore, Cr(VI) adsorption results are fitted well to the Langmuir adsorption isotherm with a high correlation coefficient value. Langmuir isotherm model shows that the adsorption process depends on a certain site sorption mechanism where chromium occupies that sites on the sheep wool.

Table 4-5 Langmuir and Freundlich isotherm constant for the biosorption of Cr(III) and Cr(VI) by chemically treated sheep wool

Chemical treatment	Langmuir isotherm			Freundlich isotherm		
	q_{max} (mmol/g)	K (L/mmol)	R^2	n	K_F (mmol/g)	R^2
Cr(III)						
none	0.045	0.95	0.8558	4.919	0.0221	0.8883
Na_2S (0.05 M)	2.388	0.02	0.9835	1.123	0.0478	0.9259
Cr(VI)						
none	0.796	0.14	0.9818	1.328	0.0606	0.9742
Na_2S (0.05 M)	0.845	0.12	0.9935	1.211	0.0561	0.911

3.3.2 Adsorption behavior of Au(III) by chemically treated sheep wool

The chemically treated sheep wool samples were applied to the gold adsorption and Langmuir and Freundlich isotherm model was used which is shown in Fig. 4-16. All the correlation coefficients and constant values obtained by the isotherm models are summarized in Table 4-6. Adsorption results of sheep wool well fitted with the Langmuir adsorption isotherm model than the Freundlich adsorption isotherm model.

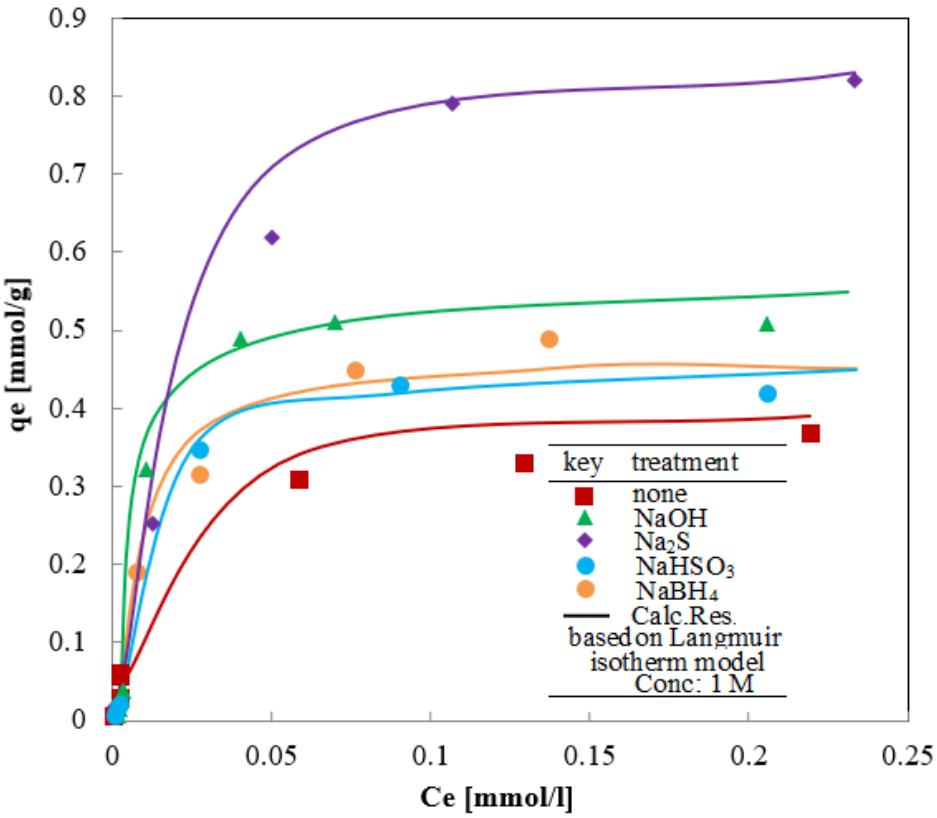


Fig. 4-16 The biosorption isotherm of Au(III)

(Experimental condition: V: 15cm^3, C_{ini}: 0.005-0.508 mmol/l, pH_{eq}=2.0)

The adsorption capacity of sodium sulfide treated sheep wool of Au(III) was determined as 0.95 mmol/g. Sodium sulfide treated sheep wool adsorbed Au(III) two times higher than untreated sheep wool and different concentration of sodium sulfide treatment selected for further adsorption study. Adsorption amounts were determined high for the chemically treated sheep wools and were also determined same in the El-Sayed's research (2004) [4.29].

Table 4-6 Langmuir and Freundlich parameters obtained from experimental data of Au(III) adsorption

Chemical treatment (Conc: 1.0 M)	Langmuir isotherm			Freundlich isotherm		
	q_{max} (mmol/g)	K (L/mmol)	R^2	n	K_F (mmol/g)	R^2
none	0.476	1.05	0.9847	0.591	0.1656	0.8115
NaOH	0.493	1.02	0.9983	1.141	0.3292	0.8624
Na_2S	0.950	1.08	0.9937	1.452	0.1642	0.8788
$NaHSO_3$	0.414	0.70	0.9969	1.056	0.2375	0.8765
$NaBH_4$	0.547	0.92	0.9938	1.315	0.2069	0.8798

3.3.3 Adsorption behavior of Cu(II) by sodium sulfide treated sheep wool

The Langmuir adsorption isotherm model was used as defining the equilibrium between sodium sulfide treated sheep wool samples and copper ion (Fig. 4-17).

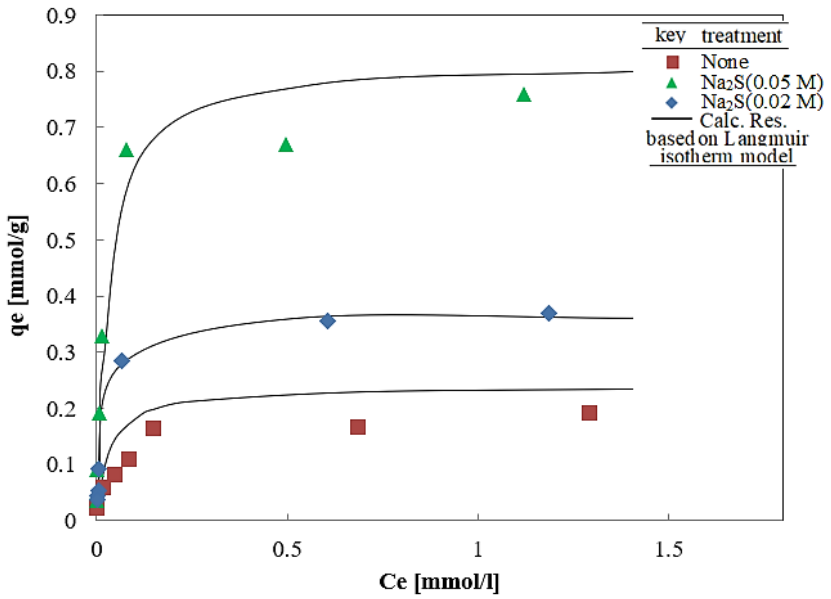


Fig. 4-17 The biosorption isotherm of Cu(II)

(Experimental condition: V: 15cm^3, C_{ini}: 0.016-1.575 mmol/l, pH_{eq}=5.8)

All the correlation coefficients and constant values obtained by the isotherm models are summarized in Table 4-7. Adsorption results of sheep wool have good agreement with the Langmuir adsorption isotherm model comparing with the Freundlich adsorption isotherm model.

Table 4-7 Langmuir and Freundlich parameters obtained from experimental data of Cu(II) adsorption

Chemical treatment	Langmuir isotherm			Freundlich isotherm		
	q_{max} (mmol/g)	K (L/mmol)	R^2	n	K_F (mmol/g)	R^2
None	0.239	0.51	0.9565	3.349	0.0806	0.6243
Na_2S (0.05 M)	0.817	0.54	0.9724	2.456	0.1095	0.7249
Na_2S (0.02 M)	0.268	1.16	0.9618	3.647	0.1290	0.9409

The sheep wool copper adsorption capacity was defined as 0.82 mmol/g in Na_2S (0.05 M) treated sheep wool and 0.27 mmol/g in Na_2S (0.02 M) treated sheep wool from the biosorption isotherm modeling. Na_2S (0.05 M) treated sheep wool had a higher copper adsorption content than Na_2S (0.02 M) treated sheep wool, indicating that the sheep wool treatment of 0.05 M of sodium sulfide is suitable for copper adsorption.

3.4 Adsorption mechanism of heavy metal

Wool fiber is a complicated material and metal adsorption, binding mechanisms of heavy metal on sheep wool was newly studied this time. Functional groups of the sheep wool surface which could be responsible for the metal binding in adsorption. Energy Dispersive X-Ray spectroscopy analysis, Fourier-transform infrared spectroscopy and X-ray photoelectron spectroscopy were used as a definition of differences in the wool structure after heavy metal adsorption.

3.4.1 EDX analysis of sheep wool

Energy Dispersive X-ray spectroscopy analysis was investigated by under operation of SEM and chemically treated sheep wool samples results are shown in Fig. 4-18. Sheep wool samples to comprise of C, N, O, and S elements. After chemical treatment, all sheep wool samples contain C, N, O, S, and Na except $NaHSO_3$ treated sheep wool. There was no significant change observed on the surface of the sheep wool after $NaHSO_3$ treatment, which explains the absence of sodium in EDX analysis.

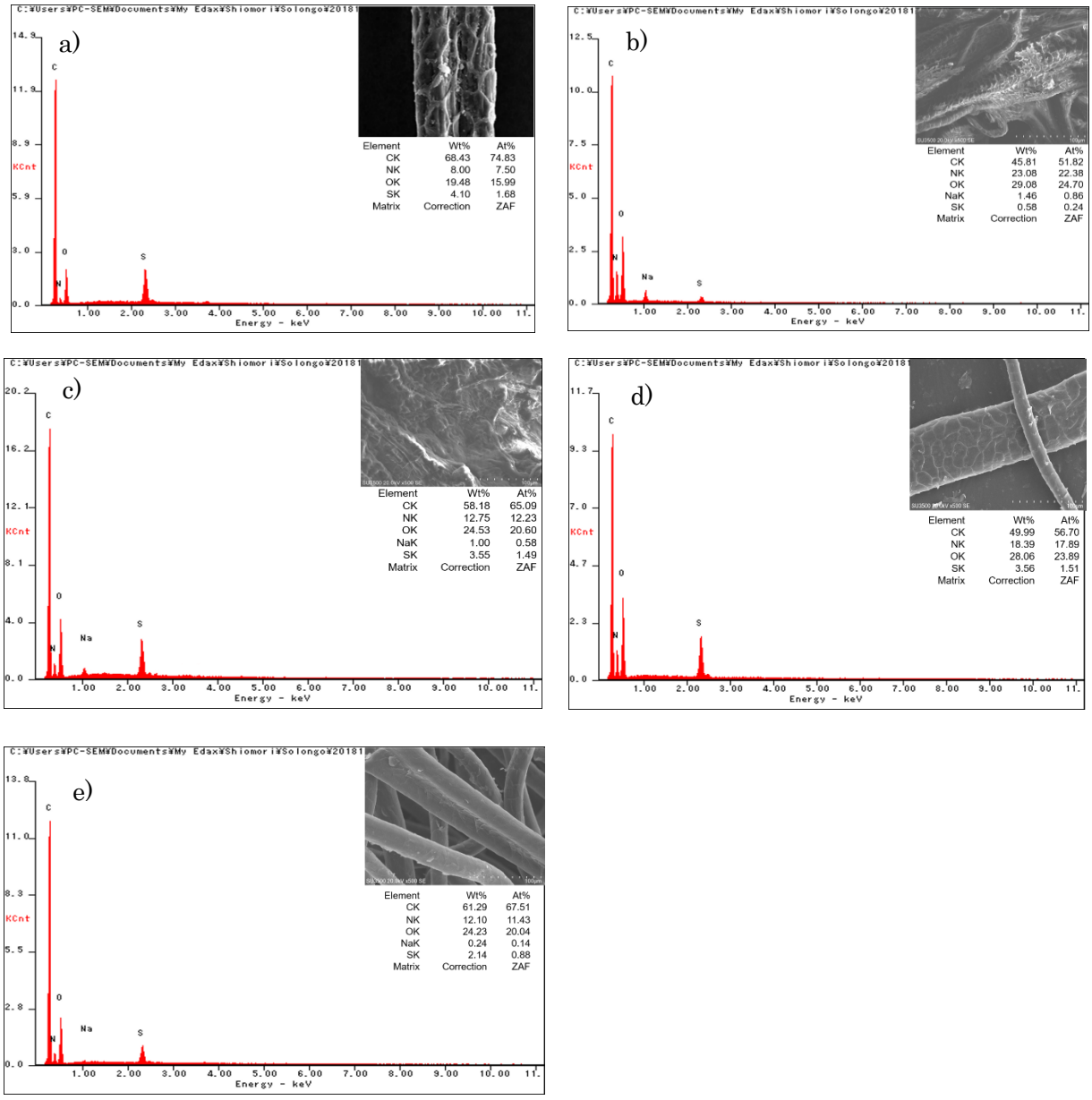


Fig. 4-18 EDX analysis of a) sheep wool, b) sodium hydroxide, c) sodium sulfide, d) sodium bisulfite, e) sodium borohydride treated sheep wool

EDX analysis of chromium adsorbed sheep wool sample results are shown in Fig. 4-19. All the sheep wool and sodium sulfide treated wool samples comprising of C, N, O, S, and Cr elements after adsorption of chromium. The amount of Cr(VI) was defined as high, 10.5 wt% in the cross-section of sheep wool, and 24.3 wt% in sodium sulfide treated wool comparing with the Cr(III) adsorption.

