Application of modified clays in nutrient removal from wastewater

Ph.D. Dissertation Booklet

Tanya Chauhan

Supervisor	Head of the Doctoral School
Dr. Zoltán Németh	Prof. Dr. Valéria Mertinger

Antal Kerpely Doctoral School of Materials Science and Technology

Faculty of Materials and Chemical Engineering

University of Miskolc

Miskolc

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1. Introduction

According to the Intergovernmental Panel on Climate Change (IPCC) Report on Climate Change 2022, nearly half of the world's population faces severe water scarcity for at least some part of the year. Water pollution is a serious threat to the existence of living beings on Earth. Population explosion, urbanisation, and industrialization over the decades have changed the pattern of land use and the hydrological cycle of the world. Increased frequency of floods, rainfall, droughts and other natural disasters in past years have proved that we are failing to maintain the ecological balance. Mobilization of bioavailable nutrients such as nitrogen and phosphorus into the water bodies is also one of the factors responsible for the degradation of water quality around the globe. A significant fraction of these elements enters the surface water via human or natural activities and are transported to coastal ecosystems through rivers. The major anthropogenic sources of nutrient water pollution are agricultural runoff, sewage, industrial and urban effluent [1]. Aquatic ecosystems are very sensitive with respect to the concentration of phosphorus. Although it is an important element for the growth of phytoplankton, but it can be a nuisance if the concentration increases up to a certain limit. Nutrient enrichment by nitrogen and phosphorus in a water body can lead to an undesirable and unavoidable situation called eutrophication. In this, the surface of the water is covered by uncontrolled growth of weeds, algae and cyanobacteria (blue-green algae) which further prevents the penetration of sunlight in the water column, thus affecting other aquatic species which are living in depth. A series of events are initiated under the influence of eutrophication starting with abundant growth of algae and plants and when these plants die a lot of oxygen is used for the decomposition of their organic matter. As a result, carbon dioxide is produced which reduces the pH of the water and makes it acidic. The biological oxygen demand (BOD) of the water body decreases and creates hypoxic conditions for aquatic plants and animals [2] [3]. Eventually either they die or move to other places and like this healthy habitat are destroyed and converted into dead zones [4]. Previous studies have demonstrated that phosphorus concentration above 0.02 mg/L can initiate the process of eutrophication [5]. Rivers, lakes, ponds, wetlands even seas are being damaged by the imbalance of nutrient levels. Another emerging pollutant detrimental to aquatic ecosystems and human health is *Escherichia coli (E. coli)* bacteria which is an indicator of faecal contamination. These are found in the intestines of human beings and animals. It is a sign of contaminated water bodies not suitable for drinking or recreational activities. The conventional methods which are used for the elimination of these microorganisms from water are chlorination, ozonation, and filtration [6]. However, these disinfection techniques have limitations for e.g. the end products which are produced during chlorination are very harmful and toxic carcinogenic chemicals. Filtration methods are better, but they are not very cost-efficient and have fouling problems. Ozonation is a very efficient method but it could also produce harmful substances and reactive free radicals [7]. Therefore, there is a need to develop cheap and alternative methods which can be applied to wastewater purification. Around the globe, researchers and scientists are working on finding a solution for the purification of water using suitable methods. Clays have been known as natural water pollutant scavengers for a long time. Interaction and migration of a pollutant with the clay depend upon its charge, cation exchange capacity and surface area [8].

2. Literature review

Clay minerals are very promising tools for the development of novel materials that are flexible and can be applied in different applications such as adsorbents, catalysts, photocatalysts, drug delivery systems and nanocomposites [9]. To improve the performance of clay adsorbents, researchers have tried to modify the structural and surface properties of clay minerals using different strategies. Different techniques are used for the modification of clay such as activation with acids [10], alcohols, surfactants or doping with metals [11]. But among all, clay pillaring or intercalation is the most promising process which can improve the porosity, create more active sites for the reaction and increase the specific surface area as well. In this method, the interlayer cations of clay minerals are exchanged with bulky polyhydroxy metal cations. Upon further heat treatment, these hydroxy metal cations are converted into metal oxide pillars. These oxide pillars keep the clay structure intact and provide stability and rigidity. These newly formed microporous structures are referred as pillared interlayered clays (PILCs). The concept of pillaring was first proposed by Barrer and Macleod in the 1950s and they used aluminium tetra alkyl ions as pillaring material [12]. The problem with using organic species for intercalation was their low thermal stability at higher temperatures. Later in the 1970s, Brindley and Sempels used a hydrolysis product of $AlCl_3$ for intercalation in sodium-saturated beidellite [13]. This was the first time when an inorganic pillared clay was produced using aluminium ions. After that, Yamanaka and Brindley used zirconium polyhydroxy cations for pillaring in 1979 [14]; later the same group produced iron-pillared clay as well. In 1973 during the time of the oil crisis, the application of these materials as catalysts was explored in the field of oil cracking [15]. In literature, other elements are also used as pillaring agents such as Ti, Ga, Cr, Cu, and La and sometimes more than one metal ion is also used such as Al-Fe, Al-Zr but among all Al ion is the most extensively studied cation because its hydrolysis properties are very well explored and understood [16]. Zr^{4+} ions started to gain attention in 1988 when Bartley threw light on the solution chemistry of zirconium ions and the factors responsible for the successful pillaring of zirconium into the clay structure [17]. Based on the field in which these pillared materials will be used and to improve the selectivity, adsorption capacity, specific surface area, porosity and surface acidity these pillared clays are modified further. Various modification methods are used on these materials to enhance their properties based on the target application. A few of the modification techniques use surfactants such as sodium dodecyl sulphate (SDS) [18], hexadecyltrimethylammonium bromide (CTAB) [19] and cetyltrimethylammonium chloride (CTAC) [46] are the most widely used surfactants used to modify the structure of pillared clays and to induce organophilic property as well [20]. After modification, the specific surface area tends to decrease because of the incorporation of surfactant molecules in between the empty spaces of clay sheets. The organic-inorganic modification of these adsorbents makes them suitable for the elimination of both organic and inorganic pollutants from water due to their structural diversity. Another type of modification which includes the usage of natural biopolymer 'chitosan' is gaining attention due to the presence of abundant amine and hydroxyl chelating functional groups within the chains. It is a linear polysaccharide composed of glucosamine and N-acetyl glucosamine linked in a β(1–4) glycosidic bond produced by deacetylation of chitin [21]. Because of its biocompatibility, low cost, biodegradability, non-toxicity, and antibacterial properties; it has been used in the food industry, cosmetics, medicine and wastewater treatment [22]. In literature, the application of pillared clays in the removal of water pollutants such as heavy metals is widely explored [23].

However, the application of Zr-pillared clays in the removal of phosphates from the water was first documented by Huang et al. The maximum phosphate adsorption capacity achieved was 13 mg P/g [24]. Furthermore, there have been few studies in which the application of clay minerals is explored in the elimination of biological pathogens as well but the use of Zr- pillared clays in the removal of *E. coli* has not been reported anywhere. Cu^{+2}-exchanged montmorillonite clays (Cu-MMT) have been prepared by Hang Xu and Sheng Xia for the elimination of *E. coli* K_{88} bacteria responsible for diarrheal disease from water [25]. Suzuki et al. prepared Fe^{3+}-loaded montmorillonite ($Fe^{3+}-MT$) for the inactivation of *E. coli* [26].

