Designing of Experiments

The biosorption experiments involve the following major steps:

Preparation of Stock Solutions: Analytical grade reagents should be used throughout the experiments. The stock solutions of desired concentrations of metals under study are to be prepared by weighing requisite amount of their parent salts and dissolving them in double distilled water. Working solutions should be freshly prepared from stock solutions for each experimental run. Accuracy of weighing should be carefully kept in mind.

Biosorbent Preparation: The sources of agricultural wastes selected for the study should be identified from authenticated taxonomy division. The biosorbents should be washed repeatedly with water to remove dust and soluble impurities, shade dried, crushed, and finally sieved through copper sieves of different mesh sizes.

Sorption Studies for Single Metal System: Batch experiments of at least three replicates should be performed in clean air-conditioned environmental laboratory using standard practices as a function of particle size, biomaterial dosage, contact time, metal concentration, and pH. After proper pH adjustments, a known quantity of biosorbents is to be added. Finally metal bearing suspensions should be kept under magnetic stirring until the equilibrium conditions are reached. After shaking, suspension is to be allowed to settle down. The residual biomaterial sorbed with metal ions should be filtered using Whatman 42 filter paper. Filtrate is to be collected and subjected for metal ion estimation.

Sorption Studies for Multi-metal System: Multi-metal solution of each metal ion under study should be prepared. After pH adjustments, requisite quantity of biomaterial is to be added and the suspension should be magnetically shaken for 40 min. The residual biomaterial sorbed with metal ions is to be filtered using filter paper, and to the filtrate, metal estimations should be carried out. Percent metal sorption by the sorbent should be computed using the same equation as employed in case of single metal system.

