

BIO-ELECTROCHEMICAL REMOVAL OF LEAD BY
EXO-ELECTROGENS AND PHOSPHATE REMOVAL USING
ZEOLITE FOR POTENTIAL INDUSTRIAL WASTEWATER
TREATMENT

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By:

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TITLE: Bio-electrochemical removal of lead by exoelectrogens and phosphate removal using zeolite for potential industrial wastewater treatment

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Abstract

Industrial wastewaters contain various contaminants depending on their sources, including phosphate, ammonium and heavy metals. To remove different contaminants, several physical, chemical or biological methods are applied in wastewater treatment plants. In this study, two contaminants of ionic lead and phosphate from two different wastewaters were considered separately with two different removal methods. Exoelectrogenic bacteria were studied for lead removal and application of zeolite for phosphate removal was also investigated. The examined methods were proposed for a relatively small-scale (maximum 5000 liters per day) and non-continuous treatment plant for achieving more than 90% removal of both contaminants.

The first part of this project was investigating Pb(II) removal using a bio-electrochemical method. Lead, as a toxic and non-biodegradable heavy metal, has been a major concern in the wastewater treatment area. Microbial electrolysis cells (MECs) are known to remove heavy metals effectively while the exoelectrogenic bacteria oxidize organics at the anode electrode and cations (like heavy metals) get reduced at the cathode electrode. A study has shown that Pb(II) can be reduced both at the cathode and the bioanode. In this lab-scale study, exoelectrogenic bacteria have been prepared on the anode fibers of an MEC and then the enriched bioanode was used individually in the system without applying any voltages. The effectiveness of the proposed system on Pb(II) removal in different initial pH condition and the consistency of this system were studied. Pb(II) removal mechanisms were also investigated. Results showed more than 90% Pb(II) removal in 24 hours for a wide range

of pH conditions (pH 5 to 9). Moreover, adding lead after each 24 hours for four times showed the high capacity of bioanode. Two mechanisms of direct reduction and biosorption were identified as the removal mechanisms of lead. Biosorption mechanism with the contribution of 82% to lead removal had a more significant role than the reduction mechanism. It was also indicated that the exoelectrogenic bacteria played the main role in both of the removal mechanisms by providing oxidation-reduction condition and biosorption capacity.

The second aspect of this project was focused on phosphate removal using zeolite. Phosphate in wastewater and the resulting eutrophication have always been a crucial concern and have resulted in developing of different technologies for its removal. Natural zeolites with the capability of cation exchanging have been extensively studied for cation removal like ammonium. However, by pretreating zeolite and making changes on its physico-chemical properties, zeolite can also be used for removing anions like phosphate. In this aspect of the thesis, a clinoptilolite zeolite was pretreated by NaCl, Mg(OH)₂ and Ca(OH)₂ and the effect of pretreated zeolites on phosphate removal efficiency was investigated. Results showed no improvement with magnesium pretreatment although zeolite pretreated by sodium and calcium improved the removal efficiency. 18% phosphate removal by natural zeolite increased to 37% and 60% for zeolites pretreated by Ca(OH)₂ and NaCl respectively. The existence of ammonium in the solution also increased the removal efficiency from 18% to 44% for natural zeolite and ammonium was removed itself by around 34%. Sodium and ammonium in the solution improved the removal efficiency due to being exchanged with calcium and increasing the calcium phosphate precipitation.

Regarding that, the type of zeolite and its amount of calcium were shown to be effective on phosphate removal efficiency. In all conditions, phosphate was removed by calcium phosphate precipitants using either the calcium hydroxide added or the calcium in zeolite structure. Calcium-pretreated zeolite despite its high capacity for phosphate removal had a low efficiency which was explained by the mass transfer mechanism and shaking results showed that better shaking condition could improve mass transferring here and get to 100% phosphate removal by calcium-pretreated zeolite. However, using only calcium hydroxide without any zeolite could also remove 100% of phosphate. Using calcium hydroxide provided a high removal efficiency but increased the final pH as well. Pretreated zeolite had lower efficiency than calcium hydroxide but did not change the pH significantly and had the ability to remove ammonium as well. Using a type of zeolite with a high amount of calcium in its structure and existence of ammonium and sodium in solution along with an appropriate shaking condition can reach high removal efficiency as well.

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1 Introduction

1.1 Background

1.1.1 Heavy metal removal from wastewater

High toxicity of heavy metals even at low concentrations puts them among the strictly regulated contaminants in wastewater effluent. Heavy metals are non-biodegradable materials and accumulation of them both in the environment and in human body remains severe toxic effects. Lead is one of the most toxic heavy metals with special characteristics such as low melting point and corrosion resistance that make it be widely used in many industries (Flora, Gupta, & Tiwari, 2012; Tchounwou, Yedjou, Patlolla, & Sutton, 2012). The lead-acid battery industry has the largest share of lead usage, followed by mining, electroplating, lead-based paint and solder industries which ultimately yields to wastewater contamination (Bahadir, Bakan, Altas, & Buyukgungor, 2007; Kaczala, Marques, & Hogland, 2009). Accumulation of lead in the environment can eventually reach human bodies through soil, dust or plants grown on contaminated soil. Lead poisoning and its potential damages have raised attention toward more efficient lead removal from industrial wastewater (Flora et al., 2012; Tchounwou et al., 2012).

Different technologies have been used for lead removal with their advantages and disadvantages. Chemical precipitation is among the widely used technologies which is relatively simple and inexpensive; however, it leads to hardships with the removal of the low-density precipitants and their disposal (Fu & Wang, 2011; Kavak, 2013). Membrane

filtration can be highly efficient for heavy metal removal and can even do selective separations. However, the high cost of membranes and their fouling problem cause less practical use of them in industrial wastewater treatment (Fu & Wang, 2011; Trivunac & Stevanovi, 2012). Adsorption is another method with a relatively high removal; however, finding the cheap but highly efficient adsorbents is still a challenge in this method. Moreover, the cost of recovering the adsorbents needs to be considered (Fu & Wang, 2011). Ion exchange method is capable of effective heavy metal removal with relatively quick speed, though high expenses caused by regenerating the ion-exchange resins and following secondary pollution have limited its use (Agustiono, Chan, Lo, & Babel, 2006; Fu & Wang, 2011). Coagulation-flocculation method improves settling time and quality, but high secondary sludge production and the high cost of chemical usage make this method less efficient (Agustiono et al., 2006; Fu & Wang, 2011).

Microbial electrolysis cells (MECs) as a type of bio-electrochemical systems have proved to be a promising method for heavy metal removal and are now attracting the researchers toward their capabilities on effective treatment and energy recovery (Fu & Wang, 2011; H. Wang & Ren, 2014). MECs consist of cathode and anode electrodes with microorganisms oxidizing organics at the anode and reduction reactions happening at the cathode. Water reduction at the cathode leads to the hydrogen gas production which is an important area of MECs to study. Additionally, other positively charged ions like heavy metal ions can get reduced to their solid form here (Lu et al., 2015). Exoelectrogenic bacteria have the main role in this whole process by providing the oxidation-reduction condition (Logan, 2009).

1.1.2 Phosphate removal from wastewater

Phosphates as essential materials in many main agricultural and industrial applications such as fertilizers, detergents, water softeners, food, and drinks are considered as of the main components in industrial wastewaters (Hosni, Ben Moussa, Chachi, & Ben Amor, 2008). High concentrations of phosphates and consequences of their accumulation in water bodies increase the importance of finding the best sustainable method for their removal. Phosphates along with ammonia and organics can directly cause eutrophication. Consequently, lack of dissolved oxygen and extreme algal growth can deteriorate the life of fishes and other organisms and contaminate freshwater resources (Correll, 1998). Since the first eutrophication problem in the 1950s, actions were taken in order to develop technologies for phosphorus removal. It was initiated with chemical precipitation method which is still a primary technology (Morse, Brett, Guy, & Lester, 1998).

Many biological, physical and chemical methods have been investigated for efficient and sustainable removal of nutrients from wastewater. Adsorption as a physical method has been proven to be effective for phosphate removal especially in low concentrations and researchers are looking for cheaper adsorbents with higher efficiency (Suresh Kumar, Korving, Keesman, van Loosdrecht, & Witkamp, 2019). Different adsorbents were investigated by researchers in different conditions. A nanostructured Fe–Al–Mn tri metal oxide was used by Lu et al. and the highest adsorption in 25 °C was 48.3 mg/g which was better than their previous results with only one component oxide (Lǔ et al., 2013). Zeng et al. used industrial waste iron oxide tailings in different pH values and found it more effective in lower pH (8.6 mg-P/g at pH 3.2) (Zeng, Li, & Liu, 2004). Alum sludge (Yang,

Tomlinson, Kennedy, & Zhao, 2006), grounded burnt patties (GBP) (Rout, Bhunia, & Dash, 2014) and calcite (Karageorgiou, Paschalis, & Anastassakis, 2007) are also among the effective adsorbents that have been studied by researchers. Kumar et al. investigated the effect of pore and particle sizes of porous metal oxide adsorbent on phosphorus adsorption kinetics (Suresh Kumar et al., 2019). Larger adsorbents (larger than 10 nm) showed faster adsorption and larger pores (larger than 10 nm) were needed for increasing the rate although smaller pores provided more surface area.

Biological methods remove phosphorus either by adsorption mechanisms or enhanced biological phosphorus removal (EBPR). In EBPR methods, specific bacteria called polyphosphate accumulating organisms (PAOs) play the main role. First, these bacteria consume the organics in an anaerobic condition, store polyhydroxyalkanoates (PHAs) and release orthophosphate. Secondly, in a following aerobic condition, PAOs use PHAs as their energy and carbon source and uptake more orthophosphates from the wastewater than the amount released in anaerobic condition (Patel, Nakhla, & Margaritis, 2005) (Luz & Bashan, 2004). However, providing the incubation conditions, organic addition in the first phase, the presence of PAOs and making a favorable condition for their enhancement make this method challenging for phosphorus removal (Luz & Bashan, 2004).

Primary methods for phosphorus removal are based on chemical precipitations usually by metal salts. Addition of iron salts is a common and conventional method for phosphorus removal, it is usually added in the aeration tank or primary sedimentation tank in wastewater treatment plants (Morse et al., 1998). Mixing condition and ferric

chloride dosage as effective parameters for phosphorus removal were studied by Thistleton et al (Thistleton, Berry, Pearce, & Parsons, 2002). Zhou et al. investigated the addition of tannic acid as a coagulant aid along with ferric chloride to see its effect on the phosphorus removal efficiency (Zhou, Xing, Liu, Cui, & Yu, 2008). Hosni et al. used calcium hydroxide to remove phosphate as calcium phosphate precipitation since it does not decrease the alkalinity and does not add any chloride or sulfide to the solution (Hosni et al., 2008). They reached 98% phosphate removal for a Ca/P ratio of 2.07.

Lots of works have been done on the precipitation of phosphates as magnesium ammonium phosphate (struvite) which removes ammonium as well as phosphorus with a high efficiency (Doyle & Parsons, 2002; Kofina & Koutsoukos, 2005; Le Corre, Valsami-Jones, Hobbs, & Parsons, 2009; You, Valderrama, & Cortina, 2017). However, the molar ratio of all three elements has to be 1:1:1 in an optimized pH condition to make it spontaneously happen (Song et al., 2007). Kumar et al. and Corre et al. provided comprehensive reviews on different areas of struvite precipitation (Kumar & Pal, 2015; Le Corre et al., 2009).

Regarding the phosphorus removal, in almost all methods it is removed as a solid fraction, either as an insoluble salt or a microbial mass (Luz & Bashan, 2004). One of the main issues regarding phosphorus removal is the possibility for its recovery. During chemical precipitation, usually some other compounds are created which makes it difficult to separate phosphorus compounds (Wilfert, Kumar, Korving, Witkamp, & Loosdrecht, 2015). Wilfert et al. studied the iron and phosphate chemistry in order to help develop methods for phosphorus recovery from iron phosphate wastes (Wilfert et al., 2015).