3. Knowledge gap

➢ In literature, the synthesis method of pillared clays has been reported but the effect of sodium pre-treatment and ratio of Zr^{4+}/clay on the process of pillaring is still not very clear. From the literature, it is difficult to optimize the Zr^{4+}/clay ratio at which the best pillaring can be achieved also there is very little information about the size of zirconium pillars which are created in between the silicate layers. A detailed comparative analysis of the prepared materials was done for a better understanding of the nature of zirconium pillars created inside the clay structure. In this work, a novel analysis about the dimensions and distribution of microstructures produced by the pillaring process was made with the help of HRTEM, STEM-EDX and HAADF imaging techniques.

➢ In literature, the usage of Zr-pillared clays in the removal of phosphates from water and the mechanism of phosphate adsorption on clay surface is not widely explored. The modified clays which are reported in the literature have shown better adsorption capacity at acidic pH, but we tried to improve the adsorption capacity of Zr-pillared clays by applying modification with a natural biopolymer chitosan. After modification, the pillared clays were working effectively even at neutral pH. To the best of our knowledge, no study has reported the application of chitosan-based zirconium pillared clays in the sequestration of phosphates at acidic and neutral pH.

➢ There is very limited research on the applicability of clay-based composites in the elimination of biological pathogens from water. The physical and chemical properties of montmorillonite clay are suitable for converting them into a sustainable, environment and budget-friendly option for water remediation. We took a novel approach to explore the ability of Zr-pillared clays for the removal of *E. coli* bacteria from an aqueous solution. There has been no study reported in the literature on the adsorption of *E. coli* bacteria using zirconium pillared clays.

4. Aim of thesis

The primary objective of this study is to create sustainable and environment-friendly clay-based adsorbent materials suitable for the elimination of water contaminants such as phosphates and bacteria. To achieve this goal, naturally occurring montmorillonite clay is used and modified using zirconium oxychloride octahydrate and chitosan. The present study focuses on the synthesis methods of Zr-pillared clays and chitosan-modified zirconium pillared clay and their application in wastewater treatment. The aim of the thesis is divided into three parts:

i.   The first part is the development and optimization of the synthesis method of zirconium pillared clays and chitosan-modified clay using a suitable precursor material (CM and Na-CM) at different Zr^{4+}/clay ratios (2.5, 5 and 10 mmol/g). CM is the naturally occurring raw montmorillonite and Na-CM is the sodium pre-treated montmorillonite. A detailed investigation will be performed to determine the influence of Na^+ pre-treatment on the process of zirconium intercalation in between the silicate layers. A thorough comparative analysis of the prepared materials will be done for a better understanding of the nature of zirconium pillars created inside the clay structure using different techniques like XRD, STEM-HAADF, EDX, FT-IR spectroscopy, TGA and CO_2 adsorption for specific surface area analysis.

ii.   The second part focuses on the potential use of prepared materials in the sequestration of phosphates from water. Comprehensive sets of experiments will be conducted in batch mode at different pH (4 and 7) and in a wide range of phosphate concentrations (1-20 mg/L) to determine the phosphate adsorption capacities of the prepared adsorbent materials. The experimental data will also be modelled with different adsorption isotherm models such as Langmuir, Freundlich and Toth models. The mechanism of phosphate adsorption on clay adsorbent will also be explored with the help of different techniques such as XPS and DLS.

iii. In the third part, the application of prepared materials will be explored in the adsorption of *E. coli* bacterial cells from an aqueous solution. *E. coli* cells are indicators of contaminated water bodies not suitable for drinking or recreational activities. In this study, a novel approach is taken for the removal of bacterial cells using zirconium pillared clays.

5. Experimental section

5.1 Synthesis

i. Preparation of Zr-pillared clays

The zirconium-pillared clays were prepared from two precursor clay materials named raw montmorillonite (CM) and sodium pre-treated montmorillonite (Na-CM). For preparing Na-CM, raw clay was saturated with 1M NaCl for 24 h. The next day, clay was recovered from the salt solution using a centrifuge followed by washing and drying at 60 ˚C. $ZrOCl_2.8H_2O$ pillaring solution (0.1 M) was prepared and aged for 24 h at room temperature. The Zr-pillared clays were synthesized using three Zr^{4+}/clay ratios (2.5, 5, 10 mmol/g). The precursor clay materials were dispersed in distilled water to make a clay suspension. The clay suspension was stirred for 2h at room temperature then the pillaring solution was added to the clay dropwise and kept at 50 ˚C under vigorous stirring. The suspension was left for stirring for 2h, followed by an ageing of 12 h at 50 ˚C. After this, the intercalated clay was centrifuged, washed thoroughly and dried at 120 ˚C. The Cation exchange capacity (CEC) of CM (56 cmol+/kg) and Na-CM (52 cmol+/kg) was also determined using the adsorption of copper ethylenediamine complex, $[Cu(en)_2]^{+2}$ [27].

ii. Preparation of chitosan-modified zirconium pillared clays

For the preparation of chitosan-based zirconium pillared clays, 1g of chitosan powder was added to 2% (v/v) acetic acid aqueous solution and mixed until it dissolved completely. After this, 2g of Na-Zr-50-2.5 was added to 100 ml of distilled water and stirred for 1 h at room temperature. To this pillared clay suspension, the chitosan solution was added dropwise at room temperature under slow stirring. The solution was left for overnight ageing. The next day it is washed thoroughly, centrifuged and dried at 80˚C for 4h. The synthesized material is named as Ch-NaZr2.5. Sodium pre-treated montmorillonite is also treated with chitosan solution and the material is named as Ch-NaCM.

iii. Preparation of LB-media, LB-agar plate, and Physiological saline solution

Luria-Bertani (LB) liquid broth is the most widely used medium for growing *E. coli* bacteria. For preparing 1 L of LB medium, 10 g of tryptone, 5 g of yeast extract and 10 g of NaCl were dissolved in ultrapure water with the help of a magnetic stirrer. After that, 35 mL of LB solution was transferred into the Erlenmeyer flask. The mouth of the flask was sealed with a cotton plug to avoid any contamination and was put into an autoclave for sterilization at 121 °C for 15 min, (103 to 117 kPa) [28]. This sterile media was used for culturing the *E. coli* and making starter bacterial suspensions. To attain this, a single colony of *E. coli* DH5α was taken from the LB agar plate with the help of an inoculation loop and put into the sterile LB liquid. Afterwards, the LB suspensions plugged with cotton were placed in an incubator overnight under continuous shaking at 160 rpm and 37 °C. The next day, the optical density of the bacterial starter solution was measured using a sterile LB medium as a blank solution. The optical density was measured at 600 nm (OD_{600}). This value helps in determining the growth stage of bacteria and the cell concentration of the starter culture. The OD measurement was crucial to check and ensure a uniform initial cell concentration for all the adsorption tests. During the experiments, the cell count of the used starter cultures was $1.00 \pm 0.05 \times 10^9$ CFU/mL.

