

**THE EFFECT OF pH AND BIOMASS CONCENTRATION ON LEAD (Pb)
ADSORPTION BY *Saccharomyces cerevisiae* FROM SIMULATED
WASTE WATER**

MOHD KAMIRUL NIZAM BIN NGAH @ OMAR

**A report submitted in fulfillment of requirements for the award of the Degree of
Bachelor of Chemical Engineering**

**Faculty of Chemical and Natural Resources Engineering
University College of Engineering & Technology Malaysia**

NOVEMBER 2006

“I declare that this thesis is the result of my own research except as cited references.
The thesis has not been accepted for any degree and is concurrently submitted in
candidature of any degree.”

Signature :.....

Name of Candidate :.....

Date :.....

DEDICATION

*Special dedication to my beloved father, mother,
brother and sisters.....*

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim

Firstly, I would like to express my gratitude to my supervisor, Mr Mior Ahmad Khushairi B. Mohd Zahari for his enduring guidance, trust, assistance and constructive ideas. Do not forget also to all lecturers who involved directly or indirectly in completing this research. Thank you very much for the knowledge and guidance.

Besides, I would like to thanks to the personnel at FKKSA clean room for their assistance and cooperation, especially to Miss Idayu and Mr Hafiz.

My special appreciation is dedicated to my father, Mr Ngah@Omar B Othman, my mother, Madam Aminah Nordin, sisters and brother for their patience, moral and financial support during the course.

Lastly, I would like to thank to my colleagues and friends for their moral support and assistants. Thank you for being there.

ABSTRACT

The contamination of wastewaters, river sediments and soil with toxic metals, is a complex problem. The removal of these contaminations has received much attention in recent years using conventional methods such as chemical reduction, ion exchange, and electrochemical treatment. The alternative method is discovered which is biosorption, refers to a physic-chemical binding of metal ions to biomass. The biosorption rate largely depends on parameters such as pH, initial biomass concentration and temperature. The biosorption of lead (Pb^{2+}) from simulated waste water using baker's yeast biomass is investigated. In this study, biosorption process was carried out in a shake flask at different initial pH and biomass concentration. Results from experiment at different initial pH show that the percent of biosorption was highest at pH 4.0, i.e. 18.884 %. For the experiment conducted at different initial biomass concentration, the highest percent of biosorption was obtained by using 2.5 mg/l of biomass. From the results also, it was observed that percent of biosorption is directly proportional to the biomass concentration.

ABSTRAK

Pencemaran air sisa, endapan sungai dan tanah oleh logam toksik adalah satu masalah yang rumit. Penyingkiran bahan tercemar ini telah mendapat perhatian sejak kebelakangan ini dengan menggunakan kaedah konvensional seperti tindak balas penurunan kimia, penukaran ion, dan rawatan elektrokimia. Kaedah alternative iaitu penjerapan bio telah digunakan dalam merawat air sisa yang mengandungi logam toksik. Kaedah penjerapan bio adalah merujuk kepada pelekatan ion logam dengan dinding sel mikroorganisma secara fizikal-kimia. Oleh itu, kadar penjerapan bio sangat bergantung kepada beberapa parameter seperti nilai pH, kepekatan awal biojisim dan logam, dan suhu. Penjerapan logam plumbum daripada simulasi air sisa telah dijalankan dengan menggunakan biojisim daripada yis ibu roti. Dalam kajian ini, process penjerapan bio telah dijalankan didalam kelalang goncang pada pH dan kepekatan biojisim yang berlainan. Keputusan daripada eksperimen yang dijalankan pada pH yang berbeza menunjukkan bahawa peratus penjerapan bio adalah paling tinggi pada pH 4.0 iaitu sebanyak 18.884%. Manakala bagi eksperimen yang dijalankan pada kepekatan biojisim yang berbeza menunjukkan bahawa peratus penjerapan bio adalah paling tinggi dengan menggunakan biojisim berkepekatan 2.5 mg/l. Daripada keputusan yang diperolehi juga, dapat dilihat bahawa peratus penjerapan bio adalah berkadar langsung dengan kepekatan awal biojisim dalam larutan.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	TITLE	i
	DECLARATION	ii
	ACKNOWLEDGEMENT	iv
	ABTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	xi
	LIST OF FIGURES	xii
	LIST OF APPENDICES	xii
	LIST OF ABBREVIATIONS	xiii
 1	 INTRODUCTION	
	1.1 Background	1
	1.2 Problem Statement	3
	1.3 Objective	4
	1.4Scope	4

