2.1 MATERIALS

2.1.1 Monomers and Crosslinker Agents

Acrylic acid (AA, 99%) and 2-(diethylamino) ethylmethacrylate (DAEMA, 99%) were purchased and used after purification by column chromatography using silica gel as stationary phase (250 mesh) and vacuum distillation (700 mm Hg) respectively to remove the inhibitors and refrigerated until use. N-isopropyl acrylamide (NIPAAm, Aldrich, 99%), and 2-acrylamido-2-methyl-1-propansulfonic acid (AMPS, 99%), trimethylolpropane triacrylate (TMPTA, 99%), N, N¹-methylene bisacrylamide (N MBA, 99%) were purchased and used as received. The initiators such as potassium persulfate (KPS, 98%), ammonium persulfate (APS, 98%), potassium persulphate (KPS, 98%) ceric ammonium nitrate (CAN, 99%) from SD fine Chemicals, India were of analytical grade and purified by recrystallization using double distilled water.

2.1.2 Simple Chemicals

NH₂CONH₂, (NH₄)₂HPO₄, DAP) and K₂O (SPIC), KOH, NaOH, NH₄OH, CaSO₄, NaCl, Na₂CO₃ (Merck), MgCl₂, KCl, NaNO₃, K₂HPO₄.3H₂O, MgSO₄.7H₂O, CaCl₂.2H₂O, C₆H₈O₇, (NH₄)₅·Fe(C₆H₄O₇)₂, EDTA (di sodium salt), Na₂CO₃·H₂O, H₃BO₃, MnCl₂·4H₂O, ZnSO₄·7H₂O, Na₂MoO₄·2H₂O, CuSO₄·5H₂O, Co(NO₃)₂·2H₂O. Na₂SO₄·10H₂O, MgSO₄·7H₂O, NH₄Cl, NaH₂PO₄·2H₂O, Na₂HPO₄·12H₂O, Na₂C₂O₄, K₂Cr₂O₇, HgSO₄, 10- C₁₂H₈N₂ ,
FeSO₄·7H₂O, FeSO₄·(NH₂)₂SO₄·6H₂O (FAS), K₂Cr₂O₇, K₂HPO₄ (KHP), reactive blue 4 (RB 4, Sigma-Aldrich, molecular weight = 637.43, colour index no= 61205), potassium iodide (KI), rhodamine-B (Rh B, Himedia, Mumbai), benzoyl chloride (BC), hydroquinone (HQ), poly(vinyl alcohol), PVA, mercury(II) chloride, sodium arsenite were of analytical grade (Merck) and used as received. Sand, red and clay soils were collected from the local fields.

2.1.3 Synthetic and Natural Polymers

The synthetic SAPs Water Lock G 500 (received as free sample from Grain processing corporation, USA) and Water Keep purchased from USA were used as received. The biopolymer Chitosan (low density, 80–85% deacetylated) of inherent viscosity 12.2 dL/g in 0.1 M acetic acid at 30°C) purchased from Kerala State Co-operative Federation for Fisheries Development Ltd, Kerala, India was purified by precipitating from its 2% acetic acid solution using 1 N sodium hydroxide solution. Sodium alginate (NaAlg, Himedia, Mumbai) of intrinsic viscosity 420-1430 dl/g in water at 30°C was purified by re-precipitation using ethanol as non-solvent.

2.1.4 Solvents

Acetone, n-heptane (NICE, Cochin), methanol, isopropanol (Rankem, New Delhi), hydrochloric acid (NICE, HCl ), sulfuric acid (NICE, H₂SO₄), analytical grade N,N¹–dimethy formamide (DMF), dimethyl sulfoxide (DMSO), acetic acid purchased from Rankem, New Delhi were used as received.
2.1.5 **Effluent**

The distillery spent wash (DSW) used for the present investigation was collected from Bannari Amman Sugars (Distillery Unit) Limited, Erode, Tamil Nadu, India.

2.1.6 **Banana Pseudo Stem Based Adsorbents**

2.1.6.1 **Pre-treated BPS**

The banana pseudo stem (BPS) collected from a local agricultural land was used as a cellulosic biomass. The collected BPS was cut into small pieces and sundried till the complete removal of water and powdered by mechanical grinding. The powdered dry BPS (2000 g) was de-lignified by digesting in 3 liters 10% KOH solution at 80°C for 3 h and repeatedly washed with water to neutrality, air dried at room temperature and at 60°C in an air oven and used as adsorbent and as a raw material for the preparation of activated and un-activated carbons.

2.1.6.2 **Carbonized BPS**

A portion of the pre-treated dry BPS powder was carbonized at 900°C in an electric muffle furnace (Genuine, India) in nitrogen atmosphere (flow rate 150 cm³ min⁻¹) for 2 h. The carbonized material was subsequently washed with 10% HCl to remove inorganic impurities and with distilled water to neutrality (Girgis et al. 1994, Djilani et al. 2012). The less porous carbonized material thus obtained was chemically activated (Girgis et al. 1994, Djilani et al. 2012) by digesting it (500g/L) in a mixture of 10% H₃PO₄ and 90% HNO₃ solution for 3 h with constant stirring and it was heated in an air oven at 120°C for 4 h and filtered. The filtered mass thus obtained was washed repeatedly with hot and cold water to ensure neutrality and complete removal of soluble phosphates and nitrates. The resulted product was finally air dried.
at 110°C in an air oven for 10 h. The tapped density values of the prepared activated and inactivated carbon powder were determined by measuring the height and hence volume of 5g each of these carbon powders taken in a flat bottomed graduated test tube (dia = 3 cm) after consolidation/compression by manual tapping the test tube to constant sample height. Keithley's electrometer (6517 B) was used for the measurement of electrical conductivity of pelletized (at 12 ton) activated carbon (3×7 mm). An average of triplicate was employed in all the experiments. The surface area of carbonized BPS and its morphology were determined by Brunauer–Emmett–Teller (BET) adsorption method and SEM respectively.