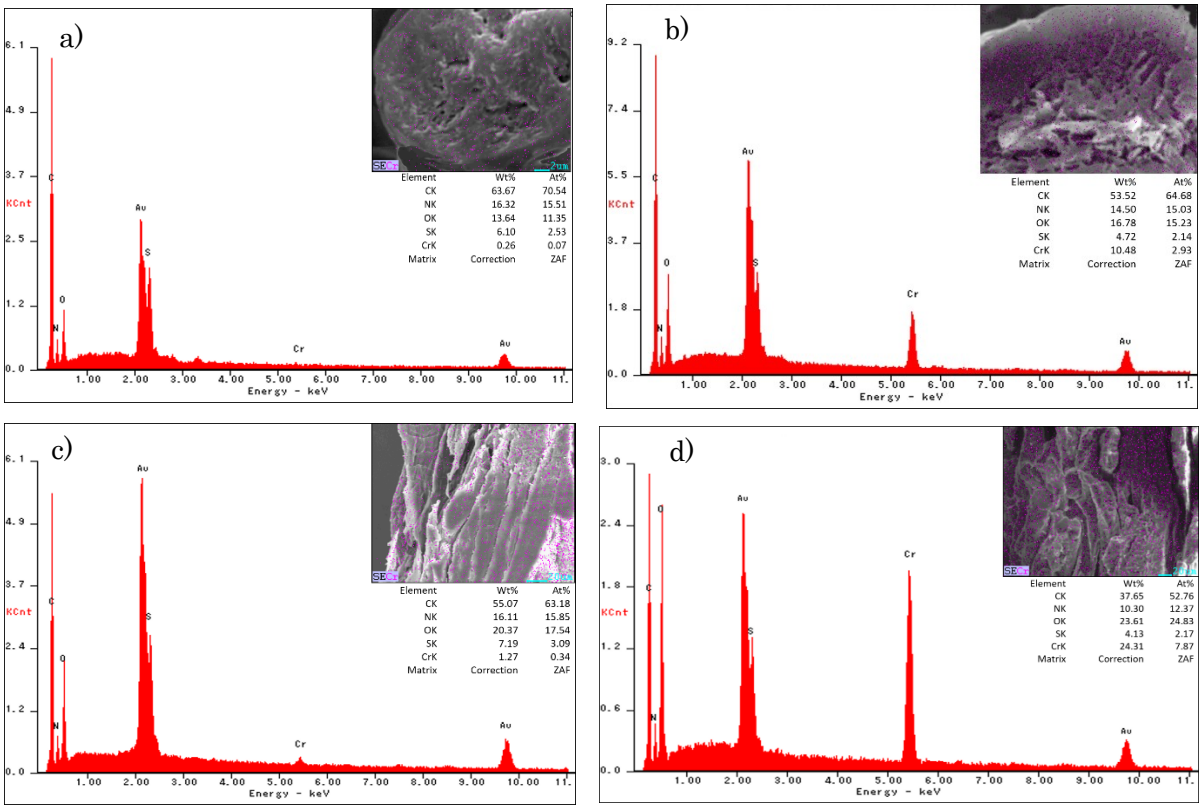
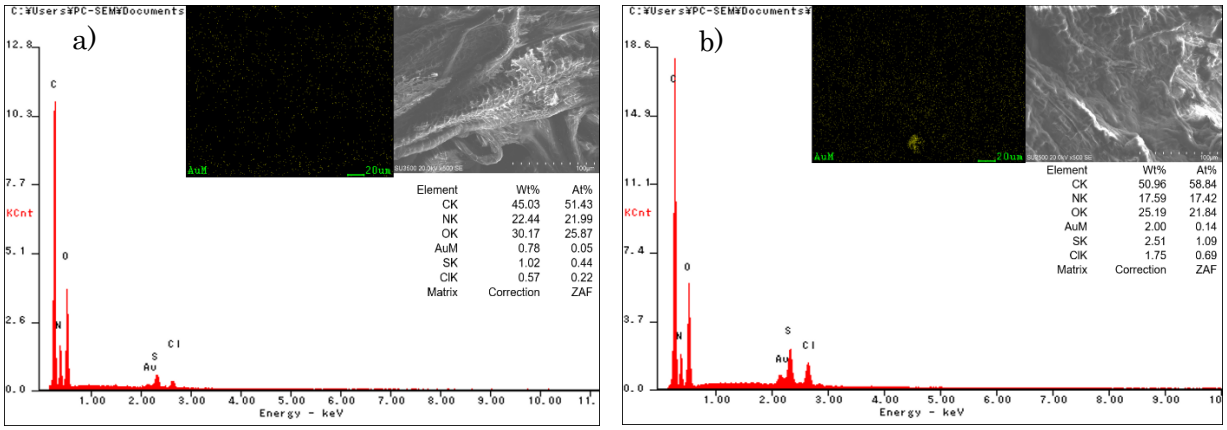


Fig. 4-19 EDX analysis of wool crosssection after chromium adsorption a) Cr(III) adsorption, b) Cr(VI) adsorption by sheep wool, c) Cr(III) adsorption, d) Cr(VI) adsorption by sodium sulfide (0.05 M) treated sheep wool

After adsorption of gold, all the chemically treated wool samples mainly contain C, N, O, Au, Cl, and S elements which are shown in Fig. 4-20. Gold and chlorine are formed after gold adsorption and gold amount were determined as 0.8 – 3.9 wt% in the chemically treated sheep wool. Moreover, chloride ion detected after the gold adsorption processes on sheep wool surfaces. Therefore, the gold ion has participated in the adsorption processes as $AuCl_4^-$ ion.



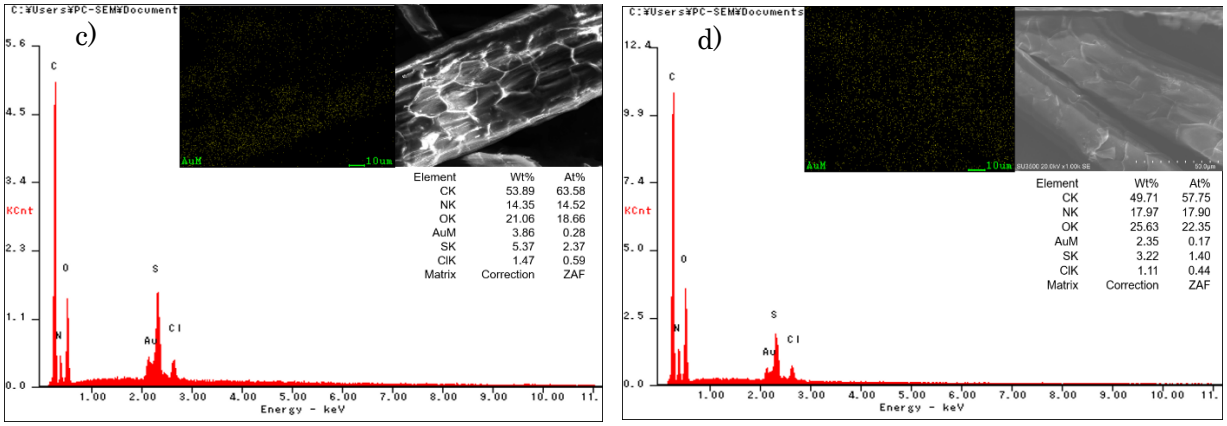
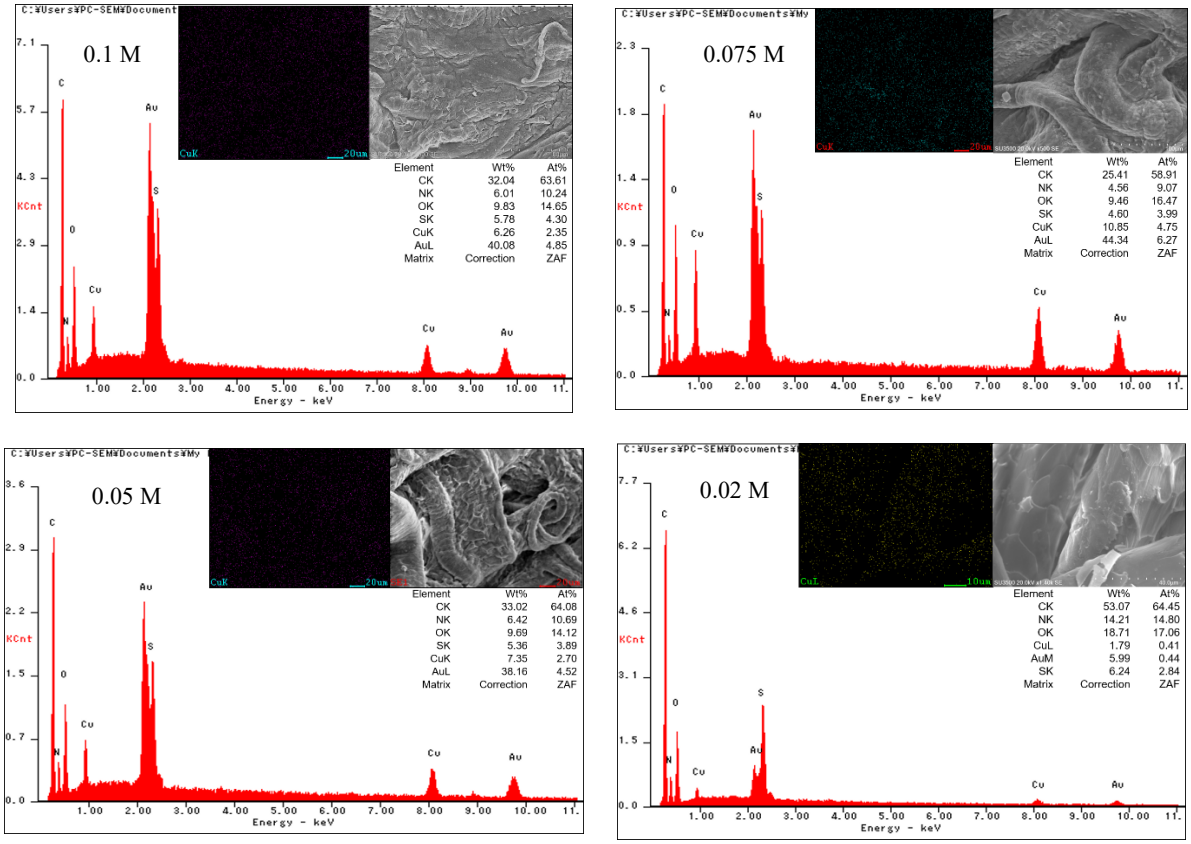


Fig. 4-20 EDX analysis of chemically treated wool after gold adsorption a) sodium hydroxide, b) sodium sulfide, c) sodium bisulfite, d) sodium borohydride treated sheep wool

In the EDX analysis, copper has been detected in sheep wool after adsorption of copper which shows in Fig. 4-21. All the sodium sulfide treated wool samples comprising of C, N, O, S, Au, and Cu elements after adsorption of copper.



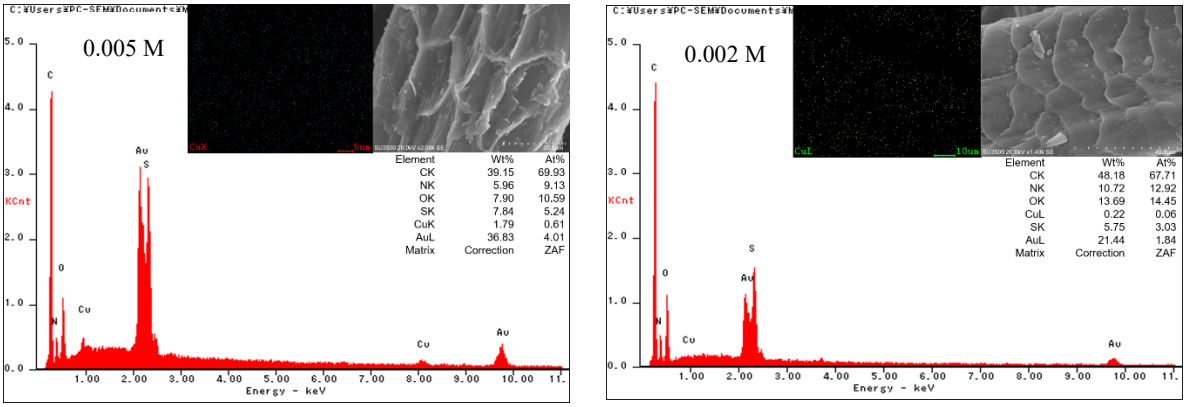


Fig. 4-21 EDX analysis of sodium sulfide treated sheep wool after copper adsorption

Sheep wool samples contain gold due to it was sputtered with gold before the EDX analysis. The copper amount was defined as 0.2 – 10.9 wt% for sodium sulfide treated wool which is treated at different concentrations.

3.4.2 FTIR spectroscopy characterization of sheep wool

The FTIR spectra of chemically treated sheep wool and metal loaded samples are shown in Figs. 4-22, 4-23.

