A new zerovalent iron chitosan-humic acid nanocomposite was prepared and tested for nitrate ion reduction in water. Humic acid was used for intramolecular cross-linking of the chitosan linear chains to increase the active sites on the chitosan biopolymer and then further used as a stabilizer to synthesize zerovalent iron nanoparticles by the reduction of iron (II) chloride with sodium borohydride. Characterization of the products was carried out using infrared spectroscopy, scanning electron microscope, energy dispersive X-ray, and X-ray diffractometer. Batch experiments were conducted for the reduction of nitrate in water using different concentrations of the products in different concentrations of nitrate ion and at different contact time. The adsorption equilibrium data for the nitrate solution gave a favorable adsorption according to the Langmuir equation. Varying the nanocomposite-to-nitrate ion ratio generally led to faster nitrate reduction, with the pseudofirst-order rate constant for the adsorption increasing with increase in nanocomposite-to-nitrate ion ratio. Nitrate removal efficiency of zerovalent iron chitosan–humic acid nanocomposite was further confirmed using real water samples obtained from drainage waste and river with an initial nitrate concentration of 18.00 ± 0.01 and 12.00 ± 0.12 ppm, respectively. The reduction of nitrate in water using the nanocomposite was concluded to be highly effective.

1. Introduction

There has been an increase in the discharge of toxic substances such as nitrate into the environment especially water bodies as a result of urbanization. Nitrate anion pollution has been attributed to agricultural runoff, leaching of nitrogen fertilizers, concentrated animal feeding operations, food processing, and industrial waste effluent discharge [1, 2]. In the area of water purification, nanotechnology offers the possibility of an efficient removal of pollutants and germs. The application of zerovalent metal nanoparticles (Nps) for environmental remediation represents one of the innovative technologies especially in the chemical reduction of some oxoanions from drinking water and destruction of some organic pollutants [3–5]. Nanoscale bimetallic particles have been shown to remove some pollutants [6].
have enormous chelation capacity due to the presence of hydroxyl-, phenoxyl-, and carboxyl-reactive groups which predominantly carry negative charges [12, 13] making it possible for further reactions with other agents. This work hitherto describes the synthesis of zerovalent iron cross-linked chitosan-humic acid nanocomposite and its effective nitrate ion reduction in waste water.

2. Materials and Method

2.1. Materials. Chitosan (average molecular weight of 4294.40 g/mol and 80% degree of deacetylation) was prepared from chitin which was obtained from snail shells as previously reported [2]. Sodium borohydride (NaBH₄), humic acid, and N-3-(dimethylaminopropyl)-N’-1-ethylcarbodiimide (CDI) were purchased from Sigma Aldrich, United State. Other reagents like ferrous chloride hydrate (FeCl₂-4H₂O), NaOH, HCl, acetone, acetic acid, and NaNO₃ were obtained from BDH. All aqueous solutions were made with ultrahigh purity water purified with an ultrapure water system Milli-Q Plus (Millipore Co.).

2.2. Characterization. The degree of deacetylation (DDA) of CTS was determined using the acid-base titration method [14]. The IR spectra were recorded on a Perkin-Elmer 100 FT-IR spectrophotometer. The XRD patterns of CTS and CTS-HMA were obtained on Philips PW04Xpert pro X-ray diffractometer. The X-ray source was Cu Ka with a voltage of 40 kV and a current of 30 mA. The morphology of the products was studied by the Tescan (TS5136ML) scanning electron microscope (SEM) operating at an accelerated voltage of 20 kV after a gold sputter coating at 20 kV after a gold sputter coating at 10 kV. The elements present in the nanocomposites determined by energy dispersive X-ray (EDX) element mapping. UV-spectrophotometer used was Spectrutmab 752S UV-Vis spectrophotometer.

2.3. Synthesis of CTS-HMA and ZvFe-CTS-HMA Nanocomposite. Following a previously described procedure [9, 15] 8 g CTS was dissolved in HMA solution and stirred with a magnetic stirrer for 2 h. Few drops of water-soluble carbodiimide were added to the solution and further stirred for 2 h at room temperature and were stored at room temperature for 24 h. The cross-linked CTS-HMA was obtained by freeze drying. The synthesis of ZvFe-CTS-HMA nanocomposite is based on borohydride reduction of Fe²⁺. To a stirred ethanolic solution of FeCl₂-4H₂O (10.68 g) CTS-HMA (3.0 g) was added. 1 M NaBH₄ solution was then added drop-wise to the Fe-CTS-HMA mixture with continuous stirring of the resulting solution (Scheme 1). The nanocomposite was collected by freeze drying.

2.4. Reduction of Nitrate in Water. Kinetic studies were carried out to evaluate the efficiency of reduction of nitrate by the products on both standards and samples collected from river and waste water. All experiments were carried out in duplicate. Control experiments were also carried out in parallel.