2.1.7 Cyanobacteria and its Growth Media

The marine cyanobacteria Oscillatoria Boryana (OB) used for this study was obtained from the National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The chemical compositions (g/L) of media (ASN III) used for cultivating this OB were given below.

ASN III Composition (g/L)

**Solution A:** NaCl: 25.0, MgCl₂: 2.0, KCl: 0.5, NaNO₃: 0.75, K₂HPO₄.3H₂O: 0.02, MgSO₄.7H₂O: 3.5, CaCl₂.2H₂O: 0.5, citric acid: 0.003, ferric ammonium citrate 0.003, EDTA (di sodium salt): 0.0005, Na₂CO₃.H₂O: 0.02.

**Solution B:** H₃BO₃: 2.86, MnCl₂.4H₂O: 1.81, ZnSO₄.7H₂O: 0.222, Na₂MoO₄.2H₂O: 0.390, CuSO₄.5H₂O: 0.079, Co(NO₃)₂.H₂O: 0.0494.

Solution A was made up-to 1 L after adding 1mL of solution B and adjusting the pH to 7.5 using 0.1 N KOH solution.
2.2 SYNTHETIC URINE

Synthetic urine supersaturated with calcium oxalate was prepared (Costa-Bauzá et al. 2006) by taking a known volume of solution ‘A’ containing reagent grade Na$_2$SO$_4$$\cdot$10H$_2$O (19.34g), MgSO$_4$$\cdot$7H$_2$O (5.93g), NH$_4$Cl (86.7g) and KCl (162.3g) per liter and mixing it with an equal volume of solution ‘B’ having NaH$_2$PO$_4$$\cdot$2H$_2$O (15.45g), Na$_2$HPO$_4$$\cdot$12H$_2$O (15.5g), NaCl (223g) and Na$_2$C$_2$O$_4$ (0.57g) per liter. The pH of both solutions was adjusted in the range of 5.5 - 6.5 using 0.1 N KOH solution. Solutions A and B prepared in deionized water were stored for a maximum of 1 week at 4°C.

2.3 REAGENTS FOR COD AND BOD

The necessary reagents for COD determination (closed reflux titrimetric method) were prepared as reported (Cuesta et al. 1996, Cuesta et al. 1998). The digestion solution was prepared using 0.0167 M K$_2$Cr$_2$O$_7$, 3.0 M H$_2$SO$_4$, 0.11 M HgSO$_4$ and keeping 5.5 g of Ag$_2$SO$_4$ per kg of H$_2$SO$_4$. The ferroin indicator was prepared by dissolving 1.485 g of 1, 10-phenanthroline monohydrate and 0.695 g of FeSO$_4$$\cdot$7H$_2$O in water and diluting to 100 mL. The titrant, 0.005 M ferrous ammonium sulphate (FeSO$_4$($\text{NH}_2$)$_2$SO$_4$$\cdot$6H$_2$O, FAS) was standardized against K$_2$Cr$_2$O$_7$. The validity of this method for COD determination was verified using potassium hydrogen phthalate (KHP) as a standard sample (0.002 mol). The required reagents for BOD estimation were prepared as reported (Uğurlu et al. 2008) and the BOD values of different DSW samples were measured after appropriate dilution.

2.4 UV RADIATION SOURCE

A 254 nm UV irradiation was employed for all photo polymerization (crosslinking and grafting) experiment from a 10W high pressure mercury vapor lamp (Techinsto, India) in air thermostat (35±0.5°C) with forced air cooling arrangements.
2.5 METHODS

2.5.1 POLYMER SYNTHESIS

2.5.1.1 Trimethylolpropane Triacrylate Crosslinked poly
(potassium/ammonium acrylate-co-N-isopropyl acrylamide)
[TMPTA crosslinked poly(KAcoor AAco-NIPAAm)] (ANT)

TMPTA crosslinked SAP hydrogels from various proportions of monomers AA, KAc/or AAc, NIPAAm were prepared by free radical copolymerization using KPS initiator, at 70°C in aqueous solution (40 mL) in a 50 mL stoppered borosilicate tube after de-aeration by oxygen free nitrogen purging. The required amount of KAc/or AAc was prepared by partially neutralizing AA, using a known equivalent of KOH /or NH₄OH under ice cold condition. After polymerization the polymer hydrogel obtained was recovered by repeatedly washing with ice cold methanol immediately to check the reaction and to remove unreacted monomers. The crosslinked gels thus obtained were cut into small pieces and dried to constant weight at 50°C under vacuum. The dry polymer gel was powdered and further purified by Soxhlet extraction using acetone–methanol (1:1 v/v), at 50°C for 3–4 days, powdered, sieved (mesh size 100 and 150 micron) and stored after vacuum drying. The synthesized polymers were designated as ANT-1 to ANT- 32, Table 3.1, Chapter 3, p-140)

2.5.1.2 DNP from TMPTA Crosslinked poly (KAc-co-NIPAAm) (ANT) and NaAlg by their Ionic and Photo Crosslinking

2.5.1.2.1 Ionic Crosslinking of NaAlg

ANT-11 (1ˢᵗ network) was allowed to equilibrium swelling in an aqueous solution containing dissolved NaAlg. CaSO₄ slurry (0.1 M) in water
was added to the reaction mixture at 30°C and thoroughly stirred to form ionic crosslinks between NaAlg and ANT-11.