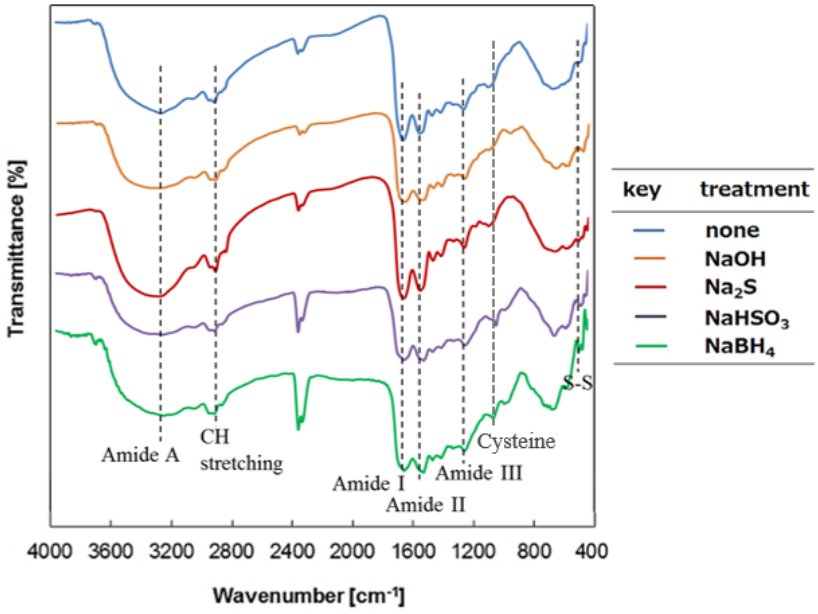


Fig. 4-22 FTIR measurements of wool and chemically treated wool

The amide A band of N-H stretching peak was determined at 3252 cm^{-1}, following 2 weak bands (-CH_3 and –CH_2- asymmetric and symmetric modes) of CH stretching at 2897 and 2905 cm^{-1} in biosorbent of sheep wool. The peaks of 1217 cm^{-1}; 1481 – 1508 cm^{-1} and 1606 –

1619 cm^{-1} are defined as the main amino acid of amide III (complex vibration contains N-H bending, C-N stretching, and C=O stretching), amide II (C-N stretching/NH bending), amide I (80% C=O stretch and a small contribution from NH bend), respectively. The disulfide bridge of the S-S bond was discriminated at 573 cm^{-1} for the untreated sheep wool sample. Weak broadband of 1030-1180 cm^{-1} represents cysteine and different intensities were observed for the chemically treated wool samples. This indicates that some part of the disulfide bond was disrupted due to the chemical treatment.

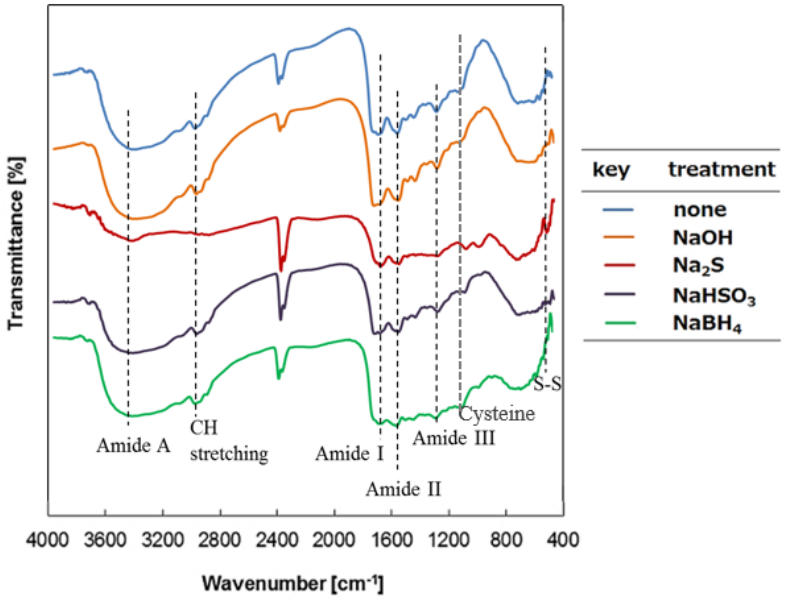


Fig. 4-23 FTIR measurements of wool and chemically treated wool after gold and copper adsorption

The main functional groups of the biosorbents had changed after gold and copper adsorption. The intensity of the amide A was decreased after Au adsorption which indicates that the functional group of amino is the main attraction site of Au(III).

FTIR analysis of sheep wool (**SW**), sodium sulfide (0.05 M) and (0.02 M) treated sheep wool (**SW-III** and **SW-IV**, respectively) after the copper analysis was investigated to know adsorption mechanisms and the result is shown in Fig. 4-24. The profile of raw wool and pre-treated wool samples showing a similar peak and a few peaks was changed after sodium sulfide treatment.

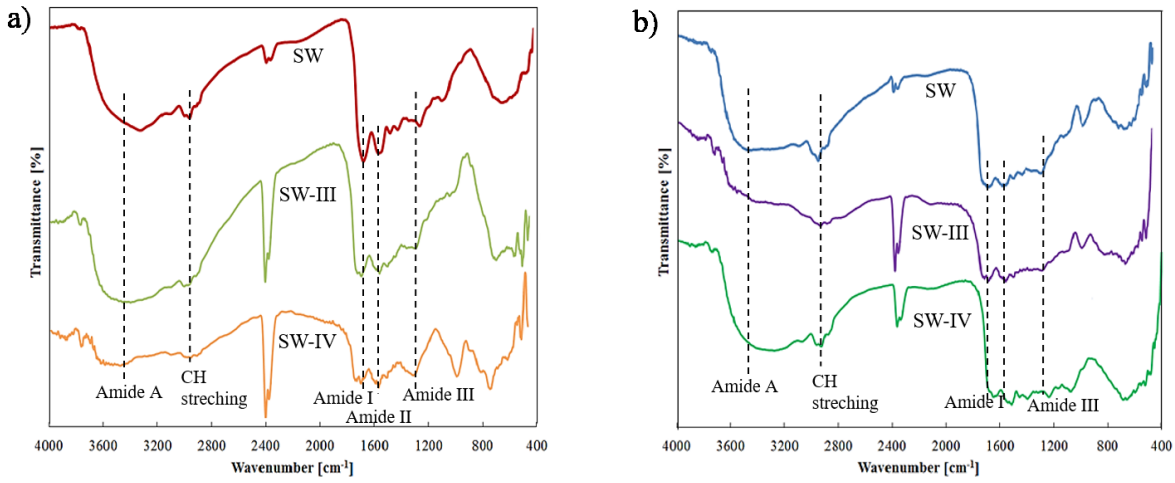


Fig. 4-24 FTIR analysis of a) sheep wool, b) after adsorption of Cu(II)

Sheep wool contains many kinds of functional groups such as amino (R-NH_2), carboxyl (R-COOH), hydroxyl (R-OH), and thiol (R-SH) group. Untreated sheep wool and alkaline treated sheep wool had the amide mode I, II, and its incidental to the α-helix and β-sheet of the structure of wool. Amide I, II, III band are usually connected to the vibration peak of C=O stretching; N-H bending deformation, and C-N stretching, respectively [4.39]. FTIR spectra in amide regions showed that the samples had higher amide-I and amide-II content. Sodium sulfide treated wool was compared with the untreated wool; functional groups of cysteine were identified at 1040 cm^{-1}. Comprehensive band assignments of the results were presented in Table 4-8.

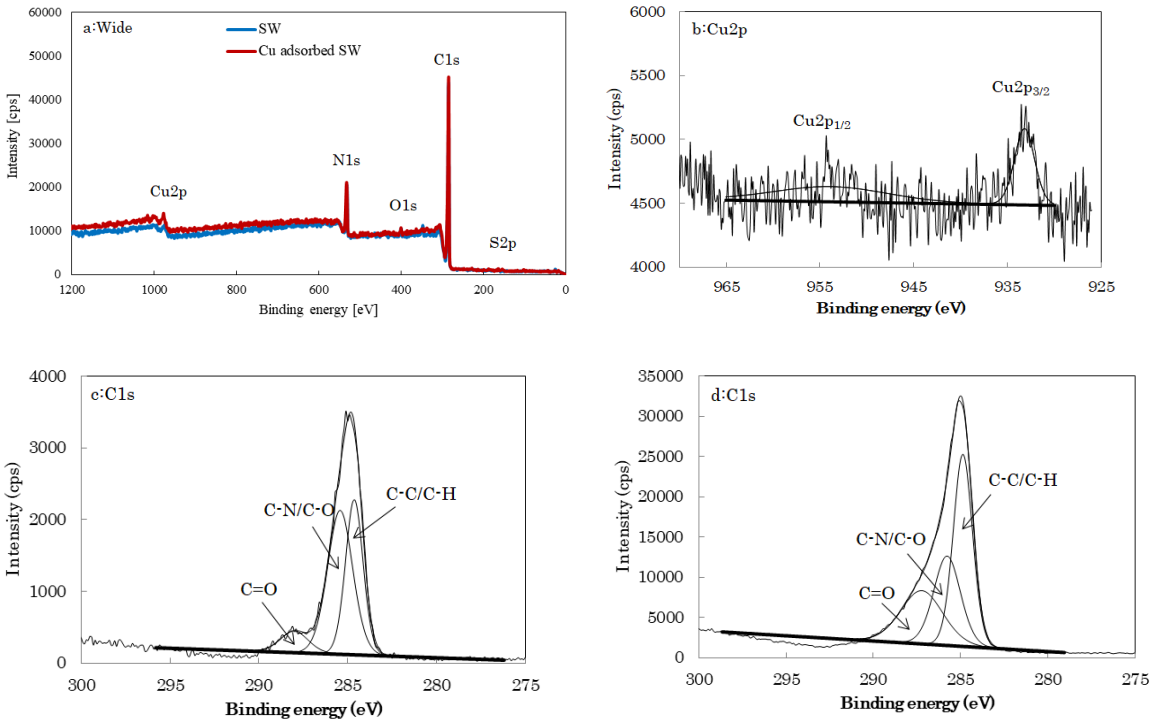
Table 4-8 The band assignment of FTIR spectra of sheep wool

	Wavenumber [cm^{-1}]		
Biosorbents / Assignments	Sheep wool	Na_2S (0.05 M) treated wool	Na_2S (0.02 M) treated wool
Amide A	3252	3279	3242
C-H stretching	2905 and 2897	2905 and 2868	2913 and 2909
Amide I	1619	1606	1618
Amide II	1502	1492	1508
Amide III	1217	1204	1195
Cysteine		1180 – 1030	

The intensity of the amide I band has changed a little since the adsorption of copper on sheep wool, indicating that copper may be absorbed to the carboxyl group as the main biosorption site.

3.4.3 XPS analysis of sheep wool

XPS analysis was performed on the heavy metal adsorbed sheep wool samples to identify the presence of heavy metals on the surface of sheep wool. The wide scan (1200 to 0 eV) of sheep wool (**SW**), sodium sulfide (0.05 M) and (0.02 M) treated sheep wool (**SW-III** and **SW-IV**, respectively) are shown in Figs. 4-25 to 4-30. It presents the single spectres of C (1s), N (1s), O (1s), S (2p), Au (4f), and Cu (2p). The main composition of sheep wool C, N, O, and S elements was confirmed on the wool surface. Au (4f) and Cu (2p) were determined on the surface of gold and copper adsorbed sheep wool samples. The analysis of the peak fitting carried out each spectral peak after narrow scan measurement.