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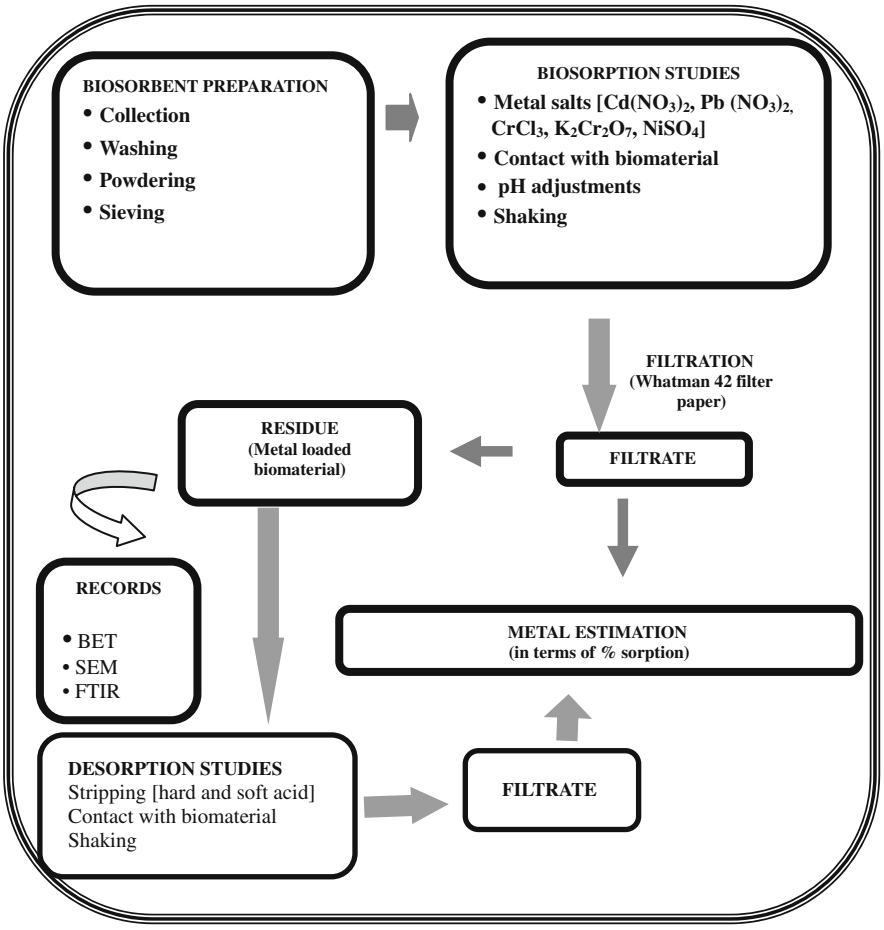
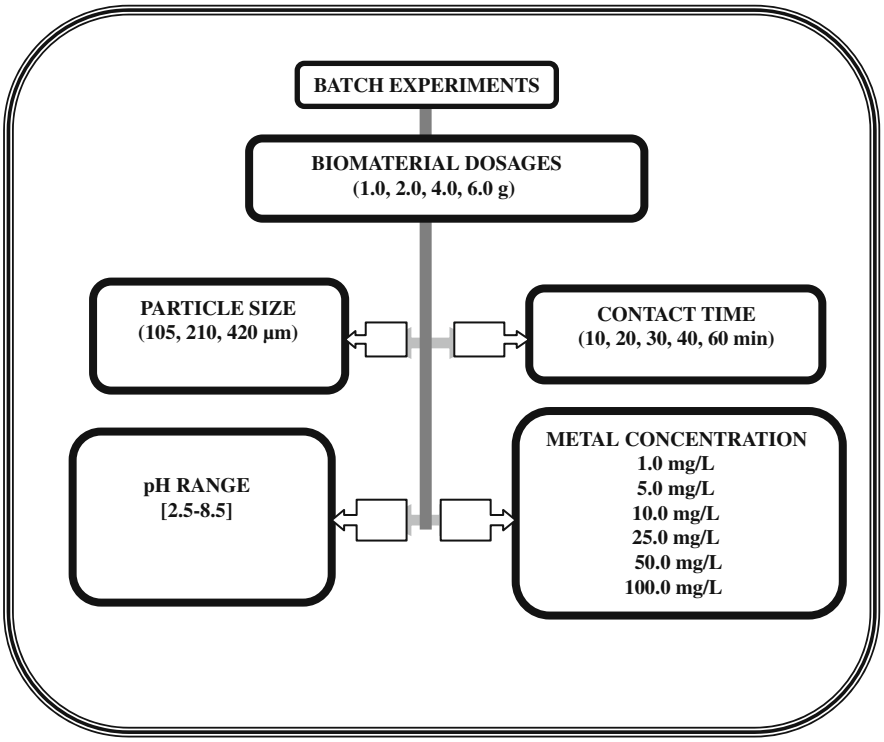


METHODOLOGY AT A GLANCE

Metal Analysis Using Various Instruments

Metal ions are analyzed using various analytical instruments like *atomic absorption spectrometer (FAAS and GFAS), ultraviolet spectrometer, gamma spectrometer,* and *neutron activation analyzer (NAA).*

Some important instruments specifically used for this purpose are discussed below.



EXPERIMENTAL CONDITIONS AT A GLANCE

Instrumentation

Atomic Absorption Spectrometer

The metal is to be estimated by *FLAME ATOMIC ABSORPTION SPECTROMETER* using its cathode lamp.

Atomic absorption spectrometer (AAS) is an efficient method from the viewpoint of chemical analysis. In this method, the element to be analyzed in a sample can be determined quantitatively by measuring the absorbance value at the specific wavelength of the analyte element. In AAS four techniques are commonly used, like

Fig. 1 Atomic absorption spectrometer

flame technique (Fig. 1), *graphite furnace technique, cold vapor technique,* and *hydride generation technique*.

Principle

Atomic absorption spectrometry is based on the principle of measurement of decrease in light intensity from a source when it passes through a vapor layer of the atoms of the analyte element. In atomic absorption spectrometry, a light beam is directed through the flame, into a monochromator, and on to a detector that measures the amount of light absorbed by the atomized element in the flame. Because each metal has its own characteristic absorption wavelength, a source lamp composed of that element is used, which makes the method relatively free from spectral or radiation interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

Sensitivity and Detection Limit

The *sensitivity* of flame atomic absorption spectrometer is defined as the metal concentration that produces absorption of 1% (an absorbance of approximately 0.0044). The *detection limit* is defined as the concentration that produces absorption equivalent to twice the magnitude of the background fluctuation. The detection limit for a given analytical procedure is the concentration that can be detected with a stated statistical certainty:

$$C = K\ \sigma/\mu$$

where C = detection limit; σ = absolute standard deviation; μ = sensitivity; K = a factor, usually taken as 2 or 3, depending on the required statistical certainty and should be stated.

