DEVELOPMENT OF MOLECULAR IMPRINTED BIOSORBENT BY USING ORANGE PEEL FOR Pb^{2+} REMOVAL FROM AQUEOUS SOLUTION

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2010

"I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)"

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Date : 26 April 2010

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A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical Engineering & Natural Resources
Universiti Malaysia Pahang

DECLARATION

I declare that this thesis entitled "Development of molecular imprinted biosorbent by using orange peel for Pb^{2+} removal from aqueous solution" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any degree.

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ABSTRACT

In this research, the investigation of the use of orange peel which has high adsorption capacity of heavy metals compare to other types of biosorbent were carried out. In order to determine the most effective adsorption for each of heavy metals, we had come out in development of molecular-imprinted technology. Molecular-imprinted technique tends to give a higher adsorption rates and higher selectivity towards target metals. In order to achieve the objectives of this research, it would based on four main scopes of study which is to know effect of imprinting ions concentration on biosorption, effect of time on biosorption , effect of initial concentration of Pb^{2+} in solution and also to compare the different type of metals on adsorption selectivity of the biosorbent. The adsorption uptake was analyzed by using Atomic Absorption Spectrophotometer (AAS). In the preparation, with the imprinted ion Pb^{2+} concentrations increasing, the more fucntional groups (-OH) were protected and the more imprinting sites on the surface were retained. So, the loading amount of imprinted ions in preparation was 2 mg/g selected as the optimum loading amount. For the effect of time in adsorption, the optimum condition was described as the shortest time that the biosorbent can absorbed the metals. So, in this experiment, the shortest time that it can achieved with a high adsorption capacity was 30 minutes. It can be said as satisfactory adsorption compare to without imprinted biosorbent. The existence of functional group of (-OH) can be proved by using FTIR analysis. It was recommended that this research was furthered by using wastewater from metal industry, to recover back the Plumbum that had attached to the biosorbent, and can be applied in adsorption column.

ABSTRAK

Dalam kajian ini, satu penyiasatan tentang penggunaan kulit oren yang mempunyai kapasiti jerapan logam berat yang tinggi berbanding dengan jenis bio-penjerap yang lain telah di jalankan. Untuk menentukan jerapan yang paling berkesan untuk logam berat, kami telah keluar dengan pembangunan teknologi molekul-dicetak. Teknik Molekul-dicetak cenderung memberikan tahap jerapan tinggi dan selektivitas tinggi terhadap logam sasaran. Dalam rangka untuk mencapai tujuan kajian ini, ia akan berdasarkan kepada empat skop utama dalam kajian iaitu untuk mengetahui pengaruh mencetak kepekatan ion pada penjerapan, pengaruh waktu pada penjerapan, pengaruh kepekatan awal Pb^{2+} dalam larutan dan juga untuk membandingkan pelbagai jenis logam pada selektivitas jerapan daripada bio-penjerap. Penyerapan jerapan dianalisis dengan menggunakan Spektrofotometer Serapan Atom (SSA). Dalam masa persiapan, apabila kepekatan ion Pb^{2+} dicetak meningkat, lebih banyak kumpulan berfungsi (-OH) yang dilindungi dan lebih banyak laman percetakan di permukaan tetap dipertahankan. Jadi, jumlah pemuatan ion dicetak dalam persiapan adalah 2 mg / g dipilih sebagai jumlah optimum. Untuk pengaruh masa dalam jerapan, keadaan optimum digambarkan sebagai waktu terpendek untuk bio-penjerap dapat menyerap logam. Jadi, dalam percubaan ini, masa terpendek yang dapat dicapai dengan kapasiti jerapan tinggi adalah 30 minit. Hal ini dapat dikatakan sebagai jerapan yang amat memuaskan berbanding dengan bio-penjerap tanpa dicetak. Kewujudan kumpulan berfungsi (-OH) dapat dibuktikan dengan menggunakan analisis FTIR. Hal tersebut boleh dianjurkan untuk meneruskan kajian ini dengan menggunakan air sisa dari industri logam, untuk mendapatkan semula logam plumbum yang telah melekat pada bio-penjerap, dan boleh dilaksanakan dalam lajur jerapan.

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LIST OF ABBREVIATIONS

AAS	=	Atomic Absorption Spectrophotometer
EDTA	=	Ethylenediaminetetraacetic acid
Pb^{2+}	=	Plumbum ion
Nm^{-2}	=	Newton per metre square
FTIR	=	Fourier Transform Infrared
Ag (II)	=	Argentum
Ni (II)	=	Nickle
Cu (II)	=	Copper
Cd (II)	=	Cadmium
DOE	=	Department of Environment
OSHA	=	Occupational Safety and Health Administration
EPA	=	Environmental Protection Agency
µg /L	=	Microgram per litre
$\mu g/m^3$	=	Microgram per metre cube
mg/kg	=	Milligram per kilogram
µg /dL	=	Microgram per desilitre
mg/m^3	=	Milligram per metre cube
IQ	=	Intelligent Quotient
DHHS	=	Department of Health and Human Services
IARC	=	International Agency for Research on Cancer
NTP	=	National Toxicology Programme
CDC	=	Centers for Disease Control
RDA	=	Recommended Dietary Allowance
NIOSH	=	National Institute for Occupational Safety and Health
HDL	=	high-density lipoprotein
HM	=	heavy metals
HNO_3	=	Nitric Acid
NaOH	=	Sodium Hydroxide
-OH	=	Hydroxyl functional group
ppm	=	Part per million
C - C	=	Carbon-Carbon bond
STD	=	Standard

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Many industrial waste water effluents, particularly from mineral processing, metal plating; electric, electronic and chemical industries are environmentally unacceptably contaminated with heavy metal. Effects from these industrial activities may lead to heavy metal contamination in surface water, groundwater or even sea which then may cause toxic effects when they enter the food chain of the ecosystem. Traditional metal removal methods like chemical precipitation, chemical redox reactions, electrochemical treatment, membrane processes and ion exchange can be extremely expensive or inefficient, especially for large solution volumes at relatively very low concentrations (S.Schiewer *et al.*, 2008). The very common cationic heavy metals that may bring harmful to us are such as Pb (II), Ag (II), Ni (II), Cu (II) and also Cd (II). This type of metals had come out with some simple methods such as precipitation. This precipitation method uses some low cost alkaline materials such as lime to

remove those heavy metals. However, this process usually produces large volumes of sludge consisting of small amounts of heavy metals in excess gypsum the recycling and reuse of which is very difficult (R.P.Dhakal *et al.*, 2005).

Due to some drawback point of view from some of the methods, we had approach other methods that is by using biosorption. The efficient adsorption was based on the adsorbent itself. A large number of different adsorbent materials containing a variety of attached chemical functional groups has been reported for this purpose, with activated carbon being the most popular, however, the high cost of this material restricts its use on large scale (F.A.Pavan *et al.*, 2006). In recent years, the natural adsorbent or biosorbent had been takes into account in order to replace activated carbon. The availability of this adsorbent had been a big advantage to use this type of adsorbent in order to remove heavy metals from industrial waste water. The potential type of biosorbent is from residuals of agricultural activities or wastes from food industries that are available in large amounts. One of the most efficient adsorbent was from fruit pectin. Pectin is the ionic plant polysaccharides, whose main structural features are the linear chains containing more than 100 (1-4)-linked α-D-galacturonic acid residue (Wong *et al.*, 2008). Common types of pectin that we may use are such as orange pectin, apple pectin or durian rind pectin.

In this, the possibility of the use of citrus peel which is from orange peel which has high adsorption capacity of heavy metals compare to other types of biosorbent were investigated. In order to develop the most effective adsorption for targeted heavy metals, we enhance the

performance of biosorbent by using molecular imprinting technique. By using surface molecular imprinting technology, this new biosorbent showed 30.0-50.0% higher uptake for Ni^{2+} in comparison to non-imprinted biosorbent (H.Huo *et al*., 2009). Besides, this type of technology had better mechanical performance and can be reused up to 15 cycles by producing the molecular templates of the heavy metals that are going to be removed.

1.2 Problem Statement

Industry which operates in heavy metals industry had introduced some heavy metals by discharging this waste into aquatic ecosystems. This problem had become a matter of concern over last few decades and had contributes to marine pollution. The pollutants of concern include silver, nickel, lead, chromium, zinc, cadmium, copper, gold and uranium. All these heavy metals bring harmful to ecosystems due to its toxicity. In order to give a better solution, the use of orange wastes from food industry that produces orange juices had been taking into account. Due to our latest technology, molecular imprinting biosorbent was prepared from orange pectin for better adsorption of heavy metals. Besides in helping to solve marine pollution which was polluted with toxic heavy metals, this research also count for the use of waste for other significant study.

Environmental contamination and exposure to heavy metals such as mercury, cadmium and lead is a serious growing problem throughout

the world. Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products. Many occupations involve daily heavy metal exposure; over 50 professions entail exposure to mercury alone. In today's industrial society, there is no escaping exposure to toxic chemicals and metals. In the United States, tons of toxic industrial waste are mixed with liquid agricultural fertilizers and dispersed across America's farmlands. This "controversial practice," which is presently legal in the U.S., has been reported in nine states. While the spreading of arsenic, lead, cadmium, nickel, mercury and uranium on soil that is utilized to produce food for human consumption is a "political and economic issue," the potential for adverse health effects is well documented.

1.3 Objectives

In this research study, the main objectives are to determine the formulation molecular imprinted based biosorbent and also to study and optimize the performance of imprinted biosorbent for heavy metal removal.

1.4 Scope of study

In order to achieve the objectives of this research, it would based on four main scopes of study which is to know effect of imprinting ions concentration on biosorption, effect of time on biosorption , effect of initial concentration of Pb^{2+} in solution and also to compare the different type of metals on adsorption selectivity of the biosorbent.

CHAPTER 2

LITERATURE REVIEW

2.1 Adsorption

The existence of industrial which involves in mineral processing, metal plating; electric, electronic and chemical industries had gives a big impact to our ecosystem and environment. These type of activities had contributes to heavy metal pollutions. In order to remove the pollutants or heavy metals, a number of methods were currently used. Some of them were chemical precipitation, chemical redox reactions, electrochemical treatment, membrane processes and ion exchange. But this method was extremely expensive and inefficient. However, there is another method that may only involve low cost usage, which is adsorption. Adsorption is one of the effective techniques for removal of heavy metal (M.Khormaei *et al*., 2007). Adsorption is a surface phenomenon and should not be confused with absorption, which refers to the penetration of substances into the porous structure within solid material Adsorption and ion exchange processes are the most useful methods to removal them, by exploring the availability of different kinds of adsorbents associated with convenient procedures for obtaining high efficiency (F.A.Pavan *et al*., 2006). Adsorption can be used to separate a

molecule from a complex mixture of molecules, or simply to separate a solute from its solvent. This is achieved by contacting the solution with the solid material which is also called the adsorbent. The molecule that binds on the adsorbent is referred to as the adsorbate.