2.5.1.2.2 DNP Preparation by Photo-crosslinking

DNPs were prepared from ionically crosslinked NaAlg (IC-NaAlg) and ANT-11 by photo crosslinking aqueous solution containing known different amounts of IC-NaAlg and ANT-11 and designated as DNP-1 to DNP-24 (Table 4.1, Chapter 4, p-169). The ingredients were taken in a 100 mL beaker and nitrogen purged for 20 min to remove the dissolved oxygen. This was irradiated with UV light for the durations of 0, 6, 12, 18, 24, 30 h by keeping a constant distance (15 cm) between the lamp and solution surface in the beaker. The second network, ionically crosslinked alginate was subsequently photo crosslinked onto the first network (Yasuda et al. 2005). The obtained gel was washed by immersing in fresh distilled water twice per day over a period of 1 week to remove any unreacted materials and then dried in hot air oven at 60°C for 24 h. The dry polymer was powdered, sieved (mesh size 100 and 150 micron) and stored after vacuum drying.

2.5.1.3 Preparation of Semi-Interpenetrating Polymer Network (semi-IPN)

Semi-IPN was synthesized by photo polymerizing (UV light) AA, KAc, and NIPAAm monomers with TMPTA crosslinker of different composition (Table 5.1) in the presence of NaAlg aqueous solution (polymerization volume 40 mL) in a quartz tube using APS (0, 3, 6, 9 and 12×10⁻³ M) as photo initiator for different irradiation times (0, 2, 4, 6, 8 and 10 h) by keeping the distance between the lamp and solution surface in the tube as 15 cm. Prior to polymerization the solution was nitrogen purged for 20 min to remove the dissolved oxygen. During irradiation, monomers and crosslinker form a polymer in the presence of preformed NaAlg (Saber-Samandari et al. 2005).
2012, Wang & Liu 2013) resulting in semi-IPN formation. The semi-IPN thus obtained was separated and cut into small pieces and dried to constant weight at 50°C under vacuum. The dry polymer was then powdered and further purified by Soxhlet extraction using acetone–methanol (1:1 v/v) at 50 °C for 3–4 days, powdered, sieved (mesh size 100 and 150 micron) and stored after vacuum drying. The semi-IPNs thus prepared for different compositions of the substrate were represented as semi-IPN 1 to Semi-IPN 24 (Table 5.1, Chapter 5, p-191)

2.5.1.4 Modified Chitosan Hydrogel (ACAD) from Acryloylated Chitosan, AMPS, DAEMA and NMBA Crosslinker

2.5.1.4.1 Synthesis of Acryloyl Chloride (ACOCl)

ACOCl was synthesized by refluxing AA (0.8 mol), BC (2.66 mol) and hydroquinone (2g, polymerization inhibitor) in a 500 mL round bottom flask at 80°C for 2 h in nitrogen atmosphere and collecting the fraction distilled at 90°C (Bharathi et al. 2010). The liberated HCl was trapped in 0.1 N NaOH solution. The collected impure ACOCl was redistilled at 72°C and the collected fraction (yield 65%) was used in subsequent experiment.

2.5.1.4.2 Acryloylation of Chitosan (AC-chitosan)

In a typical experiment chitosan solution was prepared by dissolving ten grams of purified chitosan powder using 50 mL of 1% acetic acid taken in a 250 mL round bottom flask. The flask was charged with 75 mL DMSO and the mixture was heated to 100°C to facilitate complete dissolution of chitosan. To this 20 mL of DMF and 1.5 mL of pyridine (catalyst) were added under continuous stirring over a period of 5 min and cooled to 0°C. In sequence, 3 mL of ACOCl in 5 mL DMF was added drop wise to this solution with constant stirring and left aside for 2 h at 0°C (Jayakumar et al. 2005,
Pourjavadi et al. 2010). The ACC thus formed was precipitated in acetone and repeatedly washed with acetone, air dried and stored for further use.

### 2.5.1.4.3 Synthesis of ACAD

Twenty two samples of ACADs as adsorbent materials of different compositions (Table 6.1, Chapter 6, p-222) designated by ACAD-0, ACAD-1, ACAD-2… ACAD-20 and ACAD-21 were synthesized by radical copolymerization taking various amounts of acryloylated chitosan, AMPS and DAEMA using NMBA as a crosslinker and KPS as thermal initiator in a 50 mL stoppered borosilicate tube, at 70°C after nitrogen purging. ACADs thus obtained were recovered by adding ice cold methanol and washed repeatedly with methanol. The crosslinked gels were cut into small pieces and dried to constant weight at 50°C in an air oven. Further, these ACADs were powdered and purified by Soxhlet extraction using acetone–methanol (1:1 v/v), at 70°C for 48 h. The purified ACADs were powdered, sieved and stored after vacuum drying.