1.1.3 Zeolite and its application in wastewater treatment

Zeolites are hydrated aluminum-silicate crystal-like materials with valuable properties such as cation exchanging, molecular sieving, catalysis and adsorption (Guaya, Valderrama, Farran, Armijos, & Cortina, 2015). Zeolite structure consists of three main parts which are aluminosilicate framework, exchangeable cations and water with the general formula of $M_{x/n}[Al_xSi_yO_{2(x+y)}] \cdot pH_2O$ where M is (Na, K, Li) and/or (Ca, Mg, Ba, Sr), n is cation charge; $y/x = 1-6$, $p/x = 1-4$ (S. Wang & Peng, 2010; Yuna, 2016). The main and stable part of zeolite structure is the aluminosilicate part which consists of tetrahedron with aluminum in center bounded to oxygens. Replacement of aluminum (Al^{3+}) with silica (Si^{4+}) generates the negative charge on zeolite which is balanced by cations. This negatively charged surface and the ion exchanging ability of zeolites are the main characteristics that make zeolite a great material for removing cations like ammonium and heavy metals from wastewaters. Effective properties for this ion exchanging mechanism are Si/Al ratio, cation types and their amount and location, ionic charge and concentration of the solution (Yuna, 2016).

There are many different types of natural zeolite around the world with different accessibility. Clinoptilolite, mordenite, phillipsite, chabazite, stilbite, analcime and laumontite are the most common ones among which clinoptilolite is the most abundant and commonly used one (S. Wang & Peng, 2010).

Synthetic zeolite can also be gained using silica and alumina-based raw materials. Some common raw materials are clay minerals, kaolin and also waste materials like carbon fly

ash. Carbon fly ash is the most used material for synthesizing zeolite and can be converted to over 15 types of zeolite depending on its composition (Yuna, 2016).

In order to increase the applicability of zeolite for removal of organics and anions (particularly phosphates), usually there are modification methods done on zeolite surface to change the physico-chemical characteristics and improve the removal efficiency (S. Wang & Peng, 2010). Acid pretreatment is a method that affects the pores characteristics, cleans the impurities and also exchanges hydrogen with zeolite cations until the dealumination of the zeolite structure happens eventually (S. Wang & Peng, 2010). Dealumination causes less cation exchanging; however, acid pretreatment can improve the removal efficiency of non-polar molecules (S. Wang & Peng, 2010). Zhang et al. applied acid pretreatment on synthetic zeolite in order to improve its characteristics for simultaneous ammonium and phosphorus removal at low concentrations (Bao hua Zhang et al., 2007). They found higher efficiency in mild acid pretreatment (0.01 M H₂SO₄). Over this acid concentration, there is deterioration of zeolite structure and a significant decrease in ammonium removal. Phosphorus removal capacity also decreases with higher concentrations of acid which is due to the releasing of cations such as Al, Ca, K; however, they increase the removal efficiency in low concentrations. H₂SO₄ treatment helps CaCO₃ transforming to a more soluble calcium which favors calcium dissolution and then calcium phosphate precipitation.

Surfactant modification is another method used to increase anion and organics adsorption to the zeolite. High concentration of surfactant modifies the zeolite surface by creating a

bilayer on its surface and creating positively charged sites for anions adsorption. Different ionic surfactants such as tetramethylammonium, cetyltrimethylammonium (CTMA), hexadecyltrimethylammonium (HDTMA), octadecyldimethylbenzyl ammonium (ODMBA) and n-cetylpyridinium (CPD) have been investigated by researchers (S. Wang & Peng, 2010). Dionisiou et al. investigated the adsorption and desorption of phosphorus on a natural zeolite modified by hexadecyltrimethylammonium bromide (Dionisiou, Matsi, & Misopolinos, 2013). They did a lab scale experiments and their results showed promising removal of phosphorus which was increased by increasing the pH and decreasing the temperature.

Salt treatment is another zeolite modification method which is used mostly for phosphate removal or simultaneous removal of phosphate and ammonium. In this method, cations like Ca^{2+} , Mg^{2+} , Al^{3+} and Na^{+} present in salt solutions are exchanged with zeolite cations and improve the phosphate sorption to the zeolite surface and making bonds (You et al., 2017).

1.2 Literature review

1.2.1 Application of bio-electrochemical systems (BES) for heavy metal removal

Effectiveness of BESs on removing metals such as Ni, Fe, Ag, Zn, Cr and Cu has been investigated by many researchers (Li, Zhang, & Lei, 2008; Lu et al., 2015; Oskar Modin, Fuad, & Rauch, 2017; Z. Wang, Lim, Lu, Fan, & Choi, 2010; Y. Zhang et al., 2015). Metals like copper, cobalt, silver, vanadium, and chromium due to their high redox potential get removed in microbial fuel cells (MFCs) as another type of BES methods. In MFCs, the metal ions can get the electrons from the anode electrode spontaneously without applying any voltage and they even produce electricity (Heijne et al., 2010; Mathuriya & Yakhmi, 2014; H. Wang & Ren, 2014; Baogang Zhang, Feng, Ni, Zhang, & Huang, 2012). Removal of other metals with low redox potential has been investigated in microbial electrolysis cells since they do not get reduced spontaneously and need a small voltage applied. Luo et al. used MECs to successfully remove Cu^{2+} , Ni^{2+} , and Fe^{2+} from acid mine drainage and produce hydrogen gas simultaneously (Luo et al., 2014). Qin et al. investigated the nickel removal using MECs and focused on the effect of initial nickel concentration, pH and the applied voltage. The results showed that the lower pH values were more favorable (Qin et al., 2012). Colantonio and Kim demonstrated successful removal of cadmium using MECs and recognized cathodic reduction and precipitation as the main mechanisms for cadmium removal in the cathode chamber (Colantonio & Kim, 2016a). They also investigated the effect of initial cadmium concentration in the removal efficiency and removal mechanisms.

It turned out that initial concentration controls the role of each removal mechanism (Colantonio, Guo, & Kim, 2016).

Modin et al. used BESs for metal removal and recovery from a mixed solution containing Cu, Pb, Cd, and Zn. They reached a successful recovery by controlling the cathode potential and changing the applied voltage (O Modin, Wang, Wu, Rauch, & Fedje, 2012). Tao et al. used a combined bio-electrochemical system and electrolysis cell to treat a fly ash leachate containing Cu(II), Zn(II) and Pb(II). Cu(II) was removed in BES; however, Zn(II) and Pb(II) needed applied voltage and were removed in the following electrolysis reactor. They got to 98.5%, 95.4% and 98.1% removal for Cu(II), Zn(II), and Pb(II) respectively (Tao et al., 2014). Bo et al. examined the efficiency of BES for lead removal (Bo et al., 2014). The results showed around 92% removal in 72 hours. Puig et al. focused on the effect of pH on exoelectrogenic bacteria. They observed a decrease in COD removal and power density after pH 9.5 while they were working on electricity production in the air cathode MFCs. It indicated that exoelectrogenic bacteria cannot do well in pH higher than 9.5 (Puig et al., 2010). Colantonio and Kim found out that apart from lead reduction at the cathode and adsorption at the anode, lead can also be reduced at the anode electrode and it is higher in lower voltages even open circuit condition (Colantonio & Kim, 2016b).

1.2.2 Application of zeolite for phosphate removal

Many works have been done on the application of zeolite for phosphate removal. To improve this process, there are usually pretreatment methods done on zeolite such as salt treatment which is focused more here. Hermassi et al. investigated the phosphate adsorption by synthetic zeolite and its calcium modified version and observed a substantial increase in phosphate adsorption capacity from 57 ± 5 to 203 ± 11 mg P-PO₄ /g after pretreatment (Hermassi et al., 2016). Two main mechanisms were suggested for phosphate removal based on their analysis: inner sphere and outer sphere surface complexation with AlOH or FeOH functional groups and creating calcium phosphate either by exchangeable calcium in zeolite or by CaO/ CaCO₃ present on the zeolitic structure. For calcium modified zeolite, the main mechanism was the creation of calcium phosphate as brushite. The effectiveness of both zeolites was increased in slightly basic pH condition compared to the neutral condition.

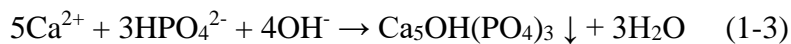
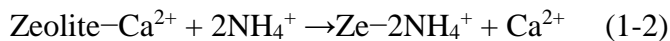
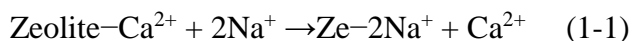
Mitrogiannis et al. worked on Ca(OH)₂ treated zeolite for phosphate removal from urine and observed the improved removal efficiency by calcium treated zeolite compared to natural zeolite (Mitrogiannis et al., 2018). Three mechanisms have been detected for phosphate removal by calcium treated zeolite which were similar to natural zeolite but in a higher extent. These mechanisms are ligand exchanging and making inner sphere complexation (dominant mechanism), surface precipitation of calcium phosphate compounds, electrostatic attraction and making outer sphere complexation.

Zeolite has mostly been studied for simultaneous removal of ammonium and phosphate. You et al. investigated the simultaneous recovery of phosphate and ammonium using zeolite modified by calcium and magnesium (You et al., 2017). The zeolite used was synthesized from coal fly ash. Based on their results, the heating step which was done for zeolite modification improved the modification process since zeolite is more towards monovalent ion exchanging and heating is providing energy for exchanging the divalent ions (Mg^{2+} , Ca^{2+}) here. Diffusion was identified as the sorption mechanism and the removal was happened by adsorption and precipitation. Precipitation was started by ion exchanging; ammonium was exchanged with calcium and magnesium and led to precipitation of calcium phosphate, magnesium phosphate or combination of them with ammonium and creating struvite or brushite. Although, the presence of struvite was not detected on loaded zeolite by XRD. Regarding the effect of pH on ammonium removal, they explained that in pH lower than 3, competition between ammonium and H^+ decreased the ammonium removal efficiency. pH above 10 also reduced the ammonium removal due to its conversion to free ammonia (NH_3).

Guaya et al. studied simultaneous removal of ammonium and phosphate using natural zeolite modified by hydrated aluminum oxide (Guaya et al., 2015). Small hydrated aluminum oxide particles can cause complications with separation if they are used alone for treatment purposes; however, impregnating them in zeolite resolves this problem and provides the chance of using them in treatment processes. They reached phosphate and ammonium sorption of 7 mg-P/g and 30 mg-N/g while natural zeolite had only 0.6 mg-P/g phosphate sorption. The removal mechanism of phosphate was found to be the creation of

inner sphere compounds with Al-OH groups and ammonium removal was caused by ion exchanging and interaction with OH groups of zeolite. They also investigated the effect of competing cations and anions on phosphorus removal efficiency and found no effect with cations and only a slight negative effect by carbonate. They also looked into regeneration ability of zeolites and their experiments showed declining in phosphate removal capacity after each regeneration cycle. So, they suggested using the zeolite as a soil amendment after usage rather than regeneration.

Lin et al. demonstrated the mechanism of simultaneous ammonium and phosphate removal by natural zeolite (Lin, Wan, Lee, Lei, & Liu, 2014). Experiments were done both as a batch and in a column. They investigated the effect of pH and initial concentrations and found more phosphate removal in higher pH (8-11) and a substantial efficiency increase with having ammonium in the phosphate solution. Based on the calcium and phosphate concentration changes in different pH, the following reactions were proposed as the supposing reactions for phosphate removal.



Sodium and ammonium were exchanged with calcium and the released calcium was precipitated with phosphate and hydroxyapatite was the most thermodynamically stable compound of calcium and phosphate. Based on these reactions the N/P ratio greater than

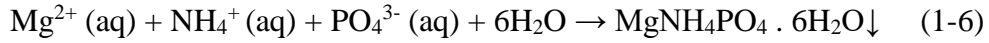
3.3 was proposed in order to reach the highest simultaneous removal of phosphate and ammonium.