2.1.1 Biosorption

Biosorption is also one of adsorption process which involves the using of biomass as their biosorbent. Biosorption is the term given to the passive sorption and/or complexation of metal ions by biomass. The mechanisms of biosorption are generally based on physico-chemical interactions between metal ions and the functional groups present on the cell surface, such as electrostatic interactions, ion exchange and metal ion chelation or complexation (C.Mack *et al*., 2007). Biosorption was really effective and involves low cost for the wastewater treatment especially for heavy metal removal. Considering the viewpoint of sustainable development and comprehensive utilization of resources, biosorption has a promising prospect and a wide application due to its low cost, abundance and good performance over other conventional treatment processes in the removal and recovery of heavy metal ions from wastewater (H.Su *et al*., 2006). Biosorption also has some advantages compare to other methods to remove heavy metals. These include low operating costs, minimization of the volume of chemical and/or biological sludge to be handled and high efficiency in detoxifying effluents (F.A.Pavan *et al*., 2006).

2.2 Adsorbent

Most adsorption processes utilize particulate adsorbents. Most of the adsorbents are made from natural or synthetic material. Commonly used adsorbents in bioseparation processes include cellulose based adsorbents, silica gel, synthetic resins; agarose based adsorbents and cross linking dextran based adsorbents. However, this type of adsorbents was very expensive. In order to minimize the cost of water treatment, low cost adsorbents will be use to remove heavy metals, dyes and others. The use of biosorbents was one of the alternative ways to replace other adsorbents. The local availability was frequently from the residues of agricultural activity, food industry, or seafood processing. A potential cheap natural source is the abundant waste from the non-profitable part of fruits that might be useful for such procedure (C.Mack *et al*., 2007). Some examples of biosorbent are such as banana peel, durian rind pectin (Wong *et al*., 2008), orange pectin (R.P.Dhakal *et al*., 2005, M.Khormaei *et al*., 2007), chitosan (H.Su *et al*., 2006), Ponkan mandarin peels (F.A.Pavan *et al*., 2006), Yellow passion-fruit shell (F.A.Pavan *et al*., 2006) and many others. Each of this biosorbent has high efficiency to remove heavy metals. Chitosan is one of the effective biosorbent. As a new kind of biosorbent, it has been prepared into different forms and widely used in the wastewater treatment because of its higher adsorption capacity and better selectivity for heavy metal ions. However, its application is limited because of its dissolution in acidic solutions and higher cost (H.Su *et al*., 2006). Then, some of the researcher gives more focus on the using of fruits pectin which is the pectin was prepared from fruits peel and was forms in gel-like form. The selectivity of these gels of alginic acid and pectic acid which show remarkable separation features for heavy metal ions (R.P.Dhakal *et al*., 2005).

2.3 Pectin

The texture of fruits and vegetables during growth, ripening and storage depends on the quantity and quality of pectin present.Pectin are the ionic plant polysaccharides, whose main structural features are the linear chains containing more than 100 (1-4)-linked α-D-galacturonic acid residue (Wong *et al*., 2008). Part of the carboxyl groups of the anhydro-galacturonic acid is esterified with methanol. Other than free carboxyl groups, pectin also possess methylated ester groups in its polymeric chain. Such methyl ester groups were saponified with saturated calcium hydroxide solution to convert them into carboxyl groups according to the following reaction.

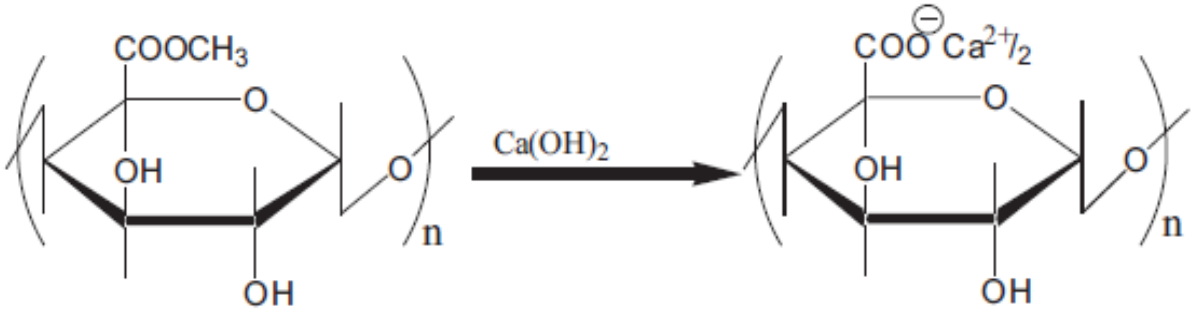

Figure 1 Methyl ester groups are saponified with saturated calcium hydroxide solution to convert them into carboxyl groups

The common types of pectin are such as citrus pectin, apple pectin, grapefruits and many others. Citrus Pectin is a complex polysaccharide obtained from the peel or pulp of the citrus fruits such as oranges. Citrus pectin has higher adsorption capacity rather than durian rind pectin (Wong *et al.*, 2008).

2.4 Molecular Imprinting

Molecular imprinting is a technique to create template-shaped cavities in polymer matrices with memory of the template molecules to be used in molecular recognition. This technique is based on the system used by enzymes for substrate recognition, which is called the "lock and key" model. The active binding site of an enzyme has a unique geometric structure that is particularly suitable for a substrate. A substrate that has a corresponding shape to the site is recognized by selectively binding to the enzyme, while an incorrectly shaped molecule that does not fit the binding site is not recognized.

In a similar way, molecular imprinted materials are prepared using a template molecule and functional monomers that assemble around the template and subsequently get crosslinked to each other. The functional monomers, which are self-assembled around the template molecule by interaction between functional groups on both the template and monomers, are polymerized to form an imprinted matrix (commonly known in the scientific community as a molecularly imprinted polymer). Then the template molecule is removed from the matrix under certain conditions, leaving behind a cavity complementary in size and shape to the template. The obtained cavity can work as a selective binding site for a specific template molecule. This technique has very high selectivity. By

using surface molecular imprinting technology, this new biosorbent showed 30.0-50.0% higher uptake for Ni^{2+} in comparison to non-imprinted biosorbents (H.Huo *et al.*, 2009). In addition, it had better mechanical performance and could be reused for up to 15 cycles.

2.5 Heavy Metals

The disposal of heavy metals into aquatic streams has been the major concern to our worldwide over last few decades. This heavy metals can be defined as a group of element between copper and lead in the periodic table of the element having atomic weight between 63.546 and 200.59 and specific gravities greater than 4.0. Living organisms require trace amount of some heavy metals including cobalt, copper, molybdenum, vanadium, strontium and zinc but excessive levels can be detrimental to the organism. However, some procedures are introduced to remove heavy metals. The commonly used procedures for removing metal ions from dilute aqueous streams include chemical precipitation, reverse osmosis and solvent extraction (K.C.Sekhar *et al*., 2003).

Heavy metals are dangerous because they tend to bioaccumulates. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Thus, the longer

the biological half-life of the substance the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high.

Heavy metals also can enter a water supply by industrial and consumer waste, or even from acid rain which then will flow into the soils and releasing the heavy metals into water streams, lakes, rivers and also groundwater. Table 1 have shown the limited parameter that has been used in Department of Environment (DOE). All the industry that use, produced or disposed heavy metals need to follow the standard before released it to the river or lakes.

Table 1 Parameter Limit for Standard A and Standard B

Parameter	Unit	Standard A	Standard B
Plumbum (II)	**mg/l**	**0.10**	**0.50**
Cadmium(II)	**mg/l**	**0.01**	**0.02**
Mangan(II)	**mg/l**	**0.20**	**1.00**
Nickle(II)	**mg/l**	**0.20**	**1.00**
Zinc(II)	**mg/l**	**2.00**	**2.00**
Ferum(II)	**mg/l**	**1.00**	**5.00**

2.5.1 Definition of Heavy Metal

Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well known toxic metallic elements with a specific gravity that is 5 or more times that

of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9;lead, 11.34; and 13.456;mercury.

2.5.2 Toxic Heavy Metal

There are more than 20 heavy metals, but four are of particular concern to human health: lead (Pb), cadmium (Cd), mercury (Hg), and inorganic arsenic (As). According to the U.S. Agency for Toxic Substances and Disease Registry, these four heavy metals are four of the top six hazards present in toxic waste sites. They are highly toxic and can cause damaging effects even at very low concentrations. They tend to accumulate in the food chain and in the body and can be stored in soft (e.g., kidney) and hard tissues (e.g., bone). Being metals, they often exist in a positively-charged form and can bind on to negatively-charged organic molecules to form complexes.

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common routes of exposures for adults. Ingestion is the most common routes of exposure for children. Children may develop toxic levels from the normal hand-to-mouth activity of small children who come in contact with contaminated soil.

The body has need for approximately 70 friendly trace element heavy metals, but there are another 12 poisonous heavy metals, such as Lead, Mercury,

Aluminum, Arsenic, Cadmium, Nickel, etc., that act as poisonous interference to the enzyme systems and metabolism of the body. No matter how many good health supplements or procedures one takes, heavy metal overload will be a detriment to the natural healing functions of the body. Some metals are naturally found in the body and are essential to human health. Iron, for example, prevents anemia, and zinc is a cofactor in over 100 enzyme reactions. Magnesium and copper are other familiar metals that, in minute amounts, are necessary for proper metabolism to occur. They normally occur at low concentrations and are known as trace metals; for example, high levels of zinc can result in a deficiency of copper, another metal required by the body. Heavy or toxic metals are trace metals that are at least five times denser than water. As such, they are stable elements (meaning they cannot be metabolized by the body) and *bio-accumulative* (passed up the food chain to humans). These include: mercury, nickel, lead, arsenic, cadmium, aluminum, platinum, and copper (metallic form versus ionic form). Toxic heavy metals have no function in the body and can be highly toxic. Heavy metals are taken into the body via inhalation, ingestion, and skin absorption. If heavy metals enter and accumulate in body tissue faster than the body's detoxification pathways can dispose of them, a gradual buildup of these toxins will occur. High-concentration exposure is not necessary to produce a state of toxicity in the body tissues and, over time, can reach toxic concentration levels.

2.5.3 Plumbum

Lead is a naturally occurring bluish-gray metal found in small amounts in the earth's crust. It has no special taste or smell. It can be found in all parts of our environment. Most of it came from human activities like mining, manufacturing, and the burning of fossil fuels. Lead is used as a construction material for equipment used in sulfuric acid manufacture, petrol refining, halogenation,

sulfonation, extraction and condensation. It is used in storage batteries, alloys, solder, ceramics and plastics. It is also used in the manufacture of pigments, tetraethyl lead and other lead compounds, in ammunition, and for atomic radiation and x-ray protection. Lead is used in aircraft manufacture, building construction materials (alloyed with copper, zinc, magnesium, manganese and silicon), insulated cables and wiring, household utensils, laboratory equipment, packaging materials, reflectors, paper industry, printing inks, glass industry, water purification and waterproofing in the textile industry. Lead itself does not break down, but lead compounds are changed by sunlight, air, and water. When released to the air from industry or burning of fossil fuels or waste, it stays in air about 10 days. Most of the lead in soil comes from particles falling out of the air. City soils also contain lead from landfills and leaded paint. Lead sticks to soil particles. It does not move from soil to underground water or drinking water unless the water is acidic or "soft". It stays a long time in both soil and water.