Sensitivity and detection limits vary with the instrument, the element determined, and the technique selected. The most widely used flame for atomic absorption spectrometry is the air–acetylene flame and the nitrous oxide–acetylene flame with premix burners. Flow rate of fuel gas is 2.2 L/min. The metal is to be directly aspirated into the air–acetylene flame of an AAS and measured at a wavelength of 232 nm at a slit width of 0.2 nm.

Neutron Activation Analyzer

Neutron activation analysis (NAA) is an important technique for quantitative multi-element analysis of major, minor, trace, and rare elements (Fig. 2).

Fig. 2 Neutron activation analyzer

Principle of Neutron Activation Analysis

The initial step in neutron activation analysis is irradiating a sample with neutrons in a nuclear reactor or sometimes in other neutron sources. The stable nucleus absorbs one neutron and becomes a radioactive nucleus. The concentration of the stable element of interest in the sample can be measured by detecting the decay of these nuclei.

In NAA, stable nuclide (^{A}Z, the target nucleus) samples undergo neutron capture reactions in a flux of neutrons. The radioactive nuclides produced in this activation process usually decay by emission of a beta particle (β^{-}) and gamma ray(s) with a unique half-life. A high-resolution gamma-ray spectrometer is used to detect these

"delayed" gamma rays in the presence of the artificially induced radioactivity in the sample for both qualitative and quantitative analyses.

NAA Detectors

There are a number of detector types and configurations used in NAA. Most are designed to detect the emitted gamma radiation. The most common types of gamma detectors encountered in NAA are the *gas ionization* type, *scintillation* type, and the *semiconductor* type. Scintillation-type detectors use a radiation-sensitive crystal, most commonly sodium iodide, NaI (TI), which emits light when struck by gamma photons. These detectors have excellent sensitivity and stability and a reasonable resolution. NAA can detect up to 74 elements depending on the experimental procedure.

Detection Limit

Activation analysis determines the total amount of an element in a sample in grams (or micrograms). A certain amount of an element, say arsenic, is needed in the sample for detection. For arsenic, under ideal conditions, 5 ng is required. To determine 5 ppb of arsenic, 1 g of the sample is enough – to determine 0.5 ppb of arsenic, 10 g of sample is necessary, etc. In some cases, it is possible to "push" detection limits by longer irradiations. If a sample is irradiated twice as long, it becomes twice as radioactive, or the detection limit is improved. This is true up to one half-life, or so, of the isotope to be determined. One disadvantage to this approach is the cost of nuclear reactor time.

Doubling the counting time can also improve detection. However, background noise from the environment limits this approach. Doubling the counting time will not help if all this does is double the background noise level.

Usually the detection limit depends on the "other" elements in the sample – the matrix. If an element in the sample becomes radioactive, besides the element of interest, the background noise may be too high to determine the desired element at low levels. This noise does not produce wrong results, just high detection limits. The signal-to-noise ratio will improve with time if the element of interest has a long half-life compared to the element(s) producing the noise.

Accuracy and Precision

Accuracy is how close the determination is to the actual value. Precision is how close replicate determinations are to each other. An analysis may be very precise (duplicates may have the same results), but not very accurate (the real value is much different). In neutron activation analysis, precision usually varies from 2 to 5% of the value obtained – independent of concentration. This precision depends on the background noise level and the concentration of the determined element. Using standards, the accuracy has been determined to be within the precision, provided

"gross" errors have been avoided. Gross errors include wrong sample weight and mathematical mistakes.

For trace element determinations, the accuracy and precision of neutron activation analysis cannot be matched. However, for major constitutions another approach should be considered.