### 2.5.1.5 Photochemical Grafting of ACAD Hydrogel on BPS (MHPS)

ACAD hydrogel grafted BPS with a percentage grafting of 64.5 was synthesized photo-chemically (Saber-Samandari et al. 2012). For photo grafting of BPS, the powdered lignin free BPS, modified chitosan hydrogel and the ceric CAN solution (0.002 m) were taken in a quartz tube and nitrogen purged for 20 min to remove the dissolved oxygen and it was irradiated with UV light (254 nm) for the durations of 0, 3, 6, 9 12, and 15 h at a distance of 15 cm from the light source. The photo crosslinked gel thus obtained was washed repeatedly with water to remove any unreacted materials and then dried at 60°C in an air oven for 24 h. The dried polymer was powdered and further purified by Soxhlet extraction using acetone–methanol (1:1 v/v) at 50°C for 3–4 days, air dried, sieved (mesh size 100 and 150 micron) and stored after vacuum
drying. Thus prepared photo grafted hydrogels were designated as MHPS-1 to MHPS-8 (Table 7.1, Chapter 7 and p-246).

2.6 ANALYTICAL INSTRUMENT

2.6.1 Ultraviolet -Visible (UV-Vis) Absorbance Measurements

A double beam Ultraviolet -Visible (UV-Vis) spectrophotometer (Perkin Elmer Lamda 35) with spectral width 200-1100 nm was used to record the absorption spectra and to measure the absorbance values at $\lambda_{\text{max}}$ for RB 4 (573 nm), Hg$^{2+}$ complex (592 nm), AsO$_2^-$ ion complex (554 nm) and DSW (475 nm). An average of triplicate measurements were taken for the absorbance value to determine amount of RB 4, pollutants in DSW, growth profiles of OB (656 nm), arsenic and mercury metal ion.

The RB 4 dye concentration in the dyed cotton fabrics were estimated using visible absorption spectroscopy by measuring the absorbance at 601 nm using Gretag Macbeth EFI ES 1000 UVcut i1 Eye-One Pro Spectrophotometer.

2.6.2 Polymer Characterization

The structural, physical and chemical characteristics of polymers are required to correlate the physico-chemical properties of the polymer, fertilizer and different adsorbates. Different methods such as Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, thermo gravimetry, scanning electron microscopy, spectrophotometer and tensile-compressive tester were employed to characterize the synthesized and modified polymer hydrogels in the present study.
2.6.2.1 Fourier Transform - Infrared (FT-IR) Spectroscopy

FT-IR spectra of virgin polymer, modified natural and synthetic polymers, RB 4, BPS carbonized carbon, residues of DSW were recorded as KBr pellet containing 5-10 mg of the sample in the frequency range 400–4000 cm⁻¹ on JASCO FT-IR-460 and Shimadzu FT-IR-8400S spectrophotometers by accumulating 48 scans at a resolution of 2 cm⁻¹.

2.6.2.2 Proton Nuclear Magnetic Resonance (¹H-NMR) Spectroscopy

Proton NMR of chitosan and acryloylated chitosan were recorded using Bruker Avance III 500 MHz multi nuclei NMR spectrometer at 500 MHz for ¹H and 125 MHz for ¹³C (proton decoupled) in CD₃COOD/D₂O mixed solvents at 26°C for the chemical shift range 0–10 ppm using TMS as internal standard. To record the ¹H NMR and ¹³C spectra of chitosan and acryloylated chitosan, only the dissolved portion of those in CD₃COOD/D₂O mixed solvents obtained by immersing 5 mg of sample in 3 mL CD₃COOD/D₂O mixed solvents for 24 h were used.

2.6.2.3 Thermogravimetry/ Derivative Thermogravimetry (TG/DTG)

TG/DTG traces of crosslinked poly(AA), poly(KAc), poly (AAc), ANTs, DNPs, semi-IPNs, ACAD, MHPS-3, chitosan, acryloylated chitosan, BPS and ACAD/BPS blend grafted were recorded on TGA Q500 V20.10 Build 36 with a sample size of 1.5–3.5 mg under air atmosphere at a heating rate of 20°C/min for the temperature range from ambient to 900°C.

2.6.2.4 Scanning Electron Microscopy (SEM)

SEM micrographs of ANT, DNP and RB 4 adsorbed DNP-16, semi-IPN, ACAD and MHPS-3 at different magnifications were recorded using ZEISS EVO Series SEM model EVO 50. The equilibrium swelled and
Lyophilized samples were used for recording SEM micrographs. The SEM micrograph of BPS and carbonized carbon was recorded after purification.

### 2.6.2.5 Tensile-Compressive Tester

The mechanical strength of the water swollen ANT-11, DNP-16 semi-IPN-21 samples were measured at room temperature in terms of elastic modulus and ultimate tensile strain using tensile-compressive tester (Tensilon RTC-1310A. Orientec Co.) and also by visual observation of the physical appearance of the samples under compression. These tests were performed using swollen cylindrical gel samples of 10 mm diameter and 5 mm thickness. DNP-16 and SAP samples were kept on the platform of the compressor and a progressive load was applied through the upper plate of the compressor till the onset of damage, and this was measured as ultimate compressive stress. Their corresponding ultimate compressive strain and modulus values were also measured. The strain under compression was defined as the change in the thickness relative to the thickness of the specimen. For the tensile tests of DNP-16, semi-IPN 20 and ANT-11 samples were stretched parallel to the sample axis using a clamp attachment at a strain rate of 10%/min. Strain rates were referenced to the initial thickness of length of specimen. Failure points of compressive and tensile tests were determined from the peak of the stress–strain curve.