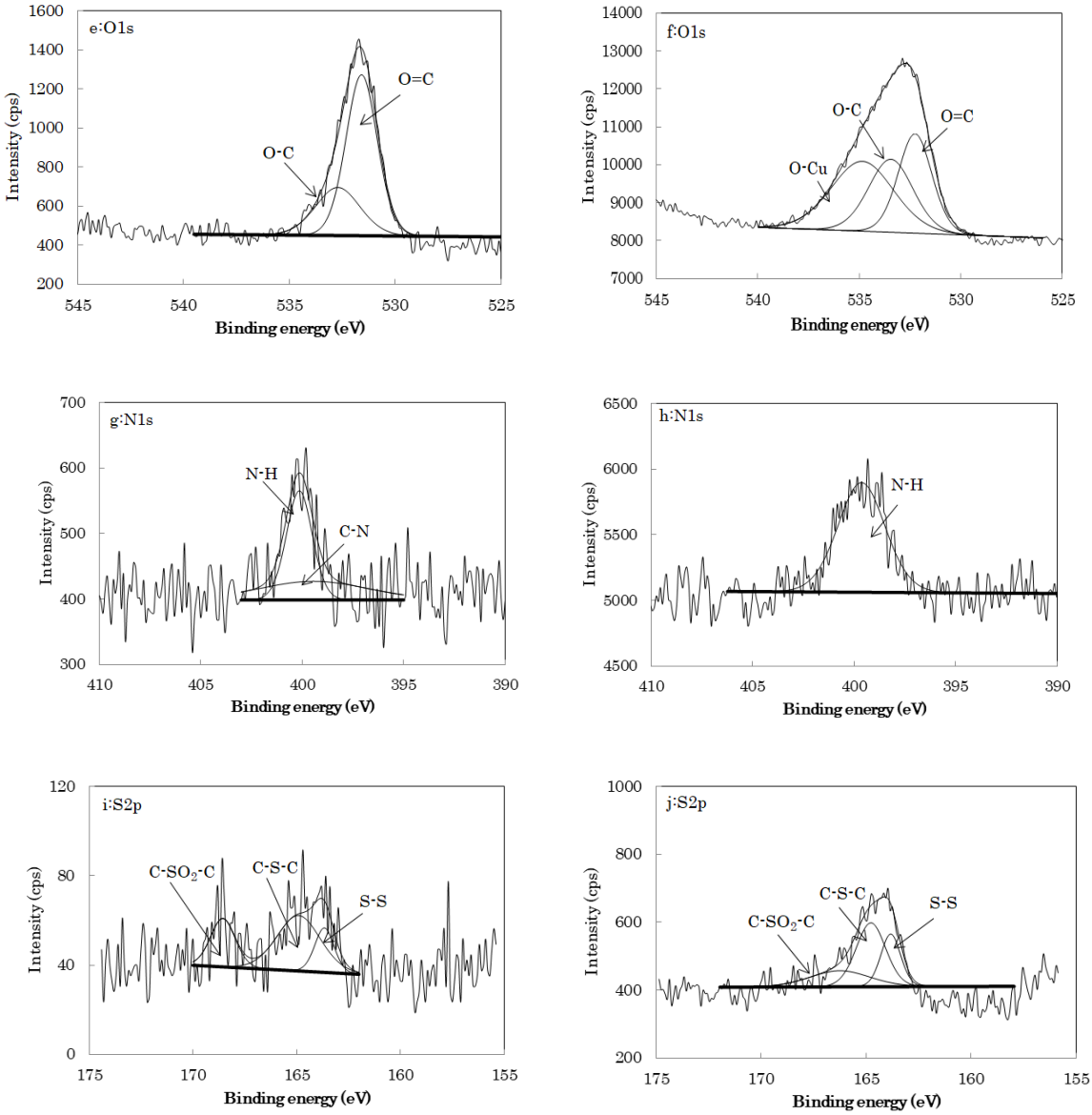
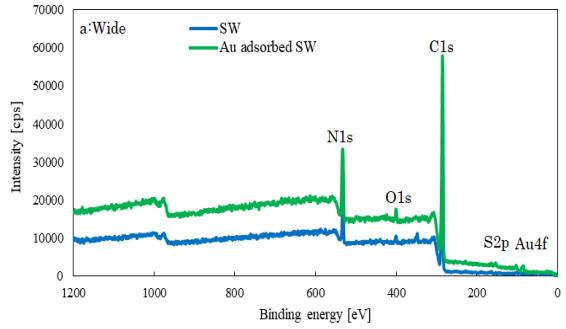
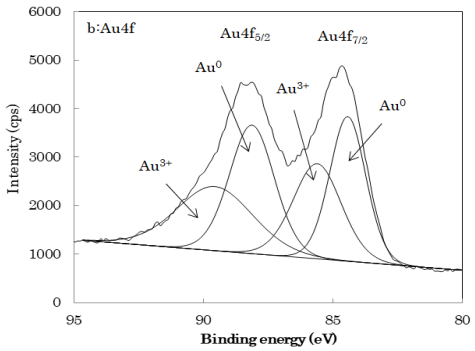
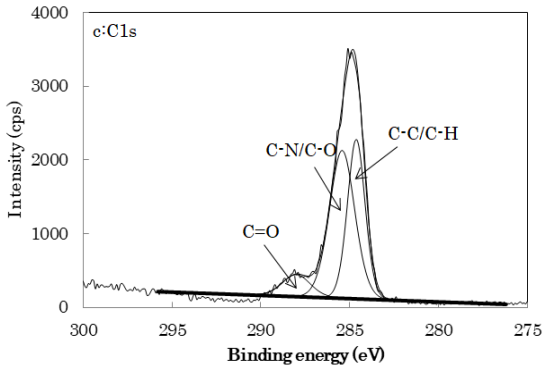
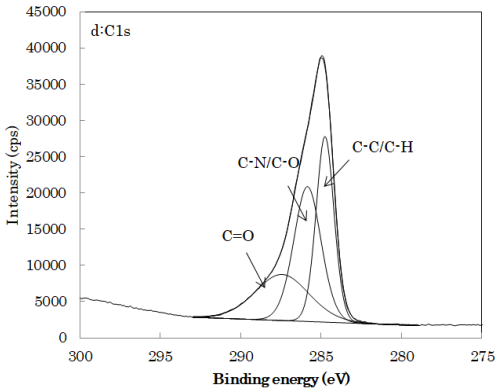


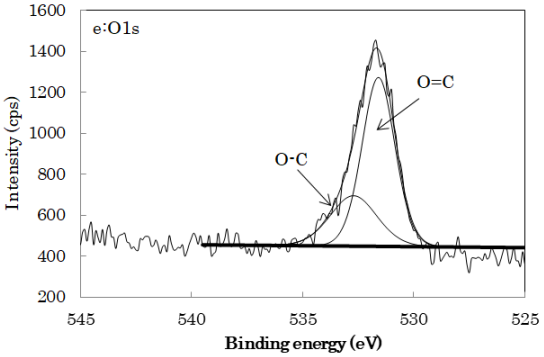
Fig. 4-25 XPS spectra of copper adsorbed SW a: Wide spectra, b: Copper adsorbed SW of Cu (2p) spectra, c: SW, d: Copper adsorbed SW of C (1s) spectra, e: SW, f: Copper adsorbed SW of O (1s) spectra, g: SW, h: Copper adsorbed SW of N (1s) spectra, i: SW, j: Copper adsorbed SW of S (2p) spectra

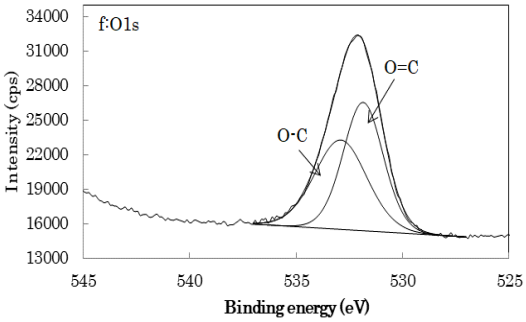

a:Wide
SW
Au adsorbed SW
C1s
N1s
O1s
S2p Au4f
Intensity [cps]
Binding energy [eV]

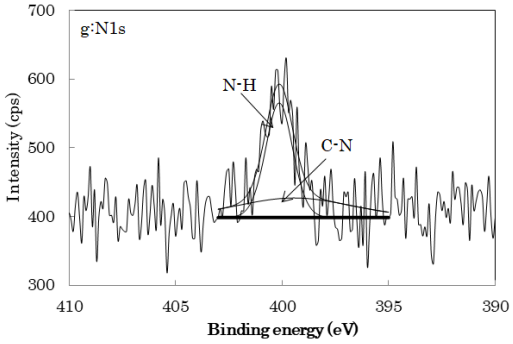

b:Au4f
Au4f5/2
Au4f7/2
Au0
Au3+
Au3+
Au0
Intensity (cps)
Binding energy (eV)

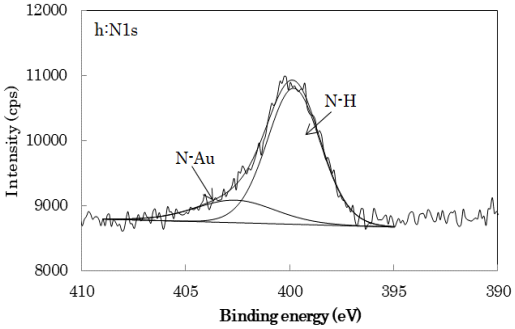

c:C1s
C-N/C-O
C-C/C-H
C=O
Intensity (cps)
Binding energy (eV)


d:C1s
C-N/C-O
C-C/C-H
C=O
Intensity (cps)
Binding energy (eV)


e:O1s
O=C
O-C
Intensity (cps)
Binding energy (eV)


f:O1s
O=C
O-C
Intensity (cps)
Binding energy (eV)


g:N1s
N-H
C-N
Intensity (cps)
Binding energy (eV)


h:N1s
N-H
N-Au
Intensity (cps)
Binding energy (eV)

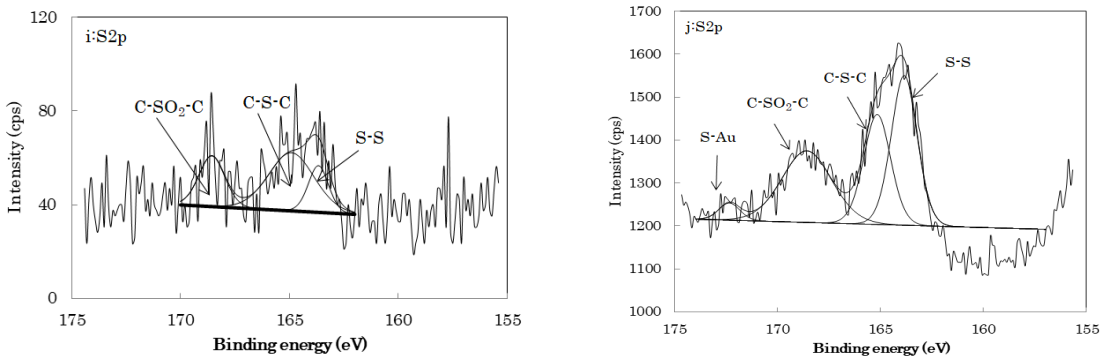
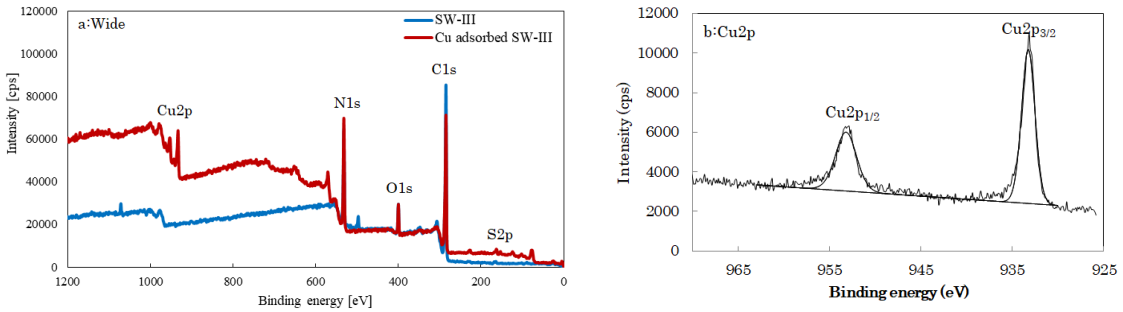


Fig. 4-26 XPS spectra of gold adsorbed SW a: Wide spectra, b: Gold adsorbed SW of Au4f spectra, c: SW, d: Gold adsorbed SW of C (1s) spectra, e: SW, f: Gold adsorbed SW of O (1s) spectra, g: SW, h: Gold adsorbed SW of N (1s) spectra, i: SW, j: Gold adsorbed SW of S (2p) spectra

Three subpeaks were attributable to carbon, C-C/C-H at 285 eV, C-N/C-O at 286.7 eV, and C=O at 288.1 eV. Comparison of the narrow scan of C (1s) of all samples, no significant changes observed before and after adsorption of gold and copper.

The amount of C-N bond in C (1s) and N (1s) spectres decreased in sodium sulfide treated wool. Additionally, a new thiol group formed as disulfide bond disruption in S (2p) spectra. Examination of the N (1s), two subpeaks attributed C-N at 400.5 eV and N-H at 401.3 eV [3.40]. After gold adsorption, a new subpeak is observed at 404 eV and small changes occurred in the fitted spectra. Hence, it shows the gold binding site on sheep wool could be the nitrogen of the amino group based on XPS result.