Environmental Protection Agency (EPA) limit in air not to exceed 1.5 µg /m³ averaged over 3 months. EPA limit in drinking water to 15 µg /L. OSHA limit in workroom air are 50 µg/m^3 for an 8-hour workday. If a worker has a blood lead level of 40 µg /dL, OSHA requires that worker to be removed from the workroom. Oral Lethal Dose (LD) for pigeon is 160 mg/kg. American Congress on General and Industrial Hygiene Threshold Value is 0.05 mg/m^3. Breathing workplace air (lead smelting, refining, and manufacturing industries), eating lead-based paint chips, drinking water that comes from lead pipes or lead soldered fittings, breathing or ingesting contaminated soil, dust, air, or water near waste sites, breathing tobacco smoke, eating contaminated food grown on soil containing lead or food covered with lead-containing dust, breathing fumes, or ingesting lead from hobbies that use lead (leaded-glass, ceramics) are how we can exposed with lead. Because of health concerns, lead from gasoline, paints and ceramic products, caulking, and pipe solder has been dramatically reduced in

recent years. Lead poisoning is one of the commonest occupational diseases, although in recent years there has been a decline in both the number of reported cases and the severity of the symptoms presented, hence lead poisoning has shifted from an industrial hazard to an environmental one.

2.5.4 Effect of Plumbum to human

Inorganic lead is not significantly absorbed through the skin, but is absorbed in small amounts from the gastrointestinal tract, which it may enter through the swallowing of inhaled particles or via tobacco, food etc. Lead dust, fumes or vapors are more easily absorbed from the respiratory tract. Once absorbed it is distributed particularly to the liver and kidneys, and is then stored in the bones. Lead affects the red blood cells (anemia and other effects on the hemopoietic system are the commonest effects) and causes damage to organs including the liver, kidneys, heart, and male gonads, as well as causes effects to the immune system. Symptoms are often precipitated by alcohol or exercise. It also affects peripheral airway function and causes lung fibrosis and emphysema.

In the central nervous system, lead causes edema, and its effects are often irreversible. Reduced IQ, learning and behavioral difficulties have been reported in children even with low blood lead levels. Neurological and behavioral effects have been reported after occupational exposure to lead, but peripheral neuropathy, (leading to weakness and palsy with wrist drop) is seen with decreasing frequency. Encephalopathy, which results from the most acute and severe exposure, has been reported in children following ingestion of lead paints, and permanent neurological effects occur if the patient survives. Lead presents a

reproductive hazard in several ways. It is gonadotoxic, causes a reduction in pregnancies in successfully mated mice, and is embryotoxic.

Lead crosses the placental barrier, and reduced fetal birth weight, neonatal body weight and motor activity, and skeletal deformities have been reported in mice. Lead induces quite specific teratogenic effects on the tail buds of hamster embryos, and these malformations tend to be potentiated by the presence of cadmium. The teratogencity and the effects of lead on women and reproduction with special reference to problems of lead in women workers. Exposure to lead is more dangerous for young and unborn children. Unborn children can be exposed to lead through their mothers. Harmful effects include premature births, smaller babies, and decreased mental ability in the infant, learning difficulties, and reduced growth in young children. These effects are more common after exposure to high levels of lead. It can cause abortion and damage the male reproductive system.

The Department of Health and Human Services (DHHS) has determined that lead acetate and lead phosphate may reasonably be anticipated to be carcinogens based on studies in animals. There is inadequate evidence to clearly determine lead's carcinogenicity in humans. Lead is listed by IARC in Group 2B ("possible human carcinogen") and by NTP as "reasonably anticipated to be a carcinogen," but is not considered to be a "select carcinogen" under the criteria of the OSHA Laboratory Standard. Although slightly higher mortality for all malignant neoplasms has been reported in smelter workers, other studies in battery workers have found no association between lead exposure and cancer. In lifetime studies, 25 ppm of lead in the drinking water of rats was not found to be tumorogenic, but renal tumors have been induced in rats following administration of large doses of lead. Also, chromosomal aberrations have been reported in

workers occupationally exposed to lead, and in monkeys. Blood test is available to measure the amount of lead in your blood and to estimate the amount of your exposure to lead. Blood tests are commonly used to screen children for potential chronic lead poisoning. The Centers for Disease Control and Prevention (CDC) considers children to have an elevated level of lead if the amount in the blood is at least 10 µg /dL. Lead in teeth and bones can be measured with X-rays.

2.5.5 Zinc

Zinc is one of the commonest elements in the earth's crust. It's found in air, soil, and water, and is present in all foods. Pure zinc is a bluish-white shiny metal. Zinc has many commercial uses as coating to prevent rust, in dry cell batteries, and mixed with other metals to make alloys like brass and bronze. A zinc and copper alloy is used to make pennies in the United States. Zinc compounds are widely used in industry to make paint, rubber, dye, wood preservatives, and ointments. Also used for galvanizing sheet iron; as ingredient of alloys such as bronze, brass, Babbitt metal, German silver, and special alloys for die-casting; as a protective coating for other metals to prevent corrosion; for electrical apparatus, especially dry cell batteries, household utensils, castings, printing plates; building materials, railroad car linings, automotive equipment; as reducer (in form of the powder) in the manufacture of indigo and other vat dyes; for deoxidizing bronze; extracting gold by the cyanide process, purifying fats for soaps; bleaching bone glue; manufacture of sodium hydrosulfite; as reagent in analytical chemistry, e.g. in the Marsh and Gutzeit test for arsenic; as a reducer in the determination of iron.

Some zinc is released into the environment by natural processes, but most comes from activities of people like mining, steel production, coal burning, and burning of waste. It attaches to soil, sediments, and dust particles in the air. Rain and snow remove zinc dust particles from the air. Zinc compounds can move into the groundwater and into lakes, streams, and rivers. Most of the zinc in soil stays bound to soil particles. It builds up in fish and other organisms, but it doesn't build up in plants. Recommended Dietary Allowance (RDA) is 15 mg a day for men ; 12 mg/day for women; 10 mg/day for children; and 5 mg/day for infants.

EPA drinking water limit is 5 ppm. EPA also requires that releases of more than 1,000 (or in some cases 5,000) pounds of zinc or its compounds into the environment be reported. Occupational Safety and Health Administration (OSHA) zinc chloride fumes limit in workplace air is 1 mg/m^3 for an 8-hour workday over a 40-hour work week and 5 mg/m^3 for zinc oxide fumes. National Institute for Occupational Safety and Health (NIOSH) has set the same standards for up to a 10-hour workday over a 40-hour workweek. Ingesting small amounts present in your food and water, drinking contaminated water near manufacturing or waste sites ,drinking contaminated water or a beverage that has been stored in metal containers or flows through pipes that have been coated with zinc to resist rust, eating too many dietary supplements that contain zinc and breathing zinc particles in the air at manufacturing sites was the way on how human may exposed to zinc in their routine. Zinc is an essential element in our diet. Too little zinc can cause health problems, but too much zinc is also harmful. Acute toxicity, inhalation of fumes may result in sweet taste, throat dryness, cough, weakness, generalized aching, chills, fever, nausea and vomiting. Zinc chloride fumes have caused injury to mucous membranes and pale gray cyanosis. Ingestion of soluble salts may cause nausea, vomiting and purging. Breathing large amounts of zinc (as dust or fumes) can cause a specific short-term disease called metal fume fever. This is believed to be an immune response affecting the lungs and body

temperature. Chronic toxicity: Harmful health effects generally begin at levels from 10-15 times the RDA (in the 100 to 250 mg/day range). Eating large amounts of zinc, even for a short time, can cause stomach cramps, nausea, and vomiting. Taken longer, it can cause anemia, pancreas damage, and lower levels of high-density lipoprotein cholesterol (HDL - the good form of cholesterol). Rats that were fed large amounts of zinc became infertile or had smaller babies. Irritation was also observed on the skin of rabbits, guinea pigs, and mice when exposed to some zinc compounds. Skin irritation will probably occur in people. The Department of Health and Human Services, the International Agency for Research on Cancer, and the EPA have not classified zinc for carcinogenicity. Level of exposure measured from blood, feces, urine, saliva, or hair samples. Amount in hair indicates long-term exposure level, but the relationship between levels in hair and the amount exposed to is not clear. These tests not routinely performed at doctors' offices, but doctor can take samples and send them to testing laboratory.

2.5.6 Effect of Heavy Metals to Human Body

Heavy metal overload in the walls of coronary arteries seems to decrease levels of *nitric oxide*, a compound known as "Endothelial Relaxing Factor,"--without this substance normal blood flow is impeded therefore increasing the risk of vascular blockages. Heavy metal overload in the adrenal glands reduce the production of hormones, which cause early aging, stress, decreased sex drive and aggravation of menopausal symptoms. Heavy metal overload can lead to unresponsiveness of diabeties to their medications. Heavy metal overload can lead to neurological diseases such as depression and loss of thinking power. It can also aggravate conditions such as osteoporosis and hypothyroidism. For obvious

reasons, removing metals from the body safely has been a concern of physicians for many years.

In general, heavy metals (HM) are systemic toxins with specific *neurotoxic, nephrotoxic, fetotoxic* and *teratogenic* effects. Heavy metals can directly influence behavior by impairing mental and neurological function, influencing neurotransmitter production and utilization, and altering numerous metabolic body processes. Systems in which toxic metal elements can induce impairment and dysfunction include the blood and cardiovascular, eliminative pathways (colon, liver, kidneys, skin), endocrine (hormonal), energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive, and urinary.

Breathing heavy metal particles, even at levels well below those considered nontoxic, can have serious health effects. Virtually all aspects of animal and human immune system function are compromised by the inhalation of heavy metal particulates. In addition, toxic metals can increase allergic reactions, cause genetic mutation, compete with "good" trace metals for biochemical bond sites, and act as antibiotics, killing beneficial bacteria. Much of the damage produced by toxic metals stems from the proliferation of *oxidative free radicals* they cause. Heavy metals can also increase the acidity of the blood. The body draws calcium from the bones to help restore the proper blood pH. Further, toxic metals set up conditions that lead to inflammation in arteries and tissues, causing more calcium to be drawn to the area as a buffer, contributing to hardening of the artery walls with progressive blockage of the arteries and osteoporosis. Even minute levels of toxic elements have negative health consequences, affecting nutritional status, metabolic rate, the integrity of detoxification pathways, and the mode and degree of heavy metal exposure. The biological half-lives for HM are

variably long; the half-life for cadmium in the kidney is decades. Most HM are readily transferred across the placenta, found in breast milk, and are well known to have serious detrimental effects on behavior, intellect and the developing nervous system in children. For adults, silent symptoms of chronic, low level HM accumulation in tissues can progress from a steady decline in energy, productivity and quality of life to accelerated cardiovascular disease, premature dementia and total debilitation. Unfortunately, the possibility of HM burden is often not considered and patients continue to suffer needlessly.