Wu et al. investigated the effect of different salts for the treatment of zeolite on simultaneous ammonium and phosphate removal (Wu, Zhang, Li, Zhang, & Kong, 2006). Their treatment method included washing the zeolite with salt solutions and then washing it with water and drying it at 45°C. Al³⁺ treated zeolite had the highest ammonium and phosphate removal. Fe³⁺, Ca²⁺, Na⁺, and Mg²⁺ took the next places in high phosphate removal efficiency respectively. However, after Al³⁺, Ca²⁺ was the best option for the simultaneous removal of ammonium and phosphate since Fe³⁺ treated zeolite did not remove ammonium. Regarding the removal mechanism of phosphate, they considered it ligand exchanging in case of Al³⁺ and Fe³⁺ treated zeolite and precipitation in case of Ca²⁺, Na⁺ and Mg²⁺ treated zeolite.

Huang et al. used magnesium modified zeolite in order to simultaneously remove ammonium and phosphate as Struvite (Huang, Xiao, Pang, Han, & Ding, 2014). Their modification method included stirring the zeolite with 2 M magnesium chloride solution for 12 hours, then washing with ample water and drying at 100 °C for 12 hours. 110 g/L of modified zeolite was used and the removal efficiency of ammonium and phosphate reached 82% and 98% after 40 minutes.

Suggested reactions based on their results are as followed:





They also mentioned Ca^{2+} as another ion being exchanged by ammonium which created calcium phosphate as the result as well. They also investigated the effect of Ca^{2+} , Na^+ , K^+ and Mg^{2+} presence in wastewater on ammonium and phosphate removal. The results exhibited the negative effect of these cations on ammonium removal but positive effect on phosphate removal except for Na^+ . The negative effect of Na^+ in the solution was due to its competition with ammonium for adsorption which resulted in less magnesium released and less ammonium and phosphate removal. They confirmed the struvite precipitation based on removal efficiency in different pH conditions, needle-shaped crystals in SEM image and struvite identification by XRD.

1.3 Objectives

1.3.1 Pb(II) removal from wastewater

Small-scale and non-continuous wastewater treatment plants with a lead concentration of 1 mg/L were targeted for reaching more than 90% lead removal with the proposed method. In MEC reactors, Pb(II) is reduced to its solid form on the cathode electrode. Since Colantonio et al. proved Pb(II) can also be removed on the anode electrode, we used only the bioanode for this purpose (Colantonio & Kim, 2016b). The bioanode enriched from an MEC reactor was used individually in a lead solution without applying any voltages. Objectives to this project were to investigate the effect of bioanode on Pb(II) removal. The effect of pH as a prime factor in MEC systems and heavy metal removal was another objective in this research. The consistency of this system for Pb(II) removal and the removal mechanisms were also examined.

1.3.2 Phosphate removal from wastewater

Phosphate-containing wastewaters from different industries are strictly required to reach a regulated limit of phosphate to get discharged. The concentration of phosphate varies among different industries. One of the local industrial wastewater treatment plants receives a type of wastewater with the phosphate concentration in a range of 20-50 mg-P/L which was the reason we focused on phosphate concentration of 30 mg-P/L. There are different regulations in different regions, for example Hamilton regulation for total phosphorus is 10 mg-P /L but we were targeting for more than 90% removal in this study (City of

Hamilton, 2014). The studied method (using zeolite) was targeted for relatively small-scale (maximum 5000 liter per day) and non-continuous industrial wastewater treatment plants.

In order to achieve high phosphate removal efficiency, natural zeolite was pretreated with different salts and their effects on phosphate removal were investigated. Zeolite pretreatment was done by NaCl, Ca(OH)₂ and Mg(OH)₂. Objectives of this project were to compare the different salt-pretreated zeolites, explore the effect of ammonium in the solution, study simultaneous removal of phosphate and ammonium and identify the removal mechanisms of them. Furthermore, we focused on the existence of sodium in the solution and its effect on improving the zeolite application for phosphate removal.

2 Bio-electrochemical removal of lead by exoelectrogens

Abstract

Non-biodegradability and carcinogenicity of heavy metals make them a major concern in wastewater treatment. Bioelectrochemical systems (BESs) are among the promising methods for their removal. Removal of lead, as one of the most toxic heavy metals, has been studied using the microbial electrolysis cells (MECs). Reduction and electrodeposition of the heavy metal at the cathode and biosorption at the bioanode were demonstrated as the main removal mechanisms in MECs. However, there is a study showing Pb(II) reduction at the bioanode as well as the cathode electrode. So, this lab-scale study demonstrated the effectiveness of using only the bioanode for the Pb(II) removal without applying any electrical energy. In this way, exoelectrogenic bacteria have been prepared on anode fibers of an MEC and then the individual bioanode was taken and put directly in the lead solution without applying any voltage. Over 90% Pb(II) removal in less than 20 hours was achieved in a wide range of pH (5-9). These results were consistent after adding lead every 24 hours for four times. Moreover, direct reduction and biosorption were identified as two mechanisms responsible for Pb(II) removal and it was identified that exoelectrogenic bacteria played the main role in both removal mechanisms.

2.1 Introduction

Lead is known as a non-biodegradable heavy metal with highly toxic properties. Accumulation of lead both in the environment and in human body remains severe toxic effects (Flora et al., 2012; Tchounwou et al., 2012). Lead has been widely used in many industries such as mining, electroplating, lead-acid battery industry, and the ones related to lead-based paint or solder (Bahadir et al., 2007; Flora et al., 2012; Tchounwou et al., 2012). Lead toxicity has raised attention toward more efficient lead removal from industrial wastewaters. Many technologies have been used for this purpose with their pros and cons. Chemical precipitation, membrane filtration, adsorption, ion exchange method, and coagulation-flocculation are among the efficient methods for lead removal; however, precipitant removal and disposal, fouling problem, finding efficient and cheap adsorbents, regenerating and secondary pollution are some serious challenges that these methods are facing respectively (Agustiono et al., 2006; Fu & Wang, 2011; Kavak, 2013; Trivunac & Stevanovi, 2012).

Microbial electrolysis cells (MECs) as a type of bio-electrochemical systems (BES) have proved to be a promising method for Pb(II) removal and energy recovery (Fu & Wang, 2011; O Modin et al., 2012; Tao et al., 2014; H. Wang & Ren, 2014). MECs consist of cathode and anode electrodes with exoelectrogenic microorganisms oxidizing organics at the anode and reduction reactions happening at the cathode. Water reduction at the cathode leads to the hydrogen gas production. Additionally, other positively charged ions like Pb(II) can be reduced to their solid forms at the cathode and this electron transferring is

happening using a relatively low external applied voltage (Lu et al., 2015). Moreover, increasing pH caused by water reduction brings precipitation as another removal mechanism at the cathode electrode (Bo et al., 2014; Colantonio & Kim, 2016a). Biosorption can also be considered as one the Pb(II) removal mechanism in MECs at the anode electrode (Colantonio & Kim, 2016b).

Apart from Pb(II) reduction and precipitation at the cathode and adsorption at the bioanode, it has been found that Pb(II) can also be reduced at the bioanode and it is higher in lower applied voltages (Colantonio & Kim, 2016b). According to those results, a directly reduced mechanism was hypothesized for Pb(II) removal using exoelectrogenic bacteria and no electric current. A simpler process was designed in the current research by removing the cathode and using only the bioanode enriched from an MEC reactor in order to confirm this hypothesis. The effectiveness of using this system on Pb(II) removal and getting an insight into the mechanisms responsible for that have also been investigated. pH as a prime factor in MECs and heavy metal removal was another objective in the current research (Bo et al., 2014). This removal method was considered for small-scale and non-continuous wastewater treatment plants with a lead concentration of 1 mg/L and the goal was reaching more than 90% lead removal.

2.2 Materials and methods

2.2.1 Bioanode enrichment.

A two-chamber MEC reactor without membrane was used in order to grow the bacteria on the anode which is a graphite fiber brush. The MEC reactor was inoculated with the effluent from an existing MEC reactor and fed with a feed solution containing 1.0 g/L CH_3COO^- , trace amount of minerals and vitamins and 10 mM phosphate buffer solution (PBS) (1.7297 g/L $\text{Na}_2\text{HPO}_4 \cdot 7 \text{H}_2\text{O}$, 0.4264 g/L NaH_2PO_4 , 0.0725 g/L NH_4Cl , 0.0259 g/L KCl) (Cheng, Xing, Call, & Logan, 2009; Colantonio & Kim, 2016a). A voltage of 0.6 V was applied to the cell using a power supplier (GW Instek, GPS-1850D, laboratory DC power supply).

2.2.2 Equipment setup

260 mL solution containing deionized water, 2 mM PBS solution and 0.3 g/L CH_3COO^- was used and the bioanode enriched from an MEC reactor was put in this solution individually (Figure 2.1). It started with the mentioned solution for 16 hours and after that 1 mg/L of Pb(II) was added to the solution. After 24 hours 1 mg/L Pb(II) and 2 mM PBS solution were added to the solution to make the volume constant and repeated for two more days with keeping the pH close to the initial value of each experiment. The temperature for the whole experiment was $23 \pm 1^\circ\text{C}$. 24 hours experiment for lead removal was picked based on the preliminary experiments done for finding the time of more than 90% Pb(II) removal (Figure 2.2).

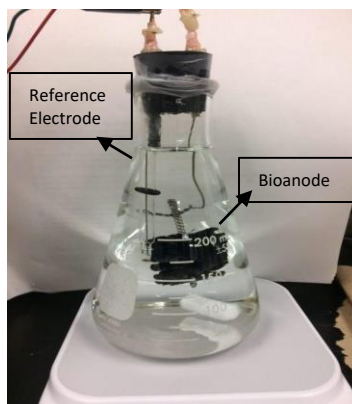


Figure 2.1: A picture of the experiment setup with the bioanode inside the solution and a reference electrode to measure the electrode potential of the bioanode

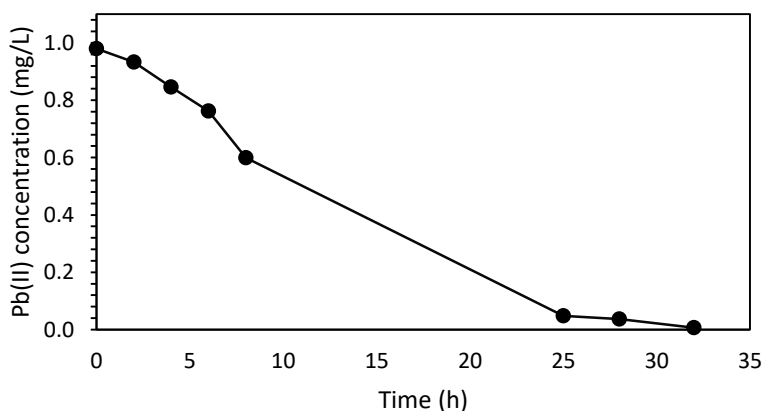


Figure 2.2: Preliminary result of using the system for removal of 1 mg/L lead at pH 7

2.2.3 Measurements and analysis

For Pb(II) concentration measurement, every sample was acidified with 5% nitric acid (70%) and then filtered using a syringe filter (pore size 0.45 μm , polyethersulfone membrane, VWR International, USA). Pb(II) concentration in the solution was measured using inductive coupled plasma-optical emission spectrometry (ICP-OES) (Vista Pro, Varian Inc., Australia). An Ag/AgCl reference electrode (Bioanalytical Systems, Inc. IN)

was also put in the solution to measure the electrode potential of the bioanode. Both electrode potential of the bioanode in the system and the MEC voltage were being recorded every 20 minutes by a multimeter (2700 multimeter/data acquisition system, Keithley Instruments, USA). The pH of the solution was measured using a pH meter (SevenMulti, Mettler-Toledo International Inc., OH).

After all the Pb(II) removal experiments were done, a part of the bioanode was put in 70% nitric acid for 48 hours to dissolve all the deposited lead. Then the amount of lead dissolved in acid was measured with ICP-OES. Another part of the bioanode was first cleaned with ethanol to remove the biofilm and reach to the electrochemically deposited lead. Then, the fibers were put in the 70% nitric acid and the amount of lead deposited on the fibers was measured with ICP-OES. These ethanol-cleaned fibers were also analyzed using scanning electron microscopy-energy dispersive X-ray Spectroscopy (SEM-EDS) (JEOL JSM-6610LV, Japan) in order to see and confirm the electrochemically deposited lead on them.