### 2.6.3 TDS Meter

Total water soluble solids in soils were measured in terms of TDS values of the water extracts of the soils (10 g each) using a known volume (100 mL) of double distilled water employing DiST 1 Hanna TDS meter with automatic temperature compensation at 27 °C.
2.6.4 Turbidity Meter

The growth profile was also monitored by measuring the turbidity on Nephelometer CL 520/ turbidity meter (Elico, India) at ambient conditions.

2.6.5 BOD Incubator

The BOD values of DSW were determined using BOD incubator (Genuine, India) by keeping samples at 20°C over a period of 5 days.

2.7 SWELLING STUDIES

2.7.1 Equilibrium Swelling (ES)

The degree of swelling of synthesized ANT, DNP, semi-IPN and ACAD were measured both in distilled water using tea bag (Pourjavadi et al. 2009) (i.e. a 100 mesh nylon screen with average mesh size in the range of 100–150 micron) method by taking 0.1 g of sieved sample in 200 mL of distilled water at 30°C under equilibrium swelling conditions (4 h). The swelling profiles were constructed by plotting the water-absorbed during various time intervals at 30°C against time. The initial slope of this plot (upto 60% swelling) was taken as the rate (R- mole/ min) of absorption. The equilibrium swelling was calculated by taking an average of three absorption measurements using the equation 1.6 (Chapter 1)

\[
ES = \frac{W_s - W_d}{W_d}
\]

(1.6)

where \(W_s\) and \(W_d\) are the weights of the swollen gel and the dry sample respectively. Similarly, the swellability of ANT samples (ANT-1 to ANT-24 & ANT – 32) in aqueous fertilizer solutions (1, 2, 3, 4 and 5 wt%) were measured by immersing ANT hydrogel in 200 mL distilled water and allowed to equilibrate (4 h) at 30°C. Then the tea bag was taken out from the solution and
the excessive water adhering to the gel surface was removed superficially with tissue paper. The swelling profiles were constructed by plotting the fertilizer uptake during various time intervals for typical samples, and at different temperature.

2.7.2 Absorbency Under Load (AUL)

Under field applications, ANT encountered significant stress from the weight of the soil which affected the swellability of ANT. Hence, the water-uptake of SAP was also evaluated under load. This was done by mounting a nylon mesh having a known weight of dry ANT-11 (0.1 g) on a porous sintered glass filter plate (dia 80 mm & thickness 7 mm) placed in a petri dish (d=118 mm, h=12 mm). The SAP was pressurized by applying a known load (1, 2, 3, 4 and 5 tons) via a stainless steel cylinder (d=74 mm, h=50 mm) kept on the SAP for 60 min (Figure 1.18). The measurement was done in an enclosure to minimize water loss. After this, the swollen particles were weighed and AUL was calculated (Marandi et al. 2008) using equation (1.32).

\[
AUL(g / g) = \frac{W_2 - W_1}{W_1}
\]

(1.32)

2.7.3 Fertilizer uptake and Release

To investigate the release of the fertilizer, 0.5 g of fertilizer loaded powdered ANT-11 was placed in 100 mL of double distilled water at 30°C to facilitate the fertilizer release. After every two days, 2 mL from this solution was pipetted and its fertilizer content was determined gravimetrically by vacuum drying at 60°C. From this the percentage of the fertilizer released was calculated (Teodorescu et al. 2009) using the equation (2.1).
% of fertilizer released

\[
\frac{(\Delta W)_n X[100 - (n - 1)X2]}{W_0} = \frac{2 + \sum_{i=1}^{n-1} (\Delta W)_i}{W_o}
\]  (2.1)

where \((\Delta W)_i\) was the weight of fertilizer released from the \(i^{th}\) 2 mL sample and \(W_0\) was the amount of fertilizer loaded in SAP.

2.8 POLLUTANTS ADSORPTION STUDIES

2.8.1 Batch Mode

The stock solution of RB 4 (7.84×10⁻³ M (= 4.9 g) was prepared using distilled water, and a series of concentrations of (1, 2, 3, 4 and 5×10⁻³ mg/L) dye solutions were prepared by appropriate dilution of stock solution in the presence NaCl (5g/L) as dye exhausting agent and Na₂CO₃ (1.5 g /L) as dye fixing agent. The adsorption studies of RB 4 were investigated as a function of the structure of adsorbent and environmental factors like time, temperature, pH, and ionic strength of the medium. All batch mode adsorption experiments were carried out by mixing 0.1g of semi-IPN 20 with 100 mL of aqueous solution (RB 4) taken in a 250 mL conical flask. The flasks were shaken in a thermostatic mechanical shaker for 24 h to provide sufficient contact time for equilibrium to be established between the solid and liquid phases at 30°C and 120 rpm. The experiments were conducted in triplicate and average values are reported. The solution was separated from the adsorbents using a 100-mesh (nylon) sieve and the concentration of RB 4 in dye effluent before and after the adsorption was measured spectrophotometrically after suitable dilutions. The equilibrium adsorption of RB 4 was calculated using the mass balance equation (2.2).

\[
q_e = (C_i - C_e) \frac{V}{W} \text{mg / g}
\]  (2.2)
where, $q_e$ was the equilibrium amount of dye adsorbed onto the adsorbents (mg/g), $C_i$ the initial concentration of dye in the dye effluent (mg/L), $C_e$ was the equilibrium concentration of the dye in solution (mg/L), $V$ was the volume of the solution (L) and $W$ was the weight of the adsorbent (g) $q_e$ was average value of three experiments.