2.6 Atomic Absorption Spectrophotometer (AAS)

2.6.1 Introduction

Atomic Absorption Spectrophotometer (AAS) is an analytical technique that measures the concentrations of elements. Atomic Absorption Spectrophotometer is so sensitive that it can measure down to parts per billion of a gram (vg dm^{-3}) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another which is higher energy level. Atomic Absorption Spectrophotometer has many uses in different areas of chemistry such as in clinical analysis, environmental analysis, pharmaceuticals, industry and mining. In clinical analysis, AAS is used to analyze metals in biological fluids such as blood and urine. For environmental analysis, it is used to monitor our environment. For example, use to finding out the levels of various element in rivers, drinking water, or seawater. In mining industry, AAS is use to trace the amount of metals such as gold in rocks to see whether it is worth to mine the rocks to extract the gold.

2.6.2 How AAS Functioning

Atoms of different elements absorb characteristic wavelengths of light. Analyzing a sample is to see if it contains a particular element by using light from that element. For example, if we want to analyze existence of lead, then a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample. In AAS, the

sample is atomized and then was converted into ground state free atoms in the vapor state while a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample.

The greater the number of atoms in the vapor, the more the radiation absorbed. The amount of light absorbed is proportional to the number of lead atoms a calibration curve is constructed by running several samples of known concentration of lead under the same conditions as the unknown. The amount of standards absorbed is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample. However, for Atomic Absorption Spectrophotometer, it needs three components such as light source, a sample to produce gaseous atoms and a means of measuring the specific light absorbed.

2.6.3 Light Source

The common source of light is a hollow cathode lamp. This contains a tungsten anode and a cylindrical hollow cathode made of the element to be determined. These are sealed in a glass tube filled with inert gas whether neon or argon at a pressure of between $1Nm^{-2}$ and $5\ Nm^{-2}$.

The ionization of some gas atoms occurs by applying a potential difference of about 300-400V between the anode and the cathode. These gaseous ions bombard the cathode and eject the metal atoms from the cathode in a process called sputtering. Some sputtered atoms are in excited states and emit radiation

characteristics of the metal as they fall back to the ground state. The shape of cathode concentrates the radiation into a beam which passes through a quartz window, and the shape of the lamp is such that most of the sputtered atoms are red positing on the cathode. A typical atomic absorption instrument holds several lamps each for a different element. The lamps are housed in a rotating turret so that the correct lamp can be quickly selected.

2.6.4 The optical system and detector

A monochromator is used to select the specific wavelength of light spectral line which is absorbed by the sample and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of others. The light selected by the monochromator is directed onto a detector that is typically a photomultiplier tube. This produces an electrical signal proportional to the light intensity.

2.6.5 Atomization of the sample

Two systems are commonly used to produce atoms from the sample. Aspiration involves sucking a solution of the sample into a flame and electro thermal atomization is where a drop of sample is placed into a graphite tube that is then heated electrically. Some instruments have both atomization systems but share one set of lamps. Once the appropriate lamp has been selected, it is pointed towards one or other atomization system.

2.6.6 Flame Aspiration

Ethyne/air which giving a flame with a temperature of 2200-2400℃ or ethyne/dinitrogen oxide with a temperature 2600-2800℃ are often used. A flexible capillary tube connects the solution to the nebulizer. At the tip of the capillary, the solution is nebulised and broken into small drops. The larger drops fall out and drain off while smaller ones vaporize in the flame. Only1% of the sample is nebulised.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Materials

Orange peel obtained from fruit juice stalls or industry; Lead Nitrate, Zinc ChlorideFerum Oxide, Copper Sulfate, Epichlorohydrin as a cross-linking agent, Edta Solution, Sodium Hydroxide, Hydrochloric acid, acetic acid was from Sigma Aldrich.

3.2 Preparation of biosorbent without imprinted

Orange peel was selected because of their high pectin content and the fact that they are generated in large quantities as residues from fruit juice production. The orange peels were washes three times with pure water to remove unwanted particles. Then it was followed by treatment with 0.1M HNO_3 for 6 hour and 12 hour drying. The fruit materials were dried at 40 °C in an

oven until they were dried enough. The dried material was then crushed and sieved.

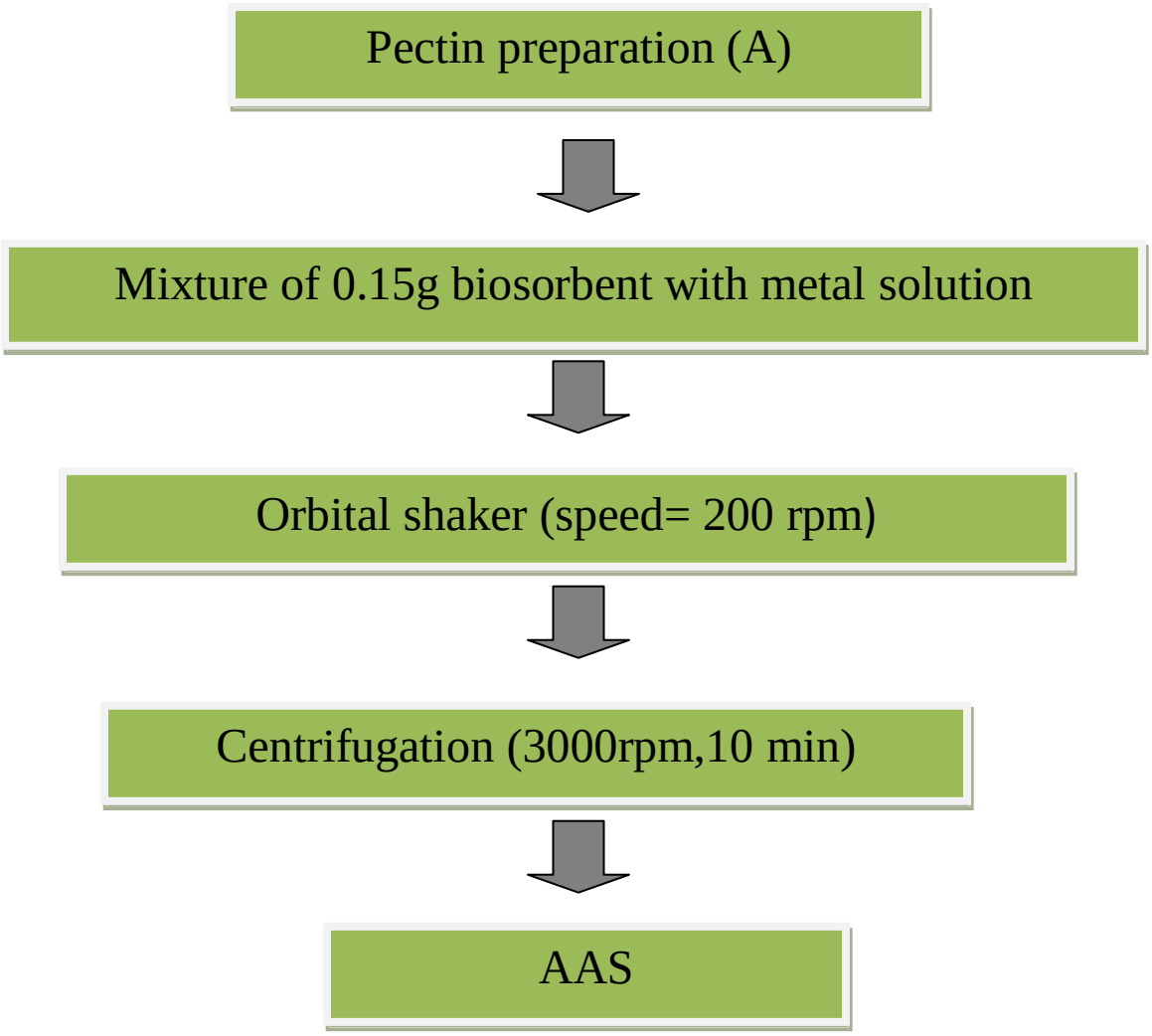

Figure 2 Process Diagram for control biosorption preparation and evaluation

3.3 Preparation of surface Pb^{2+} imprinted biosorbent

$PbNO_3$ was dissolved in 2.0 ml dilute acetic acid solution 2.5% v/v to give a Pb^{2+} solution of 2.0mg/L. 0.1g dried orange peel was dissolved in this solution and stir for 20 minutes. Epichlorhydrin as a cross-linking agent was added into mixture and allowed to carry out for 8 hour at room temperature (27°C). The imprinted Pb^{2+} on biosorbent was removed by treating with a 0.2g/l EDTA solution for 12 hour. Regeneration was carried out by washing the biosorbent with 0.2M NaOH for 2 hour and was shake on orbital shaker at (200rpm). The surface Pb^{2+} imprinted biosorbent was washed twice with running water and 5 times with deionized water. The washed biosorbent was sun dried at room temperature (27°C) for 24 hour or can be dried in an oven at 50°C for 20 hour. Then the dried biosorbent was crushed with analytical mill, sieve and stored in a sealed bottles until it is used. **Figure 3** shows how the preparation of biosorbent in the laboratory.

Figure 3 Preparation of biosorbent from orange peel

3.4 Heavy Metals biosorption

0.1g biosorbent thoroughly mixed with 150ml of metal solution and was shaken on the orbital shaker at speed 200rpm as shown in **Figure 4** Then, to separate the liquid and solid phase, it is put into centrifuge tubes and was centrifuge for about 10 minutes with speed of 3000rpm. The supernatants of heavy metals were then analysed using AAS as in **Figure 5**. The biosorption equilibrium uptake capacity for each sample was calculated according to mass balance on the metal ion expressed as

$$\mathbf{Q = \frac{(C_0 - C_e)\, V}{W}}$$

where Q is adsorption capacity (mg/g), C_0 and C_e are the initial and equlibrated concentrations of metal ion (mg/L), respectively. V is volume of added solution (L) and W is the weight of the dry biosorbent.

Figure 4 Orbital shaker that was used to shake the mixture of biosorbent and metal solution at speed 200 rpm.