The LB agar plates provide a nutrient-rich surface for the growth of bacteria. To prepare this, 15 g of bacteriological agar was added to 1000 mL of the LB medium. For proper mixing of agar in LB medium, the solution was autoclaved and sterilized properly. The prepared transparent LB agar solution was cooled to ~50 °C with the help of a water bath and then it was spread evenly on sterile plastic Petri dishes having a diameter of 90 mm. The prepared plates were left to solidify in a biological safety cabinet under aseptic conditions. These plates were later used in the colony counting method.

The *E. coli* adsorption experiments were carried out in a physiological saline solution. In this solution, the bacterial cells could survive with minimal cell division thus ensuring a constant cell density throughout the study. It was prepared by dissolving 8.5 g NaCl in ultrapure water (1L) followed by sterilization in an autoclave at 121°C.

6. Results

6.1 Application of Zr-pillared and chitosan-modified zirconium pillared clays in phosphate removal from water

The application of prepared materials was explored in the adsorption of phosphates from water. In the below sections the experimental conditions, adsorption isotherms used and mechanism of the phosphate adsorption on clay adsorbents is discussed.

6.1.1 Experimental conditions of phosphate adsorption tests

All phosphate adsorption experiments were carried out in a batch mode at pH 4 and 7 at room temperature with an adsorbent loading of 1 g/L. To ensure adequate contact between clay samples and phosphates in the water, the solutions were put in an orbital shaker at 400 rpm for different time intervals (30-240 minutes). The experiments were conducted in a phosphate concentration range of 1-20 mg P/L. After adsorption, the solutions were filtered with syringe filters, and phosphate concentration in the filtrate was determined using Varian 720-ES axial view inductively coupled plasma-optical emission spectrometer (ICP-OES) and expressed as mg P/L in this study (1 *mg P/L=3.06 mg of phosphate/L). The adsorption capacity of the adsorbent in this study is shown in mg P/g (1 mg P/g = 3.06 mg of phosphate/g).*

The phosphate adsorption capacity of Zr-pillared clays was calculated using the following equation:

$$Q_t = \frac{(C_i - C_t) * V}{m}$$

where Q_t (mg P/g) is the phosphate adsorption capacity at different time periods, C_i and C_t were the phosphate concentration in solution at initial and different time periods (mg P/L), m (g) was the weight of adsorbent, V(L) was the volume of solution taken.

The equilibrium adsorption capacity was calculated by,

$$Q_e = \frac{(C_i - C_e) * V}{m}$$

where C_i and C_e were the initial and equilibrium phosphate concentration in solution (mg P/L) respectively, m (g) was the weight of adsorbent, and V(L) was the volume of solution taken.

6.1.2 Phosphates adsorption kinetics

Fig. 1 shows the adsorption capacity of raw clay (Na-CM) was almost zero throughout the 240 minutes. However, in the Na-Zr-50-2.5 sample, the phosphate adsorption was very rapid in the first 90 minutes and then changed gradually until Q_t reached 11.3 ± 0.17 mg P/g after 210 minutes where the adsorption equilibrium was achieved (**Fig. 2A**). At the beginning of the adsorption phase a high standard deviation was also observed because there were many accessible vacant adsorption sites available in the adsorbent and there were many free adsorbates in the solution as well. During the later phase of adsorption, the saturation of available adsorption sites took place and equilibrium was achieved (**Fig. 2A**). However, in the Na-Zr-50-5 sample, in the first 120 minutes the adsorption was very rapid after that a sudden drop was observed at 150 minutes and after that, it started to increase again (**Fig. 2B**). In this samples equilibrium was not achieved and a high standard deviation was observed throughout the whole adsorption process. The maximum phosphate adsorption capacity achieved at 240 minutes was 11.5±1.34 mg P/g. In literature, it is well documented that Zr-based adsorbents

have shown excellent properties in the sequestration of phosphates from water based on the principle of Lewis-acid base interaction [29]. However, the higher zirconium loading in the clay structure does not confirm more adsorption of phosphate ions. Among both the pillared clays, the sample having less concentration of zirconium ions i.e. 2.5 mmol/g has shown a better adsorption curve.

To gain more insight into the adsorption mechanism and kinetics, the pseudo-first-order kinetic model (1), pseudo-second-order kinetic model (2) and Elovich models (3) were applied to kinetic data. The following equations were used to study adsorption kinetics [30].

$$Q_t = Q_e (1-\exp{}^{-k_1 t}) \tag{1}$$
$$Q_t = k_2 Q_e^2 t/1 + k_2 Q_e t \tag{2}$$
$$Q_t = \frac{1}{\beta}\ln(1 + \alpha\beta t) \tag{3}$$

Where t was the adsorption time (min); Q_t (mg/g) and Q_e (mg/g) are the phosphate adsorption capacities at time t (min) and at equilibrium, respectively; k_1 (1/min) and k_2 (g/mg min) are rate constants of a pseudo-first-order kinetic model and pseudo-second-order kinetic model, respectively; α is the initial adsorption rate (mg/g min) and β is the desorption constant (g/mg).

The non-linear fitting plots of pseudo-first-order, pseudo-second-order and Elovich models are depicted in **Fig. 2A and B.** The kinetic model parameters are shown in **Table 1**. Based on the correlation coefficient (R^2) values, it was found that for Na-Zr-50-2.5 the Elovich model (0.93) gave the best fit to the kinetic data followed by pseudo-second-order (0.87) and pseudo-first-order (0.73). The well-fitting of the Elovich model suggests that the chemisorption took place between phosphates and clay surfaces [30]. However, for Na-Zr-50-5 there was a slight difference between the Elovich kinetic model (0.88) and pseudo-second-order kinetic model (0.89).

Table 1: Kinetics parameters of the pseudo-first-order kinetic model, pseudo-second-order kinetic model and Elovich kinetic model for phosphate adsorption onto Na-Zr-50-2.5 and Na-Zr-50-5.

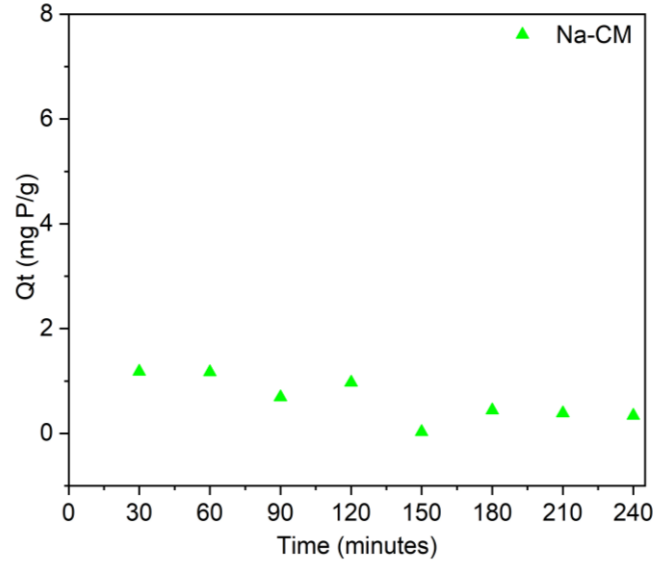| Sample	pseudo-first-order kinetic model			pseudo-second-order kinetic model			Elovich kinetic model		
	R^2	k_1 (1/min)	Q_e (mg/g)	R^2	k_2 (g/mg min)	Q_e (mg/g)	R^2	α (mg/g min)	β (g/mg)
Na-Zr-50-2.5	0.73	0.0169	11.42	0.87	0.0021	13.02	0.93	1.72	0.46
Na-Zr-50-5	0.83	0.0266	10.73	0.89	0.0027	12.42	0.88	1.26	0.43