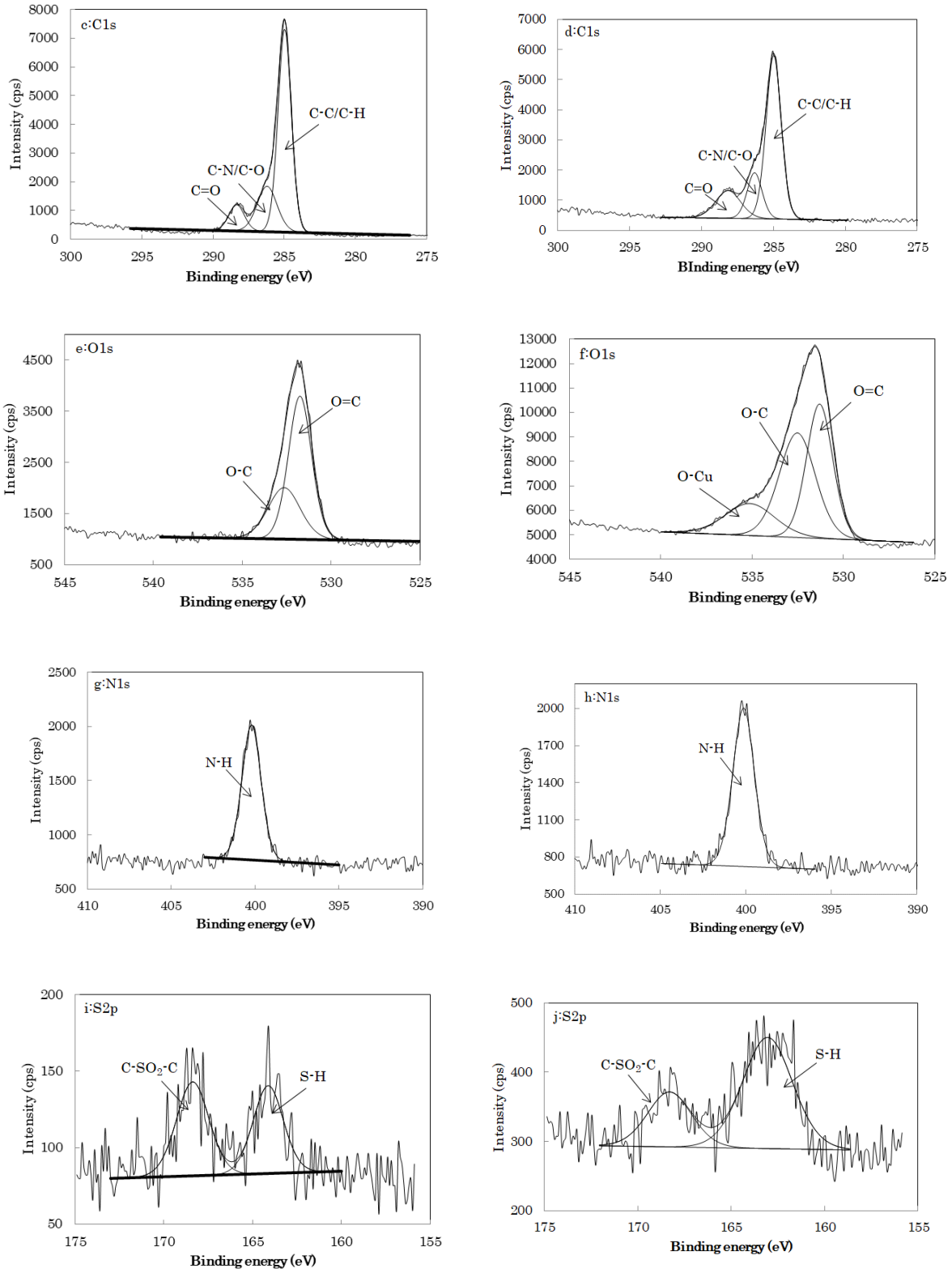
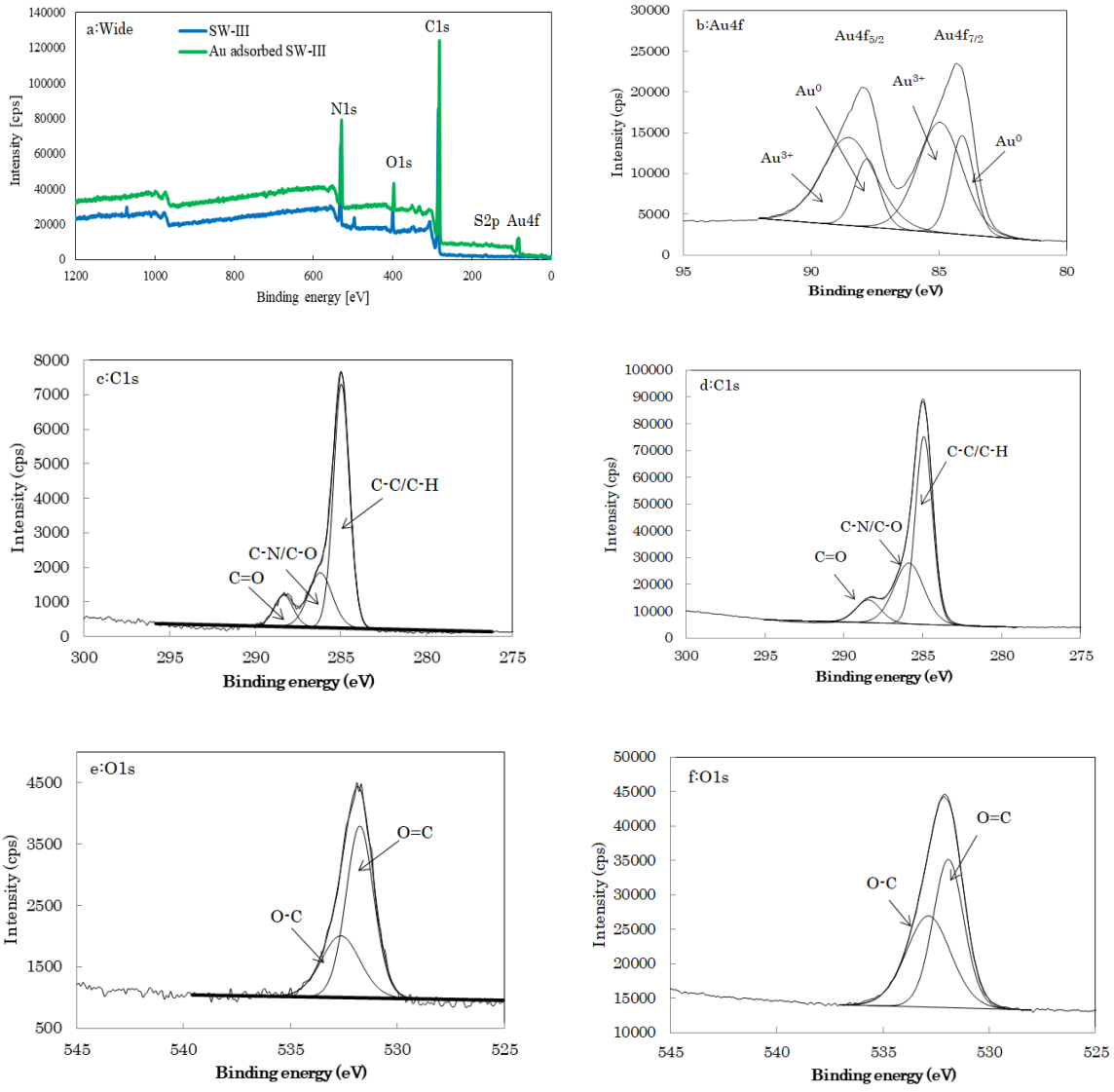


Fig. 4-27 XPS spectra of copper adsorbed Na_2S (0.05 M) treated sheep wool a: Wide spectra, b: Copper adsorbed SW-III of Cu (2p) spectra, c: SW-III, d: Copper adsorbed SW-III of C (1s) spectra, e: SW-III, f: Copper adsorbed SW-III of O (1s) spectra, g: SW-III, h: Copper adsorbed SW-III of N (1s) spectra, i: SW-III, j: Copper adsorbed SW-III of S (2p) spectra

The element of copper is determined for the copper adsorbed sheep wool samples (Fig. 4-27). To compare with the C (1s) spectra of the SW, the subpeaks of C-C/C-H, C-N/C-O, and C=O for copper adsorbed SW is shifted from 284.63, 285.43, and 288.01 eV to 284.88, 286.33, and 288.09 eV, respectively. Also, two subpeaks at 531.59 (O=C) and 532.71 eV (O-C) are shifted to 532.61 and 534.28 eV. Meanwhile, a new subpeak identified at the binding energies of 528.63 eV for the SW-III O (1s) peak.



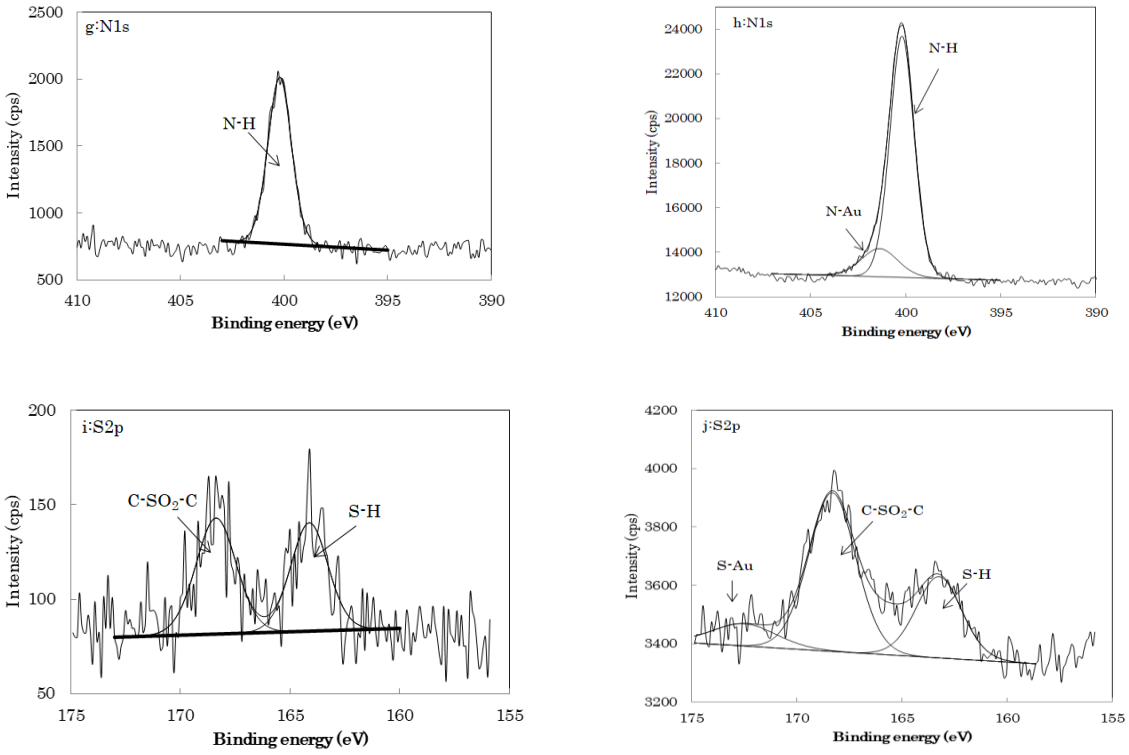
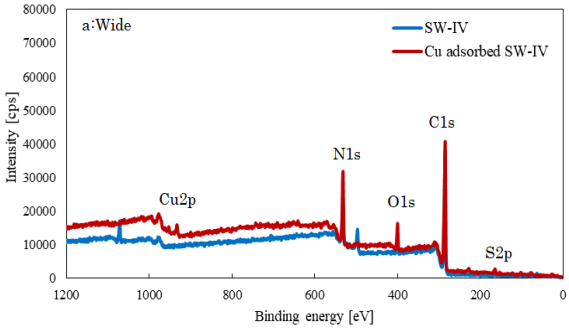
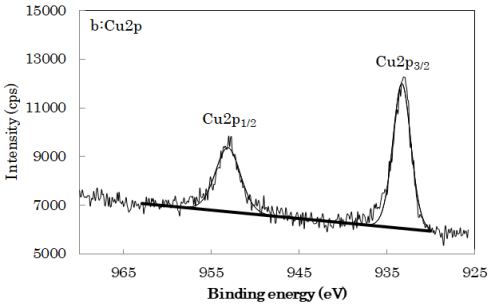


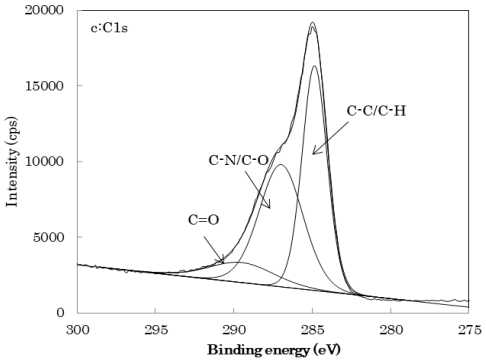
Fig. 4-28 XPS spectra of gold adsorbed SW-III a: Wide spectra, b: Gold adsorbed SW-III of Au (4f) spectra, c: SW-III, d: Gold adsorbed SW-III of C (1s) spectra, e: SW-III, f: Gold adsorbed SW-III of O (1s) spectra, g: SW-III, h: Gold adsorbed SW-III of N (1s) spectra, i: SW-III, j: Gold adsorbed SW-III of S (2p) spectra

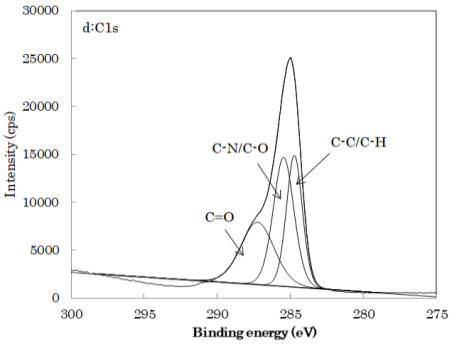
Investigation of the O (1s) spectra, two subpeaks observed in sheep wool sample that are O=C at 532 eV and O-C at 534.2 eV. After copper adsorption, a new subpeak is observed at 535.5 eV and little changes occurred in the fitted peaks. Therefore, the main attraction site for copper is determined as the oxygen of carboxyl groups.

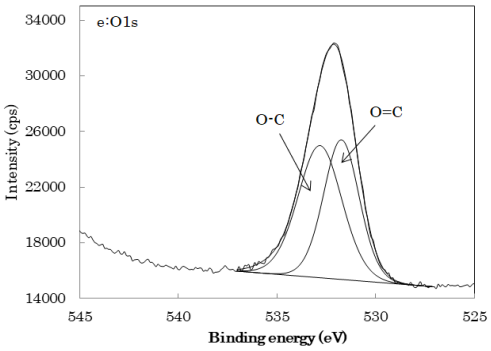
Three subpeaks at 163.5 eV, 165.2 eV, and 166.6 eV are attributed to S-S, C-S-C, and C-SO_2-C of S (2p) spectra in sheep wool sample. After chemical treatment, the thiol group formed as disruption of disulfide group and two subpeaks observed in S (2p) spectra, which are S-H and C-SO_2-C at 163.8, 166.6 eV, respectively. Further, a new subpeak is observed by S-Au bond at 172.5, S (2p) spectra were broadened after gold adsorption in sheep wool samples.