Figure 5 Atomic Absorption Spectrometer (AAS) for analysis

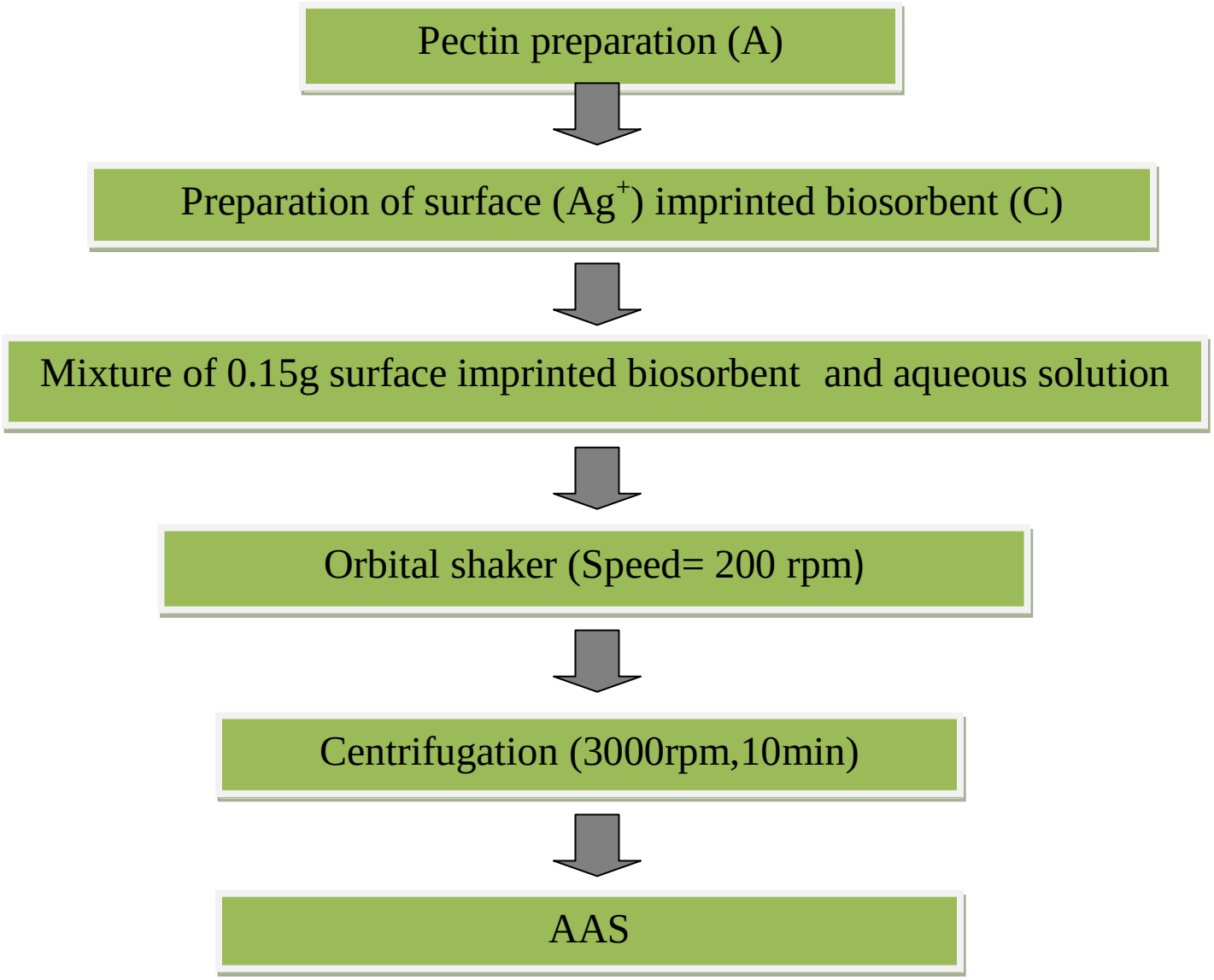

Figure 6 Process Diagram for molecular imprinting biosorption preparation and evaluation

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of the loading amount of imprinted ions on biosorption

As shown in **Figure 7**, the concentration of the imprinted Pb^{2+} ions was an important parameter to adsorption ability in the range of 1.0-4.0 (mg/g). The imprinted Pb^{2+} concentration used in preparation of the biosorbent had an optimal value of 2.0mg/g and the adsorption capacity of the loading amount of 2.0mg/g is 277.5 mg/g at initial concentration of 200mg/L which was about 35% higher than non-imprinted biosorbent. One reason is that more functional groups (-OH) are retained at higher imprinted Pb^{2+} concentrations. The maximum adsorption capacity is 300mg/g. The surface imprinted biosorbent prepared by using molecular imprinting method has higher selectivity towards target metals. When the heavy metal ions selected are reversibly bound on the surface of biosorbent in the preparation and subsequently desorbed, the imprinting sites or the specific spaces of the imprinted metal ion are retained on the surface of biosorbent, so enhancing the selectivity for imprinted ions.

When Pb^{2+} was used as the imprinted metal ion in the preparation, the adsorption ability for Pb^{2+} increased considerably compared with the surface non-

imprinted biosorbent. For the loading amount of 1.0mg/g, the adsorption capacity was lower than adsorption capacity of 2.0mg/g which is about 273.9 mg/g. This was probably because when the Pb^{2+} concentration in preparation was lower, there are less functional group (-OH) on the surface of biosorbent. When the Pb^{2+} concentration in preparation was greater than 2.0mg/g, the adsorption capacity of the surface-imprinted biosorbent decreased. It is because the specific binding sites on the surface-imprinted biosorbent was insufficient at higher Pb^{2+} concentration. The other reason was may be because of the incomplete desorption during the preparation of the surface Pb^{2+} imprinted biosorbent. Therefore, 2.0 mg Pb^{2+}/g biosorbent was selected as the optimal Pb^{2+} imprinted concentration in this experiment.

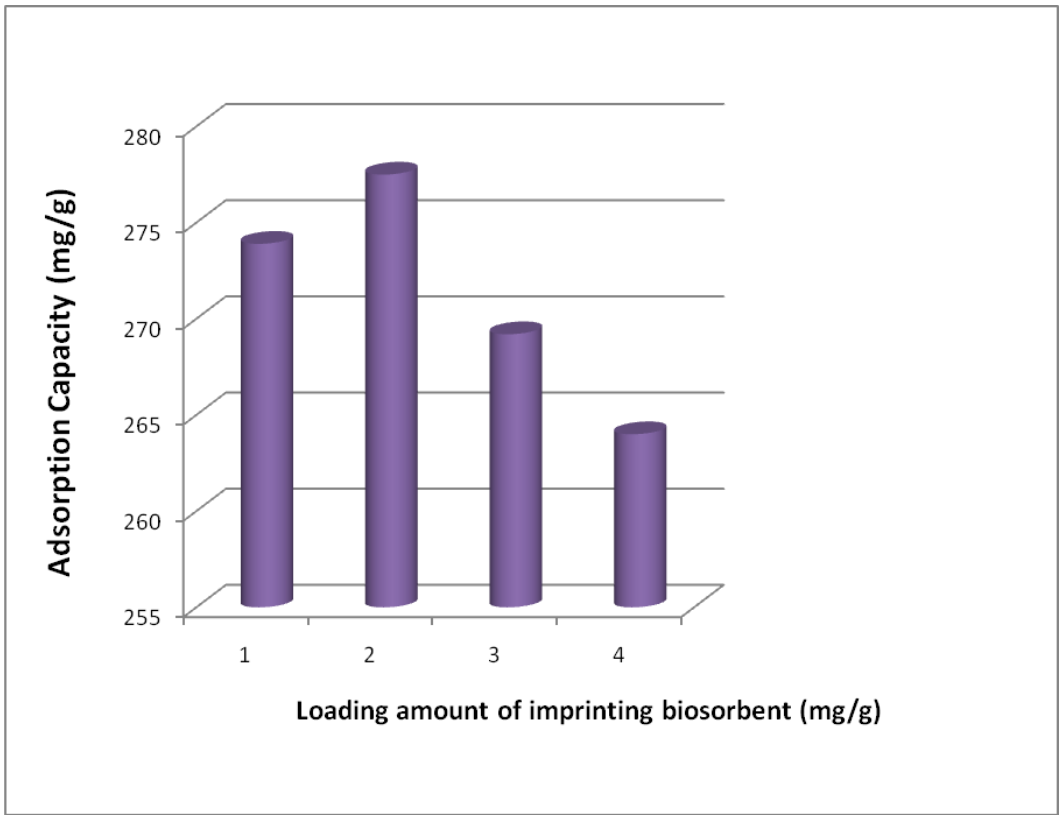

Figure 7 Effect of loading amount of imprinted ions on adsorption capacity 150 ml single Pb^{2+} solution (200 mg/l)was mixed with 3 g/l of Pb^{2+} imprinted biosorbent at pH4 and room temperature (27°C)

4.2 Effect of adsorption time

Effect of time on adsorption capacity for Pb^{2+} by the surface Pb^{2+} imprinted biosorbent was shown in **Figure 9.** The amounts of Pb^{2+} adsorbed were calculated using adsorption capacity equation.

The slope of the lines joining the data points in the figure reflected the adsorption rates. As can be seen, the adsorption capacity for Pb^{2+} increased abruptyly with the adsorption time increasing and the optimum value can be reached around 30 minutes which was 284.25mg/g while the maximum adsorption capacity can be reached at 90 minutes which was 293.7 mg/g. This can be compared with the adsorption of lead by using orange peel without imprinting which takes around 2-3 hours of adsorption time to reach optimal adsorption rate. The adsorption rate seemed to be very satisfactory.From the graph also we can see that after it has reach maximum adsorption capacity after 90 minutes, the data got almost constant value of adsoprtion capacity. This was because the surface of the biosorbent has reach maximum binding whereas all the binding site on surface was fully bind by the Pb^{2+} from the solution. So, that is why the adsorption capacity was constant after it reached maximum adsorption rates.

Due to the preference of short adsorption time for the minimum energy consumpion, the surface Pb^{2+}imprinted biosorbent could be accepted as a good candidate for Pb^{2+} removal in effluent treatment. So, 30 minutes was chosen as adsorption time in the following adsorption experiments.

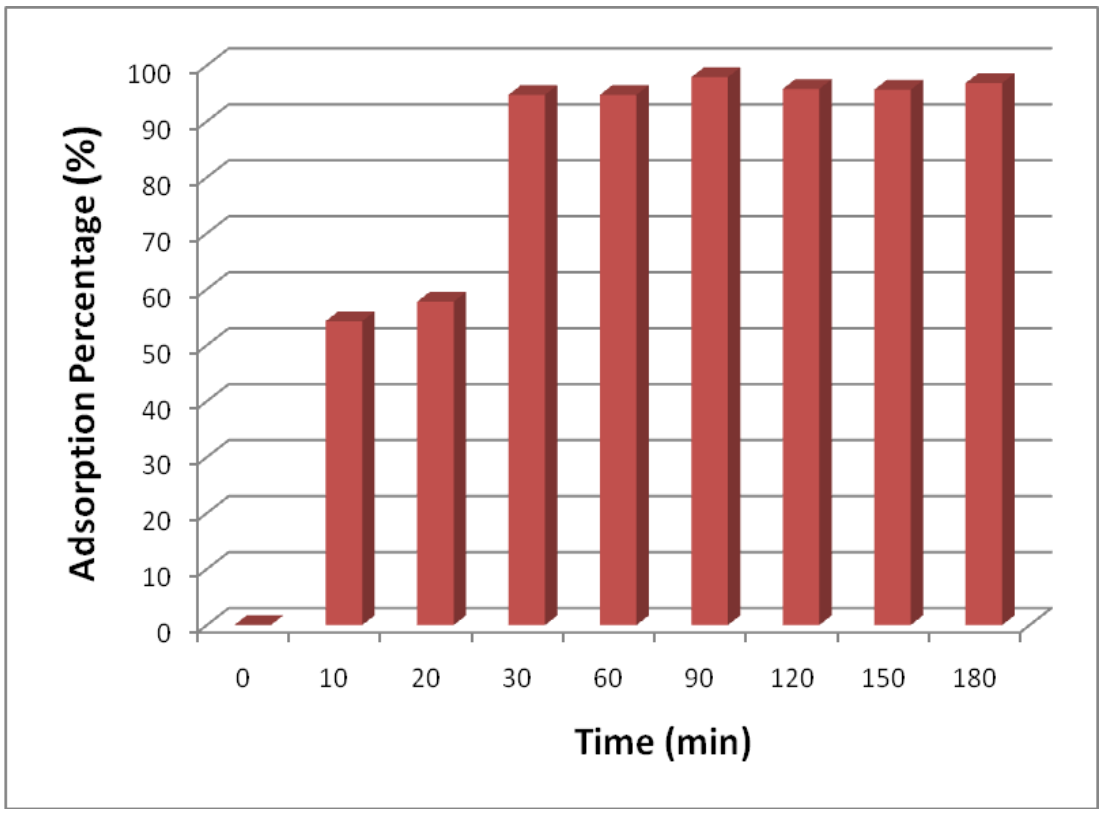

Figure 8 Effect of time on adsorption percentage 150 ml single Pb^{2+} solution (200 mg/l) was mixed with 3 g/l of Pb^{2+} imprinted biosorbent at pH4 and room temperature (27°C)