2.8.2 Column Mode

Separate stock solutions of AsO$_2^-$ and Hg$^{2+}$ were prepared by dissolving 1.733 and 1.350 g of sodium arsenite and mercury (II) chloride, respectively in 1000 mL deionized water. A series of RB 4 solution of different concentrations (1, 2, 3, 4 and 5×10$^3$ mg/L) were prepared. The adsorption studies of AsO$_2^-$ and Hg$^{2+}$ from their respective solutions were performed on ACAD samples at different swelling time, temperature and pH. But the adsorption studies of RB 4 dye were performed on all DNP-16, semi-IPN 20 and ACAD samples. The adsorption experiments were done using separate identical glass columns of 2 cm diameter and 86 cm length. The columns were filled with 10 g of powdered and sieved adsorbents (ACADs) without voids. Then, the synthetic effluent prepared using metal ion/or dye were eluted through these columns at a constant flow rate (2 mL/min) at 30 °C. The effluent solutions were collected at different time intervals and the concentration of metal ion and dye in the collected fractions were estimated spectrophotometrically. An average of triplicate was taken for metal ion and dye concentrations. Similarly the adsorption capacity of virgin chitosan was tested for these adsorbates. The simultaneous removal (Taştan et al. 2010) of dye and metal ion using ACAD was also tested using a synthetic composite effluent containing RB 4 (5.3 ×10$^{-4}$ mol), AsO$_2^-$ (1.1 ×10$^{-3}$ mol) and Hg$^{2+}$ (1.23 ×10$^{-3}$ mol) Besides, the adsorption capacity of ACADs (showed maximum uptake behavior) were also compared to the reported (Bayramoglu et al. 2007, Pourjavadi et al. 2010) values on other adsorbents and virgin chitosan. The
amount of dye and metal ion uptake were calculated as per the mass balance equation (2.2).

\[ q_e = (C_i - C_e) \frac{V}{W} \text{mg/g} \]  

(2.2)

2.9 ESTIMATION OF ADSORBED DYE AND METAL ION FROM EFFLUENTS

2.9.1 Reactive blue (RB 4)

The recovered RB 4 residue from the treated was dissolved in 100 mL double distilled water and the concentration of RB 4 in the residue was determined spectrophotometrically after appropriate dilution by measuring it absorbance at 573 nm.

2.9.2 AsO\textsubscript{2}\textsuperscript{-}

To estimate the amount of AsO\textsubscript{2}\textsuperscript{-} ion, 20 mL of eluate containing residual AsO\textsubscript{2}\textsuperscript{-} ion was taken in 50 mL standard flask. To this 4 mL KI and con HCl solution and were added and the mixture was shaken gently, followed by the addition of 8 mL of 0.05% Rhodamine-B (Rh B). The solution was made up-to 50 mL with distilled water and left aside for 15 min. The absorbance of the complex was measured at 554 nm. The amount of metal ion, dye uptake and its molar absorption coefficient values were calculated using Beer–Lambert law equation (Pillai et al. 2000, Loo et al. 2012).

2.9.3 Hg\textsuperscript{2+}

For the estimation of Hg\textsuperscript{2+}, 2 mL each of 10.8 M H\textsubscript{2}SO\textsubscript{4} and 0.15 M KI solution were added to 20 mL of eluate containing residual Hg\textsuperscript{2+} was taken in a 50 mL standard flask and shaken for a min. Subsequently 2 mL of Rh B
(5×10^{-4} M) and 5 mL of 1% PVA were added to the above solution and made up-to 50 mL, and left aside for 10 min and the absorbance of complex formed was measured at 592 nm (Pillai et al. 2000, Loo et al. 2012). The amount of metal ion, dye uptake and its molar absorption coefficient values were calculated using Beer–Lambert law equation (Pillai et al. 2000, Loo et al. 2012).

### 2.10 Textile Dying Using Virgin and Recovered RB 4

The dyebath (2%) for dying process was prepared using cold brand RB 4 dye and keeping material: liquid ratio as 1:20. Then 5 g of well scoured, bleached and wetted cotton fabric was dipped in the dyebath for 10 min. The dye exhausting agent (NaCl, 3.5 g) was added in two installments and the dyebath was set aside for another 20 min, and subsequently 20 mL dye fixing agent (Na_{2}CO_{3}, 0.45 g) was added (Riera-Torres & Gutiérrez 2010) to the dyebath and allowed for dye fixation for another 60 min. The cold and hot water rinsing followed by detergent washing removed the unfixed dye from the fabric and these fabric washings with water were added into the dye bath solution. The visible absorbance for dyed and air dried fabric were recorded. The unfixed dye in dyebath after dying was recovered by passing through semi-IPN 20 in a column followed by elution using isopropanol eluent, and the dye was recovered through by distilling out of isopropanol. The adsorption-desorption of RB 4 was performed over five times for the same adsorbent to determine the frequency of reusability of DNP-16 and semi-IPN 20 in effluent treatment. After each cycles of adsorption-desorption, both DNP-16 and semi-IPN 20 were washed with distilled water thrice and then dried in an air oven at 60°C for reuse. The amount of total residue along with RB 4 in the solution was quantified after evaporation. Another dye bath was prepared with same dyebath concentration using recovered RB 4 for dying and the solid state absorption spectra were recorded for comparing the absorption spectra. The
color fastness properties of the dyed fabric with virgin and recovered RB 4 were also determined (under sunlight and 0.2% soap solution containing 0.5% Na$_2$CO$_3$ solution) over a period of 12 h by recording and comparing the absorption spectra.