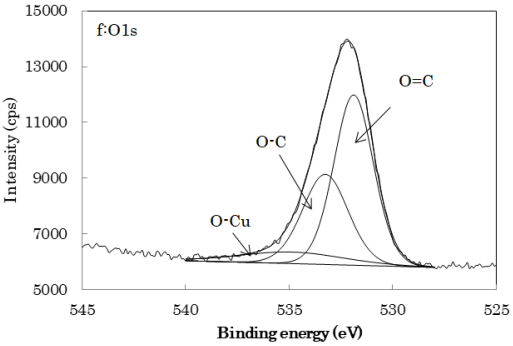

a:Wide
SW-IV
Cu adsorbed SW-IV
Cu2p
N1s
O1s
C1s
S2p
Intensity [cps]
Binding energy [eV]

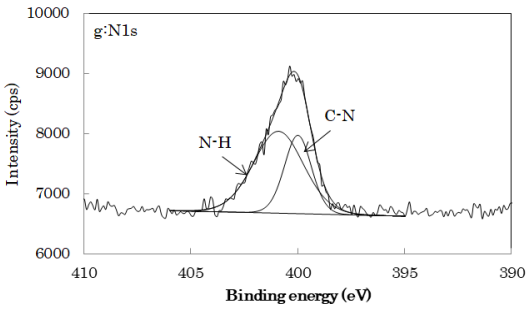

b:Cu2p
Cu2p1/2
Cu2p3/2
Intensity (cps)
Binding energy (eV)

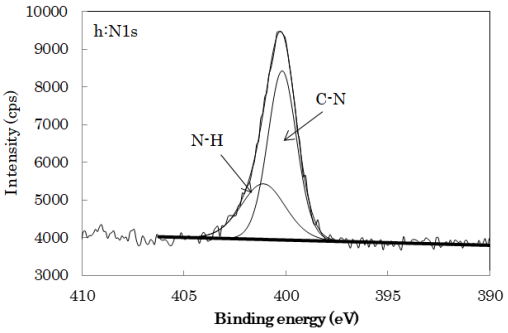

c:C1s
C-C/C-H
C-N/C-O
C=O
Intensity (cps)
Binding energy (eV)


d:C1s
C-N/C-O
C-C/C-H
C=O
Intensity (cps)
Binding energy (eV)


e:O1s
O-C
O=C
Intensity (cps)
Binding energy (eV)


f:O1s
O=C
O-C
O-Cu
Intensity (cps)
Binding energy (eV)


g:N1s
C-N
N-H
Intensity (cps)
Binding energy (eV)


h:N1s
C-N
N-H
Intensity (cps)
Binding energy (eV)

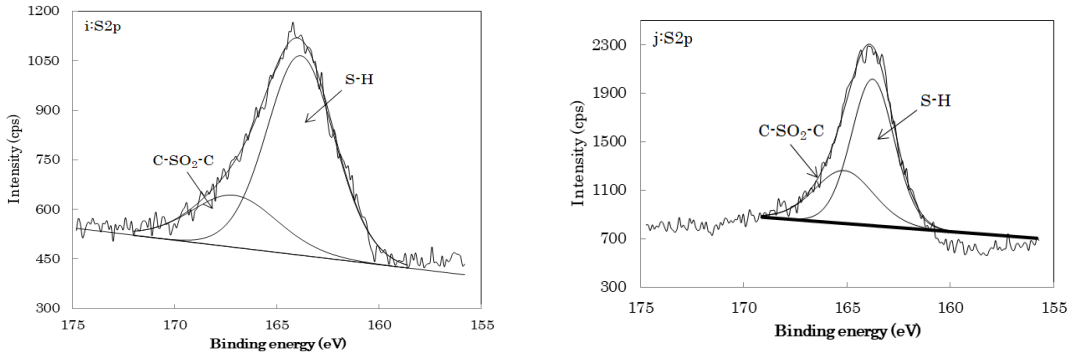
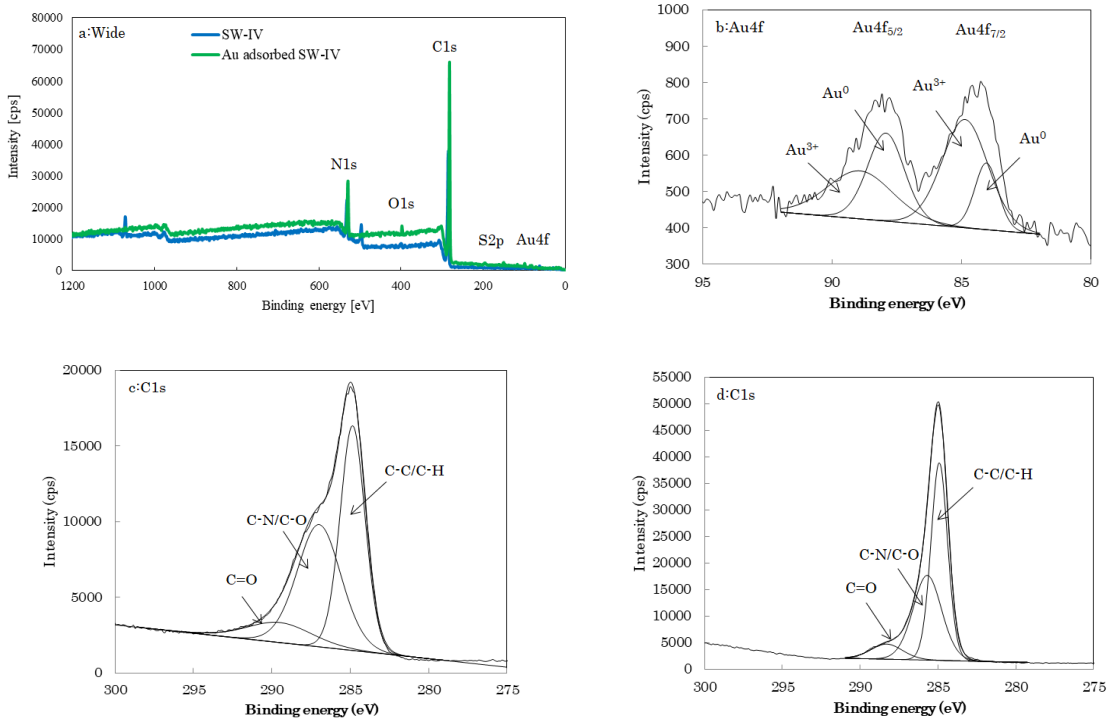


Fig. 4-29 XPS spectra of copper adsorbed SW-IV a: Wide spectra, b: Copper adsorbed SW-IV of Cu (2p) spectra, c: SW-IV, d: Copper adsorbed SW-IV of C (1s) spectra, e: SW-IV, f: Copper adsorbed SW-IV of O (1s) spectra, g: SW-IV, h: Copper adsorbed SW-IV of N (1s) spectra, i: SW-IV, j: Copper adsorbed SW-IV of S (2p) spectra



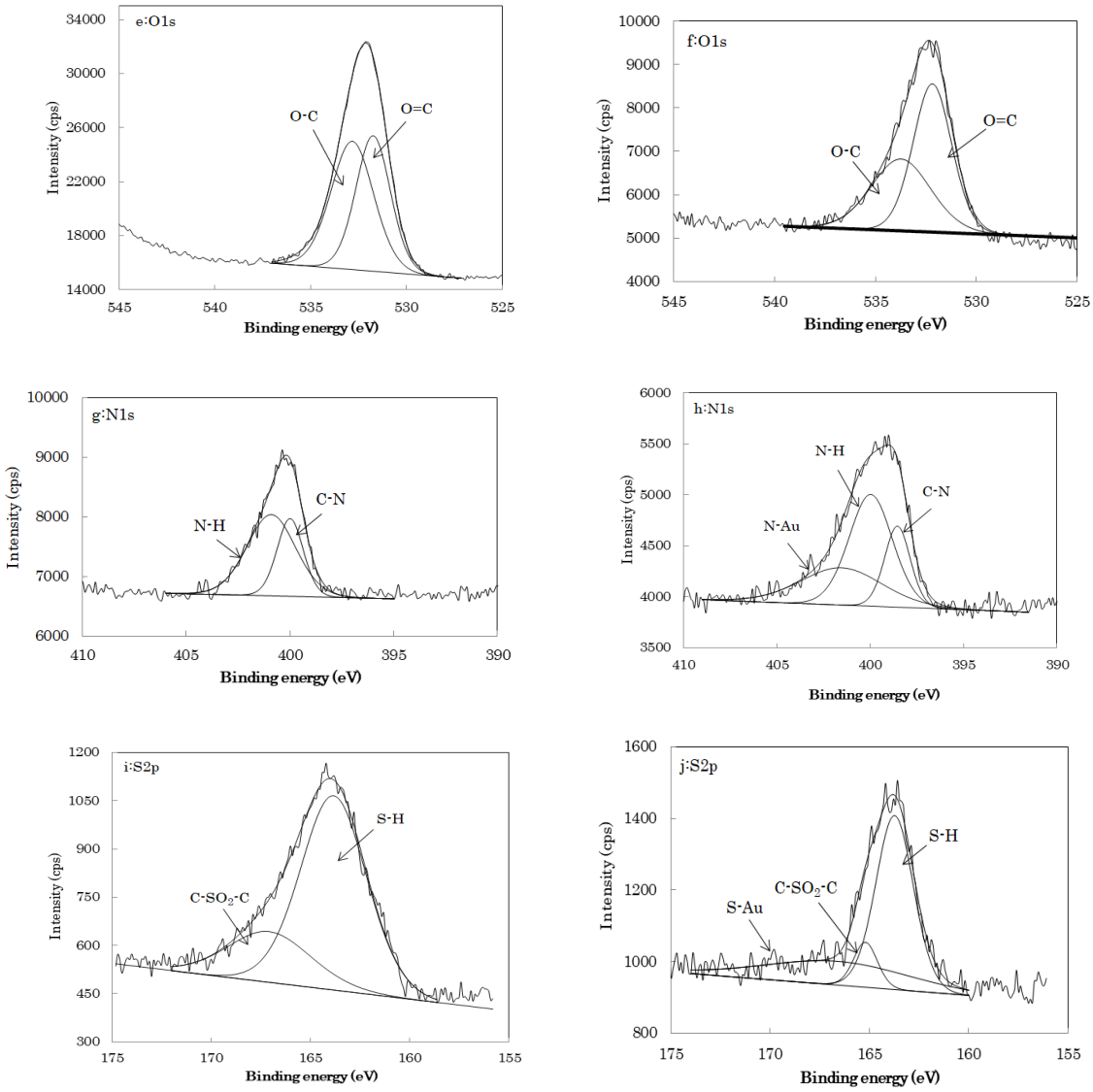


Fig. 4-30 XPS spectra of gold adsorbed SW-IV a: Wide spectra, b: Gold adsorbed SW-IV of Au (4f) spectra, c: SW-IV, d: Gold adsorbed SW-IV of C (1s) spectra, e: SW-IV, f: Gold adsorbed SW-IV of O (1s) spectra, g: SW-IV, h: Gold adsorbed SW-IV of N (1s) spectra, i: SW-IV, j: Gold adsorbed SW-IV of S (2p) spectra

The O (1s), N (1s), and S (2p) spectres were widened by adsorption of gold and copper. It was concluded that gold is adsorbed on the functional group containing nitrogen and sulfur, and copper is attached to the functional group containing oxygen of sheep wool, respectively.

3.4.4 Heavy metal and precious metal adsorption mechanism

The heavy metal adsorption process of wool is complex, and it seems that functional groups are binding metal ions during the adsorption process [4.41]. Sheep wool could remove

heavy metals by both physisorption and chemisorption. Nano-porous network of wool fiber is binding heavy metal ions in physisorption. Carboxylic and other binding groups of wool fiber are binding metal ions in chemisorption [4.42]. Cr(III) and Cr(VI) adsorption mechanism is shown in Fig. 4-31.