2.3 Results and discussion

2.3.1 *Pb(II) removal*

Substantial decrease in Pb(II) concentration and its consistency for all four cycles is indicated in Figure 2.3. Rate of Pb(II) removal in the first 8 hours of each cycle was more than the rest of the time after that. After the first cycle, the removal was even better, around 80% of Pb(II) removal (76-81%) was achieved in the first 8 hours and more than 90% removal was achieved in 24 hours of the experiment. The first cycle did not reach 90% removal which could be because of adaptation of the system to the new condition but the rest of the cycles were consistent.

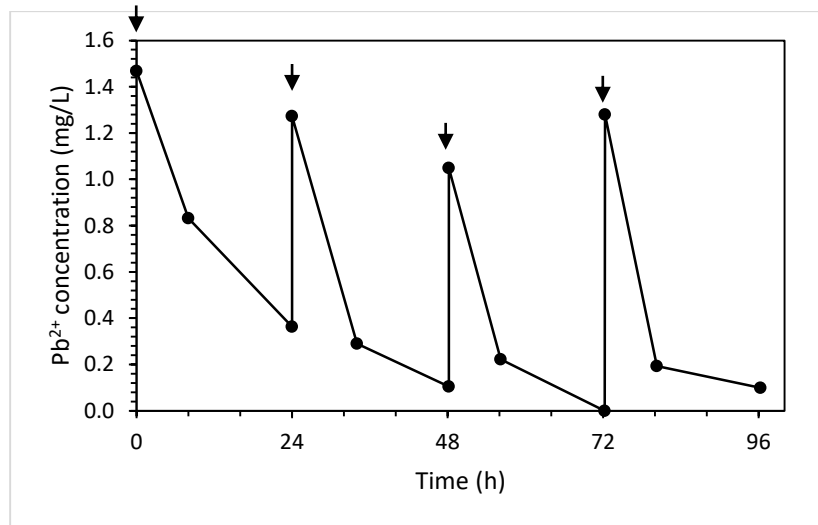
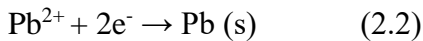


Figure 2.3: Pb(II) removal in the system using only the bioanode enriched from an MEC reactor for four cycles of 24 hours experiment in neutral pH (1 mg/L lead was added at the beginning and then every 24 hours after that- lead injection times are indicated by arrows)

Exoelectrogenic bacteria played the key role here by reducing Pb(II). Respiration of these bacteria in nature leads to the reduction of some positive ions like Fe³⁺ (Colantonio & Kim, 2016b). Thus, Pb(II) reduction can be expected from these bacteria. In addition, the

spontaneous redox reaction was expected here as well. Based on Nernst equation (Eq 2.1), the electrode potential needed for reducing 1 mg/L of lead (-0.286 V vs. SHE) in 296 K is more positive than anode potential (-0.33 V vs. SHE) which means the coupled redox reactions can be done spontaneously. In Nernst equation (Eq 2.1), E° is the standard potential for Pb(II) reduction (-0.126 V vs. SHE), T is the temperature, R is the gas constant ($8.314 \text{ J mole}^{-1} \text{ K}^{-1}$), n is the number of electrons exchanged per reaction mole and F is the Faraday's constant (96485 C mol^{-1}). The reaction of Pb(II) reduction is also shown in Equation 2.2. Using MEC reactors for reducing ions, usually a voltage must be applied to help the redox reactions (Lu et al., 2015). However, Pb(II) reduction can be spontaneous here and the previous study showed it can be removed successfully in an open circuit condition in MEC on both cathode and bioanode (Colantonio & Kim, 2016b).

$$E = E^\circ - \frac{RT}{nF} \ln \frac{1}{[\text{Pb}^{2+}]} \quad (2.1)$$



The electrode potential of the bioanode in different pH conditions is shown in Figure 2.4. This figure is also proving Pb(II) reduction as a mechanism for Pb(II) removal. For the neutral pH, once Pb(II) was added to the solution, the electrode potential started to increase which means reduction reaction was happening. The simultaneous change in the electrode potential with adding Pb(II) confirms that a part of Pb(II) was removed by a reduction reaction.

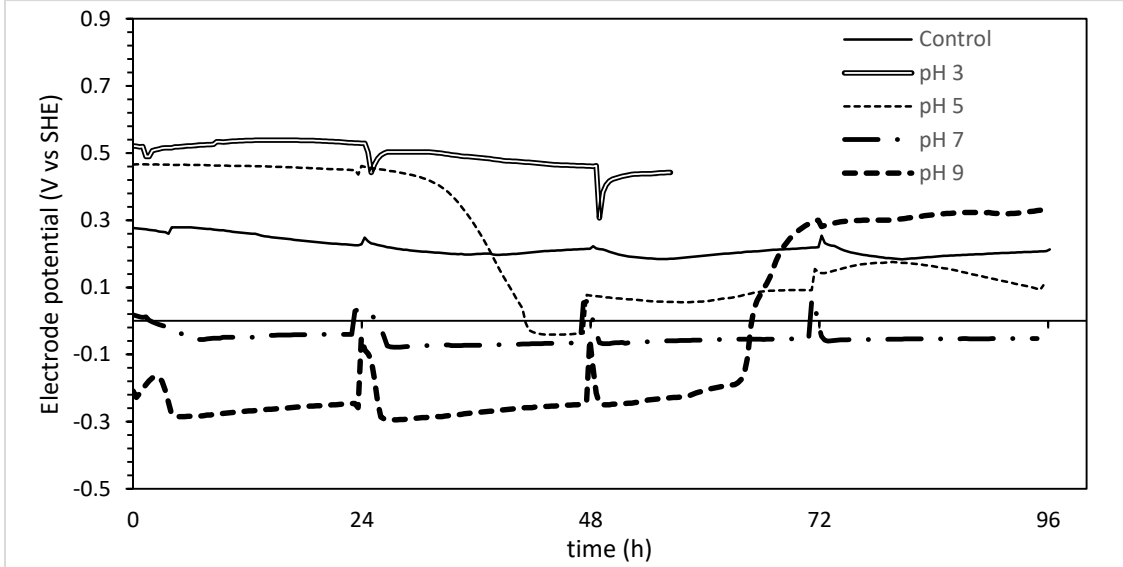


Figure 2.4: Electrode potential of the bioanode using in the system for four cycles of 24 hours experiment in different pH conditions-1 mg/L lead was added at the beginning and then every 24 hours after that (a part of data for pH 3 condition was missed due to power outage and since the bacteria were not alive, it could not be repeated)

2.3.2 Effect of pH

Figure 2.5, which shows the average of Pb(II) concentration in 4 cycles in different pH conditions, indicates that pH 5 had the highest rate of removal. pH 5, 7 and 9 were all working properly and having around 66%, 70% and 82% Pb(II) removal after 8 hours respectively. Low pH, due to having more protons in the solution, could work better than the other pH values. Also, higher pH means fewer electrons, so it led to more oxidation reaction and producing more electrons for reduction reactions. Nonetheless, acidic pH lower than 5 was not favorable for bacteria anymore and the rate of removal dropped after a certain pH value. It can even be seen in Figure 2.4 that the electrode potential of pH 9 was more negative than the others and the pH 3 had the highest electrode potential.

According to Figure 2.4, in pH of 7 and 9 bacteria were in a healthy condition with a constant electrode potential which started to increase by the time of adding lead. However, in pH 5, electrode potential was acting differently which showed this was not a very healthy condition for them, but they were still participating in reduction reactions for Pb(II) removal with having more protons. pH lower than 5 was unhealthy for bacteria and reduction reactions on Pb(II) were not happening. Both kinetics of reactions and the bacteria condition matter here, that is why the acidic condition worked better in terms of Pb(II) removal but bacteria limit this condition. Regarding the effect of pH, Qin et al. have investigated that on the nickel removal using MECs, and the results showed that the lower pH values were more favorable (Qin et al., 2012). Furthermore, Puig et al. observed less efficiency of exoelectrogenic bacteria in pH higher than 9.5 in terms of COD removal and power density in the air cathode MFCs (Puig et al., 2010). Regarding both kinetics and bacteria condition, the current system can work successfully in a wide range of pH values; however, more values need to be investigated in the future. The abiotic condition with a clean fiber brush as a control experiment was also done and according to Figure 2.5, there was not much removal in this condition and the slight removal was for adsorption to the clean fibers. This results showed the major role of exoelectrogenic bacteria for lead removal.

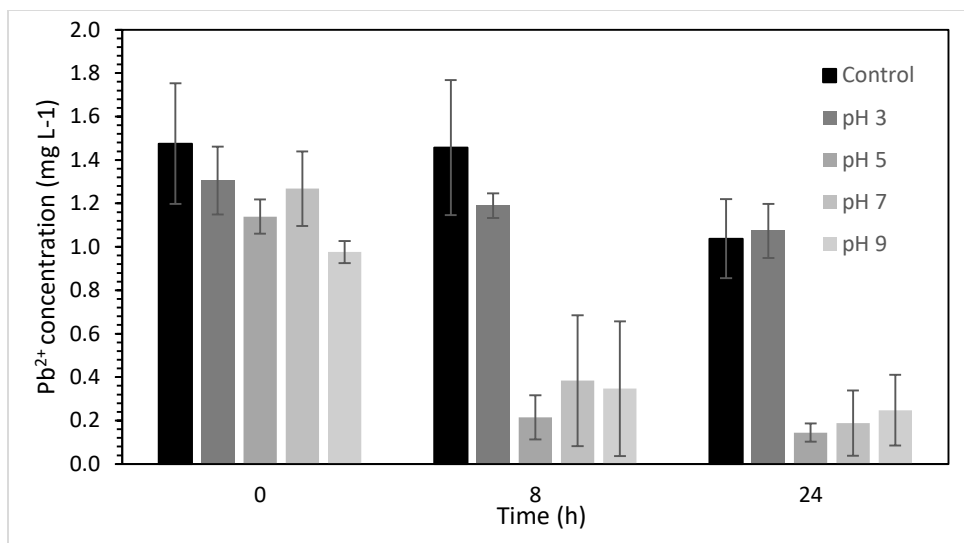


Figure 2.5: Effect of pH on Pb(II) removal for four cycles of 24 hours experiment (data for each pH condition is the average of 4 experiments which were done right after each other by adding 1 mg/L after every 24 hours- for each time adding lead, 3 samples were taken one at the beginning, one after 8 hours and one after 24 hours and the results shown are the averages of these three samples for all 4 cycles)

2.3.3 Pb(II) deposited on bioanode brush

Analyzing the amount of lead deposited on the bioanode, both biofilm and fibers were considered. Biofilm was removed with an ethanol wash and the deposited lead on fibers was analyzed using scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS). These ethanol-cleaned fibers were then soaked in nitric acid (70%) to measure the amount of deposited lead on the fibers using inductive coupled plasma-optical emission spectrometry (ICP-OES). A non-ethanol-cleaned part of the fibers was also soaked in nitric acid (70%) in order to measure the total amount of lead on fibers.

Eventually, the amount of lead on biofilm was 2.57 mg (82% of total) and 0.56 mg lead was deposited on fibers (18% of the total amount). SEM-EDS has also been done on the

ethanol cleaned fibers. SEM result (Figure 2.6a) showed some bright spots which were proved by EDS (Figure 2.6b) that they were lead deposited spots. According to the above-mentioned results, it was confirmed that Pb(II) can be removed by both electrochemical and biosorption mechanisms.