2 LITERATURE REVIEW

2.1 Biosorption	
2.1.1 Introduction	5
2.1.1 Biosorbent material	6
2.1.2 Choice of metal for biosorption process	8
2.2 AAS	
2.2.1 Introduction	10
2.2.2 The light source	10
2.2.3 The optical system and detector	11
2.3 <i>Saccharomyces cerevisiae</i>	
2.3.1 Introduction	12
2.3.2 Lifecycle	13
2.4 Heavy Metal	
2.4.1 Definition of Heavy Metal	15
2.4.2 Toxic Heavy Metal	15
2.4.3 Lead (Plumbum)	16
2.4.4 Act Relevant to Heavy Metal Discharges	16

3 MATERIALS AND METHODS

3.1 Introduction	18
3.2 Materials and Methods	
3.2.1 Chemical	18
3.2.2 Microorganism	19
3.2.3 Simulated Wastewater preparation	19
3.2.4 pH Profile Experiment	20
3.2.5 Biomass Concentration Profile Experiment	20
3.3 Analysis lead ions	21
3.4 Summary of Methodology	22

4	RESULTS AND DISCUSSIONS	
4.1	Introduction	23
4.2	pH Profile Experiment	23
4.3	Concentration Profile Experiment	25
5	CONCLUSION AND RECOMMENDATIONS	
5.1	Conclusion	26
5.2	Recommendations	27
	REFERENCES	28
	APPENDICES	31

LIST OF TABLES

TABLE NO	TITLE	PAGE
2.1	Physical and Chemical Adsorption Differences	6
2.2	Permissible Concentrations for Effluents (Malaysian Environmental Quality Act)	16
2.3	Permissible Concentrations for Direct Discharges in Receiving Streams Limiting Value of Drinking Water	17

LIST OF FIGURES

FIG. NO	TITLE	PAGE
1.1	Malaysian waste under trade 2004 (Exported : 3354 tonnes)	3
2.1	Schematic Diagram of Atomic Absorption Spectrometer (AAS)	9
2.2	Hallow Cathode Lamp	10
2.3	Some sputtered atoms are in excited states and emit radiation	11
2.4	Shows electrical signal proportional to the light intensity	12
2.5	Yeast cells	12
2.6	<i>Saccharomyces cerevisiae</i> cell	13
2.7	Yeast Cycle	14
3.1	Yeast	19
3.2	Simulated Wasterwater	19
3.3	Yeast and metal binding samples	20
3.4	AAS	21
3.5	Methodology of Biosorption Experiment	22
4.1	Effect of initial pH on the biosorption capacity	24
4.2	Biosorption capacity of lead for different biosorbent concentration (pH 4.0).	25

LIST OF APPENDIES

APP. NO.	TITLE	PAGE
A	Data and calculation	31
B	Data Sheet from AAS	37

LIST OF ABBREVIATIONS

AAS	=	Atomic Absorption Spectrometry
T	=	Temperature
t	=	Time
Pb	=	Plumbum (Lead)
g/ml	=	gram per milliliter
K	=	rate constant
C_o and C (mg/ l)	=	the initial and equilibrium (residual) concentrations of metals in the solution

CHAPTER 1

INTRODUCTION

1.1 Background

The conventional process of heavy metal removal from industrial wastewater involves chemical precipitation of metals usually by lime followed by settling of the metal precipitates in a pond and/or a clarifier. The major shortcoming of conventional treatment includes low efficiency at low concentration of heavy metals and expensive handling and safe disposal of toxic sludge.