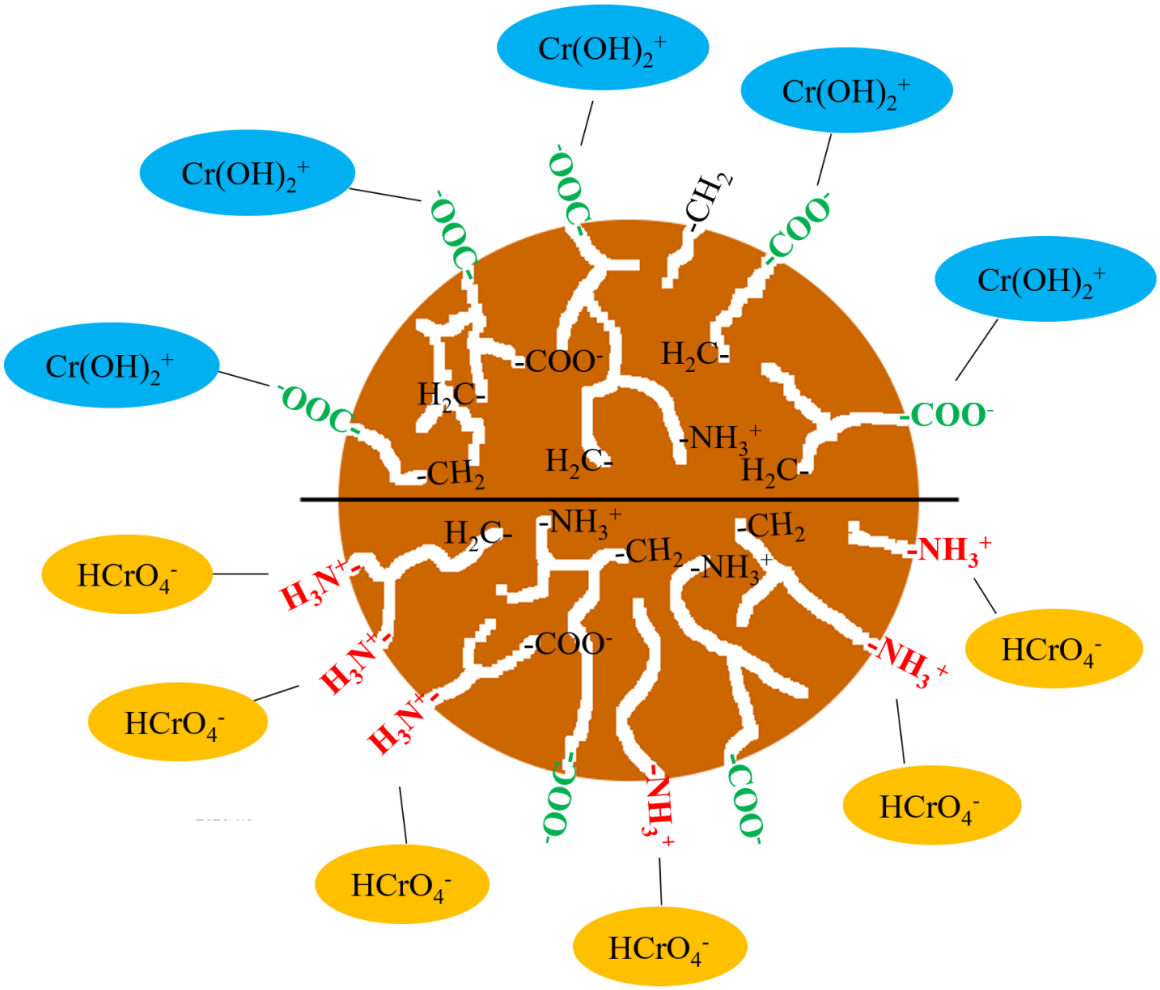


Fig. 4-31 Adsorption mechanism of Cr(III) and Cr(VI) on sheep wool

The metal binding properties of gold ion on sheep wool: Wool contains a substantial amount of cystine, glutamic acid, and serine. The side chain of these amino acids which were thiol, amino, and hydroxyl groups are defined as highly reactive with metal ions. Researchers studied heavy metals, toxic wastes are binding on thiol group of cystine amino acid of natural and treated keratin-based materials. Though keratin-based materials, especially wool has other reactive sites such as NH and OH groups [4.43].

A substantial amount of Au(III) adsorption was performed by sheep wool and chemically treated wool at low pH value. $–NH_3^+$ and $AuCl_4^-$ were charged negatory at low pH and it can be determined that low pH is best suited for gold adsorption. Au(III) can be selectively adsorbable when positively charged metal ions co-existing could not affect the adsorption because of the positive charge of $–NH_3^+$.

The metal binding properties of copper ion on sheep wool: It is important to estimate the number of binding sites and coupling constant when the complex is formed with the protein and metal ions. It should calculate the number of binding sites when the minimum concentration of metal.

$$R - COO^- + M^{2+} \leftrightarrow R - COOM^+$$

Guthrie and Laurie have shown that mohair keratin forms complexes with Cu(II) ions, probably two types, one stable at pH < 9 (type I complex, the green Cu(II) complex) and the other stable at pH > 9 (type II complex the brown Cu(II) complex). Same as our result, like the first one, the dissociated groups (carboxyl groups of glutamyl and aspartyl residues) on wool keratin are thought to occupy coordinated positions on Cu(II) ions.

The copper metal is related to borderline Lewis acid and it can be bind to Lewis bases of –OH, $–NH_3$, and –CO. These can be found widely in wool fiber proteins and the order of binding preferences are determined as OH < NH_3 < CO [4.21]. Kadokura et al. [4.6] study, the volume of complexes with metal ions determined as Cu^{2+} > Pb^{2+} > Zn^{2+} > Cd^{2+} with R – COOH. R – COOH has interacted strong complex with Cu^{2+} ion. The COOH functional group was determined as high adsorption characteristics compared with others such as R – COOH >> R – SO_3H. Adsorption mechanism of the copper on sheep wool can be explained as the formation of Cu(II) complexes with carboxylic acid. The formation of complexes between copper and carboxyl groups in proteins was suggested to be the reason for high levels of sorption [4.23].

The main active sites for the adsorption of Au(III) and Cu(II) are determined as different for each metal. The method of appropriate chemical treatment can be selected depending on which heavy metal is to be adsorbed. There are also many advantages, such as the ability to extract only precious metals without the influence of co-existing ions in the aquatic environment.

Gold is adsorbed on the amino and thiol groups of the sheep wool sample. Hence, this method may be the preferred method of gold adsorption, as the sodium sulfide treatment of

sheep wool forms a thiol group, which increases the adsorption capacity of gold. Au(III) and Cu(II) adsorption mechanism is shown in Fig. 4-32.

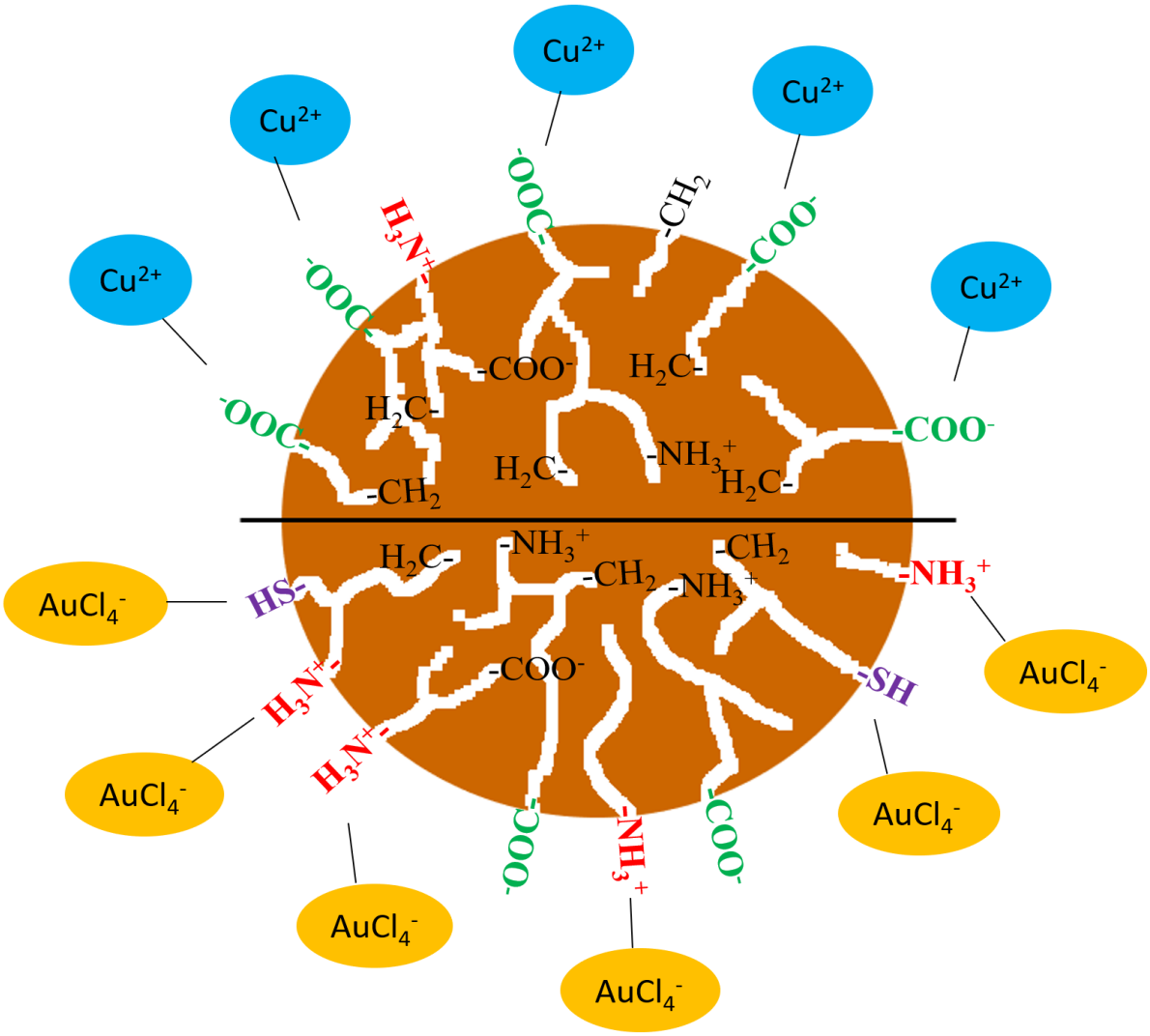


Fig. 4-32 Adsorption mechanism of Au(III) and Cu(II) on sheep wool

The following conclusions could drawn from the adsorption mechanism of heavy metal:

- Na_2S treatment is **necessary**: Au(III) and Cr(III) adsorption
- Na_2S treatment is **not necessary** but adsorption amount will increase after treatment: Cu(II) and Cr(VI) adsorption

3.5 Gold adsorption from barge and process water of the "Oyu Tolgoi" mine, Mongolia

Generally, precious metals are found less in wastewater like 0.05-0.20 mmol/l. The wastewater contains strong acid and base (copper, iron), therefore precious metals can not be

fully processed. Practical studies on the adsorption of precious metals even its low amount of aqueous solutions and can be effectively extracted in acidic conditions.

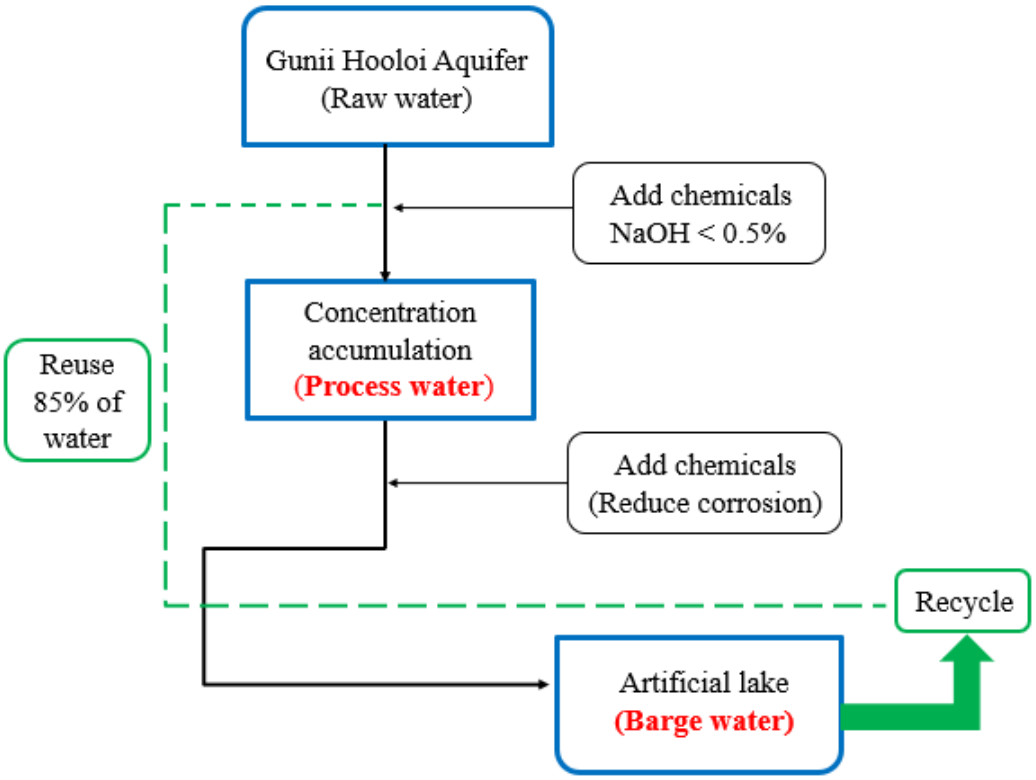


Fig. 4-33 Water flow of the Oyu Tolgoi mining area

The mining wastewater samples of barge and process water were collected from the Oyu Tolgoi mining area. Samples were analyzed field analysis and transferred to the laboratory to do heavy metal adsorption.

Gold and arsenic were selected as representative of precious metals and heavy metals, adsorption studies were conducted for each wastewater sample and results are shown in Figs. 4-34, 4-35.