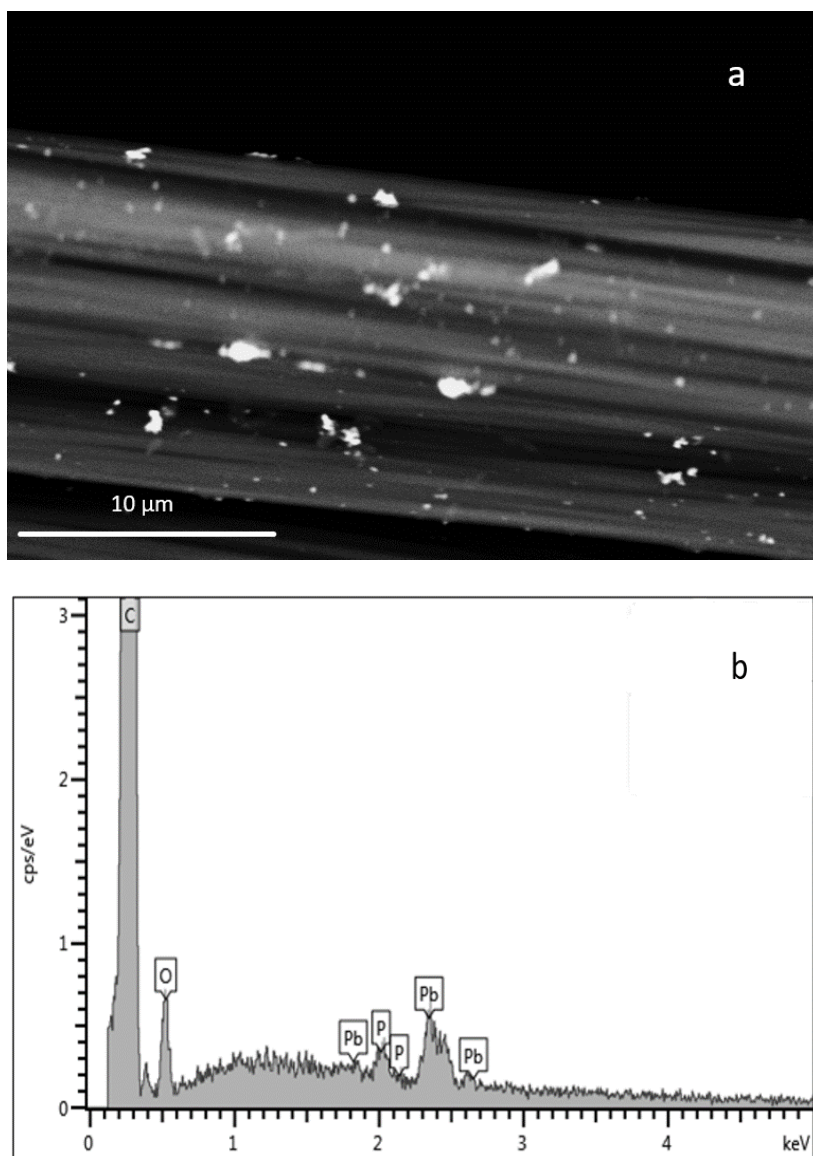


Figure 2.6: (a) SEM image of the bioanode fibers after Pb(II) removal experiment and cleaning them with ethanol, (b) EDS spectrum of the bright spots on the SEM image of the bioanode fibers which shows they contain Pb (s)

2.4 Conclusions

The method studied here was considered for small-scale and non-continuous wastewater treatment plants. Lead concentration was considered 1 mg/L and the objective was more than 90% removal of it. This study showed that bioanode enriched from an MEC reactor was able to remove Pb(II) without applying any external voltages. More than 90% Pb(II) removal was achieved in less than 24 hours. Pb(II) removal efficiency was consistent after 4 cycles of adding Pb(II) to the same bioanode (1 mg/L after each 24 hours for 4 days). Moreover, the system could work effectively in a wide range of pH (pH 5 to 9). Direct reduction and biosorption were two main mechanisms responsible for Pb(II) removal in this system and it was proved that exoelectrogenic bacteria played the key role in both mechanisms. Moreover, the contribution of biosorption mechanism to lead removal was much higher than the direct reduction mechanism.

3 Phosphate removal using zeolite

Abstract

High quantity of phosphates in wastewaters and their entrance to water bodies along with organics and other nutrients can lead to algal bloom and its hazardous consequences to aquatic wildlife and water shortage. Zeolite as a natural substance with special physico-chemical properties such as ion exchanging and molecular sieving has been studied for its application in wastewater treatment. Zeolites are usually used for cation exchanging purposes; however, pretreating its surface with salts can improve its ability for phosphate removal. The effect of three different salts (NaCl , $\text{Mg}(\text{OH})_2$ and $\text{Ca}(\text{OH})_2$) were investigated on improving the zeolite capability for phosphate removal. Zeolites pretreated by calcium and sodium were shown to be improved for phosphate removal and magnesium pretreatment had no effect on that. Sodium and calcium-pretreated zeolites increased the removal efficiency of natural zeolite from 18% to 60% and 37% respectively. Also, the presence of ammonium in the solution improved the phosphate removal efficiency in all conditions. In case of using only natural zeolite, phosphate removal efficiency increased from 18% to 44% in presence of ammonium. Phosphate removal was identified to have happened as calcium phosphate. Ammonium and sodium in solution assisted with calcium exchanging and improved removal efficiency of phosphate. It was also shown that the type of zeolite had a significant impact due to its concentration of calcium which can be exchanged and remove phosphates as calcium phosphate precipitants. Moreover, it was

shown that using only calcium hydroxide without any zeolite could also remove the whole phosphate. Although calcium-pretreated zeolite had a high capacity for phosphate removal, its efficiency was lower than using only calcium hydroxide which could be because of slow mass transferring. Shaking condition was recognized as another important factor for improving the removal efficiency which improved mass transferring condition for calcium-pretreated zeolite and increased the removal efficiency to 100%. Using zeolites with high calcium concentrations in their structure for removing phosphate in a wastewater containing ammonium and sodium in a proper mixing condition can provide a high removal efficiency.

3.1 Introduction

Phosphate accumulation in water bodies can cause eutrophication and huge consequences after that will put the aquatic life in danger and cause water shortage (Correll, 1998). Phosphate is a primary component in many industrial and agricultural usages like fertilizers, detergents, food, and drink (Hosni et al., 2008). A large amount of phosphate released by all of these industries and agricultures intensifies the importance of finding the best sustainable method for its removal from wastewater.

Many physical, chemical and biological methods were applied and studied for phosphate removal. Regarding the physical methods, many researchers worked on phosphate adsorption and investigated the cheaper and more efficient adsorbents. Some of these adsorbents are like industrial waste iron oxide (Zeng et al., 2004) and Alum sludge (Yang et al., 2006). However, they are more effective in low concentrations and for polishing step (Suresh Kumar et al., 2019). Enhanced biological phosphorus removal (EBPR) is a common biological method. Polyphosphate accumulating organisms (PAOs) play the main role in the EBPR process (Patel et al., 2005). Providing the incubation conditions for bacteria, providing organics in the first phase, making a favorable condition for PAOs make this method challenging for phosphorus removal (Luz & Bashan, 2004). Primary methods used for phosphorus removal are based on chemical precipitations which are usually done by metal salts. Addition of iron salts is a common and conventional method for phosphorus removal in wastewater treatment plants (Morse et al., 1998).

Zeolite is a cost-effective and abundant material with specific properties that is usually used for cation exchanging. However, modification of zeolite makes it a potential method for phosphate removal (S. Wang & Peng, 2010). Zeolites are hydrated aluminum-silicate crystalline materials. There are many different types of natural zeolite around the world with different accessibility among which clinoptilolite is the most abundant and commonly used one (S. Wang & Peng, 2010). Synthetic zeolite can also be created by silica and alumina-based raw materials among which carbon fly ash is the most commonly used one (Yuna, 2016).

Specific physico-chemical characteristics of zeolites make them a suitable material for cation exchanging. Zeolite structure consists of three main parts: aluminosilicate framework, exchangeable cations, and water (S. Wang & Peng, 2010). Zeolite surface is negatively charged which attracts the cations to itself for cation exchanging. In order to use zeolite for anion removal, some modifications need to be done on zeolite (Yuna, 2016). Acid pretreatment is mostly done for improving the removal of non-polar materials (S. Wang & Peng, 2010). However, Bao-hua et al. applied acid pretreatment on synthetic zeolite to improve simultaneous removal of ammonium and phosphorus at low concentrations (Wu et al., 2006). They found that mild H_2SO_4 (0.01 M) treatment helped CaCO_3 transforming to a more soluble calcium which improved calcium phosphate precipitation. Surfactant modification is another method for increasing anion and organics adsorption to the zeolite by creating a bilayer on zeolite surface and making positively charged sites for anions adsorption (S. Wang & Peng, 2010).

Salt treatment method which is used mostly for phosphate removal is another zeolite modification method. Salt treatment method uses a salt solution to exchange the zeolite cations with the cations like Ca^{2+} , Mg^{2+} , Al^{3+} and Na^+ present in the salt solutions. This improves the phosphate sorption to the zeolite surface and making bonds. This pretreatment step is improved with heat since divalent or trivalent ions are exchanged with monovalent ones (You et al., 2017). Salt treated zeolites are also common for simultaneous removal of phosphate and ammonium. Researchers worked on different salts for modification and looked into the phosphate removal mechanism.

Guaya et al. modified a natural zeolite by hydrated aluminum oxide for simultaneous removal of ammonium and phosphate (Guaya et al., 2015). Phosphate sorption increased from 0.6 to 7 mg-P/g after the modification. The phosphate removal mechanism was concluded to be the formation of inner sphere compounds with Al-OH groups and ammonium was removed by ion exchanging and interaction with OH groups of zeolite.

Hermassi et al. and Mitrogiannis et al. worked on calcium modified zeolite and observed a substantial increase in phosphate removal (Hermassi et al., 2016; Mitrogiannis et al., 2018). Mechanisms for this removal were identified as inner sphere and outer sphere surface complexation and precipitation of calcium phosphate compounds.

Wu et al. modified zeolite with different salts to compare their effects on simultaneous ammonium and phosphate removal (Wu et al., 2006). Al^{3+} modified zeolite had the highest efficiency for ammonium and phosphate removal. After that, Fe^{3+} , Ca^{2+} , Na^+ and Mg^{2+} had

higher phosphate removal efficiency respectively. However, after Al^{3+} , Ca^{2+} was the best option for the simultaneous removal. Fe^{3+} modified zeolite did not remove ammonium.

Huang et al. modified a type of zeolite with magnesium for simultaneous removal of ammonium and phosphate as Struvite (Huang et al., 2014). 110 g/L of modified zeolite was used and the removal efficiency of ammonium and phosphate reached 82% and 98% after 40 minutes.

In this project, a clinoptilolite zeolite was used for phosphate removal. Zeolite pretreatment with NaCl , $\text{Ca}(\text{OH})_2$ and $\text{Mg}(\text{OH})_2$ and their effect on phosphate removal were investigated. Objectives of this project were to compare the different salt pretreated zeolites, identify the removal mechanism of phosphate and improve its removal by ammonium. Moreover, the effect of sodium in the solution, the type of zeolite and the mixing condition were specifically investigated. Targeted wastewater for this method was an industrial type of wastewater with a phosphate concentration of 20-50 mg-P/L and the objective was more than 90% phosphate removal. Zeolite method was aimed to be applied for a small-scale and non-continuous wastewater treatment plant.

3.2 Materials and methods

3.2.1 Zeolite samples

The zeolite used in this project was clinoptilolite zeolite obtained from Zeocem company, Slovakian Republic. Zeolite purchase is usually based on the particle size and the size of zeolite ordered in this study was 0.2 mm. It was passed through 100 mesh sieve (0.149 mm) and the fraction of zeolite on top of the sieve was used in the experiments. The chosen mesh size was according to the previous papers on zeolite application. This Slovakian zeolite was the main type of zeolite that was used in all of the experiments. In only one experiment, another type of zeolite was used to compare two different types of zeolites. This other zeolite was another clinoptilolite zeolite from Castle Mountain Zeolites, Australia. It was ground to be as approximately the same size as Slovakian zeolite.

The chemical characteristics of these zeolites are provided in Table 3.1. In order to measure the chemical compounds in Table 3.1, 1 g of zeolite was soaked in 40 mL of HCl solution (2M) for 12 hours to release all the cations and dealuminate the zeolite. Then the acid solution was analyzed using inductive coupled plasma-optical emission spectrometry (ICP-OES) (Vista Pro, Varian Inc., Australia).