Adsorption on the ion exchange or activated carbon is a recognized method for the removal of heavy metals from wastewater. However, high costs of them have limited their use in the real electroplating wastewater treatment. Innovative methods for heavy metal removal including biosorption have been investigated in an ongoing effort to develop a better treatment for wastewater containing toxic heavy metals.

A search for a low-cost and easily available adsorbent has led to the investigation of materials of agricultural and biological origin, along with industrial byproducts, as potential metal sorbents. The variety of materials tested as metal adsorbents includes coal, crushed coconut shell, peat, bark and straw. Recent developments in the field of environmental biotechnology include the search for microorganisms as sorbents for heavy metals. Bacteria, fungi, yeast and algae can remove heavy metals from aqueous solution in substantial quantities. The uptake of heavy metals by biomass can take place by an active mode (dependent on the

metabolic activity) known as bioaccumulation or by a passive mode (sorption and/or complexation) termed as biosorption. (W. Jianlong, 2001)

The uptake of heavy metals by biomass can in some cases reach up to 50% of the biomass dry weight. New biosorbents can be manipulated for better efficiency and multiple re-use to increase their economic attractiveness. In the concept of biosorption, several physical or chemical processes may be involved such as physical and/or chemical adsorption, ion exchange, coordination, complexation, chelation and microprecipitation. Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids offer many functional groups which can bind metal ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. In addition to these functional binding groups, polysaccharides often have ion exchange properties. Non-living biomass appears to present specific advantages in comparison to the use of living microorganisms. (B. Volesky *et al*, 2003)

Killed cells may be stored or used for extended periods at room temperature, they are not subject to metal toxicity and nutrient supply is not necessary. Moreover pretreatment and killing of biomass either by physical or chemical treatments or cross linking are known to improve the biosorption capacity of biomass. It has also been reported that cell wall soluble proteins, which make complexes with metal ions can be fixed by some denaturation processes such as heat and ethanol treatment. Deactivated yeast cells do not release protein and exhibit higher metal ion removal capacity than live yeast.

Among the microorganisms used for biosorption, *Saccharomyces cerevisiae* is an inexpensive, readily available source of biomass for heavy metal removal from wastewater. Investigations conducted by several researchers demonstrated that *S. cerevisiae* is capable of accumulating heavy metals such as Cu^{2+} , Cd^{2+} , Pb^{2+} , Zn^{2+} , Cr^{2+} and Ni^{2+} (Y.Goksungur, 2004).

1.2 Problem Statement

The discharge of heavy metals into aquatic ecosystems has become a matter of concern over the last few decades. These pollutants are introduced into the aquatic systems significantly as a result of various industrial operations. The pollutants of concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper and nickel. These toxic materials may be derived from mining operations, refining ores, sludge disposal, fly ash from incinerators, the processing of radioactive materials, metal plating, or the manufacture of electrical equipment, paints, alloys, batteries, pesticides or preservatives. Heavy metals such as zinc, lead and chromium have a number of applications in basic engineering works, paper and pulp industries, leather tanning, organ chemicals, petrochemicals fertilizers, etc. Major lead pollution is through automobiles and battery manufacturers.

