CHAPTER 2

MATERIALS AND METHODS

This chapter outlines the materials used in the production process and experimental methodologies used in this thesis to evaluate the physicochemical properties and performance of the biomaterial. It covers the procedures used for modification process of the adsorbent material, as well as the procedure applied for assessing their phosphate adsorption capacities. Additionally, it details the characterization techniques utilized.

2.1 Materials

Reagent bottles (125 mL), beakers, conical flasks, measuring cylinders, pipettes, burettes, volumetric flasks, round-bottom flask, test-tubes, mortar-pestle, and micropipette have been used during the experiments. All chemicals were used as received, without further purification, and deionized water was used for all the experiments. Table 2.1 details the list of chemical name, formula, molecular weight, physical appearance and manufacturers.

Table 2.1 List of chemical name, formula, molecular weight and manufacturers.

S. no.	Chemical name	Chemical formula	Molecular weight (g/mol)	Manufacturer
1	Potassium dihydrogen phosphate	KH_2PO_4	136.08	Sigma-Aldrich
2	Glycerol	$C_3O_3H_8$	92.09	Sigma-Aldrich
3	Xylitol	$C_5H_{12}O_5$	152.14	Sigma-Aldrich
4	Citric acid	$C_6H_8O_7$	192.12	Sigma-Aldrich
5	Tartaric acid	$C_4H_6O_6$	150.087	Sigma-Aldrich
6	Acetic acid	CH_3COOH	60.052	Sigma-Aldrich
7	Cetyltrimethylammonium bromide	$C_{19}H_{42}BrN$	364.45	Sigma-Aldrich

8	Bentonite	Al_2O_3 • SiO_2 •H_2O	180.1	SRL
9	Magnesium chloride	$MgCl_2$	95.21	Sigma-Aldrich
10	Sodium hydroxide	NaOH	40	SRL
11	Hydrochloric acid	HCl	36.46	SD Fine
12	Ethanol	C_2H_5OH	46.06	Sigma-Aldrich

2.2 Analytical instruments

2.2.1 Fourier-transform infrared spectroscopy (FT-IR)

The infrared spectra of the synthesized solvents with different initial materials, NFCs and modified NFCs were obtained through Fourier Transform Infrared Spectrometer (FTIR) (Nicolet iS5, THERMO Electron Scientific Instruments LLC) operated using KBr pellets in the range from 4000 to 400 cm^{-1}. It was performed to investigate the stretching and bending molecular vibration of functional groups moieties that exist in the materials.

2.2.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

^1H NMR analysis was employed to investigate the ionization states of protons and ascertain the molecular interactions within the NADES system**.** This technique quantitatively reveals the proton distribution through the detection of proton spectra. ^1H NMR analysis of the synthesized NADES was recorded at 40 °C on a JEOL AL500 FT-NMR spectrometer with D_2O as the solvent.

2.2.3 Dynamic Light Scattering (DLS)

To estimate the average particle size and size distribution of NFCs, DLS was performed with Zetasizer nano-ZS Zen 3600 (Malvern Instruments Ltd.). Before conducting the analysis, the NFCs were dispersed in ethanol (refractive index: 1.3614 & viscosity: 1.1

Cp) and subjected to ultrasonication in triplicates for 10 minutes in temperature-regulated cell set to a temperature of 25 °C, and measurements were taken to determine the particle size within the range of 5 nm to 250 nm.

2.2.4 Determination of pH_{PZC} and zeta potential of adsorbent

The pH at which surface charge was zero i.e., pH_{PZC} was calculated by plotting a graph between initial pH along x-axis and difference of initial and final pH (ΔpH) on y-axis using pH drift method [132,133]. Typically, 100 mL of 0.01 M of NaCl solution was prepared and divided into five different reagent bottles, each having 20 mL of NaCl solution. 0.01 g of synthesized adsorbent was added to it and initial pH values was adjusted with the help of 0.1 M HCl/NaCl. The solution was kept for 24 hrs in water shaker under stirring at 150 rpm and then final pH values was recorded with the help of pH meter (Orion star A211, Thermo scientific). To evaluate the impact of modification on the surface charge of CTAB@NFC, zeta potential measurements were performed across different pH ranges using a Zetasizer Ultra (ZSU5700) (Malvern Panalytical, UK).