2.11 CYANOBACTERIA (OB) GROWTH

Photo grown cyanobacteria was chosen for the bio remediation of pre-treated DSW. To optimize the irradiation time for the maximum growth of OB, growth curve was constructed using the following procedure.

ASN III was steam sterilized (110°C) and incubated at 30 °C. To this the cyanobacteria was added and photo cultivated by irradiation (40 W/m$^2$h) using fluorescent lamps. 200 mL of DSW 3 was taken in 500 mL conical flask and 1 mg of OB was added and photo incubated over a period of 18 days. The growth rate of OB in DSW 3 was monitored by spectrophotometry, turbidimetry and gravimetry by measuring the optical density of the inoculum at 656 nm, turbidity (turbidity meter reading) and dry weight of the pellet respectively for every two days. The biomass obtained was determined gravimetrically by centrifuging the inoculum and vacuum drying the pellet at 40 °C.

2.12 PHYSICOCHEMICAL TREATMENT OF DSW

The raw DSW was first eluted through a sand bath (15x10x15 cm) initially to remove the floating and suspended impurities if any. The top layer of sand was periodically removed from the bath in-order to avoid the slow filtration rate due to chocking of interstitial pathways of sand particles. The effluent thus obtained was subsequently treated by eluting through BPS to remove the coloring material of DSW partially, an activated carbon column (60x2 cm) at a constant flow rate (1 mL/min) at 30°C to adsorb the colored
melanoidin and other recalcitrant compounds to the extent of 95%, bio-
remediation using cyano bacteria, constant current electrolysis and finally
eluting through MHPS-3. The effluents coming out from these stages were
designated as DSW-1, DSW-2, DSW-3, DSW-4, DSW-5 and DSW-6,
respectively. The schematic diagram for the multistep bio-physicochemical
remedial processes followed in DSW treatment is displayed in Figure 2.1. The
adsorption capacity of un-activated BPS carbon was also tested under identical
conditions.

The afore mentioned sequence of DSW treatment in the present
study was followed on the ground that, (i) for bioremediation with OB to be
effective and efficient without dilution of virgin DSW, the dissolved impurities
and colored pollutant in DSW have to be minimized initially by the
economically viable physical methods such as filtration and adsorption; (ii) the
electro degradation could be effectively and economically employed to remove
only the traces of electro active chemical pollutants present in the partially
treated DSW and as well as those formed if any via bioremediation step, this
step was implemented only after bio-physical treatment of DSW; (iii) effective
removal of last traces of pollutant can be done on MHPS-3 adsorbent as it will
capture the pollutants by facilitating adsorption and entrapment mechanisms at
low concentrations of contaminants, and this sequence of treatment is briefly
given in Table 2.1.
Figure 2.1 Multistep methods in DSW treatment

1 Sand bed
2 BPS Column
3 Activated carbon column
4 Bioremediation via OB
5 Electrolysis
6 MHPS column
Table 2.1 Different steps/ methodology followed in DSW treatment

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Name of the treatment</th>
<th>Purpose</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Preliminary treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(i) Sand filtration</td>
<td>To remove the floating and suspended impurities</td>
<td>Water washed sand bed with periodical removal of filtered mass on top of sand bed</td>
</tr>
<tr>
<td>2</td>
<td>(ii) BPS as an adsorbent</td>
<td>To eliminate coloring material of DSW partially</td>
<td>De-lignified BPS</td>
</tr>
<tr>
<td>3</td>
<td><strong>Primary treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(i) Activated carbon as adsorbent</td>
<td>Removal of melanoidin and other recalcitrant impurities by adsorption</td>
<td>Activated carbon obtained by carbonization of BPS</td>
</tr>
<tr>
<td>4</td>
<td>(ii) Bio-remediation</td>
<td>Biodegradative removal of residual melanoidin and other recalcitrant compounds</td>
<td>Cyano bacteria (OB) grown photo chemically in ASN(III) medium</td>
</tr>
<tr>
<td>5</td>
<td><strong>Methods to remove final traces of pollutants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(i) Electrolysis</td>
<td>Electro-degradative removal of residual pollutants</td>
<td>Constant current electrolysis with graphite anode and aluminum cathode</td>
</tr>
<tr>
<td>6</td>
<td>(iii) Modified chitosan hydrogel as adsorbent</td>
<td>To remove the final trace impurities if any present in collected effluent in stage five</td>
<td>Modified chitosan hydrogels grafted BPS are excellent adsorbent capable of adsorb trace impurities</td>
</tr>
</tbody>
</table>
2.13 COLOR REMOVAL OF DSW

2.13.1 Bio-degradation by OB

The effluent DSW-3 collected after initial three different stages of the treatment processes was still colored and this warranted further treatment. Hence DSW-3 was subjected to biochemical treatment by incubating it with OB at 30°C under fluorescent light over a period of 18 days after sterilization, and adjusting the pH to 7.5 (Kalavathi et al. 2001, Prasad & Srivastava 2009c). The OB had grown by consuming the trace level of nutrients present in the partially treated DSW-3. Then known small volume of samples were withdrawn from the incubated DSW samples periodically after every 2 days for absorbance measurement (@475 nm). The percentage of decolorization was calculated using the equation (2.3).

\[
\text{% Decolourization} = \frac{\text{Initial absorbance} - \text{absorbance after remediation}}{\text{Initial absorbance}} \times 100 \tag{2.3}
\]