Figure 1: Phosphate adsorption capacity of raw montmorillonite (Na-CM).
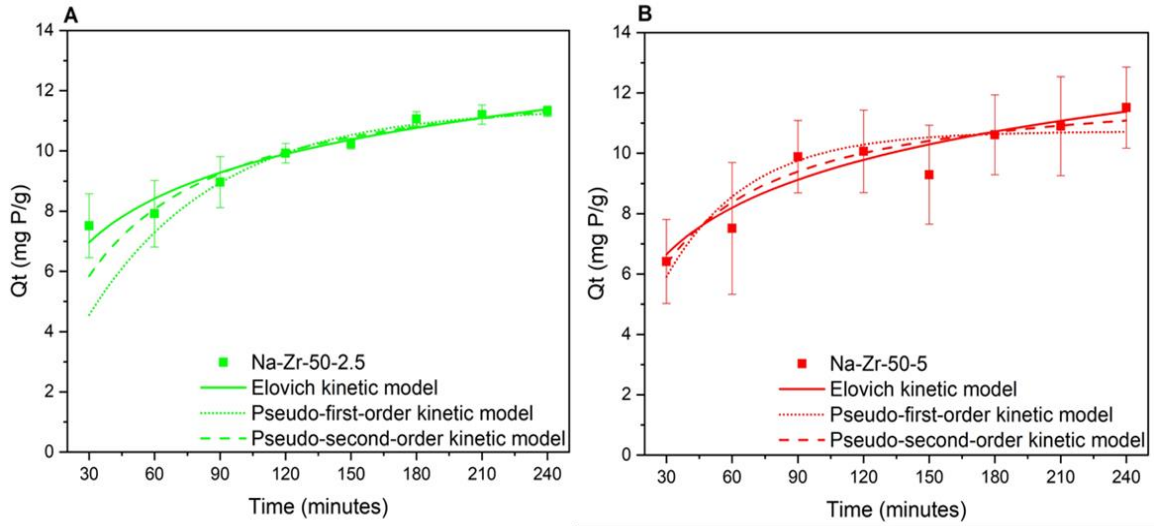

Figure 2: Pseudo-first order, pseudo-second order and Elovich kinetic fitting plots of phosphate adsorption on Na-Zr-50-2.5 **(A)** and Na-Zr-50-5 **(B)** at pH 4.

6.1.3 Adsorption isotherms

The adsorption experimental data were fitted with Langmuir (4), Freundlich (5) and Toth (6) adsorption isotherm models.

$$Q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e} \qquad (4)$$

$$Q_e = K_F C_e^{1/n} \qquad (5)$$

$$Q_e = \frac{Q_{max} K_T C_e}{[1 + (K_T C_e)^t)]^{1/t}} \qquad (6)$$

Where Q_e (mg P/g) is the phosphate adsorption capacity at equilibrium, C_e (mg P/L) is the equilibrium phosphate concentration in the solution, K_L (L/mg) is the Langmuir constant related to the energy of adsorption, Q_{max} (mg P/g) is the maximum adsorption capacity, K_F (mg/g·(L/mg)$^{1/n}$) is the Freundlich constant related to adsorption capacity, and 1/n is a dimensionless indicator measuring the adsorbent surface heterogeneity, K_T is the Toth equilibrium isotherm constant (L/mg), t is the dimensionless Toth exponent [31].

Figures 3-4 depict the equilibrium adsorption capacities of phosphates for Na-Zr-50-2.5, Na-Zr-50-5 and Ch-NaZr2.5 as a function of equilibrium concentrations at pH 4 and 7. For all three samples, phosphate adsorption data were best fitted with the Toth model based on regression coefficient (R^2) values (**Table 2**). Toth model is a three-parameter isotherm used to study the adsorption on heterogeneous surfaces [32]. It is an extended modification of the Langmuir model. By comparing the values of Q_{max} (mg P/g) obtained from the Toth model, the values were very deviated from the experimental data, and also the standard deviation was very high. However, values obtained from the Langmuir isotherm were much closer to the experimental data. The Langmuir model is the most widely applied sorption isotherm which fits well with different types of experimental data [33]. It assumes that adsorption is homogenous, in which all active sites are energetically equivalent, and the surface of the adsorbent is covered with a monolayer [31]. If we compare the Q_{max} values obtained from the Langmuir model (**Table 5**), then the chitosan-modified sample has shown the highest phosphate adsorption capacity at both pH.

Table 2: Parameters of isotherm models for phosphate adsorption onto different Zr-PILCs and chitosan-modified zirconium pillared clay (Ch-NaZr2.5).