2.2.5 Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

The morphological properties of both the raw samples as well as synthesized samples were analysed using SEM instrument (Carl Zeiss, Evo-18 Research Model, Germany) with an operating voltage of 20 kV. As the samples were non-conducting in nature, the samples were sputtered with gold particles prior to conducting SEM analysis. For elemental analysis, energy dispersive X-ray spectroscopy (EDS) (EDAX TEAM, AMETEK, Version 4.5, EDAX Inc, 2017) was performed in tandem with SEM imaging

[134]. FESEM was done using (SEM) (Nova Nano SEM 450), in conjunction with energy dispersive X-ray spectroscopy (EDX) (Team Pegasus Integrated EDS-EBSD with Octane Plus and Hikari Pro EDAX Inc) for Mg@NFC/BN samples.

2.2.6 High Resolution-Transmission Electron Microscopy (HR-TEM)

Nanofibrillated cellulose micrographs were visualized using TEM instrument (Tecnai G2 20 TWIN, FEI Company of USA, EDS: TEAM EDS SYSTEM with Octane Plus SDD; EDAX Inc.). The size distributions of the particles were subsequently analysed. For all the samples, ethanol was used as a dispersing medium, and one or two drops of the sample were placed onto a carbon-coated copper grid with a mesh size of 200. High-resolution images were then acquired using a TEM instrument operating at 200 kV, with a magnification of 40 kx, by transmitting a high-energy electron beam.

2.2.7 X-ray Diffraction (XRD)

XRD analysis was employed for pristine NFC and modified NFC samples using PANalytical X'Pert Pro diffractometer (fitted with a copper tube (Cu Kα radiation) and xenon X-ray detector) and Rigaku Miniflex 600. The samples were scanned over a range 2θ = 5 - 90° with a step size of 0.020, scan rate of 5º / min at 45 kV and 40 mA, and the resulting diffractogram was analysed using JCPDS database to identify and determine the crystal structure of the materials.

Crystallinity index (CrI) was determined using Segal method [135].

$$\text{Crystallinity index (CrI) \%} = \frac{Icb - Iam}{Icb} \times 100 \qquad (2.1)$$

Here, I_{cb} denotes the maximum intensity of the crystalline band, I_{am} is the amorphous diffraction intensity band.

2.2.8 Thermogravimetry Analysis (TGA) / Differential Thermogravimetric Analysis (DTG)

Thermal properties of NFCs were investigated by performing thermogravimetry analysis. The thermal examinations i.e., weight loss with increasing temperature were evaluated using TGA 4000, Perkin Elmer. The samples were heated from 40 °C to 600 °C in a high-pure nitrogen atmosphere with a steady flow rate of 20 mL per minute and heating (ramp) rate of 10 °C per minute.

2.2.9 BET (Brunauer-Emmett-Teller) surface area analysis of adsorbent

BET analysis is a widely utilized gas adsorption technique for determining the specific surface area and porosity of adsorbent materials. This non-destructive method involves the physical adsorption of an inert gas, typically nitrogen or argon, onto the surface of a solid sample. By measuring the amount of gas adsorbed at varying relative pressures, the surface area and pore characteristics of the material can be accurately evaluated. BET analysis was conducted using Bellsorp Max II & Belcat-II, MicrotracBEL Corp. Prior to adsorption measurements, the sample was degassed under vacuum for a minimum of 12 h at 363 K and freeze-dried before analysis.

2.2.10 X-ray Photoelectron Spectrometry (XPS)

XPS is a highly sensitive surface characterization technique widely employed to investigate adsorption. XPS enables the identification of elemental chemical states, quantification of adsorbed species, and detection of changes in surface composition and electronic structure. Prior to analysis, the prepared samples were compressed into pellets. The samples were analysed using XPS, K-Alpha Thermo Fisher Scientific.

2.3 Analytical procedures

2.3.1 Compositional analysis of SCB and residual fractions

Cellulose, hemicellulose, total lignin and ash contents were determined in pristine biomass as well as in residual fractions using National Renewable Energy Laboratory (NREL) protocol [136]. Each sample was subjected to a minimum of three trials, and their average values were used in calculation.

2.3.2 Batch adsorption experiments

Potassium dihydrogen phosphate (KH_2PO_4) was dissolved in distilled water to prepare standard solutions of phosphate. Batch adsorption experiments were conducted to thoroughly assess the adsorption performance of a series of synthesized adsorbents. These experiments were conducted in a shaker at an ambient temperature of 25 ± 2° C and an agitation speed of 150 rpm. An initial phosphate concentration of 20 mg L^{-1}, representing typical levels in municipal wastewater, was used for all experiments. The initial pH for each system was adjusted to 7 ± 0.02 with 0.01 M of NaOH and HCl prior to the adsorption experiments excluding those performed for pH optimization. To evaluate the phosphate adsorption performance of Mg@NFC/BN in complex solutions containing interfering anions (Cl^-, SO_4^{2-}, NO_3^-, HCO_3^-, and F^-) at different molar ratios, batch experiments were conducted with their respective sodium salts. In CTAB@NFC study, concentrations of interfering ions reflecting typical levels in wastewater were utilized: 50 mg/L Cl^- (NaCl, 1.4 mM/L), 15 mg/L NO_3^- ($NaNO_3$, 0.24 mM/L), 80 mg/L HCO_3^- ($NaHCO_3$, 1.3 mM/L), and 20 mg/L F^- (NaF, 0.47 mM/L). The ratio of adsorbent to initial adsorbate ions solution remained constant with that used in the kinetics/isotherm experiment. In contrast, for Mg@NFC/BN, the influence of interfering ions was evaluated at three concentrations; 1 mM, 10 mM, and 100 mM