Sensitivity

The sensitivities for NAA are dependent on the irradiation parameters (i.e., neutron flux, irradiation, and decay times), measurement conditions (i.e., measurement time, detector efficiency), nuclear parameters of the elements being measured (i.e., isotope abundance, neutron cross section, half-life, and gamma-ray abundance). The accuracy of an individual NAA determination usually ranges between 1 and 10% of the reported value.

All methods for detection of radioactivity are based on the interactions of the charged particles or the electromagnetic rays with matter traversed. Electromagnetic radiations like X rays and gamma rays lose their energy in a stopping material mainly through three mechanisms, namely (i) photoelectric effect, (ii) Compton scattering, and (iii) pair production. These processes are strongly dependent on the energy of photon and atomic number (Z) of the stopping material. Other effects such as Rayleigh scattering and Thompson scattering are much less important and can be ignored in the detection process (Fig. 3).

Fig. 3 NaI (TI) gamma-ray detector coupled to a 4 K MCA

Photoelectric Effect

In this process, a photon is absorbed in the medium and energy is transformed to one of the electrons (normally tightly bound orbital electron); then the velocity of

that electron will be too high to remain in the orbital resulting in its emission. The difference between the incident photon energy (E_i) of the electron and the binding energy of the photon (E_b) appears as the kinetic energy of the ejected electron (E_e):

$$E_e = E_i - E_b$$

The probability of the emission of the electron is in the order of *K>L>M>N*... electrons if the energy of the photon is high enough. Photoelectric effect is characterized by the total absorption of the photon energy within the medium and is the predominant mode of interaction of low-energy gamma rays.

Compton Scattering

In this process, the photon interacts with an electron that may be loosely bound or free. The incoming photon is deflected through an angle with respect to its original direction and a fraction of its energy is transferred to the electron. The energy of the electron and the scattered photon is

$$E_g = E_o/[1 - E_o(1 - \cos\theta)/mc^2]$$

$$E_e = E_o - E_g$$

where E_o is the energy of the incident photon, E_g is the scattered photon energy, E_e is the scattered electron energy, m is the electron rest mass, and c is the velocity of light. θ is the angle between scattered and incident gamma-ray spectra.

Pair Production

This process involves the complete absorption of a photon in the vicinity of an atomic nucleus with the formation of an electron–positron pair. In accordance with the momentum conservation, this interaction mainly occurs in the field of the nucleus of the absorbent material. Pair production cannot occur when the energy of gamma radiation is less than 1.02 MeV that is equivalent to the rest mass of $e^- - e^+$ pair with zero kinetic energy. The cross section for pair production is also proportional to Z^2. At high energies, where pair production is the predominant process, gamma-ray energies can be best determined by measurement of the total energies of electron–positron pairs. Pair production is always followed by annihilation of the positron, usually with the simultaneous emission of two 0.51 MeV photons. The absorption of quanta by the pair production process is, therefore, always complicated by the appearance of this low-energy secondary radiation with its associated Compton.

In summary, all the three processes produce moving electrons in matter that can be detected directly or can initiate other electronic processes to obtain an electric

charge pulse that represents the initial photon energy. The recorded pulse is proportional to the energy lost in all the three processes. Full energy photopeak results from the complete energy deposition of the photon in the detector by any one or the combination of the three processes mentioned.

Simple Counting System

A basic measurement in many physics experiments is a simple counting of the number of pulses from a detector. In this setup, the analog signal from a detector is amplified by the preamplifier, shaped, and further amplified by the amplifier. The resulting analog signal is then sent through the low-level discriminator that delivers a signal for every input pulse with amplitude greater than the threshold value. The signal is then sent to the timer/scalar that counts each arriving pulse for a giving counting period *T* preset in the timer section. Discriminator serves the dual purpose of excluding low-level noise in analog suit and shaping the accepted signal to a form suitable for the scalar to accept.