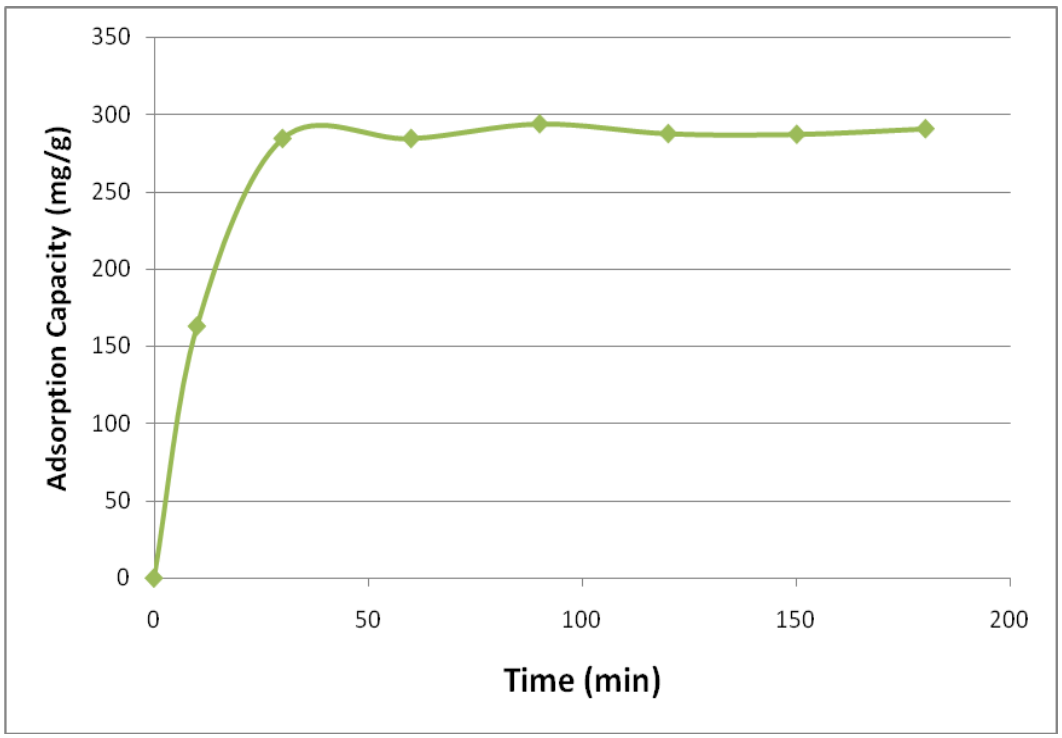

Figure 9 Effect of time on adsorption capacity 150 ml single Pb^{2+} solution (200 mg/l) was mixed with 3 g/l of Pb^{2+} imprinted biosorbent at pH4 and room temperature (27°)

4.3 Effect of heavy metal Pb^{2+} concentration on adsorption capacity

Experiments conducted with different initial Pb^{2+} concentrations, showed that the adsorption capacity of the surface Pb^{2+}imprinted biosorbent increased with the initial concentration of Pb^{2+} ions as in **Figure 10**. This increase continued up to 1000mg/L Pb^{2+} and there was not significant change at the amount of adsorbed Pb^{2+} ions beyond this value. As we can see in the graph, as the concentration increased, the adsorption capacity also increased. This was due to the initial metal concentration provides necessary driving force to overcome the resistance to the mass transfer between the aqueous solution and the solid phase of the surface imprinted biosorbent. As the concentration of Pb^{2+} increased in metal concentration, it makes more contact between metal solution and the biosorbent. The availability of the Pb^{2+} molecules in the surrounding of the biosorbent also increases while increasing the concentration would results a higher uptake of $PbNO_3$ at higher concentration.

However, in order to get more good results, it was recommended that this experiment was conducted start from 200 mg/L to 2000 mg/L. This was because it was easy for us to know the maximum initial concentration of Pb^{2+} that can be adsorp by the 2mg/g surface imprinted biosorbent. Mostly in industry handling with metals, they would probably disposed highly concentrated of metals. So for some researcher, it was suggested that we conducts with high concentrated heavy metals to know the ability of the biosorbent reacts with the concentrated metals. From previous journal, the initial concentration also increased from 200 mg/L to 1000 mg/L but then start from 1200 mg/L, the adsorption capacity get to have a constant value until 1400 mg/L. At 2000 mg/L the adsorption capacity would be decreased because of insufficient binding to the surface of imprinted biosorbent and also the contact

between aqueous solution and surface biosorbent had achieved maximum contact.

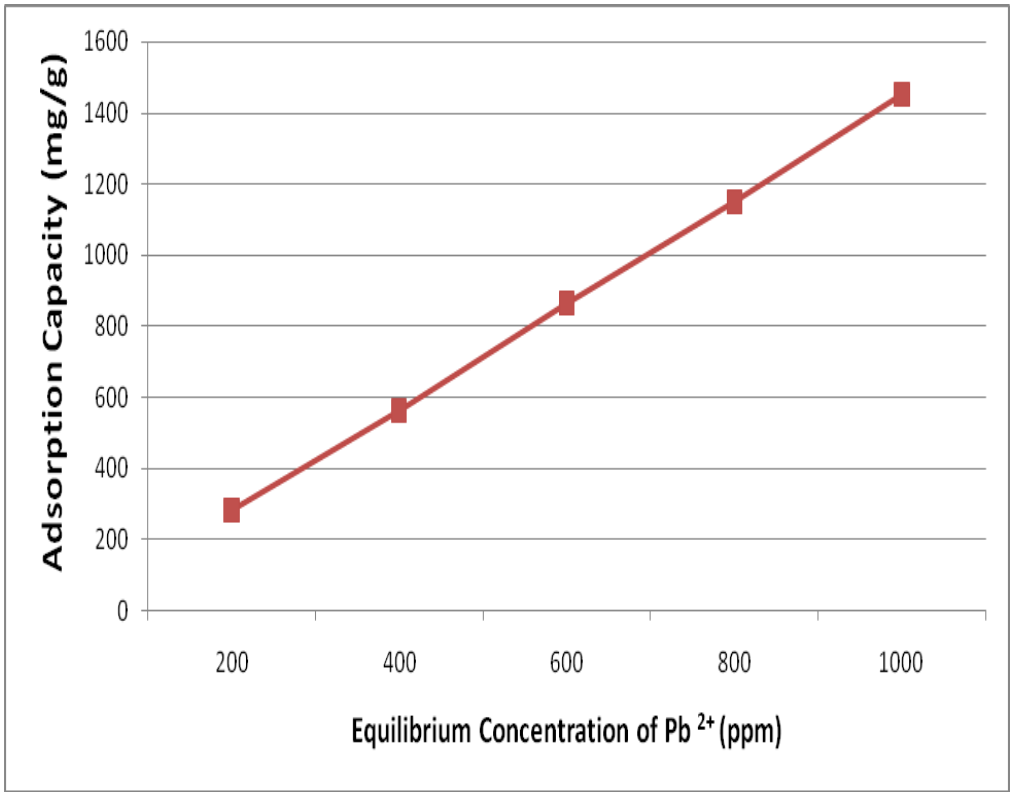

Figure 10 Effect of initial Pb^{2+} concentration (200-1000mg/L) on adsorption capacity 150 ml single Pb^{2+} solution (200 mg/l) was mixed with 3 g/l of Pb^{2+} imprinted biosorbent at pH4 and room temperature (27°C)

4.4 Selectivity ability of Pb^{2+} molecular imprinting biosorbent

The adsorption selectivity of the Pb^{2+} imprinted and other types of metal ions were investigated as in **Table 2**. The Pb^{2+} imprinted biosorbent was exhibited higher adsorption affinity and selectivity towards Pb^{2+} itself and get the highest selectivity which is 1.0. The reasons were because by the surface template polymerization technique, Pb^{2+} selective binding sites could be effectively created on the surfaces of the Pb^{2+} imprinted biosorbent. But the surfaces imprinted for the adsorption of other type of metal ion would provide less suitable binding sites for Pb^{2+}.

For other types of ions such as Fe^{2+}, Cu^{2+}, and $Zn^{2+,}$ the affinity towards the binding sites of Pb^{2+} surface imprinted is lower compare to Pb^{2+} itself. Ferum has the second highest adsorption capacity compare to the other two type of ions because the electron affinity was greater. From Figure 11 also clearly shown that the Pb^{2+} surface imprinted biosorbent mostly would adsorbed the Lead Nitrate solution and gives the highest adsorption capacity compare to the other types of ions.

So, as a conclusion here, the Pb^{2+} surface imprinted biosorbent has a higher selectivity for Pb^{2+} metal ions solution compare to the other metal ions solution.

Table 2: The adsorption for Pb^{2+} imprinted biosorbents 150 ml single Pb^{2+} solution (200 mg/l) was mixed with 3 g/l of Pb^{2+} imprinted biosorbent at pH4 and room temperature (27°C)

The types of ions adsorbed	Final Concentration of metal solution (mg/l)	Amount of metal adsorbed (mg/l)	Percentage of adsorption (%)	Adsorption capacity, Q (mg/g)	Selectivity coefficient, α
Pb^{2+}	**0**	**200**	**100**	**300**	**1.0000**
Fe^{2+}	**6.9**	**193.1**	**96.55**	**289.65**	**0.9655**
Cu^{2+}	**14.7**	**185.3**	**92.65**	**277.95**	**0.9265**
Zn^{2+}	**44.4**	**155.6**	**77.8**	**233.4**	**0.7780**