| Model	Parameter	Na-Zr-50-2.5		Na-Zr-50-5		Ch-NaZr2.5	
		pH 4	pH 7	pH 4	pH7	pH 4	pH 7
Langmuir	Q_{max} (mg P/g)	11.5 ± 0.98	8.3 ± 0.64	9.6 ± 0.93	6.19 ± 0.21	14.5 ± 0.42	11.8 ± 0.35
	K_L	3.1 ± 1.16	4.05 ± 1.8	5.6 ± 3.86	3.7 ± 0.88	7.1 ± 0.58	4.05 ± 0.48
	R^2	0.93	0.93	0.93	0.93	0.98	0.97
Freundlich	K_F	8.16 ± 0.61	4.93 ± 0.69	8.04 ± 0.09	4.53 ± 0.18	11.4 ± 0.24	9.25 ± 0.03
	1/n	0.19 ± 0.06	0.26 ± 0.07	0.34 ± 0.10	0.16 ± 0.02	0.15 ± 0.01	0.12 ± 0.01
	R^2	0.81	0.89	0.89	0.93	0.95	0.96
Toth	Q_{max} (mg P/g)	7.9 ± 3.71	5.07 ± 4.97	6.9 ± 8.5	3.5 ± 1.26	11.13 ± 3.82	7.23 ± 2.95
	K_T	7.52 ± 8.97	8.04 ± 13.7	8.27 ± 14.7	13.7 ± 14.4	12.3 ± 9.7	18.12 ± 27
	t	0.93 ± 0.08	0.90 ± 0.16	0.9 ± 0.30	0.89 ± 0.04	0.94 ± 0.05	0.92 ± 0.04
	R^2	0.95	0.94	0.94	0.98	0.98	0.98
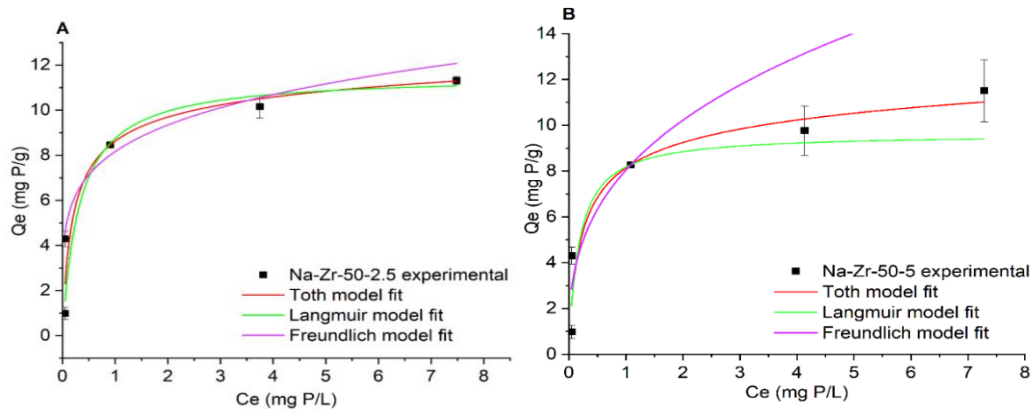
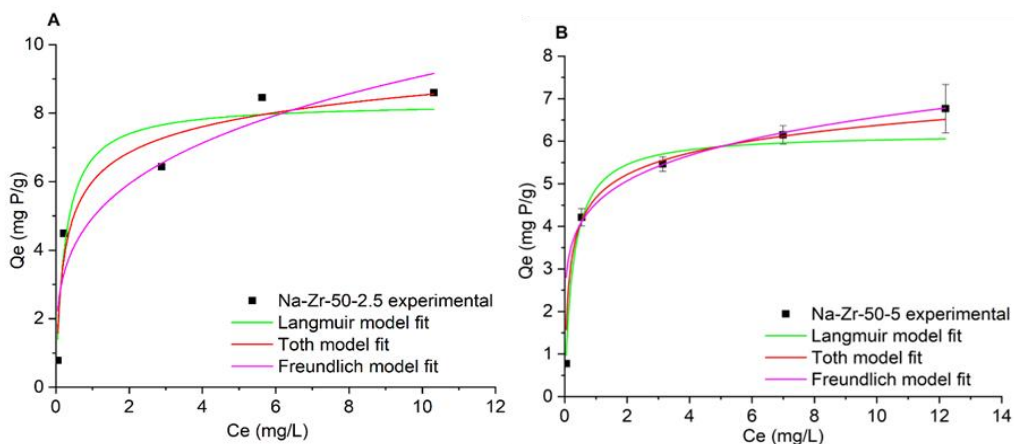**Figure 3:** Adsorption isotherms of phosphates on Zr-pillared clays at pH 4.

Figure 4: Adsorption isotherms of phosphates on Zr-pillared clays at pH 7.
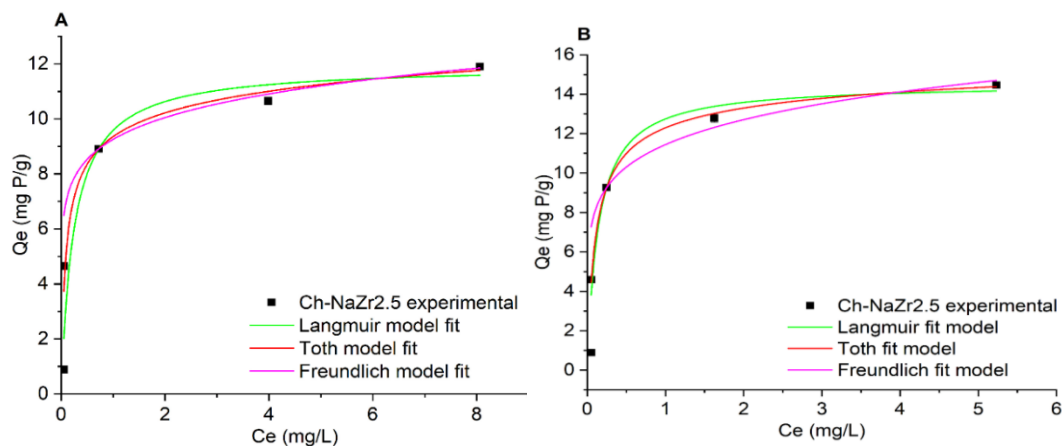

Figure 5: Adsorption isotherms of chitosan-modified zirconium pillared clay (Ch-NaZr2.5) at pH 7 (A) and 4 (B).

6.1.4 Mechanism of phosphate adsorption

The initial pH of the solution is one of the important parameters influencing the adsorption of phosphates on the clay adsorbent. In the crystal lattice of clay, silicon and aluminium ions are present. When these ions are replaced by other less positive valence ions, a pH-independent permanent negative charge is created on the basal surface of clay. This negative charge can be balanced by exchangeable interlayer cations. Along with this, the edges of clay particles contain polarisable Al–OH and Si–OH groups which became positively or negatively charged depending on the pH of the solution [34]. The intercalation of positively charged zirconium ions facilitates the charge neutralization or blocking of negative charge, as a result, the effect of negative charges on the reactivity of edge groups is neutralized [35]. **Fig. 6** shows the effect of pH on the zeta potential of raw clay and modified clays.
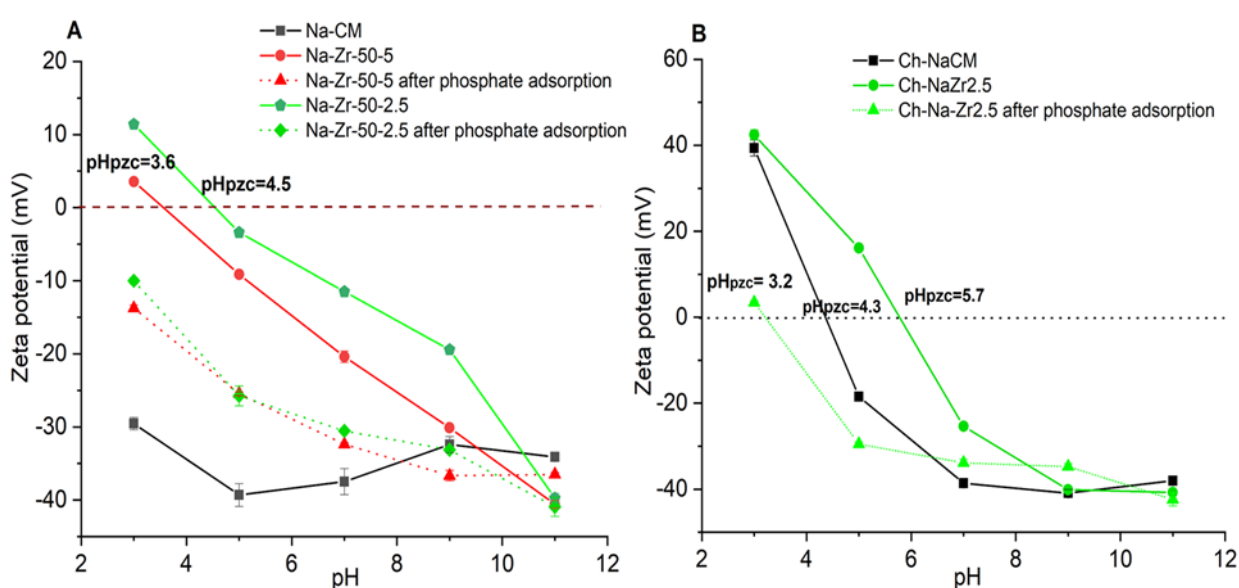

Figure 6: Effect of pH on zeta potential of raw montmorillonite (Na-CM) and Zr-pillared clays **(A)** chitosan-modified Zr-pillared clay **(B)** before after phosphate adsorption.