Table 3.1: Chemical composition of natural zeolites

Elements	Slovakian zeolite Measured by ICP (mg/g-zeolite)	Slovakian zeolite Provided by company (mg/g-zeolite)	Australian zeolite Measured by ICP (mg/g-zeolite)
Al	177.40	119.5	56.66
Ca	10.60	27.1	3.36
Na	1.04	3.79	3.61
K	17.81	35	0.13
Mg	0.49	7.1	0.12
Fe	0.09	12.9	0

3.2.2 Zeolite Pretreatment

Zeolite pretreatment was carried out using three different salt solutions: NaCl (0.1 M), Mg(OH)₂ (0.01 M) and Ca(OH)₂ (0.01 M). Sodium pretreatment was done by boiling 20 g of zeolite with 200 mL of sodium solution, it was mixed and boiled until it was dried (in around 30 minutes). For magnesium and calcium pretreatment, 4 g of zeolite was boiled with 100 mL of salt solutions until it was dried (in around 20 minutes). For sodium-calcium-pretreated zeolite (Na-Ca-Z) and sodium-magnesium-pretreated zeolite (Na-Mg-Z), 4 g of sodium-pretreated zeolite (Na-Z) was heated with 100 mL calcium/magnesium solution.

3.2.3 Experimental process

Phosphate and ammonium solutions were made by NaH₂PO₄ and NH₄Cl. Ammonium in the solution was in a molar ratio of 3.5 to phosphate which was according to the results of Lin et al. (Lin et al., 2014). 30 mL of phosphate or ammonium/phosphate solution with the initial pH of 10.5 was mixed with 0.5 g modified zeolite in centrifuge tubes by a shaker at 210 rpm (Force benchtop orbital open air shakers, model No. 4633, Lab-Line, IL). 10.5 is the initial pH of solutions before adding zeolite to them which was adjusted by NaOH (1 M) solution, the final pH was measured after the experiment by a pH meter (SevenMulti, Mettler-Toledo International Inc., OH).

A synthetic solution of phosphate (30 mg-P/L) and ammonium (49 mg-N /L) with pH of 10.5 contained a sodium amount of 141.3 mg/L which needed to be considered in the experiment. Shaking condition was same in all experiments except for one experiment

which examined the effect of shaking condition and the solutions were shaken with the same shaker but in larger containers (250 mL Erlenmeyer flask).

Based on the amount of zeolite and salt solution used for its pretreatment, the amount of salt that had to be on zeolite surface after pretreatment was calculated. According to these calculations, the amount of Na^+ , Mg^{2+} , and Ca^{2+} contained in 0.5 g pretreated zeolite were approximately 10.63, 2.96 and 4.96 mg respectively. Afterward, control experiments were done by adding only these amount of salts to the solution to see their effect without zeolite. Also, the same amount of salts (10.63 mg Na^+ , 2.96 mg Mg^{2+} , and 4.96 mg Ca^{2+}) was added with zeolite to the phosphate solutions in order to observe the effect of pretreatment step. All these experiments were done in 3 hours and at 25 °C and in duplicate. 3 hours of the experiment was selected based on the preliminary results done on phosphate removal (Figure 3.1).

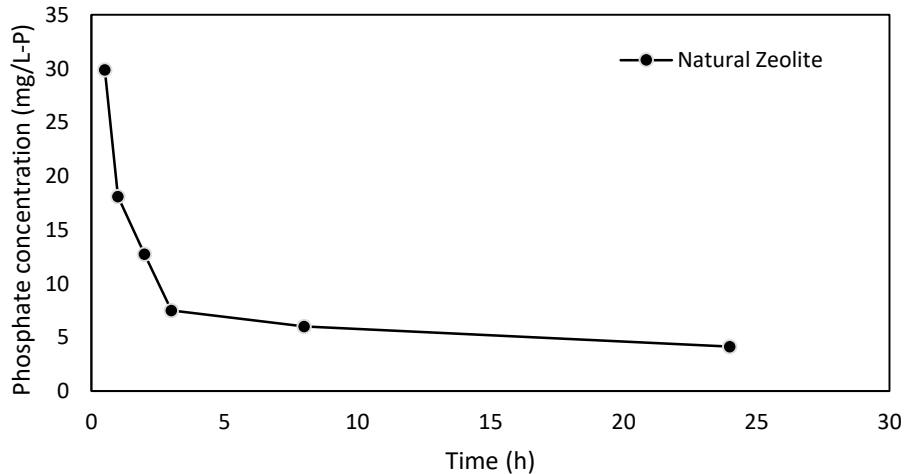


Figure 3.1: Preliminary experiment on the solution of phosphate and ammonium at pH 10.5 using only natural zeolite (P: N=1:3.5- P=30 mg/L)

3.2.4 Analytical methods

Total phosphorus and ammonium were measured according to the standard methods (APHA, 1998). Molybdovanadate with acid persulfate digestion method was the method used for phosphate determination. Salicylate method was also used for ammonium measurement.

Calcium and sodium were measured using inductive coupled plasma-optical emission spectrometry (ICP-OES) (Vista Pro, Varian Inc., Australia). For measuring them in the solid phase, all the solution and solid parts were passed through 0.45 μm filter papers and the solid phase on top of the filter were dissolved in HCl solution (2M) after being dried. Acid solutions were then analyzed to measure the elements in the solid phase.

3.3 Results

3.3.1 Effect of pretreated zeolite on phosphate removal

The effect of different salt-pretreated zeolites on phosphate removal is shown in Figure 3.2. As can be seen, pretreating the natural zeolite with Ca(OH)_2 and NaCl increased the phosphate removal efficiency from 18% for untreated zeolite to 37% and 60% for zeolite pretreated by Ca(OH)_2 and NaCl respectively. Pretreating the zeolite with both Ca(OH)_2 and NaCl increased the removal efficiency up to 94%. However, no significant changes were observed for zeolite pretreated by Mg(OH)_2 .

In order to see the effect of the pretreatment process, salt and zeolite were added to the solution without pretreatment step, then they were compared with the salt-pretreated zeolite. Based on the NaCl results, pretreated zeolite by NaCl had higher removal efficiency compared to the addition of untreated zeolite and NaCl salt which showed the positive effect of the heating process done at the pretreatment stage. Both magnesium-pretreated zeolite and the addition of Mg(OH)_2 with zeolite without pretreatment did not make any changes on removal efficiency. So, pretreatment step did not have any effect here. According to Ca(OH)_2 results, although pretreated zeolite improved the removal efficiency of natural zeolite, the addition of salt and zeolite without pretreatment step had a higher efficiency than zeolite pretreated by Ca(OH)_2 .

In order to see the role of salts in phosphate removal, only salts were added to the solution without any zeolite. NaCl and Mg(OH)_2 salt did not result in any removal. However, Ca(OH)_2 salt removed the whole phosphate even without zeolite. Yet, the calcium-

pretreated zeolite had a higher capacity than $\text{Ca}(\text{OH})_2$ salt for phosphate removal, since there was calcium in zeolite structure as well.

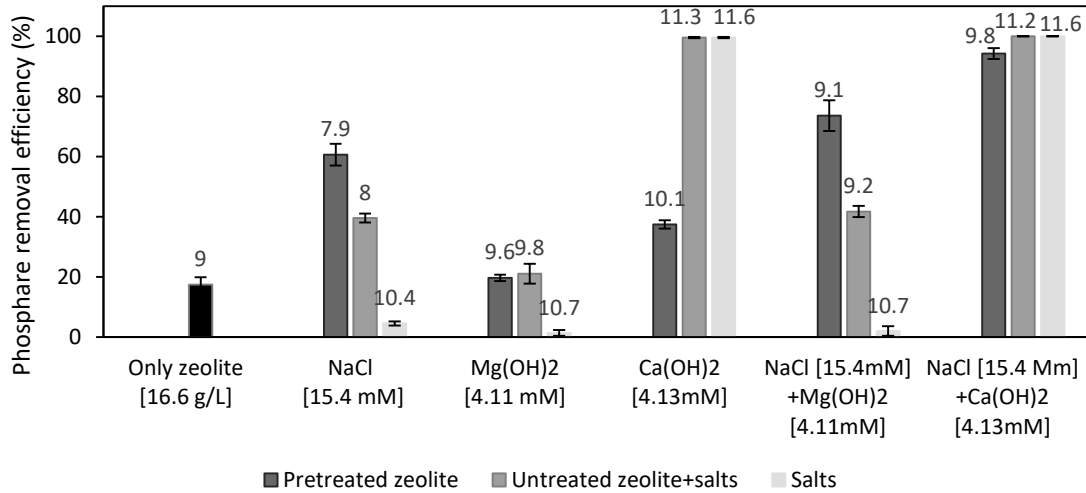


Figure 3.2: Effect of natural zeolite and salt pretreated zeolite on phosphate removal in a synthetic phosphate solution (30 mg-P /L), effect of the addition of only salt to the solution is shown as the control- the final pH of each condition is shown on top of each column

3.3.2 Simultaneous removal of phosphate and ammonium

Presence of ammonium in the solution significantly improved the phosphate removal efficiency which can be seen in Figure 3.3a. Comparing the results with Figure 3.2, phosphate removal efficiency for only natural zeolite improved from 18% to 44% in presence of ammonium and pretreated zeolites by NaCl and $\text{Ca}(\text{OH})_2$ reached even higher removal efficiencies. Insignificant effect of $\text{Mg}(\text{OH})_2$ and the effect of pretreatment step were the same as the previous section (3.3.1).

According to $\text{Mg}(\text{OH})_2$ results, since magnesium-pretreated zeolite did not improve the removal efficiency and ammonium was sufficiently available in the solution, it was concluded that there was no struvite precipitation.

pH results showed an increase in pH to around 11.5 for the conditions of having calcium hydroxide salt directly which was because of accumulation of Ca(OH)_2 . No changes were seen in pH for control experiments with only NaCl and Mg(OH)_2 salts which were expected since they did not remove any phosphate. Moreover, in all pretreated zeolite conditions and also only natural zeolite, pH decreased during the experiment which could be due to the creation of calcium phosphate.

Ammonium was also removed using zeolite which is done by ion exchanging (Figure 3.3b). Around 34% removal was achieved using only natural zeolite. Moreover, pretreatment of zeolite or adding a salt with it did not change the ammonium removal significantly. Only when there was zeolite pretreated by Ca(OH)_2 or by both NaCl and Ca(OH)_2 , lower ammonium removal was observed which could be because of ion exchanging competition. Around 10% ammonium removal was attained by adding only salts without zeolite which can be considered as the amount that was removed by volatilization.