General Principle of Detection

In all radiation detectors, deposition of radiation in its volume causes ionization releasing electric charge (in the case of scintillator emitted light is converted to electric charge in photomultiplier tube) and effective collection of this charge, under the applied electric field, forms the basic signal of the interacting radiation. The ionization results in a very low current or voltage pulse that needs proper amplification. The amplified pulses are further processed to obtain the number of radiations (counting) or the energy and the intensity of the radiations (spectrometry). Interaction time is very small, a few nanoseconds in gases and a few picoseconds in solids. These times are so short that the deposition of the radiation energy can be considered instantaneous. The detector acts like a capacitor and the charge collected in the capacitor can be discharged through a resistance giving a voltage pulse.

NaI (TI) Scintillator Detector

Scintillation detectors are based on the conversion of the absorbed energy into light and detection by the use of photomultiplication. Among the inorganic scintillators, NaI, activated with 0.1–0.2%, thallium is, by far, the most widely used. The high density (3.7 g/cm^3) of NaI and the high molecular weight of iodine make this a very efficient gamma-ray detector. Approximately 30 eV of energy deposition in a NaI (TI) crystal is required to produce one light photon, and it takes on an average about 10 photons to release one photoelectron at the photocathode of the multiplier. These photoelectrons are then accelerated by a potential of the order of 100 V to the first dynode where each one produces "*n*" secondary electrons; these

secondary electrons are then similarly accelerated and multiplied n-fold at the second dynode, and so on. With 10 dynodes and with n typically about 3 or 4, the total multiplication factor is n^{10} or of the order of 10^5 or 10^6. Thus a 0.3 MeV gamma ray absorbed in a NaI (TI) crystal might produce 10^4 light photons giving 10^3 photoelectrons and leading eventually to an output pulse of about 10^8 electrons or 1.6×10^{-11} coulomb (C). In an output circuit of about 10^{-10} F capacity this would be a pulse of about 0.16 V requiring further amplification. There is a good correlation between the energy absorbed in the scintillator and the size of the output pulse.

An additional feature in the NaI (TI) spectra is the so-called iodine escape peak about 28 keV. It results from the absorption of gamma ray near the surface of the detector and the subsequent escape of the K–X ray of the iodine. This effect becomes less pronounced with increasing gamma-ray energy because fewer of the initial interactions take place near the surface.

Multi-channel Analyzer

Multi-channel analyzer (MCA) is used widely for pulse height analysis. The basic function of the MCA is to sort out the incoming pulses from the detector according to the pulse height and keep the count of the number of pulses at each pulse height in a multi-channel memory. The content of a large number of channels is known as pulse height spectrum which can be displayed on the visual display unit for monitoring.

MCA works by digitizing the amplitude of the detector pulse heights with a nuclear analog to digital converter (NADC). The MCA then takes this channel address and increments by one of the corresponding memory channel contents whose address is proportional to the digital number. In this way pulses are sorted out according to the height of the analog pulse and the number at each pulse height stored in the corresponding memory locations. As a result of this histogram process, one builds up the pulse height spectrum. The total number of channels into which the voltage range is to be digitized is known as the "conversion gain" and it determines the resolution of the MCA.

Interference in Gamma Counting

There are several factors such as background radiations, sample geometry, and dead time which affect the count rate or counting efficiency of the detector. These factors are as follows.

Background Radiations

Interfering background in gamma spectra originates from either within the sample being counted (Compton produced or due to the presence of other radionuclides) or the environment. The Compton increases if the sample being analyzed has a high

content of high-energy gamma-emitting radioisotopes. For extremely weak samples, the environmental background becomes more significant. This can be reduced using massive shielding generally made of lead.

Sample Geometry

The sample size should be selected as large as possible for maximum efficiency. The sample was distributed uniformly so as to minimize the distance between the sample volume and the detection itself.

Dead Time

Radiation produces a pulse in the detector subsequent to its interaction. While this pulse is being processed, detector is not available for processing the next pulse that might be generated during this interval. Because of the random nature of radioactive decay, there is always some probability that a true event will lose because it occurs too quickly following a preceding event. The minimum time separation required to record two successive events as two separate pulses is usually called the dead time of the counting system. The dead time loss increases with increase in the count rate. The detector should have small dead time so that many counts are not lost just because the detector is still processing an earlier pulse. The percentage dead time is given by

$$\%\,\text{Dead Time} = (\text{True Time} - \text{Live Time}) \times 100/\text{True Time}$$

Energy and Efficiency Calibration

The detector should be calibrated for the gamma-ray energy of a particular isotope using gamma reference standards (Module Disc Type, provided by Electronics Corporation of India Ltd.). Details of gamma reference standards are tabulated below.