2.4.1. Batch Experiment. Batch tests for nitrate reduction were conducted in a 20 mL sterilized, disposable centrifuge tubes. Different concentrations of nitrate were prepared from a stock solution of 1000 ppm ranging from 10 ppm to 50 ppm using double distilled and deionized water. CTS, HMA, CTS-HMA, and ZvFe-CTS-HMA of 0.1 g dosage were added to 20 mL each of varying concentrations of nitrate at different selected time intervals of 15, 30, 45, 60, 75, and 90 minutes; the mixtures were shaken and centrifuged at 400 rpm. At selected time intervals, 2 mL samples were taken using a gas tight syringe and nitrate concentration was determined using UV-visible spectrophotometer [16]. An increase in dosage to 0.2 g and 0.4 g was also carried out using high concentration of nitrate of 50 ppm at different time intervals.

2.4.2. Nitrate Reduction in River and Waste Water Samples. Samples were collected randomly in polyethylene bottles. Nitrate concentration was determined before and after the reduction using the synthesized zerovalent iron cross-linked chitosan at 0.1 g and 0.2 g dosages as carried out in the batch experiments.

2.4.3. Reaction Kinetics and Adsorption Isotherms. A pseudo-first-order reaction with respect to nitrate concentration was used to fit the kinetic data as given in (1), where k is the observed first-order reaction rate constant, which is the slope of the regression lines by plotting a natural log graph with respect to nitrate concentration and reaction time (ln[NO₃⁻]) versus time) according to (2). A pseudosecond-order reaction with respect to nitrate concentration was also used to fit the observed kinetic data as given in (3). Thus, plot of ln[NO₃⁻] versus time should yield a straight line, and the slope k is the pseudosecond rate constant.

\[ r = \frac{-d[A]}{dt} = k[A], \]  
\[ \ln[A] = -k_1t[A]_0, \]  
\[ r = \frac{-d[A]}{dt} = k_2[A]^2, \]  
\[ \frac{1}{[A]} = \frac{1}{[A]_0} + kt. \]

To examine the relationship between sorbed (q) and aqueous concentrations (Cₑ) at equilibrium, sorption isotherm models are employed. Freundlich equation was tested by plotting log qₑ against log Cₑ; according to (3), such a plot should yield a straight line with intercept equal to log Kₑ and slope equal to 1/n. The Langmuir isotherm was also tested by plotting Cₑ/qₑ against Cₑ according to (5). The applicability of the isotherm to the adsorption was judged by comparing the values of regression coefficient (R²).

\[ Cₑ = \frac{1 + Cₑ}{q₀kₐt₀}, \]  
\[ Rₑ = \frac{1}{1 + bCₑ}. \]
The equilibrium parameter, $R_L$, of the Langmuir isotherm was calculated (6) as has been previously defined [17], where $C_0$ is the initial NO$_3^-$ concentration (ppm) and $b$ is Langmuir adsorption equilibrium constant (mL mg$^{-1}$).

### 3. Results and Discussions

#### 3.1. Synthesis and Characterization

The zerovalent iron chitosan-humic acid nanocomposite was precipitated out as a powder and therefore could be dispersed into aqueous solutions. The synthesized ZvFe-CTS-HMA was stable when exposed to open atmosphere for long period of hours. It is believed that, in the humic acid-chitosan system, the zerovalent iron nanoparticles are better protected by the CTS-HMA and oxidation prevented as previously reported with other cross-linked chitosans [9].

IR spectrum of chitosan obtained in this study was comparable to those previously reported [2, 9, 18]. Figure 1 compares the infrared assignment to functional groups of CTS, HMA, CTS-HMA, and ZvFe-CTS-HMA. The broad peak around 3448–3373 cm$^{-1}$ in the spectrum of CTS, HMA, and CTS-HMA samples indicated the absence of free OH groups [19]. A new weak peak at 1654 cm$^{-1}$ in chitosan-humic acid was assigned to be characteristic -COO$^-$ symmetric stretching vibration of carboxylate anion. This is comparable to that observed by Santosa et al. [18], where chitosan was cross-linked with glutaraldehyde. It was suggested that the amine groups of chitosan reacted with the carboxylic groups of humic acid electrostatically, this is because carboxylic groups of humic acid release protons (H$^+$) and convert to COO$^-$ anions, whereas amine groups of chitosan as a base can accept protons and convert to cations. Hence, cross-linking between humic acid and chitosan can be considered to occur chemically. Comparing the IR spectra of CTS-HMA and ZvFe-CTS-HMA, it is evident that there is a slight band shift of absorption peaks of carbonyl stretching occurring at 1831 cm$^{-1}$ and the amide I and amide II bands occurring at 1475 and 1391 cm$^{-1}$, respectively. A weak peak of free -OH appeared, which is attributed to electrostatic interaction of Fe with OH and NH$_2$ group of CTS-HMA. The observed shift in
the position of the absorption bands and the appearance of two novel bands in the IR spectra of CTS-HMA and ZvFe-CTS-HMA showed that ZvFe-CTS-HMA was successfully synthesized.