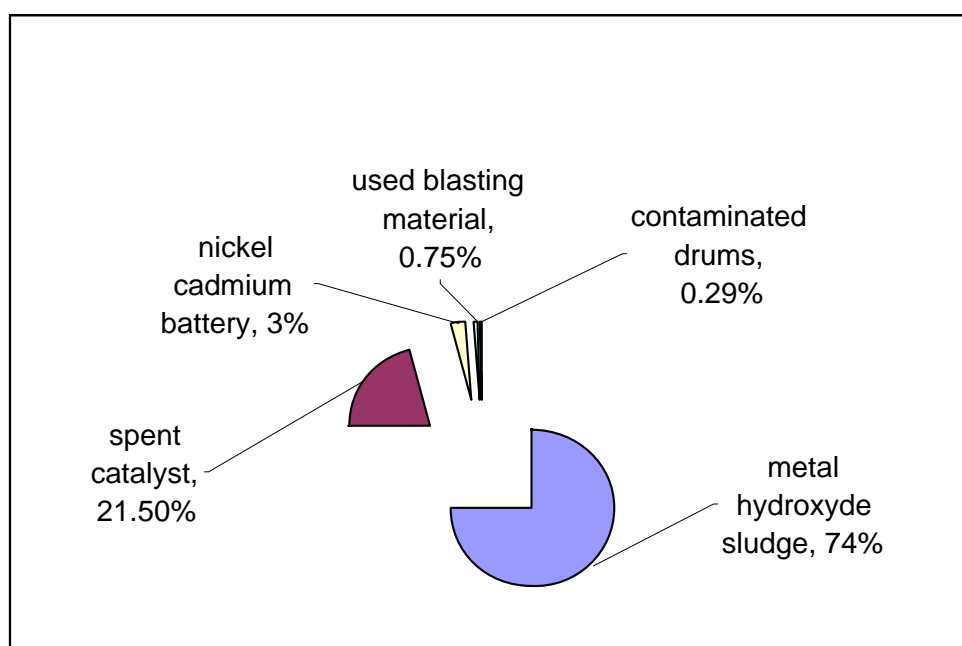


Figure 1.1 Malaysia waste under trade 2004[Exported: 3354 tonnes]
(The Star, 2006)

1.3 Objectives

The focal purpose of this project is to study the effect of pH and biomass concentration on **Lead (Pb)** adsorption by *Saccharomyces cerevisiae* from simulated waste water.

1.4 Scopes of The Research

This research consists of two components which are:

- i Study on effect of pH on lead adsorption by *Saccharomyces cerevisiae* from simulated waste water
- ii Study on effect of initial biomass concentration on lead adsorption by *Saccharomyces cerevisiae* from simulated waste water

CHAPTER 2

LITERATURE REVIEW

2.1 Biosorption

2.1.1 Introduction

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake. Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (N. Ahalya *et al*, 2001). The major advantages of biosorption over conventional treatment methods include

- Use of renewable biomaterials, which can reduce production costs
- High selectivity of biosorbents (possible to recover valuable metals)
- Cleansing of aqueous solution with low metal concentration
- High capacity by small equilibrium concentration
- Easy desorption of metals by pH swing
- Low affinity with competing cations(calcium and magnesium)

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different

mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases. (H.-J Rehm, 1999):

Physical Adsorption	Chemical Adsorption
The adsorbate adheres to the surface only through Van der Waals force interactions.	Molecule adheres to a surface through the formation of a chemical bond
Low temperature	High temperature
Low activation energy	High activation energy
Low enthalpy	High enthalpy
Adsorption takes place in multilayer	Adsorption takes place only in a monolayer

Table 2.1 Physical and Chemical Adsorption Differences

2.1.2 Biosorbent material

Strong biosorbent behavior of certain micro-organisms towards metallic ions is a function of the chemical make-up of the microbial cells. This type of biosorbent consists of dead and metabolically inactive cells.

Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Some laboratories have used easily available biomass whereas others have isolated specific strains of microorganisms and some have also processed the existing raw biomass to a certain degree to improve their biosorption properties;

Recent biosorption experiments have focused attention on waste materials, which are by-products or the waste materials from large-scale industrial operations.

For e.g. the waste mycelia available from fermentation processes, olive mill solid residues, activated sludge from sewage treatment plants, biosolids, aquatic macrophytes, etc. (Tae-Young Kim *et al*, 2005)

Another inexpensive source of biomass where it is available in copious a quantity is in oceans as seaweeds, representing many different types of marine macro-algae. However most of the contributions studying the uptake of toxic metals by live marine and to a lesser extent freshwater algae focused on the toxicological aspects, metal accumulation, and pollution indicators by live, metabolically active biomass. Focus on the technological aspects of metal removal by algal biomass has been rare. Although abundant natural materials of cellulose nature have been suggested as biosorbents, very less work has been actually done in that respect.