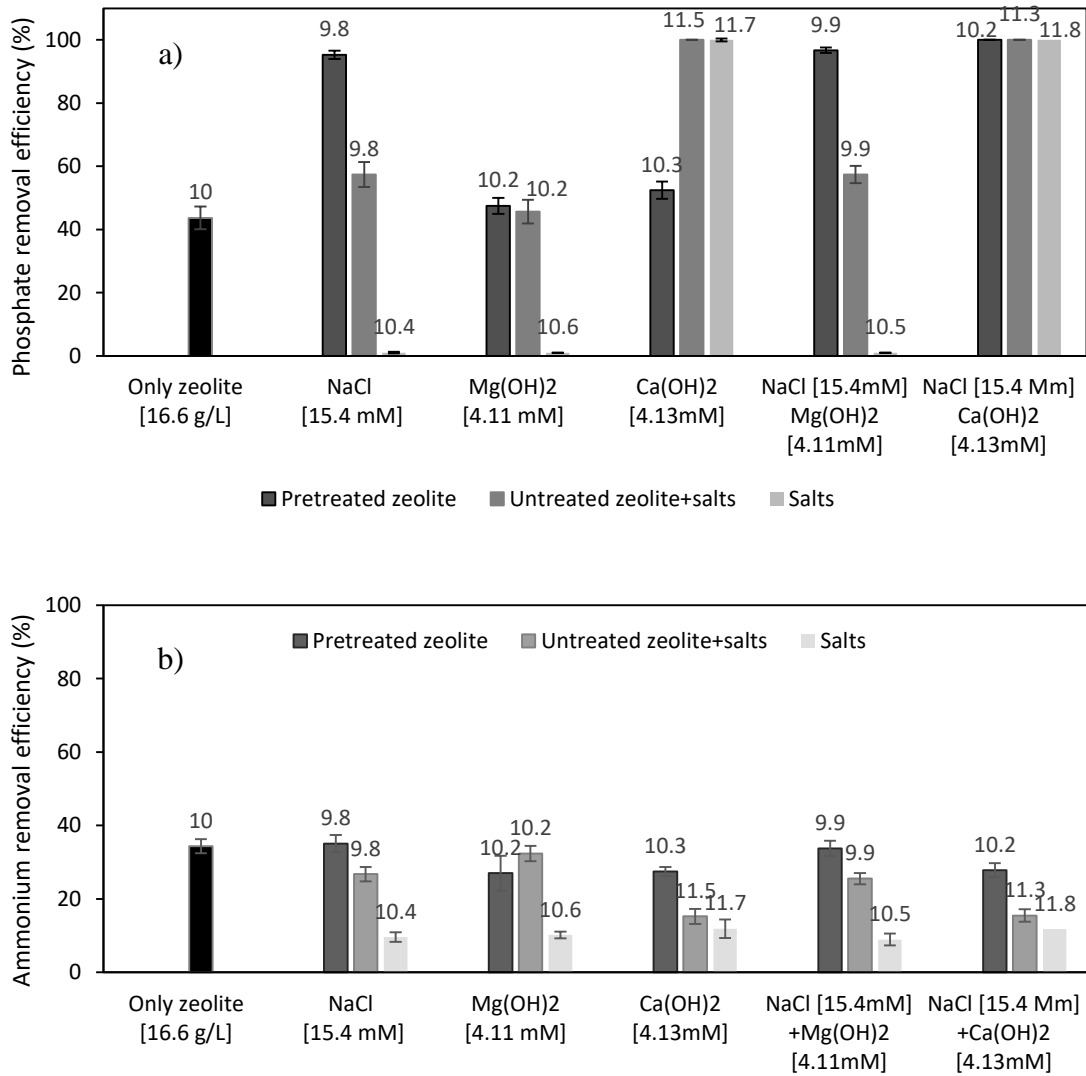


Figure 3.3: Effect of natural zeolite and salt modified zeolite on phosphate and ammonium removal in a synthetic solution (P: N=1:3.5- P=30 mg/L), effect of the salt addition on phosphate removal is shown as the control- the final pH of each condition is shown on top of each column, a) phosphate removal efficiency, b) ammonium removal efficiency

Calcium, sodium and phosphate concentrations in the solution and solid phase are shown in Figure 3.4. Figure 3.4a shows the original amount of sodium in zeolite was low (0.02 mmole in 0.5g zeolite) and it increased in the solid phase during the experiment. The

existence of more sodium in solid phase proved that sodium was exchanged with the cations of zeolite.

According to Figure 3.4b, all the calcium remained in the solid phase. Only when untreated zeolite and $\text{Ca}(\text{OH})_2$ salt were added to the solution, around 20% of the whole amount of calcium was detected in the solution. This amount of calcium in solution could be the residual amount of calcium hydroxide added. Figure 3.4c shows the phosphate amount that was precipitated (solid phase) and the rest which was left in the solution. This figure also clearly shows the effect of different salt-pretreated zeolites and untreated zeolite added with different salts.

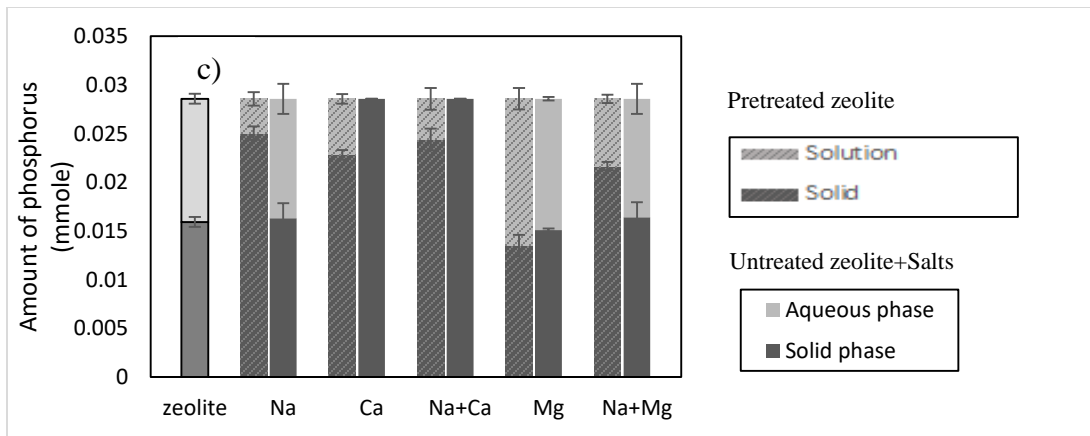
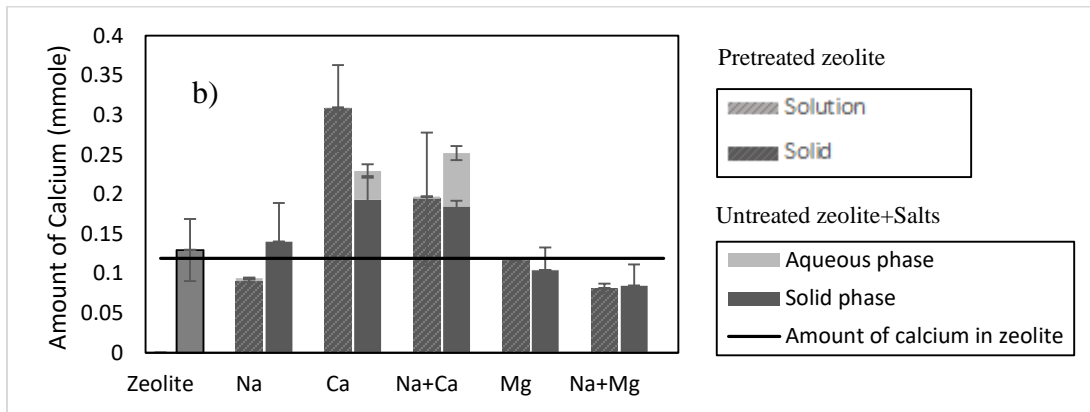
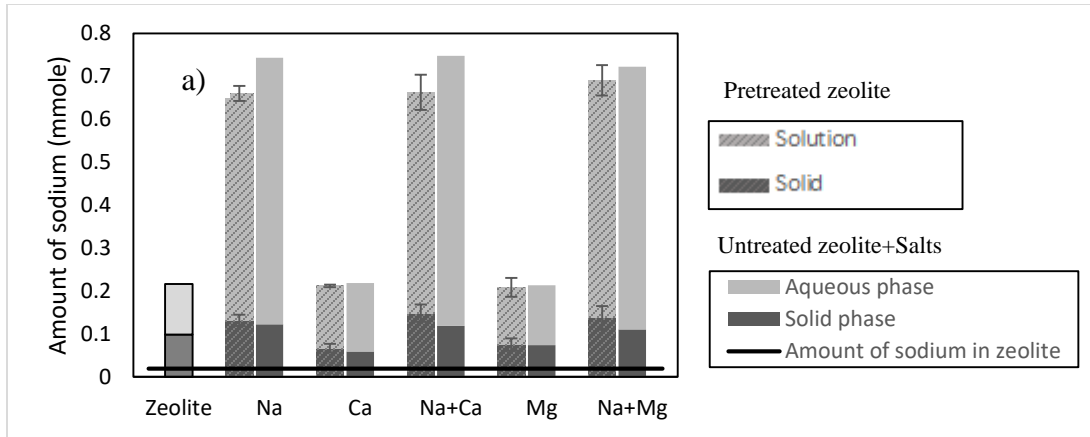


Figure 3.4: a) Amount of sodium in the solution and solid phase after the experiment, b) amount of calcium in the solution and solid phase after the experiment, c) amount of phosphate in the solution and solid phase after the experiment- amount of sodium and calcium in zeolite structure before treatment are displayed by a line- all experiments were done in duplicate

In order to confirm the effect of calcium concentration of zeolite on phosphate removal, two different zeolites were tested with and without sodium in order to see the effect of sodium on releasing the calcium as well. As can be seen in Figure 3.5, Australian zeolite had a substantial lower efficiency than Slovakian zeolite. After measuring the amount of calcium of each zeolite (table 3.1), it was found that Australian zeolite had much less calcium compared to Slovakian zeolite which can explain its lower efficiency in phosphate removal.

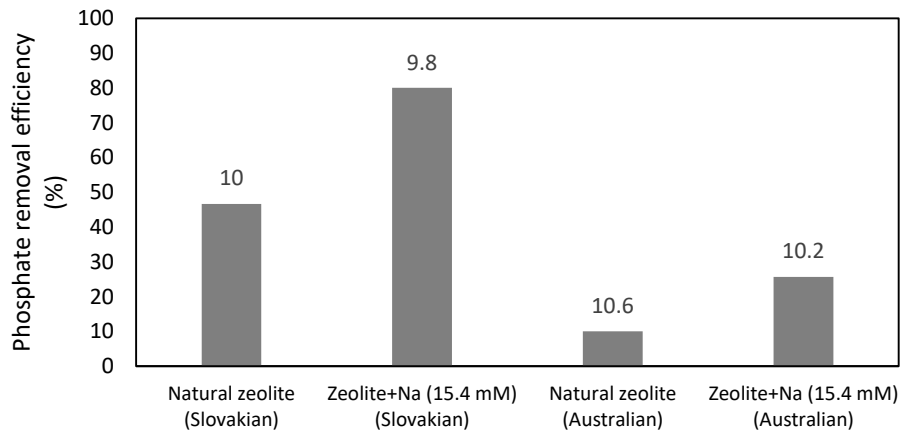


Figure 3.5: Effect of two different natural zeolites on phosphate removal in a solution containing 30 mg-P/L- the final pH of each condition is shown on top of each column- This experiment was done once

3.3.3 Effect of sodium addition on phosphate removal

Addition of sodium to the solution along with the zeolite increased the phosphate removal efficiency and it is shown in Figure 3.6. Sodium was added once at the beginning and once at 20 minutes to the calcium-pretreated zeolite and the results were compared with the

condition of no sodium. Based on these results, increasing the removal rate was clear after addition of sodium. For the results of adding sodium after 20 minutes it can be seen in Figure 3.6 that before 20 minutes, the removal rate is same as the control experiment without any sodium and after adding sodium the rate was changed and followed the same trend as the results of zeolite and sodium from the beginning. The final concentration also became the same.

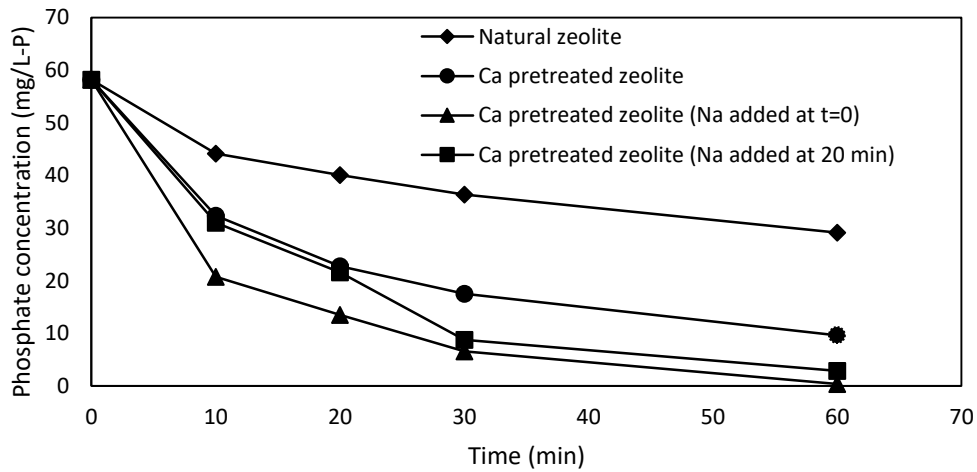


Figure 3.6: Effect of sodium on phosphate removal rate in a solution of phosphate and ammonium (P:N=1:3.5)

Following the effect of sodium on increasing the removal rate, more sodium was added to the solution and results are shown in Figure 3.7. It can be seen that more sodium reached more phosphate removal.