Decay characteristics of few radionuclides used for calibration:

GAMMA REFERENCE STANDARDS – R 2300 B			
ISOTOPE	GAMMA ENERGY (MeV)	NOMINAL ATIVITY	HALF-LIFE
Co	0.123	3.0	273 days
Co	1.17; 1.33	1.0	5.3 years
Cs	0.662	1.0	30 years

The gamma-ray intensity (A) is given by the following expression: Net Count Rate (P) = Abundance (A) × Efficiency (E_f) × Disintegration Rate (D), where "P"

is the photo peak area, "E_f" is the efficiency for detection of a gamma ray with energy "E," and "D" is the disintegration rate of the sample. The efficiency of the detector depends on the gamma-ray energy and the detector type, its shape, and size. At low energies, the attenuations of the gamma rays in the window and dead layer are predominant. This effect reduces as the energy of the gamma ray increases. For high-energy gamma ray for a given size, detector efficiency again falls due to greater chance escape of Compton-scattered gamma ray. Thus depending on the type and size of the detector, efficiency peaks at certain energy falls on both sides of this value.

Calculation for Metal Uptake

Metal uptake by biosorbent should be calculated using the mass balance equation for the biosorbent: $q = [V(C_i - C_f)]/W$, where q = metal uptake (mg metal/g dry weight), V = volume of metal-bearing solution contacted (batch) with the biosorbent (L), C_i = initial concentration of metal in solution (mg/L), C_f = final concentration of target seed biomaterial in solution (mg/L), W = dry weight of biosorbent added (g).

Sorption Isotherm

The adsorption capacity and affinity of biosorbent for metal ions are to be determined with two well-known isotherm models.

Freundlich sorption isotherm is given in the form of a linearized equation as follows: $\log q = \log K_f + 1/n \log C_e$, where q is the metal uptake per unit weight of biosorbent, C_e is the equilibrium (residual) concentration of metal ion in solution, K_f and n are the characteristic constants. *Langmuir equation* has the general form as $C_e/q_e = (1/Q_o b) + (C_e/Q_b)$, where C_e is the equilibrium concentration, q_e is the amount of adsorbed metals at equilibrium, Q_o and b are the Langmuir constants related to adsorption capacity and energy of adsorption, respectively. The biosorption capacity (K_f and Q_o) and biosorption intensity/energy ($1/n$ and b) are to be estimated from the slope and intercept of the Freundlich and Langmuir isotherms.

Considerations for Desorption Experiment

Desorption experiments are to be carried out in order to explore the feasibility of recovering the metals and reuse the metal-treated biosorbent for further cycle of sorption. Desorption studies (batch process) are to be conducted to restore the biomaterial as a function of concentration of different desorption reagents: *hard acid* [0.05 M HNO_3 and HCl], *soft acid* [0.5 M citric acid], *base* [0.05 M NaOH], and distilled water. Metal-loaded biosorbent obtained from sorption experiments should be transferred to Erlenmeyer flasks and shaken with 50 mL of each desorption reagent as a function of time (20, 40, 60, and 80 min) at room temperature. At the end of each time interval the suspension should be stirred for 5 min. The suspension is to

be filtered using Whatman 42 filter paper and in the filtrate estimation of metal ion concentration is to be carried out.

The amount of metal ion remaining on the biomaterial as a function of time is to be calculated using the mass balance equation: $q_t = q_e - c_t$ (v/m), where q_t and q_e are the biomaterial phase metal ion concentration (mg/L) and c_t solution phase metal ion concentration (mg/L) at time t (min), respectively.

Statistical Analysis

Batch experiments should be conducted in three replicates ($N = 3$) and data represent the mean value. Correlation coefficient and standard deviations should be calculated using SPSS PC^{+TM} statistical package. For the determination of intergroup mean value differences, each parameter should be subjected to the student-t test for significance level ($p < 0.05$).