Fig. 6A depicts that the zeta potential of raw montmorillonite (Na-CM) was negative in the whole pH range and point of zero charge (pH$_{PZC}$) was not achieved. This confirms the dominance of structural negative charge on the basal surface. However, in the Zr-pillared samples, a significant change in zeta potential values was observed. It has increased to 11 mV and 3 mV in samples Na-Zr-50-2.5 and Na-Zr-50-5 at pH 3, respectively (**Fig. 6A**). The former sample shifted the zeta potential in a more positive range than the latter, favouring the strong interaction between the clay surface and phosphate species. One of the possible explanations for achieving higher zeta potential in Na-Zr-50-2.5 could be the arrangement and distribution of ZrO$_2$ pillars in the interlayer region of montmorillonite clay. In STEM-HAADF and EDX analysis of these samples it was found that the Zr structures were separated by a distance of 1.6-2 nm and 2.8-1.6 nm in Na-Zr-50-2.5 and Na-Zr-50-5 respectively. Through adsorption experiments, it was revealed that a Zr^{4+}/ clay ratio of 2.5 mmol/g is the optimum concentration for preparing adsorbent materials suitable for targeting phosphate ions. Furthermore, the composition of the prepared clay materials can affect not only the adsorption properties but also the orientation of the ZrO$_2$ pillars. It is assumed that the increased Zr^{4+} amount caused the

formation of a less regular clay structure in the Na-Zr-50-5 sample. The ZrO_2 pillars in the Na-Zr-50-5 sample could have negatively affected the adsorption test results.

In the case of the chitosan-modified Zr-pillared sample (Ch-NaZr2.5) it was increased to 42 ± 1.2 mV (**Fig. 6B**). The zeta potential of Zr-pillared clay shifted to a much higher positive value after the treatment of chitosan. This confirms the introduction of more positive reactive sites in the clay structure. This can favour the electrostatic attraction between modified clays and negatively charged phosphate species. After phosphate adsorption, the zeta potential of all three samples (Na-Zr-50-2.5, Na-Zr-50-5 and Ch-NaZr2.5) became negative in the whole pH range. The pH_{PZC} of Ch-NaZr2.5 was 5.7 before adsorption, after being loaded with phosphates, it shifted to a lower value (3.2) (**Fig. 6B**). The adsorption mode of ions on a mineral surface is mainly divided into inner and outer complex formation. The chemical interaction in the former is stronger than in the latter, and generally, the value of pH_{PZC} does not change by the formation of outer sphere complexes because of insufficient sorbate-sorbent interaction [36]. The result thus indicates that phosphates were adsorbed on modified clays by forming inner-sphere surface complexes.

Another possible mechanism for adsorption could be ligand exchange. During the process, the hydroxyls (-OH) groups of the adsorbent are exchanged by phosphates. This results in an increase of aqueous solution pH due to the release of -OH ions [37]. However, we checked the pH of the solutions before and after phosphate adsorption. After adsorption, the pH of the solutions was found to be increased slightly. Because there is very little pH increase during the phosphate adsorption process, it is possible that ligand exchange is not the primary driving force.

6.2 Application of precursor (Na-CM) and zirconium pillared clay (Na-Zr-50-2.5) in *E. coli* adsorption from aqueous solution

Table 7 summarizes the concentration of *E. coli* cells before and after the adsorption by using different separation techniques. In the reference sample, a reduction of 5.67% in the cell concentration was observed after sedimentation. However, in Na-CM, a 34.7% reduction was observed and the dispersion of Na-CM in a physiological saline solution was found to be stable even after 30 minutes. This suggests that the dispersed sodium-saturated montmorillonite clay is capable of adsorbing a certain amount of bacteria. In comparison to Na-CM, the Na-Zr-50-2.5 particles showed rapid sedimentation and therefore it was required to shake the sample at 160 rpm to maintain the dispersion during incubation to use them as efficient adsorbents for bacterial cells. Na-Zr-50-2.5 sample was able to reduce the bacterial cell concentration up to 62.64% which is almost twice that of unmodified clay (Na-CM).

The separation of cells from suspension after adsorption is very sensitive as it can be affected by external factors such as centrifugation which is the standard method reported in literature used for the separation of adsorbed and non-adsorbed microorganisms [38]. Therefore, to verify the results of adsorption, we extended our experimental work and included the usage of centrifugation at 1000 rpm for 1 minute. It was found that after applying centrifugation, in the reference sample, only a 2.59% reduction in bacterial cell concentration was achieved which is not a significant amount. This led to the conclusion that centrifugation at 1000 rpm for 1 minute did not significantly separate the *E. coli* bacterial cells from the liquid phase. Therefore, while using an adsorbent material any notable difference in cell concentration between the initial and

the post-centrifugated dispersion would indicate the adsorption capacity of the clay particle. However, in the case of Na-CM, only 9.74% of the initial cell concentration was missing from post-centrifugation dispersions. This reveals that sedimentation is a more suitable and effective method for demonstrating the adsorption capacity of the Na-CM particles than 1-minute centrifugation. However, in the Na-Zr-50-2.5 sample, a significant reduction in bacterial cell concentration (60.33%) was observed during centrifugation. In this case, the application of gentle centrifugation (1000 rpm for 1 minute) did not alter the overall observations obtained in the case of sedimentation. This validates that the adsorption capacity of Zr-modified clay is neither affected by sedimentation nor centrifugation.

The possible reason for achieving a higher *E. coli* adsorption capacity in the Zr-pillared clay sample is the stronger electrostatic interaction between PO_4^{3-} and COO^- groups which are present in the bacterial cell wall, and positive groups of pillared clays. The gram-negative bacteria have an outer covering of phospholipids and lipopolysaccharides, which impart a negative charge to the bacteria's surface [39]. The zeta potential values of Na-CM and Na-Zr-50-2.5 in saline solution were found to be -50.4± 2.8 and -5.31± 0.21 mV at pH 5.6. After the intercalation of zirconium ions, the surface of montmorillonite clay becomes more positive, thus facilitating the attachment of negatively charged bacterial cells on its surface. However, despite having a very high negative zeta potential, Na-CM was able to capture a few percentages of *E. coli* cells. The reason could be other non-electrostatic forces such as hydrogen bonding, van der Waals forces and hydrophobic interactions acting between bacteria and clay surface [40].

Table 3: Comparison of the cell concentrations obtained before and after adsorption for adsorbent-free (reference), Na-CM, and Na-Zr-50-2.5 samples using different separation techniques.