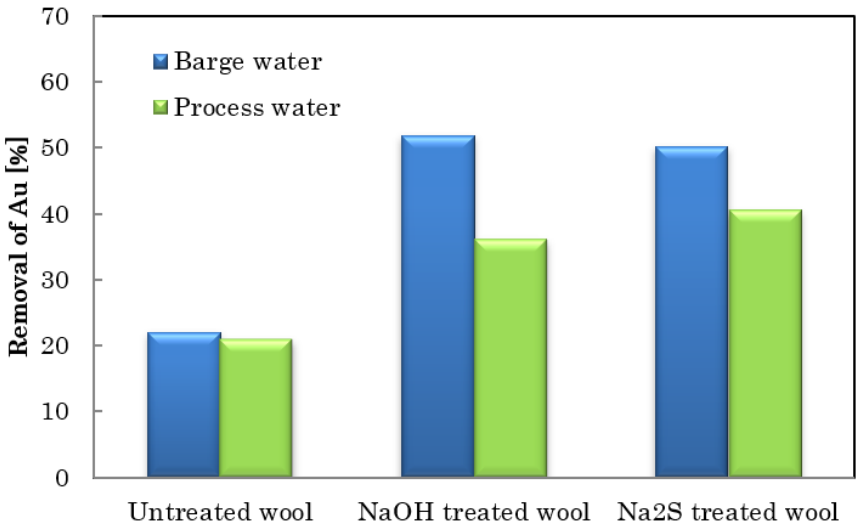


Fig. 4-34 Removal of gold from the barge and process water, %
[pH(barge)=4.73, pH(process)=5.97]

Removal of Au(III) from the Oyu Tolgoi mining water was determined as 21% for the sheep wool. However, removal percentages were increased to 52% after the chemical treatment of sheep wool. In the process water sample, removal of Au(III) was determined 21% in wool samples, 36% in sodium hydroxide treated wool samples and 41% in sodium sulfide treated wool samples. It can be increased more by appropriate treatment of biosorbent and suitable adsorption conditions such as pH and dose of biosorbent.

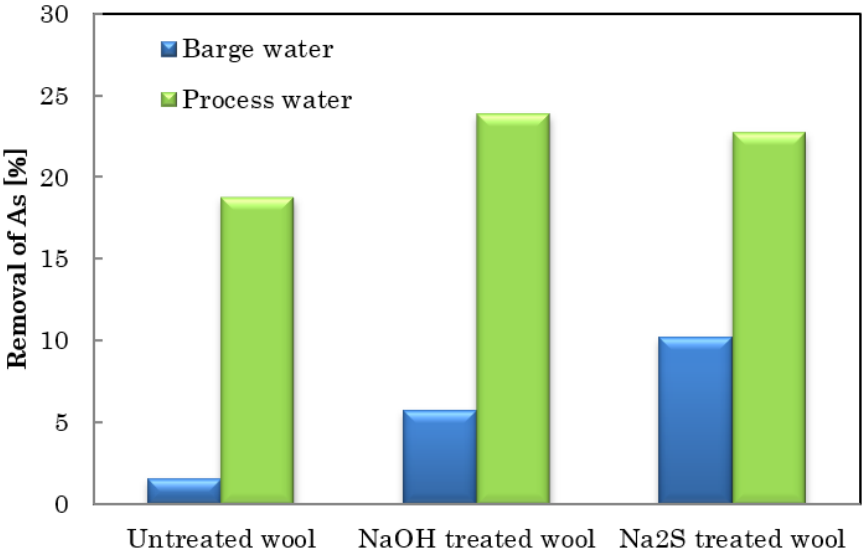


Fig. 4-35 Removal of arsenic from the barge and process water, %
[pH(barge)=4.73, pH(process)=5.97]

The adsorption amount of As(V) from the Oyu Tolgoi wastewater was determined lower comparing to Au(III). In the case of barge water, As(V) removed 2-10.2% in wool, sodium hydroxide and sodium sulfide treated wool. In the process water sample, As(V) removed 18.7-23.8% by wool, sodium hydroxide, and sodium sulfide treated wool.

4. Summary

The adsorption of chromium, gold, and copper from their aqueous solution by chemically treated sheep wool systems was investigated. The sodium sulfide treated wool showed better biosorption capacity of Cr(III) adsorption than untreated sheep wool. In the case of Cr(III), the adsorption value increased as the pH increased, while in the case of Cr(VI), the adsorption value decreased as the pH increased. The adsorption capacities of the chromium determined as 2.39 mmol/g in Cr(III), 0.85 mmol/g in Cr(VI) adsorption by Na_2S treated sheep wool.

The pH of aqueous solutions, contact time, and initial metal concentration were strongly affected to the adsorption capacity. In the case of Au(III), the adsorption value decreased as the pH increased, while in the case of Cu(II), the adsorption value increased as the pH increased. The sodium sulfide treated wool showed the highest adsorption of Au(III) than sodium hydroxide, sodium bisulfite, and sodium borohydride treated sheep wool.

Gold, arsenic adsorption from barge and process water of the Oyu Tolgoi mine were evaluated. The removal of gold, arsenic increased after sodium hydroxide and sodium sulfide treatment. The removal percentage was increased to 52% for gold removal from barge and process water.

The adsorption mechanism of gold and copper was evaluated by EDX, FTIR and XPS analysis. In EDX analysis, after the adsorption of chromium, gold, and copper, the content of these elements was observed on the surface and cross-section of the sheep wool. From the FTIR and XPS result, it was approved that, gold is attached to the amino and thiol groups of the sheep wool, while copper is attracted to the carboxyl group of the sheep wool.

5. References

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Chapter 5
General Conclusion

Expeditious technique and technology development have resulted in harmful effects on the environment due to the mining of natural resources and industrial production. All types of waste material go to ends up at water sources causing pollution such as rivers and underground water. Various adsorption studies of heavy metals and the reduction of pollution from the aquatic environment are being conducted around the world, which is often expensive. Therefore, a method of highly effective and low-cost adsorption of heavy metals from the aqueous solution using the biosorption method was studied. In this study, keratin biomaterial of sheep wool have been tested as heavy metal, precious metal biosorbent and its adsorption performance have been investigated by batch adsorption methods.

Cr(III), Cr(VI), Au(III), and Cu(II) ions adsorption analysis were investigated by natural biomass of sheep wool and chemically treated sheep wool. Sheep wool was treated by using NaOH, Na_2S, $NaHSO_3$, and $NaBH_4$ solution. The Na_2S treatment determined as the most effective treatment of sheep wool to enhance the removal of heavy metal ions. The chemical composition and morphology analysis of sheep wool were analyzed by Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDX) analysis respectively, after the chemical treatment as well as after the heavy metal adsorption process.

The adsorption capacities of sheep wool and chemically treated sheep wool for heavy metal, precious metal ions have been carried out at different pH values, initial adsorbate concentration, and adsorption time. The adsorption results were characterized using the isotherm and kinetic models. The heavy metal adsorption mechanism and identification of functional groups of the sheep wool surface proposed from FTIR and XPS measurement.

Cuticle scale changes on the surface of sheep wool were observed by using SEM after each chemical treatment. A significant change was observed when the wool fibers transformed into a sheet-like structure after the chemical treatment of Na_2S comparing with the others.

Batch experiments for removal of Cr(III) and Cr(VI) were varied due to the pH value. Cr(III) has the highest adsorption amount at pH of 5-7, while Cr(VI) has the highest removal value at pH of 2-4. Kinetic results indicated that the removal of chromium followed pseudo-second order kinetic model for the whole contact time range. The experimental data of all system fitted well the Langmuir isotherm with high correlation coefficient R^2, except for

the adsorption of Cr(III) by sheep wool and Na_2S treated sheep wool.

The adsorption amounts of Au(III) and Cu(II) were increased after the chemical modification and the adsorption capacity of the chemically modified wool was carried out in the following order; Na_2S treated wool > $NaBH_4$ treated wool > NaOH treated wool > $NaHSO_3$ treated wool. Based on these high adsorption results, sheep wool was treated by Na_2S at different concentrations to do further study. The appropriate concentration of modification was determined at 0.02 M and 0.05 M Na_2S concentration. Sheep wool changed into sheet-like structures after 0.05 M of Na_2S treatment; however, it keeps fiber structure after treatment of 0.02 M of Na_2S. The adsorption amounts of Cu(II) were increased after the Na_2S treatment.

Biosorption is being described to be a useful method for the removal of heavy metals from the aquatic environment, such as mining and industrial wastes. Waste sheep wool was effectively used as adsorbent of heavy metals.

The chemically treated sheep wool, especially Na_2S treated wool can enhance the adsorption of heavy metals and precious metals. Effective treatment methods to each metal adsorption determined as follow: Na_2S treatment is necessary for Au(III) and Cr(III) adsorption; Na_2S treatment is not necessary but adsorption amount will increase after treatment for Cu(II) and Cr(VI) adsorption.

Sheep wool is a cheap, eco-friendly material, and has a high capacity of adsorption, which is expected to be applied to the mining and industrial wastewater treatment in near future.

Presentation and discussion review of research work

Participated conferences and symposiums:

1. Enkhzaya Solongo, Koichiro Shiomori, Bolormaa Oyuntsetseg. Heavy metal removal from aqueous solution using Na_2S treated sheep wool at different concentration. International Symposium on Earth Science and Technology 2019, December 5-6, 2019, Shiiki Hall, Kyushu University, Fukuoka, Japan
2. Enkhzaya Solongo, Koichiro Shiomori, Bolormaa Oyuntsetseg. Chemically Treated Sheep Wool as Biosorbent for the Adsorption of Cu(II) and Au(III) from wastewater. 8th Joint Student Conference of National University of Mongolia and University of Miyazaki, November 18-19, 2019, Ulaanbaatar, Mongolia
3. Enkhzaya Solongo, Koichiro Shiomori, Bolormaa Oyuntsetseg. Effect of Chemical Treatment on Adsorption Properties of Wool Fibers for Au(III) and Cu(II). Japan / Taiwan / Korea Chemical Engineering Conference 2019, November 13-15, 2019, Housensou, Beppu, Japan
4. Enkhzaya Solongo, Koichiro Shiomori, Bolormaa Oyuntsetseg. Adsorption Properties of Au(III) and Cu(II) from aqueous solution using chemically treated sheep wool. 18th Asian Pacific Confederation of Chemical Engineering Congress, September 23-27, 2019, Sapporo Convention Center, Sapporo, Japan
5. Enkhzaya Solongo, Koichiro Shiomori, Bolormaa Oyuntsetseg. Adsorption of heavy metals by chemically treated Mongolian livestock biomass. The 24th Symposium of the Japanese Arsenic Society, November 23-25, 2018, Prefectural University of Kumamoto, Kumamoto, Japan
6. Enkhzaya Solongo, Koichiro Shiomori, Bolormaa Oyuntsetseg. Recovery of Au from mining wastewater using Mongolian natural biomass of wool. NOKOH Student Seminar in English 2018, November 6, 2018, University of Miyazaki, Miyazaki, Japan
7. Enkhzaya Solongo, Koichiro Shiomori, Bolormaa Oyuntsetseg. Au(III) adsorption from aqueous solution using Mongolian sheep wool. SCEJ Chemical Engineering Society 50th Annual meeting, September 18-20, 2018, Kagoshima University, Kagoshima, Japan
8. Enkhzaya Solongo, Koichiro Shiomori and Bolormaa Oyuntsetseg. A study on the heavy metal adsorption of Mongolian livestock biomass. UOM-NUM Symposium on Environmental Sciences and Technologies & The Seventh Joint Student Conference of

University of Miyazaki and National University of Mongolia, August 27, 2018, University of Miyazaki, Miyazaki, Japan

9. Enkhzaya Solongo, Koichiro Shiomori, Bolormaa Oyuntsetseg. A study on the heavy metal adsorption of Mongolian livestock biomass. 5th International Arsenic Symposium in MIYAZAKI 2018, Environmental Impact and Health Hazards, June 22-25, 2018, University of Miyazaki, Miyazaki, Japan
10. Enkhzaya Solongo, Koichiro Shiomori and Bolormaa Oyuntsetseg. Mongolian biomasses used as sorbents to remove Cu^{2+}, Pb^{2+}, Cd^{2+} ions from aqueous solution. UOM-NUM Symposium on Environmental Sciences and Technologies & The Sixth Joint Student Conference of University of Miyazaki and National University of Mongolia, February 26-27, 2018, University of Miyazaki, Miyazaki, Japan

Published papers:

1. Solongo Enkhzaya, Koichiro Shiomori, and Bolormaa Oyuntsetseg; "Effective adsorption of Au(III) and Cu(II) by chemically treated sheep wool and the binding mechanism", J. Environ. Chem. Eng., **8**:104021, (2020), DOI: 10.1016/j.jece.2020.104021
2. Solongo Enkhzaya, Koichiro Shiomori, and Bolormaa Oyuntsetseg; "Removal of Heavy Metals from Aqueous Solution by Adsorption using Livestock Biomass of Mongolia", J. Environ. Sci. and Technol., **10**:107-119, (2017), DOI: 10.3923/jest.2017.107.119
3. Solongo Enkhzaya, Kaoru Ohe, Koichiro Shiomori, Bolormaa Oyuntsetseg, Ochirkhuyag Bayanjargal and Makiko Watanabe; "Assessment of heavy metals in mining tailing around Boroo and Zuunkharaaa gold mining areas of Mongolia", J. Environ. Sci. and Technol., **9**(5):379-389, (2016), DOI: 10.3923/jest.2016.379.389

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