The mechanism of biosorption is complex, mainly ion exchange, chelations, adsorption by physical forces, entrapment in inter and intrafibrillar capillaries and spaces of the structural polysaccharide network as a result of the concentration gradient and diffusion through cell walls and membranes. (N. Ahalya *et al*, 2001)

There are several chemical groups that would attract and sequester the metals in biomass: acetamido groups of chitin, structural polysaccharides of fungi, amino and phosphate groups in nucleic acids, amido, amino, sulphhydryl and carboxyl groups in proteins, hydroxyls in polysaccharide and mainly carboxyls and sulphates in polysaccharides of marine algae that belong to the divisions Phaeophyta, Rhodophyta and Chlorophyta. However, it does not necessarily mean that the presence of some functional group guarantees biosorption, perhaps due to steric, conformational or other barriers.

2.1.3 Choice of metal for biosorption process

The appropriate selection of metals for biosorption studies is dependent on the angle of interest and the impact of different metals, on the basis of which they would be divided into four major categories:

- i. Toxic heavy metals
- ii. strategic metals
- iii. precious metals and
- iv. Radio nuclides.

In terms of environmental threats, it is mainly categories (i) and (iv) that are of interest for removal from the environment and/or from point source effluent discharges. A whole new family of suitably "formulated" biosorbents can be used in the process of metal removal and detoxification of industrial metal-bearing effluents. The sorption packed-column configuration is the most effective mode of application for the purpose. Recovery of the deposited metals from saturated biosorbent can be accomplished because they can often be easily released from the biosorbent in a concentrated wash solution which also regenerates the biosorbent for subsequent multiple reuse. (N. Ahalya *et al*, 2001). This and extremely low cost of biosorbents makes the process highly economical and competitive particularly for environmental applications in detoxifying effluents of e.g.

- i. metal-plating and metal-finishing operations,
- ii. mining and ore processing operations,
- iii. metal processing, battery and accumulator manufacturing operations,
- iv. thermal power generation (coal-fired plants in particular),
- v. nuclear power generation

Apart from toxicological criteria, the interest in specific metals may also be based on how representative their behavior may be in terms of eventual generalization of results of studying their biosorbent uptake (N. Ahalya *et al*, 2001). The toxicity and interesting solution chemistry of elements such as chromium, arsenic and selenium make them interesting to study. Strategic and precious metals though not environmentally threatening are important from their recovery point of view. Atomic absorption spectrometry (AAS)

2.2 Atomic Absorption Spectrometry (AAS)

2.2.1 Introduction

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (g 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, higher, energy level.

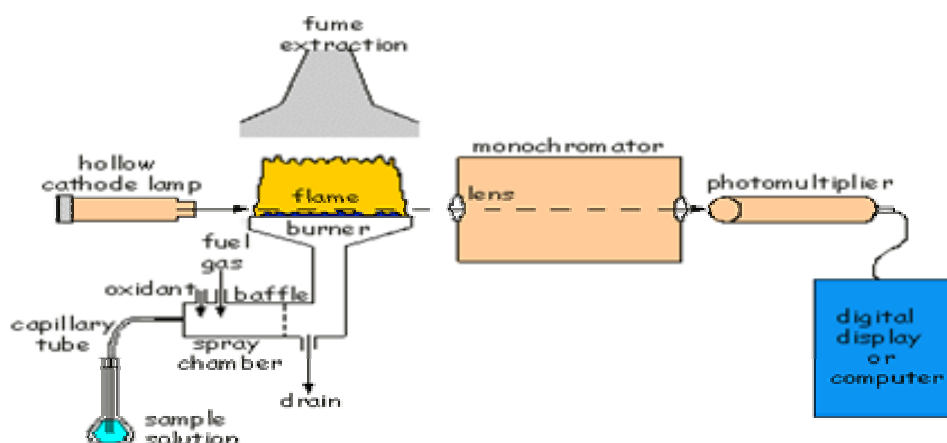


Figure 2.1 Schematic Diagram of Atomic Absorption Spectrometer (AAS)

(R. Levinson *et al* 1998),

Atoms of different elements absorb characteristic wavelengths of light. Analyzing a sample is to see if it contains a particular element means using light from that element. For example with lead, 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 – i.e. converted into ground state free atoms in the vapor state – and 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 there is in the vapor, the more radiation is 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 lead concentration under the same conditions as the unknown. The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample. Consequently an atomic absorption spectrometer needs the following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed

2.2.2 The light source

The common source of light is a 'hollow cathode lamp' (Fig. 2.2). 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 an inert gas – e.g. neon or argon at a pressure of between 1 Nm⁻² and 5 Nm⁻².