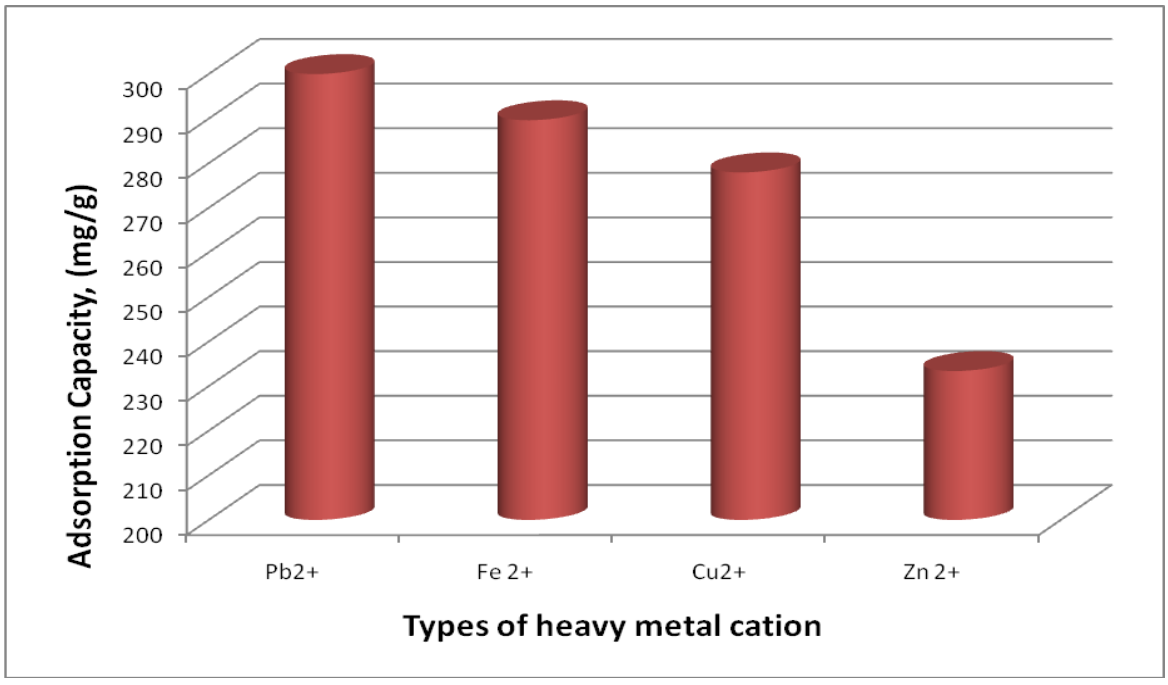

Figure 11 Comparison of different cation heavy metal uptake on adsorption capacity 150 ml single Pb^{2+} solution (200 mg/l), Fe^{2+} solution (200mg/L), Cu^{2+} solution (200mg/L). Zn^{2+} solution (200mg/L) was mixed with 3 g/l of Pb^{2+} imprinted biosorbent at pH4 and room temperature (27°C)

4.5 Effect of molecular imprinting technique on biosorbent performance

From the **Figure 12**, it was clearly shown that the molecular-imprinted biosorbent was preferable for the adsorption of Pb^{2+} solution due to its highest adsorption capacity which is 293.7 mg/g. the maximum adsorption capacity that can be reached was 300 mg/g . For the biosorbent without imprinted technique, its adsorption capacity was just only 191.6 mg/g which contribute to 34.76% lower than imprinting biosorbent.

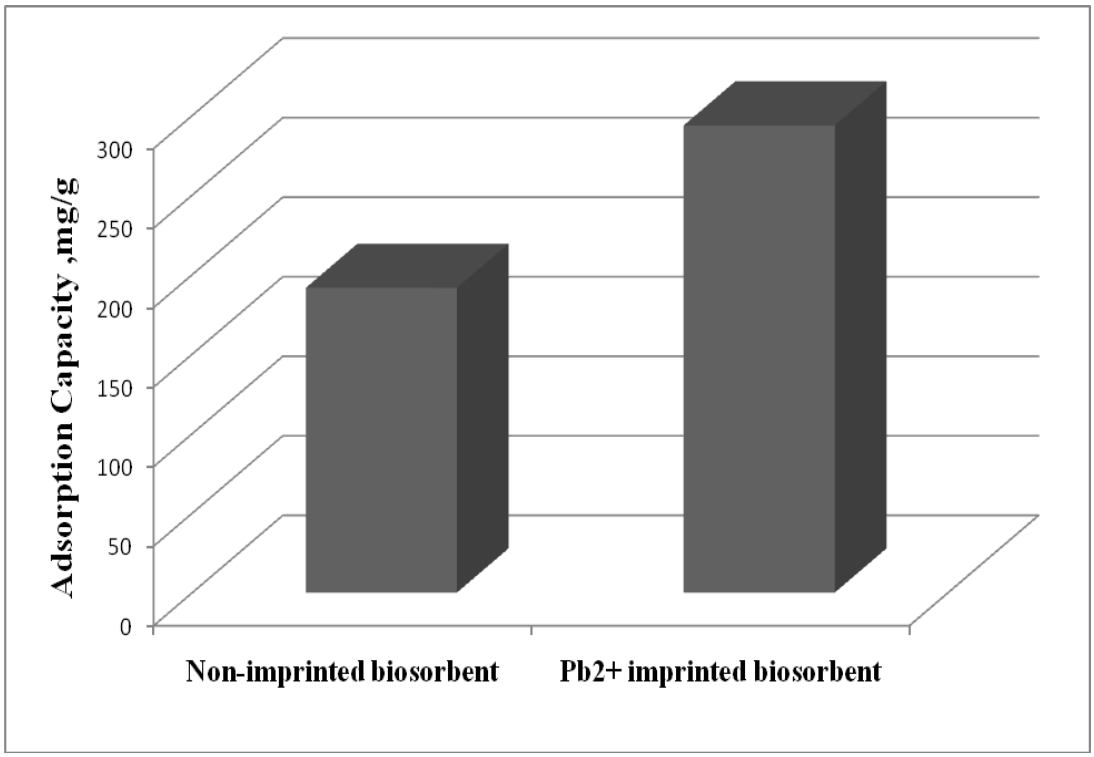

Figure 12 Comparison between non- imprinted and Pb^{2+}imprinted biosorbent 150 ml single c solution (200 mg/L) at pH4 and room temperature (27°C)

4.6 Characterization of Pb^{2+} imprinted biosorbent

FTIR analysis is done also in this research which is to know the existence of functional groups in the molecular imprinted biosorbent. So, before starts any experiment, the biosorbent was analysis using FTIR. FTIR is commonly used in order to see the functional groups of biosorbents. It is as a method of characterization of dried orange peel with the molecular imprinted technique before the experiment in order to know what functional group exist in the biosorbent . Figure 13 shows the adsorption peak of several compounds that found in the dried orange peel. The adsorption site indicated maximum wave number at 3100 cm^{-1} to 3550 cm^{-1}, representing stretching of –OH groups while the absorption peaks around 3300 cm^{-1} to 3600 cm^{-1} represented the presence of NH_2 which is the amine group The stretching at 1644 cm^{-1} was represented C - C bending. So, it was clearly shown that the existence of –OH in the biosorbent can attracts the positively charged heavy metals and the selectivity of heavy metals towards biosorbent due to the molecular-imprinted technique.

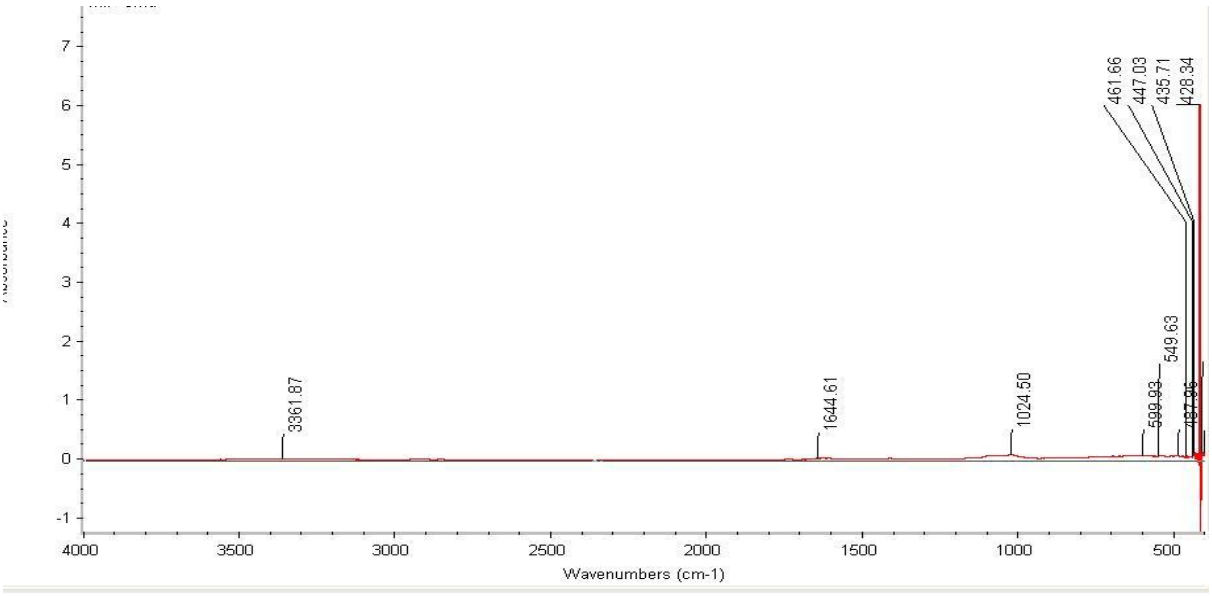

Figure 13 FTIR analysis result for molecular imprinted biosorbent

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusions

Pb^{2+} imprinted biosorbent prepared by using molecular imprinted technique, showed higher adsorption ability and selectivity towards plumbum ions solution compare to other types of metal ions. The results indicated that the adsorption process was influenced by the factors such as loading amount of targeted ions during preparation and also adsorption time. In the preparation, with the imprinted ion Pb^{2+} concentrations increasing, the more fucntional groups (-OH) were protected and the more imprinting sites on the surface were retained. So, it was found that 2mg/g loading amount of imprinted ions during preparation was selected as the optimum loading amount. For the effect of time in adsorption, the optimum condition was described as the shortest time that the biosorbent can absorbed the metals. So, in this experiment, the shortest time that it can achieved with a high adsorption capacity was 30 minutes. It can be said as satisfactory adsorption compare to without imprinted biosorbent. The existence of functional group of (-OH) can be proved by using FTIR analysis.

5.2 Recommendations

It was recommended that this research was furthered and use the waste water from metal industry. This was very important because we may showed to the industry and commercialized these molecular imprinted biosorbent from orange peel to them so that they would fulfill the standard of Environmental Act 1954 before disposed it to water stream. If we can proved that the molecular imprinted biosorbent can achived those objective in this research by using wastewater from industry, then we might have a big chance to develop it in large scale production for commerical reason. Other than that, due to our waste to wealth, it would be very low cost in the production of molecular-imprinted biosorbent besides having the shortest time compare to the other type of adsorbent.

It is also recommended that to further the research to recover back the Plumbum that had attached to the biosorbent. Besides, in this further study also would choose which type of desorbents had the highest desorption rates to recover to plumbum. This was also important in real industry that disposed the wastewater containing plumbum to recover the plumbum back and can be sent to metal industry for further processing or reused again the metal. It also can cut the cost in the processes. If the efficiency of the desorption process can achieved 99% with the shortest time, then we can select the desorbent as the best desorbent to recover the plumbum.

Lastly, it was recommended that the adsorption process in this experiment can be applied in adsorption column. So, with the existence of adsorbent which is the surface molecular imprinted biosorbent and the desorbents to regenerate the

Plumbum, it is for sure that it can be applied in adsorption column and very useful for metal industry and can be predicted to get a high demand from those metal industries.