	Reference				
	C_i	C_a	C_d	difference	C_{ads}
Experiment	×10⁹ CFU/mL	×10⁹ CFU/mL	×10⁹ CFU/mL	%	×10⁹ CFU/mL
sedimentation	2.35 ± 0.90	2.22 ± 0.19	0.13	5.67	n.a
centrifugation	2.13 ± 0.28	2.07 ± 0.30	0.06	2.59	n.a
sedimentation + resuspension	2.40 ± 0.27	2.48 ± 0.28	-0.08	-3.33	0.04 ± 0.02
	Na-CM				
	C_i	C_a	C_d	difference	C_{ads}
Experiment	×10⁹ CFU/mL	×10⁹ CFU/mL	×10⁹ CFU/mL	%	×10⁹ CFU/mL
sedimentation	2.62 ± 0.35	1.71 ± 0.21	0.91	34.71	n.a
centrifugation	2.16 ± 0.19	1.95 ± 0.13	0.21	9.74	n.a
sedimentation + resuspension	2.25 ± 0.05	1.53 ± 0.11	0.72	33.05	0.65 ± 0.06

	Na-Zr-50-2.5				
	C_i	C_a	C_d	difference	C_{ads}
Experiment	**$\times 10^9$ CFU/mL**	**$\times 10^9$ CFU/mL**	**$\times 10^9$ CFU/mL**	**%**	**$\times 10^9$ CFU/mL**
sedimentation	2.27 ± 0.50	0.85 ± 0.28	1.42	62.64	n.a
centrifugation	2.00 ± 0.04	0.79 ± 0.05	1.20	60.33	n.a
sedimentation + resuspension	2.26 ± 0.25	1.07 ± 0.04	1.19	52.65	0.88 ± 0.13

C_i: Initial bacterial cell concentration in the adsorbent-free and adsorbent-containing dispersions after the incubation period (CFU/mL).

C_a: Bacterial cell concentration after adsorption (CFU/mL).

C_d: Difference in the initial and post-adsorption cell concentrations (CFU/mL).

C_{ads}: Cell concentration in the residual precipitate phase during confirmation tests (CFU/mL).

7. Summary

The goal of this research was to create and optimize the synthesis process of sustainable clay-based adsorbent materials which can be used in the removal of water contaminants. Montmorillonite clay which is used as a base material is found in abundance in nature. This clay was selected for the study because of its unique chemical structure and physical properties. The structure is made up of aluminosilicate layers and in between these layers (interlayer space), water molecules and small cations are present such as Na^+, Ca^{2+} and Mg^{2+}. These ions are mobile and exchangeable in nature. For modification of Mt clay, zirconium and chitosan were used because of their distinctive properties favourable for the adsorption of target molecules. Zr^{4+} possesses a high coordination ability and more selectivity towards phosphates. It can immobilize the phosphates on the clay surface by making inner-sphere complexes. Chitosan is a natural biopolymer with no toxicity and when it is combined with clay more active sites are generated on the clay surface which helps to catch phosphates from water at different ranges of pH. In this study, interlayer ions of naturally occurring montmorillonite clay were replaced and fixed with zirconium cations and the process is known as intercalation or pillaring. The study is divided into two parts, the first part consists of the synthesis method used for the development of zirconium pillared clays and chitosan-modified Zr-pillared clays, and the second part mainly focuses on the application of these modified clays in the adsorption of phosphates and *E. coli* from aqueous solution.

To begin with, a comprehensive study was conducted to determine the optimum amount of zirconium uptake into the interlayer space of montmorillonite clay and the effect of sodium pre-treatment on the process of pillaring. Two precursor materials were taken pure raw clay (CM) and sodium ion-saturated montmorillonite clay (Na-CM). From each precursor, three zirconium pillared clays were developed using three different Zr^{4+}/clay ratios (2.5, 5 and 10 mmol/g). The samples were investigated with different techniques such as XRD, FT-IR, TGA, HRTEM and STEM-EDX to confirm the process of intercalation and to get more insight into the zirconium microstructures created during the process of pillaring. After investigation, it was found that pillared clays which were prepared from Na-CM, have shown better intercalation of zirconium

ions. Also, samples which were prepared with 2.5 and 5 mmol of zirconium per gram of clay (Na-Zr-50-2.5 and Na-Zr-50-5) showed an even distribution of zirconium pillars in the interlayer space. The thickness of these pillars was found to be in the range of **1-2 nm,** and they were separated by a distance of **1.5-3 nm**. These two samples were further used in the adsorption of phosphates from water. Among two of them, the **Na-Zr-50-2.5** sample was found to be more effective in the sequestration of phosphates. The Langmuir phosphate adsorption capacity of Na-Zr-50-2.5 and Na-Zr-50-5 was found to be **11.5 ± 0.98** and **9.6 ± 0.93** mg/g respectively at pH 4. A possible reason for this could be that the distribution and arrangement of zirconium pillars created in between the silicate layers are influenced by zirconium concentration which further affects the surface charge of pillared clays. Zeta potential measurements revealed that the Na-Zr-50-2.5 sample was able to shift the zeta potential to a more positive value i.e, 11 mV than Na-Zr-50-5 (3 mV) thus favouring the strong interaction between the clay surface and phosphate species. Therefore, zirconium loading of 2.5 mmol per gram of clay is found to be the optimum concentration for achieving maximum adsorption capacity.

In the beginning, the application of clay samples was explored only at acidic pH because in literature it is well documented that phosphate adsorption is favoured at acidic pH because the surface hydroxyl groups of pillared clays get protonated which facilitates the attraction of negatively charged phosphate species. However, to enhance the performance of these materials at higher pH as well further chitosan modification was applied. Positively charged amino groups of chitosan introduced more reactive sites on the surface of clay which are easily accessible to negatively charged phosphate groups. As a result, **Ch-Na-Zr-50-2.5** was perfectly working at pH 4 and pH 7. After chitosan modification, the maximum phosphate adsorption capacity of Ch-NaZr2.5 increased from **11.5 ± 0.98 to 14.5 ± 0.42** and **8.3 ± 0.64 to 11.8 ± 0.35** mg/g at **pH 4 and 7** respectively. The possible major governing mechanism responsible for the adsorption of phosphates was the formation of inner-sphere complexes.

Another application of **Na-Zr-50-2.5** was explored in the removal of *E. coli* from an aqueous solution. As per our knowledge, these zirconium pillared clays have not been used in the removal of biological pathogens so far. In the study, *E. coli* adsorption properties of raw montmorillonite (Na-CM) and Na-Zr-50-2.5 were investigated and compared. To conduct the *E. coli* adsorption experiments, a novel approach was taken for the optimization of the procedure suitable for calculating the adsorption capacity of powder clay materials. Three approaches (sedimentation, centrifugation and residual pellet investigation) were taken to verify the efficiency of clay samples in the removal of *E. coli*. For Na-CM, the sedimentation technique was found to be more accurate in determining the number of *E. coli* cells adsorbed. The *E. coli* removal percentage of Na-CM was found to be 34.7%. In the case of Na-Zr-50-2.5, the *E. coli* removal percentage achieved from sedimentation and centrifugation techniques was 62.64 and 60.33 % respectively. Both sedimentation and centrifugation techniques produced almost similar results in Na-Zr-50-2.5. To validate these results, the precipitate phase (adsorbent containing attached bacteria) was also examined and plated on agar plates to determine the number of cells attached to the clay particles. The amount of *E. coli* cells attached to the Na-CM and Na-Zr-50-2.5 particles was found to be 0.65 ± 0.06 and 0.88 ± 0.13 CFU/mL**.** The prepared clay-based adsorbent materials have shown satisfying results in the adsorption of phosphates and *E. coli* from an aqueous solution. Application of these newly formed clay

materials in wastewater treatment plants can open an alternative pathway which is economically feasible and non-toxic.