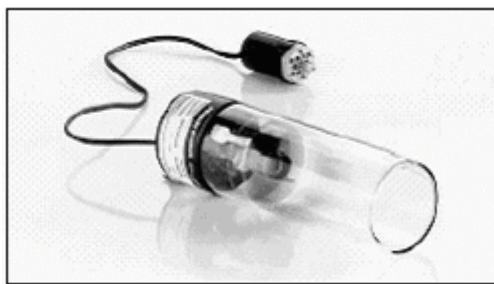


Figure 2.2 Hollow Cathode Lamp (Source: (R. Levinson *et al* 1998))

The ionization of some gas atoms occurs by applying a potential difference of about 300–400 V between the anode and the cathode. These gaseous ions bombard the cathode and eject metal atoms from the cathode in a process called sputtering. Some sputtered atoms are in excited states and emit radiation characteristic of the metal as they fall back to the ground state – e.g. Pb^* . Pb^{+h} (Fig.2.3). The shape of the 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 redepositing on the cathode. (R. Levinson *et al* 1998)

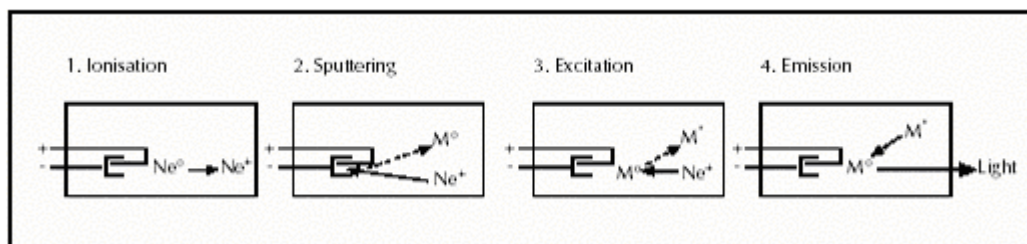


Figure 2.3 Some sputtered atoms are in excited states and emit radiation
(R. Levinson *et al* 1998)

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

2.2.3 The optical system and detector

A monochromator is used to select the specific wavelength of light – ie 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 (Fig. 2.4).

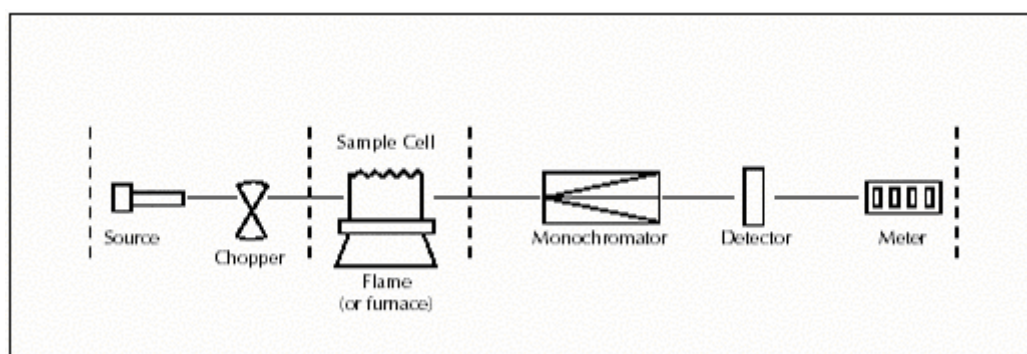


Figure 2 4. Shows electrical signal proportional to the light intensity
(R. Levinson *et al* 1998)