while maintaining a constant phosphate concentration throughout the tests. All the experiments were conducted at 25 ± 2 °C.

Water samples were collected at consistent intervals and immediately filtered through 0.22 μm pore size nylon membrane filters for subsequent analysis. The concentrations of residual phosphate were assessed using the ammonium molybdate spectrophotometric methods respectively, using UV-vis spectrophotometer (UV-1800, Shimadzu). Each adsorption experiment was conducted in triplicate, and the results reported are the averages of the three trials. The percentage of phosphate removal by the adsorbent at equilibrium was calculated using the following equations:

$$\text{Adsorption capacity (q)} = (C_0 - C_e)V/M \quad (2.2)$$

$$\text{Percentage phosphate removal (\%)} = \frac{(C_0 - C_e)}{C_0} \times 100\% \quad (2.3)$$

Here, in equation (2) and (3), C_0 and C_e denote the initial and equilibrium concentrations (mg/L) of adsorbate, respectively, and V and M indicate the volume of solution (L) and weight of adsorbent (g), respectively.

2.3.2.1 Stability experiments of CTAB@NFC

The dried adsorbent was added to a 100 mL beaker, followed by the addition of 50 mL of deionized water. The mixture was stirred at 150 rpm for 24 h at pH 7. The concentrations of phosphate in the solution were then determined. This desorption experiment was repeated three times to analyse the retention capacity of CTAB@NFC for phosphate.

2.3.2.2 Synthesis and evaluation of water-retention capacity of soil with PLC (phosphate loaded Mg@NFC/BN composite)

Phosphate-loaded composite (PLC) was prepared as follows: 0.25 g of Mg@NFC/BN was added to a flask containing 250 mL of 100 mg/L of $H_2PO_4^-$ aqueous solution. The mixture was maintained at 25 °C and agitated for 2 h, followed by filtration and drying at 80 °C until a constant weight was achieved.

To evaluate the soil water-retention capacity with PLC, sandy loam soils were collected from cultivated fields at IIT BHU, Varanasi (Eastern India). The samples were amended with varying ratios of PLC and soil and assessed for water-retention ratios. The experiment included four groups: (i) 100 g of air-dried soil (control); (ii) 0.5 g of PLC mixed with 100 g of dry soil; (iii) 1 g of PLC mixed with 100 g of dry soil; and (iv) 2 g of PLC mixed with 100 g of dry soil. The samples were packed into PVC tubes with a diameter of 4.5 cm, where the bottom of each tube was sealed with four layers of 200-mesh nylon fabric.

The initial weight of the tubes was recorded as M_0. Tap water was gradually added from the top of each PVC tube until water percolated out from the bottom. Once the soil and PLC mixture were thoroughly saturated with water and no further percolation was observed, the tubes were weighed again and recorded as M_i. Subsequently, the tubes were maintained under identical environmental conditions and weighed at different time interval (M_F) alternately over a monitoring period of 30 days. The water-retention capacity (WRC %) for the soil samples was calculated using the following equation:

$$WRC\ \% = \frac{M_F - M_0}{M_i - M_0} \times 100\ \% \qquad (2.4)$$

2.3.2.3 Adsorption isotherm

The analysis of adsorption isotherms is crucial for understanding the sorption mechanism, surface characteristics, and affinity between the adsorbent and adsorbate, as well as for designing effective adsorption process models. Equilibrium data obtained from batch adsorption experiments enable the correlation between the amount of adsorbate adsorbed per unit mass of adsorbent and the residual adsorbate concentration in the solution.

For isotherm studies, a fixed amount of each adsorbent was introduced into reagent bottles containing 50 mL of adsorbate solutions at varying initial concentrations. These bottles were then placed in a thermostatic water-bath shaker and agitated at controlled temperatures (298.5 K, 308.5 K, and 318.5 K) until equilibrium was reached. For CTAB@NFC, adsorption isotherm studies were conducted at a constant (ambient) temperature. To interpret the equilibrium behaviour of the adsorbent-adsorbate system, various isotherm models are applied. Among them, the Langmuir, Freundlich and Sips models are the most widely used to describe the adsorption equilibrium.