2.13.2 Electrochemical Degradation

Graphite (G) rod (length 80 mm and diameter 8 mm, Sigma Aldrich) anode and aluminium (Al, Sigma Aldrich) rod (length 80 mm and diameter 8 mm) cathode were used for the electrolysis of partially treated DSW using constant DC power source (GWINSTEK, GPS 4303 India, 0-3A, 0-30 V) after converting into a constant current source (Galvanostat). 200 mL of the DSW-4 effluent was electrolyzed at different constant current values (constant current density with reference to anode surface area) in an undivided electrolytic cell using G rod anode and Al rod cathode at 30°C taking NaCl (0.07 M) as supporting electrolyte for various pH values (3, 5, 7, 9 and 11) under constant stirring (500 rpm magnetic stirrer) rate. The anode and cathode were positioned vertically and paralleled with an inter electrode gap of 3 cm. The non-
submersible portions of the electrodes (20 mm) were insulated using Teflon tape (Khandegar & Saroha 2012). The insoluble residue (DSW-5R) formed during electrolysis was collected and characterized using FT-IR spectroscopy (Figure 7.3). The absorbance (@475 nm) of DSW samples before and after electrolysis were measured (Prasad & Srivastava 2009a, Thakur et al. 2009) and the degree of decolorization was calculated as per the equation (2.3), and compared with PCU.

2.14 COD, BOD AND PLATINUM COBALT COLOUR UNIT (PCU) DETERMINATION

The COD and BOD determine the quantum of oxygen required for chemical and biochemical oxidization respectively of the organic matter in the effluent under specific experimental conditions such as temperature, time, etc. These values were determined using the necessary reagents as per standard procedure reported (closed reflux titrimetric method, (Cuesta et al. 1996, Cuesta et al. 1998, Uğurlu et al. 2008)). The BOD values of different DSW samples (virgin DSW, DSW 3, DSW 4, DSW 5 and DSW 6) were measured after appropriate dilution if required. The color intensities of the DSW samples were also measured in platinum–cobalt color unit (PCU) scale using standard chloroplatinate solution (Hazen 1896).

2.15 KINETICS AND ISOTHERM MODELING OF ADSORPTION

2.15.1 Isotherms

In any adsorption experiment the equilibrium adsorption isotherm data could explain the interactive behavior between adsorbate and adsorbents, and the isotherm data would be useful for the selection of an adsorbent for a particular adsorbate. The equilibrium relationship between an adsorbate and adsorbent was correlated by several expressions (Banerjee & Sharma 2013)
which were used to describe isotherm modeling. In the present investigation the isotherm data were fitted for the Langmuir and Freundlich models as per the equation (2.4) and (2.5) respectively, since majority of adsorbent-adsorbate systems obeyed these two models.

\[
\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{q_mC_L}
\]

(2.4)

\[
\log q_e = \log K_F + \frac{1}{n} \log C_e
\]

(2.5)

where \( q_m \) - the equilibrium monolayer adsorption capacity (mg/g), \( K_L \) - Langmuir constant, (L/mg.), \( K_F \) - Freundlich constant, which predicts the quantity of adsorbate (mg) per g of polymer under equilibrium and “n” – a temperature dependent parameter indicating quantitatively the heterogeneity of the energetic sites on the adsorbent surface. The variation of \( K_L \) with temperature can be used to estimate the enthalpy change accompanying adsorption and the affinity between the adsorbent and adsorbate.

2.15.2 Kinetics

The RB 4 dye, metal ion and pollutants in DSW adsorption kinetics will be influenced by adsorption reactions, and the mass transfer steps that govern the transfer of metal ion/dye from the respective effluents to the adsorption sites. Hence the kinetics of RB 4, AsO_2^- and Hg^{2+} adsorptions were tested using pseudo-first and second order kinetic models (Banerjee & Sharma 2013) based on equation (2.6) and (2.7), respectively.

\[
\log(q_e - q_t) = \log q_e - \left(\frac{k_1}{2.303}\right)t
\]

(2.6)

\[
\frac{dq_t}{dt} = k_2(q_e - q_t)^2
\]

(2.7)
where $q_e$ and $q_t$ (mg/g) are amounts of AsO$_2^-$ / or Hg$^{2+}$/or RB 4 adsorbed at equilibrium and at time ‘t’ respectively, $k_1$ (min$^{-1}$) and $k_2$ (g mg$^{-1}$ min$^{-1}$) are the rate constants for pseudo-first and second order kinetics, respectively.

2.16 THERMODYNAMICS OF ADSORPTION

The practical applicability and spontaneity of the adsorption process was evaluated by measuring the changes in thermodynamic parameters such as $\Delta G^0$, $\Delta H^0$ and $\Delta S^0$, since they were the actual indicators of adsorption process. The values of $\Delta G^0$, $\Delta H^0$ and $\Delta S^0$ for AsO$_2^-$, Hg$^{2+}$ and RB 4 adsorptions were determined (Bhattacharyya & Gupta 2007) graphically using the equation (2.8)

$$\ln k_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT}$$

where $K_d$ was the distribution coefficient of the adsorbate ($K_d = q_e/C_e$), which was a measure of adsorption capacity of ACAD, $R$ is the universal gas constant (8.314 J K$^{-1}$ mol$^{-1}$) and $T$ was the absolute temperature (K). The plot of $\ln K_d$ vs. $1/T$ yields a straight line with $\frac{\Delta H^0}{RT}$ and $\frac{\Delta S^0}{R}$ as slope and intercept, respectively.