CHAPTER IV

EXPERIMENTAL MATERIAL AND METHODS
4.1. Analytical procedures of batch biosorption studies

The biomass was collected from a treatment plant of brewery industry, washed several times with distilled water and then dried by vacuum drying in an oven at 100°C for 3 hours. Then its dried form was grounded with a mortar and pestle. The powdered form was stored in a sealed bottle with a silica gel to prevent re-adsorption of moisture. Heavy metal stock solutions (1000mg/l) were separately prepared by dissolving analytical grade nitrate salts of copper, cadmium, chromium and nickel, in deionized distilled water. Solutions of different concentrations were obtained by diluting the stock solutions. Batch experiments for determining metal biosorption isotherms were carried out with the initial cadmium concentrations ranging from 10 to 100 mg/l and biomass concentrations ranging from 0.5 to 3.0 grams per litre. The total reaction volume was 50 ml in 250 ml flasks. The pH range chosen for the sorption is also based on avoiding metal precipitation and was adjusted to the required value with 0.1 mol/L HNO₃ or 0.1 mol/L NaOH hourly, throughout the experiment. The mixtures were shaken on a rotary shaker (agitation rate, 200 rpm) for 2.5 hours, and then set still 1 hour to reach equilibrium. Reaction bottles were agitated on an orbital shaker at 25°C for 2.5 hours to reach metal biosorption equilibrium. All experiments were performed in duplicate. At designated intervals, samples were taken and the biomass was separated by filtration. Samples were then filtered through 0.2 millipore filters in order to remove solid particles and then were analyzed to determine the ion concentration by an atomic absorption spectrophotometer (Model GBC 932 Plus Australia). For the kinetic experiments, 25-200 mg of biomass was contacted with 50 ml of the metal ion solutions of initial concentration between 10-100 mg/l, keeping the same conditions as described above. At scheduled time intervals, 8 ml solution samples were drawn out and the concentrations of the residual heavy metals were analyzed. The depleted metal solutions were then analyzed to assess the metal concentration decline. Equilibrium isotherms were obtained using sample doses of 0.5g/50 ml solution and a range of initial metal concentrations between 10 and 100 mg/l. The general procedure depicted above was followed, applying the same experimental conditions. The suspensions were stirred for the time required to attain
equilibrium, as determined from kinetic measurements. Duplicate experiments were carried out for all the experiments. Average values are reported. In all the tests, metal and sorbent free blanks were also used for control. Sorption capacity “q” is the amount of metal ion (mg) biosorbed per g (dry weight) of biomass. \( X_m \) (qmax) is the maximum amount of metal which can be up taken by biosorbent. Maximum metal sorption capacity (q) was determined by the decrease in metal concentration in the solution after addition of different amounts of biomass and equilibration. The q value was calculated using the concentration difference method.

The amount of metal ions sorbed at equilibrium per unit mass of biosorbent was determined according to the following equation;

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q = \frac{v(C_i - C_f)}{m}
\]

4.2. Dynamic metal biosorption studies in continuous flow packed bed column

A series of experiments were conducted with various influent simulative wastewater and dried sludge columns. The experiments involved two sections: biosorption of single-metal and competitive biosorption of dual-metal ions. A 2.0 cm bed glass wool was placed at the bottom and top of the column prior the active biomass bed, in order to ensure homogenous distribution of the feeding solution. The biomass was first washed by slowly flooding the column with distilled water from the bottom. A known quantity of dried sludge was packed in the column to yield the desired bed height of the sorbent. The metal solution was pumped from a storage tank into the column, whose pH value was adjusted to desired optimum values, using a 1.0 M HCL acid solution. The metal solution was pumped to the column in a down-flow direction by a peristaltic pump at a certain rate, at a flow rate 3.0-5.0 mL/min, which allowed different detention times within the column. Liquid samples of the column effluent were collected at predefined time intervals. In each operation phase of the column, metal sampling and analysis were being continued until the breakthrough curve was being formed. Samples of column effluent were collected from the bottom by means of a fraction collector and were analyzed for heavy metals content using the flame atomic absorption spectrophotometer. (AAS) Model GBC 932 Plus Australia). The experiments
were terminated when the concentration of the treated solution reached the inlet concentration of the metal-bearing solution, the column feed was switched to distilled water for a defined time interval, in order to elude the heavy metals. All the experiments were conducted at room temperature (24 °C). The breakthrough time \( (t_b) \), the time at which dye concentration in the effluent reached 5% of the influent) and bed exhaustion time \( (t_e) \), the time at which the metal concentration in the effluent reached 95% of the influent) were used to evaluate the breakthrough curves. The slope of the breakthrough curve \( (\text{dc/dt}) \) was determined from \( t_b \) to \( t_e \). Each experiment was repeated twice. All chemicals used in this study were of analytical grade.

4.3: XRD diffractogram, Infra-red/spectrum, TGA thermogram analysis

The structure of various sludge used as biosorbent was studied using X-ray diffractograms (XRDs) obtained from an X-ray diffractometer (Brueker AXS, Diffractometer D8, Germany). The X-ray diffraction analysis was done by using Cu Kα as a source and Ni as a filter media, and K radiation maintained at 1.542 Å. Goniometer speed was kept at 2°min⁻¹. The range of scanning angle \((2\theta)\) was kept at 10–90°. The intensity peaks indicate the values of \(2\theta\), where Bragg's law is applicable. The identification of compounds was accomplished by using the ICDD library.

FTIR spectrometer (Thermo nicolet, Model Magna 760) was employed to determine the presence of surface functional groups in ACC, before and after the adsorption of metal ions, at room temperature over a spectral wave number range of 4000–400 cm⁻¹. Pellet (pressed-disk) technique was used for this purpose.

The thermal analysis [Thermogravimetric analysis (TGA), differential Thermal analysis (DTA), and Derivative thermogravity dTG)] of sludge samples were performed using a Perkin-Elmer Pyris Diamond TG/TGA instrument at a fixed heating rate of 20 K/min over a temperature range of 25–1000 °C.