The XRD data of ZvFe-CTS-HMA presented in Figure 2 shows a very broad peak and scattered features. The broad and scattered peak reveals the existence of an amorphous phase of the nanoparticle; therefore this cannot be defined by a crystalline model. The characteristic broad peak at 2θ of 25° indicates that the zerovalent iron is predominantly present in the sample in an amorphous phase [18, 20]. This is not surprising as humic acid is amorphous in nature and also the diffraction pattern of sorbent obtained by cross-linking between chitosan and humic acid showed an amorphous form.

Figure 3 compares the scanning electron micrographs of CTS, CTS-HMA, and ZvFe-CTS-HMA. The micrograph of chitosan indicates a smooth surface with some straps confirming high degree of chitin deacetylation as previously reported [2, 9]. Chitosan loses its smooth surface on cross-linking with humic acid as indicated in the SEM images showing that intramolecular cross-linking between humic acid and chitosan was obtained and that secondary interactions
caused surface aggregation. This observation is in accord with our previous studies where chitosan has been cross-linked with notable cross-linkers [9]. The scanning image of ZvFe-CTS-HMA showed significant morphological changes thereby confirming a very strong bond between the cross-linked CTS-HMA and the zerovalent iron.

The characteristic peak of iron was observed in the EDX spectra ZvFe-CTS-HMA (Figure 4(b)) but was not seen in the EDX spectra of cross-linked CTS-HMA (Figure 4(a)). The electron dense part on surface condition of cross-linked chitosan was proved as iron. This method of elemental mapping of nanoparticle has been previously reported where it was used to confirm the existence of mercury ions before and after sorption on the electron dense part surface of aminated chitosan bead [21].

3.2. Reduction of Nitrate in Water. Batch experiment showed that rates of reduction of $\text{NO}_3^-$ increased as the loadings of synthesized nanoparticle increased (Figure 5) and are attributed to the increase in the active sites on the chitosan-zerovalent iron surface [22]. It was observed that the efficacy of humic acid to reduce nitrate showed a positive trend but at
a lower rate compared to chitosan, cross-linked chitosan, and zerovalent iron cross-linked chitosan (Figure 6). The zwitterionic character of humic acid allows the interaction of anions with its positively charged groups and cations with its negative charged groups [23]. This result confirmed the use of humic acid in fertilizer production to prevent nitrate leaching out into the groundwater [24].

3.3. Adsorption Isotherms. Equations (2) and (4) were performed for each data set of high nitrate concentration. The plots of the kinetic reaction models are displayed in Figure 7. The good fit of linear model was observed in the case of pseudofirst order with regression coefficient ($R^2$) of 0.980, 0.978, and 0.983, while the pseudosecond order gave $R^2$ value of 0.777, 0.831, and 0.896. The observation implies that, under these conditions, rate reduction of nitrate is directly proportional to the concentration of the reactant; doubling the concentration increases the rate by a factor of 2. This is comparable with some similar studies [25]. The Langmuir isotherm represents the experimental data better than the Freundlich isotherm (Figure 8). The regression coefficient of the Langmuir plot moving closer to unity provides strong evidence that the surface of ZvFe-CTS-HMA consists of adsorption sites. All adsorbed species interact only with the sites and not with each other with the adsorption being limited to a monolayer and independent of the presence of adsorbed species on the other sites.

Table 1 shows the model-fitted pseudofirst-order rate constants of nitrate reduction under various experimental conditions. For adsorption of the different $NO_3^-$ concentration by ZvFe-CTS-HMA, $R_L$ values were calculated and the values are shown in Table 2. In line with the isothermal shape
obtained by $R_L$ values, a favorable adsorption of NO$_3^-$ by ZvFe-CTS-HMA took place (where all the calculated values fall within the favorable range of $0 < R_L < 1$) [17].

**4. Conclusions**

ZvFe nanoparticles were synthesized and dispersed on intramolecular cross-linked CTS-HMA stabilizer. The SEM micrograph of ZvFe-CTS-HMA nanocomposites distinctly showed interactions between the nanoparticles and the new amide chain of the chitosan–humic acid as well as the free amino groups on the chitosan outer surface. In line with previous studies, the use of cross-linked chitosan–humic acid as ZvFe stabilizers has greatly enhanced the reduction of nitrate ion reactivity. It was observed that reduction rate is directly proportional to the concentration of the reactant; doubling the concentration increases the rate by a factor of 2. This nontoxic and biodegradable organic and inorganic polymeric composite can serve as a new material for permeable reactive barrier walls as well as a material for ex situ and in situ water treatment.

**Competing Interests**

The authors declare that they have no competing interests.

**Acknowledgments**

This work was supported by the Department of Chemistry, Federal University of Agriculture, Abeokuta, Nigeria.

**References**