8. Claims

The modified clays were synthesized by intercalating zirconium ions in the interlayer region of montmorillonite clay and the surface of the prepared materials was further modified using chitosan. The powder materials were tested for their applicability as adsorbents in wastewater treatment. These nanoporous clay-based materials were found to be suitable for targeting more than one pollutant from water i.e. phosphates and *E. coli*.

Claim 1: Effect of Na$^+$-pretreatment and zirconium concentration on the nature of pillars created inside the clay structure.

The Zr-pillared clays were synthesized using two precursor materials; naturally occurring montmorillonite (CM) and sodium-exchanged montmorillonite (Na-CM). I observed that samples which were prepared from Na-CM have shown better pillaring. A possible explanation for this could be when CM was treated with NaCl, sodium ions entered the interlayer space and interlayer water was removed as an effect of heating. This makes the clay structure homogenized and the sodium ions facilitate the subsequent exchange with zirconium ions. Also, the mobility of the interlayer ions depends on the stability of the hydration shell around them. Sodium ions have a less stable hydration shell therefore they have more mobility. In CM, sodium ions are present along with other cations such as calcium and potassium therefore the moving region of Na$^+$ ions are constrained by the hydration shell of other cations.

I confirmed that Na$^+$-treatment enhanced the process of pillaring. I established that the optimum concentration for pillaring was found to be 5 mmol/g above this concentration the pillaring was non-uniform. The thickness of the ZrO$_2$ pillars varied between 1-2 nm and the structures were separated by a distance of 1.5-3 nm in Na-Zr-50-2.5 and Na-Zr-50-5 samples.

Claim 2: Effect of Zr^{4+}/clay ratio and chitosan modification on phosphate adsorption property of Zr-pillared clays

During the phosphate adsorption study, it was observed that the pillared clay having a Zr^{4+}/clay ratio of 2.5 mmol/g (Na-Zr-50-2.5) showed better phosphate adsorption capacity than 5 mmol/g (Na-Zr-50-5). At pH 4, the Langmuir phosphate adsorption capacity was found to be 11.5 ± 0.98 and 9.6 ± 0.93 mg/g respectively. A possible reason for this could be that the distribution and arrangement of zirconium oxide pillars created in between the silicate layers are influenced by zirconium concentration which further affects the surface charge of pillared clays. Zeta potential measurements revealed that the Na-Zr-50-2.5 sample was able to shift the zeta potential to a more positive value i.e, 11 mV than Na-Zr-50-5 (3 mV) thus favouring the strong interaction between the clay surface and phosphate species.

I confirmed that zirconium loading of 2.5 mmol per gram of clay is found to be the optimum concentration for achieving maximum adsorption capacity. To enhance the performance of Na-Zr-50-2.5 at higher pH as well a further chitosan modification was applied. Positively charged amino groups of chitosan introduced more reactive sites on the surface of clay which are easily accessible to negatively charged phosphate groups. I established that the Ch-NaZr2.5 sample can work effectively in the removal of phosphates from water at both pH.

Claim 3: *E. coli* **adsorption capacity of zirconium pillared (Na-Zr-50-2.5) and precursor clay (Na-CM)**

Precursor clay (Na-CM) and zirconium pillared clay (Na-Zr-50-2.5) were tested against the removal of *E. coli* bacteria from the saline solution. A novel approach was developed to conduct these experiments which include the usage of different separation techniques for separating powder adsorbent materials from saline solution such as sedimentation and centrifugation. For further validation of the results, the adsorbent materials collected at the bottom of the centrifuge tubes after the adsorption of *E. coli* cells were also investigated separately and the results were in favour of the sedimentation technique. Na-CM was able to reduce the *E. coli* level up to 34.71% and Na-Zr-50-2.5 was able to remove 62.64% which is almost double of precursor material.

I confirmed that Na-Zr-50-2.5 is more effective in the removal of *E. coli* **cells from aqueous solution than raw clay due to stronger electrostatic interaction between clay surface and bacterial cells.**

9. Publications

Publications connecting to dissertation

1. **Chauhan, T.,** Udayakumar, M., Shehab, M.A., Kristály, F., Leskó, A.K., Ek, M., Wahlqvist, D., Tóth, P., Hernadi, K. and Németh, Z., 2022. Synthesis, characterization, and challenges faced during the preparation of zirconium pillared clays. *Arabian Journal of Chemistry*, *15*(4), p.103706. **I.F – 6.212 (Q1)**

2. **Chauhan, T.,** Németh, Z.,2022. Intercalation of zirconium ions into the montmorillonite clay using stir-age-stir technique.PhD students Almanc, ISSN 2939-7294,vol I

3. **Chauhan,T.,**SzőriDorogházi,E.,Muránszky,G.,Kecskés,K.,Finšgar,M., Szabó,T., Leskó ,M., Németh, Z., Hernadi, K.2024. Application of modified clays in the removal of phosphates and E. coli from aqueous solution. *Environmental Nanotechnology, Monitoring and Management* **(Q1).**

Other publications

1. El Mrabate, B., Szőri-Dorogházi, E., Shehab, M.A., **Chauhan, T**., Muránszky, G., Sikora, E., Filep, Á., Sharma, N., Nánai, L., Hernadi, K. and Németh, Z., 2021. Widespread applicability of bacterial cellulose-ZnO-MWCNT hybrid membranes. *Arabian Journal of Chemistry*, *14*(7), p.103232. (**Q1; IF= 6.212**).

2. M., Shehab, Sharma, N.,Gabor,K., Kristály, F., El Mrabate, B**., Chauhan, T**., Tamás,K., Leskó, A.K.,Katalin,A., Árpád,P.B., Hernadi, K., Németh, Z.,2021.Effect of the synthesis parameters on the formation of TiO2 nanostructures: controllable synthesis and adsorption properties of nanowires and nanotubes. *Circular Economy and Environmental Protection*, vol. 5, issue 3.

3. Shehab, M.A., Szőri-Dorogházi, E., Szabó, S., Valsesia, A., **Chauhan, T**., Koós, T., Muránszky, G., Szabó, T., Hernadi, K. and Németh, Z., 2023. Virus and bacterial removal ability of TiO2 nanowire-based self-supported hybrid membranes. *Arabian Journal of Chemistry*, *16*(1), p.104388. (**Q1; IF= 6.212**).

Conference presentations

1. Presented oral presentation on the topic: synthesis of Zr-pillared montmorillonite clays'' in INTERNATIONAL IGDIR CONFERENCE ON APPLIED SCIENCE on April 14-15, 2021 / Igdır, Turkey.
2. Presented oral presentation in 8th International Renewable and Sustainable Energy Conference (IRSEC'20) on 25-28, 2020, Morocco.
3. Presented oral presentation in 9th Interdisciplinary Doctoral Conference (IDK2020) on 27-28 November 2020 organised by the Doctoral Student Association of the University of Pécs.
4. Presented oral presentation LATIN AMERICAN CONFERENCE ON NATURAL AND APPLIED SCIENCES held on October 4-6, 2022 / Villahermosa, Tabasco, Mexico Universidad Autonoma de Guadalajara.

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