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APPENDICES

APPENDIX A RESULTS FROM ANALYSIS

4.1 Effect of the loading amount of imprinted ions on biosorption

Table 3 Effect of the loading amount of imprinted ions on biosorption

Concentration of imprinted Pb^{2+}(mg/g)	Final Concentration of Pb solution (mg/l)	Amount of Pb^{2+} adsorp (mg/l)	Percentage of adsorption (%)	Adsorption capacity, Q (mg/g)
1	17.4	182.6	91.3	273.9
2	15.0	185.0	92.5	277.5
3	20.5	179.5	89.75	269.2
4	24.0	176.0	88.0	264.0

4.2 Effect of adsorption time

Table 4 Effect of adsorption time

Time (minutes)	Final Concentration of Pb solution (mg/l)	Amount of Pb^{2+} adsorbed (mg/l)	Percentage of adsorption (%)	Adsorption capacity, Q (mg/g)
10	91.4	108.6	54.30	162.87
20	84.44	115.56	57.78	173.34
30	10.5	189.5	94.75	284.25
60	10.5	189.5	94.75	284.25
90	4.2	195.8	97.9	293.7
120	8.4	191.6	95.8	287.4
150	8.7	191.3	95.65	286.95
180	6.3	193.7	96.85	290.55

4.3 Effect of heavy metal Pb^{2+} concentration on adsorption capacity

Table 5 Effect of heavy metal Pb^{2+} concentration on adsorption capacity

Initial Concentration (ppm)	Final Concentration of Pb solution (mg/l)	Amount of Pb^{2+} adsorbed (mg/l)	Percentage of adsorption (%)	Adsorption capacity, Q (mg/g)
200	12	188	94	282
400	24.3	375.7	93	563
600	24.6	575.4	95.9	863.1
800	34.8	765.2	95.65	1147.8
1000	32.7	967.3	96.73	1450.95

Meas. Date : 3/11/2010 5:32 PM
Element : Pb STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0002	-0.0065
STD2		1.00	0.00	0.0092	-0.0100
STD3		2.00	0.00	0.0161	-0.0149
STD4		4.00	0.00	0.0293	-0.0178

Coefficient : K3=--
K2=--
K1=7.165714E-003
K0=1.160000E-003
Corr.Coef. : 0.9974

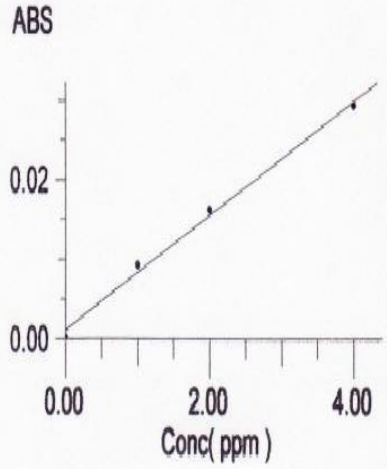
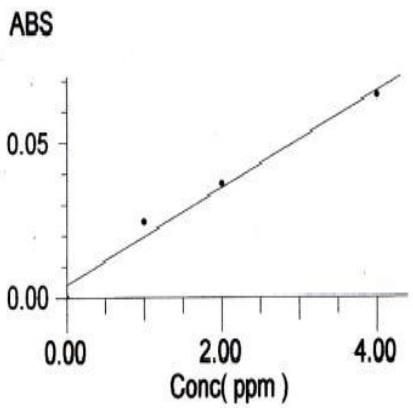


Figure 14 AAS analysis result for Standard Curve

Meas. Date : 3/12/2010 3:13 PM
Element : Pb STD Unit : ppm UNK Unit : ppm

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0001	-0.0310
STD2		1.00	0.00	0.0241	-0.0461
STD3		2.00	0.00	0.0364	-0.0543
STD4		4.00	0.00	0.0649	-0.0608

Coefficient : K3=--
K2=--
K1=1.568286E-002
K0=3.880000E-003
Corr.Coef. : 0.9907



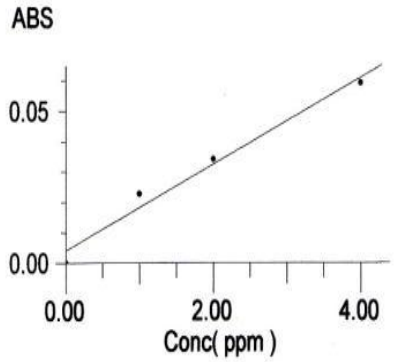
UNK-001	1mg/g	0.58	0.00	0.0129	-0.0726
UNK-002	2mg/g	0.50	0.00	0.0117	-0.0804
UNK-003	4mg/g	0.80	0.00	0.0165	-0.0868

Figure 15 AAS analysis result for loading amount of imprinted biosorbent

Meas. Date : 3/15/2010 4:31 PM
Element : Pb STD Unit : ppm UNK Unit : ppm

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0000	-0.0354
STD2		1.00	0.00	0.0227	-0.0513
STD3		2.00	0.00	0.0339	-0.0664
STD4		4.00	0.00	0.0588	-0.0763

Coefficient : K3=--
K2=--
K1=1.414286E-002
K0=4.100000E-003
Corr.Coef. : 0.9881



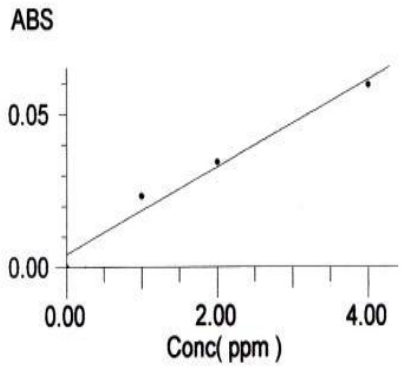
UNK-001	0.5 hour	0.35	0.00	0.0091	-0.0883
UNK-002	1.0 hour	0.35	0.00	0.0091	-0.0938
UNK-003	1.5 hour	0.14	0.00	0.0061	-0.1023
UNK-004	2.0 hour	0.28	0.00	0.0081	-0.1079
UNK-005	2.5 hour	0.29	0.00	0.0082	-0.1130
UNK-006	3.0 hour	0.21	0.00	0.0071	-0.1188
UNK-007	3.5 hour	0.04	0.00	0.0046	-0.1241
UNK-008	4.0 hour	0.06	0.00	0.0032	-0.1276

Figure 16 AAS analysis result for effect of biosorption time

Meas. Date : 3/19/2010 3:09 PM
Element : Pb STD Unit : ppm UNK Unit : ppm

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0000	-0.0206
STD2		1.00	0.00	0.0231	-0.0402
STD3		2.00	0.00	0.0342	-0.0543
STD4		4.00	0.00	0.0592	-0.0709

Coefficient : K3=--
K2=--
K1=1.422000E-002
K0=4.240000E-003
Corr.Coef. : 0.9874



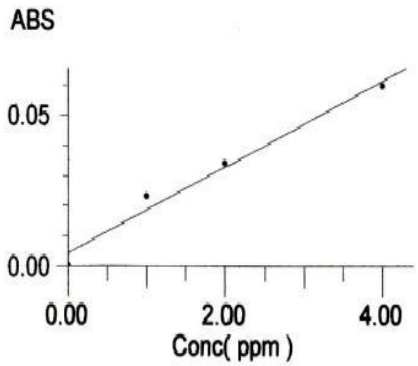
UNK-001	200 ppm (DF=30)	0.40	0.00	0.0099	-0.0874
UNK-002	400 ppm (DF=30)	0.81	0.00	0.0158	-0.0968
UNK-003	600 ppm (DF=30)	0.82	0.00	0.0159	-0.1041
UNK-004	800 ppm (DF=30)	1.16	0.00	0.0207	-0.1107
UNK-005	1000 ppm (DF=30)	1.09	0.00	0.0198	-0.1166

Figure 17 AAS analysis result for effect of heavy metal Pb^{2+} concentration on adsorption capacity

Meas. Date : 3/19/2010 10:34 PM
Element : Pb STD Unit : ppm UNK Unit : ppm

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0003	-0.0067
STD2		1.00	0.00	0.0230	-0.0095
STD3		2.00	0.00	0.0341	-0.0126
STD4		4.00	0.00	0.0597	-0.0139

Coefficient : K3=--
K2=--
K1=1.429429E-002
K0=4.260000E-003
Corr.Coef. : 0.9890



UNK-001	plumbum ion	0.00	0.00	0.0042	-0.0222

Figure 18 AAS analysis result for biosorption of plumbum on imprinted biosorbent

Meas. Date : 3/19/2010 10:52 PM
Element : Zn STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0101	-0.0009
STD2		1.00	0.00	0.2627	0.0387
STD3		2.00	0.00	0.4878	0.0833
STD4		4.00	0.00	0.7087	0.1530
UNK-001	zinc ion	0.49	0.00	0.1506	0.0292

Coefficient : K3=--
K2=--
K1=1.716371E-001
K0=6.696000E-002
Corr.Coef. : 0.9778

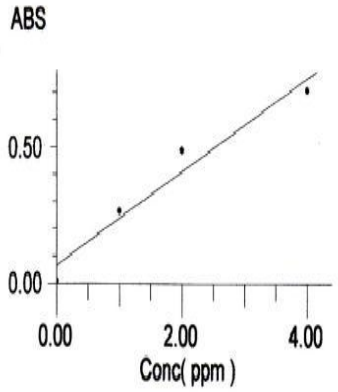
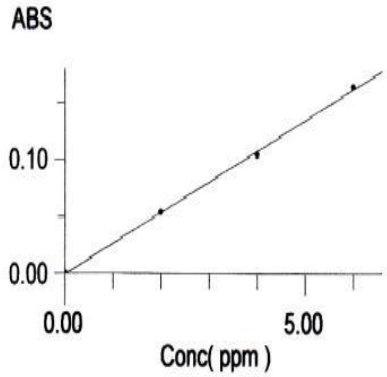


Figure 19 AAS analysis result for biosorption of zinc on imprinted biosorbent

Meas. Date : 3/19/2010 10:40 PM
Element : Cu STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0002	-0.0059
STD2		2.00	0.00	0.0534	0.0347
STD3		4.00	0.00	0.1039	0.0732
STD4		6.00	0.00	0.1635	0.1173

Coefficient : K3=--
K2=--
K1=2.702000E-002
K0=-8.099997E-004
Corr.Coef. : 0.9994



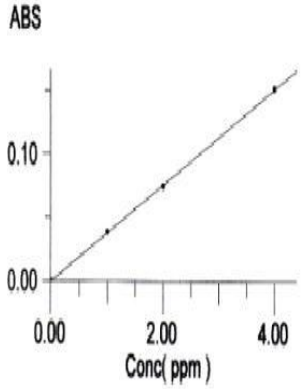
UNK-001	copper ion	1.48	0.00	0.0391	0.0109

Figure 20 AAS analysis result for biosorption of copper on imprinted biosorbent

Meas. Date : 3/19/2010 10:47 PM
Element : Fe STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0001	-0.0043
STD2		1.00	0.00	0.0383	-0.0043
STD3		2.00	0.00	0.0743	-0.0032
STD4		4.00	0.00	0.1513	0.0011

Coefficient : K3=--
K2=--
K1=3.772571E-002
K0=-1.999987E-005
Corr.Coef. : 0.9999



UNK-001	Ferum ion	0.23	0.00	0.0087	-0.0078

Figure 21 AAS analysis result for biosorption of Ferum on imprinted biosorbent