2.3.2.4 Adsorption kinetics

Adsorption kinetics is essential for the design and modelling of industrial adsorption systems. Kinetic studies provide valuable insights into the rate at which adsorbate molecules are taken up by the adsorbent over time, following a specific mechanism. These studies help in determining the kinetic parameters that characterize the adsorption process. Typically, adsorption may involve multiple steps, including chemical interactions, diffusion, and mass transfer phenomena. To investigate the underlying mechanisms and identify potential rate-controlling steps, models such as the pseudo-first-order, pseudo-second-order, and Elovich models are commonly applied.

The best-fit model was selected based on the correlation coefficient (R^2) value for regression.

Kinetic experiments were conducted under conditions similar to those used in equilibrium studies. Specifically, 50 mL of the adsorbate solution was agitated with a measured quantity of adsorbent. Samples were withdrawn from the water bath at predetermined time intervals, and the residual concentrations were analysed to evaluate the adsorption kinetics.

The equations of kinetic and isotherm models are provided in following table:

Table 2.2 Kinetic and isotherm equations [137–139]**.**

Models	**Equation**	**Parameters**
Kinetic models		
Pseudo-first-order (PFO)	$q_t = q_e(1 - e^{-k_1 t})$	q_t ($mg \cdot g^{-1}$)is the adsorbed amount of phosphate at time t; q_e ($mg \cdot g^{-1}$) is the adsorbed amount of phosphate at equilibrium; k_1 (min^{-1}), k_2 ($mg \cdot g^{-1} \cdot min^{-1}$), and k_e($g \cdot min^{-0.5} \cdot g^{-1}$) are the rate constants of Pseudo-first-order, Pseudo-second-order, and Elovich, respectively; α ($mg \cdot g^{-1} \cdot h^{-1}$) is the initial adsorption rate, and β ($g \cdot mg^{-1}$) is the desorption constant.
Pseudo-second-order (PSO)	$q_t = \frac{q_e^2 kt}{1 + q_e k_2 t}$	
Elovich	$q_t = \frac{1}{\beta}\ln(1 + \alpha\beta t)$	
Isotherm models		
Langmuir	$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$	q_e ($mg \cdot g^{-1}$) is the adsorbed amount of phosphate at equilibrium; q_m ($mg \cdot g^{-1}$) is the maximum adsorption capacity of adsorbent; C_e ($mg \cdot L^{-1}$) is the concentration of phosphate at equilibrium; K_L ($L \cdot mg^{-1}$), K_F ($mg \cdot g^{-1}$), K_S ($L^{n_s} \cdot mg^{-n_s}$) and K_T ($L \cdot g^{-1}$) are the adsorption constants of Langmuir, Freundlich, and Sips models, respectively; n_F n is heterogeneity factor (dimensionless);
Freundlich	$q_e = K_F C_e^{1/n_F}$	
Sips	$q_e = \frac{q_m K_S C_e^{n_S}}{1 + K_S C_e^{n_S}}$	

	n_S is the heterogeneity factor that indicates the deviation from Langmuir isotherm (when n=1, the Sips equation reduces to the Langmuir isotherm). R (8.314 J· mol^{-1} · K), T (298 K) are the universal gas constant and experimental temperature, respectively.

2.3.2.5 Adsorption thermodynamics

Thermodynamics describes the temperature dependence of the process [140]. The thermodynamic data of the phosphate adsorbed by the selected adsorbent was calculated at different temperatures (298.5, 308.5 318.5 K) at different initial concentrations of phosphate. The parameters such as change in standard Gibbs free energy (ΔG^0), enthalpy(ΔH^0) and entropy (ΔS^0) were determined with the given equations.

$$\Delta G^0 = -RT\ lnK \tag{2.5}$$

Where, R (8.314 J/mol/K) is the ideal gas constant, T (K) is the Kelvin temperature and K_d is the thermodynamic equilibrium constant of the adsorption process, reflecting phosphate distribution between the solid and liquid phases at equilibrium. The values of $\Delta H^0$and ΔS^0can be evaluated using Van't hoff equation as follows

$$lnK_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \tag{2.6}$$

Values of the standard changes of enthalpy (ΔH^0) and entropy (ΔS^0) are calculated by the equation:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{2.7}$$

Thus, the slope and intercept of the linear plot of ln(K_d) vs. 1/T can be used to determine the values of ΔH^0 and $T\Delta S^0$.