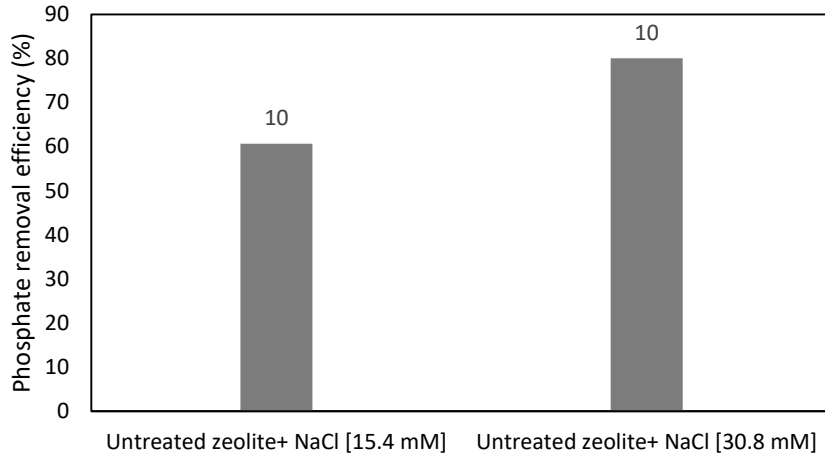


Figure 3.7: Effect of sodium amount on phosphate removal efficiency in a solution of phosphate and ammonium (P: N=1:3.5- P=30 mg/L)- the final pH of each condition is shown on top of each column- This experiment was done once

3.3.4 Effect of calcium hydroxide on phosphate removal

Calcium hydroxide could remove phosphate on its own as calcium phosphate (Figure 3.8).

Using only $\text{Ca}(\text{OH})_2$ led to high final pH and difficulties with the precipitants separation.

Ca/P ratio of 1.5 and above reached 100% removal.

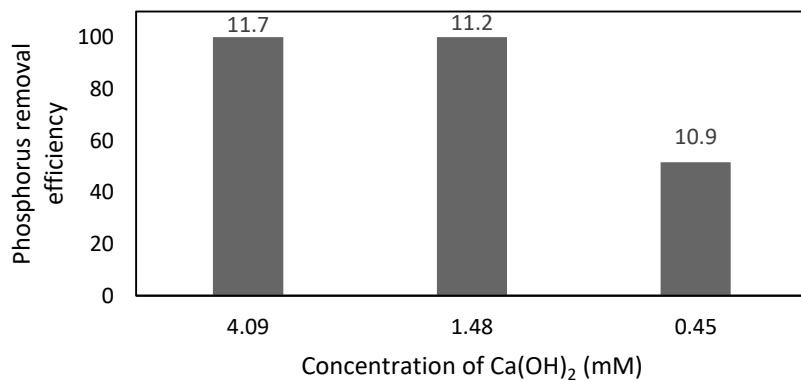


Figure 3.8: Effect of adding different dosages of calcium hydroxide on phosphate removal in a solution of ammonium and phosphate (P=1mM - P: N=1:3.5)- the final pH of each condition is shown on top of each column- This experiment was done once

3.3.5 Effect of shaking on phosphate removal

All the experiments were done in 50 ml centrifuge tubes. In order to see the effect of shaking, a few conditions were chosen and shaken in Erlenmeyer flasks. The same shaker with the speed of 210 rpm was used for both conditions. Using a larger container made the solution and zeolite mix better which could lead to a better mass transfer condition. Figure 3.9 shows the improved removal efficiency for the ones shaken in Erlenmeyer flasks. Even the removal efficiency of natural zeolite without any pretreatment increased from 32% to 94%.

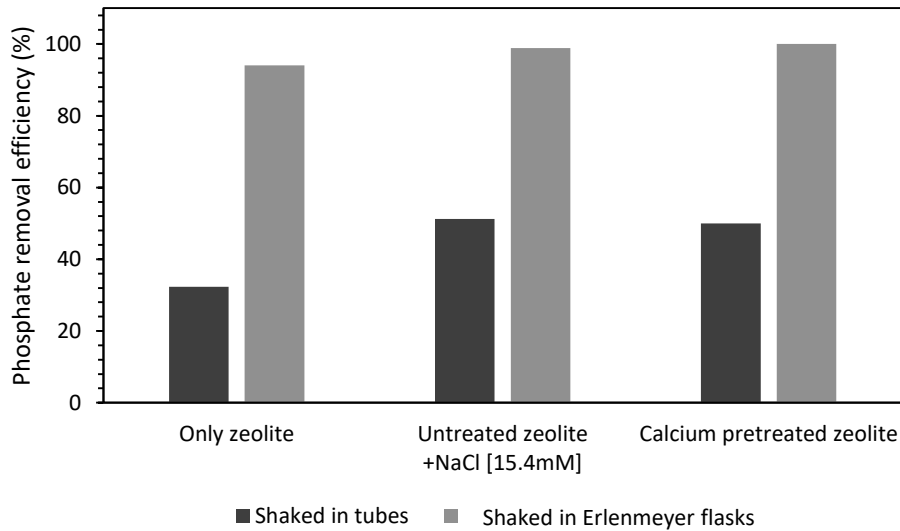


Figure 3.9: Effect of shaking on phosphate removal efficiency in a solution of ammonium and phosphate (P=1mM - P: N=1:3.5)- This experiment was done one time

3.4 Discussion

3.4.1 Phosphate removal mechanism for sodium-pretreated zeolite

According to NaCl results in Figure 3.2 and 3.3, the zeolite pretreated by NaCl improved the removal efficiency. Considering the low amount of NaCl in the zeolite, Figure 3.4a shows that the amount of sodium in the solid phase was increased during the experiment. It shows that sodium was exchanged with another cation during the experiment. Potassium, iron, and magnesium were also measured and only a slight release of potassium was seen. However, the amount of released potassium was still less than the amount of exchanged sodium (results are not shown). So, sodium was exchanged with calcium. Based on Figure 3.4b, calcium remained in solid phase and since phosphate was also removed, calcium phosphate was the compound created.

Comparing the results of Figure 3.2 and Figure 3.3a, a significant removal improvement was achieved in presence of ammonium for all conditions which could be due to assisting of ammonium in releasing calcium by ion exchanging. Sprynskyy et al. investigated the selectivity of zeolite exchangeable cations for exchanging with ammonium (Sprynskyy, Lebedynets, Terzyk, & Kowalczyk, 2005). They found the order to be $\text{Na}^+ > \text{Ca}^{2+} > \text{K}^+$. When the ammonium concentration is high, calcium exchanging increases as well. This result was consistent with our finding since the sodium concentration in zeolite was low, ammonium was exchanged with calcium here and increased the phosphate removal efficiency.

pH results were also consistent with the possibility of sodium and ammonium exchanging with calcium. Except for the conditions of adding Ca(OH)_2 salt directly, pH decreased in the other conditions. That could be because of the creation of calcium phosphate which can be in the form of $\text{Ca}_5\text{OH(PO}_4)_3$, also when Na^+ and NH_4^+ get exchanged with Ca^{2+} , H^+ is released based on the reaction and it decreases the pH (Hermassi et al., 2016).

According to NaCl results in Figure 3.2 or 3.3a, the pretreating step had a positive effect on removal efficiency compared to adding zeolite and NaCl without pretreating (around 20% higher for phosphate solution and 30% higher in phosphate and ammonium solution). Regarding that, You et al. investigated the effect of heating and concluded that heating accelerates the cation exchanging process by providing the required energy for exchanging divalent ions with monovalent ions and more calcium are released and then more phosphate is removed (You et al., 2017).

In order to prove the effect of sodium on releasing calcium and improving the phosphate removal efficiency, an experiment was done on calcium-pretreated zeolite by adding sodium at different times. As can be seen in Figure 3.6 adding NaCl at any time dropped the phosphate concentration at that point. The final concentration of phosphate remained the same for the conditions containing NaCl (either at the beginning or after 20 minutes) and lower than the condition of no NaCl added. So, adding sodium could clearly increase the rate of phosphate removal by releasing the calcium and favoring calcium phosphate precipitation. Then, more sodium was added in order to see its effect on calcium exchanging. Figure 3.7 shows that by increasing the NaCl to a double amount, the

phosphate removal efficiency increased by 20%. It is not a very high improvement but it still shows the positive effect of sodium in the solution.

Wu et al. compared different salt treated zeolites for phosphate removal and their results for sodium-pretreated zeolite resulted in a low phosphate removal (Wu et al., 2006). It could be due to the difference of pretreatment methods. After heating the zeolite with salt, they washed the zeolite with ample water which resulted in a zeolite with mostly Na^+ as its exchangeable cation. However, in the pretreatment method used in this project, there was no washing step after heating and the exchanged cations (Ca^{2+}) were kept on zeolite and improved removal efficiency.

3.4.2 Effect of different salt-pretreated zeolites on ammonium removal

Regarding the ammonium removal results, pretreatment of zeolite or adding a salt with untreated zeolite did not change the ammonium removal significantly (Figure 3.3b). Only when there was zeolite pretreated by $\text{Ca}(\text{OH})_2$ or by both NaCl and $\text{Ca}(\text{OH})_2$, lower ammonium removal was observed. Results of Lei et al. also showed the negative effect of sodium and calcium and insignificant effect of magnesium in the solution on ammonium removal because of being competing cations with ammonium (Lei, Li, & Zhang, 2008). Their results also are consistent with our results. Natural zeolite provided the highest ammonium removal efficiency here due to the lack of competing cations.

Ammonium removal with the only zeolite (Figure 3.3b) showed it was happening by ion exchanging. Figure 3.3b also shows that there was almost same ammonium removal when zeolite was untreated or treated with different salts. This same removal efficiency showed

that ammonium was not removed by precipitation as a salt. There was a slight removal in case of having only sodium, magnesium and calcium salts which was probably due to the ammonium volatilization.

3.4.3 Phosphate removal mechanism for magnesium-pretreated zeolite

According to $\text{Mg}(\text{OH})_2$ pretreatment results (Figure 3.2, 3.3a), no improvement in phosphate removal was achieved and it was concluded that no struvite was created. However, Huang et al. observed struvite precipitants on magnesium-pretreated zeolite as well as calcium phosphate (Huang et al., 2014). It could be due to the higher concentration of phosphate in their solution (5 mM) which made it more favorable for precipitation since our concentration was 1 mM. Wu et al. also did not get a good removal with magnesium-pretreated zeolite in low concentrations of phosphate (Wu et al., 2006). Their results started to be improved only in high concentrations (>2 mM) and they related this to the precipitation properties that require high concentrations to happen.

3.4.4 Phosphate removal mechanism for calcium-pretreated zeolite

Regarding the $\text{Ca}(\text{OH})_2$ results, $\text{Ca}(\text{OH})_2$ salt could remove the whole phosphate as calcium phosphate precipitation (Figure 3.2, 3.3a) since the pH was high and the calcium phosphate solubility was low (25 mg/L) (Ullmann et al., 1985). Hosni et al. also studied the effect of calcium hydroxide on phosphate removal and optimized its operating condition (Hosni et al., 2008). They found Ca/P ratio of 2.07 as an optimum amount which can reach 98% phosphate removal in 30 minutes. Figure 3.8 also shows phosphate removal with different amount of calcium hydroxide. When the molar ratio of calcium to phosphate was 1.5 or

more, 100% phosphate removal was achieved which confirmed the effectiveness of calcium hydroxide as a method for phosphate removal.

Results also showed that pretreated zeolite by calcium had lower efficiency than only $\text{Ca}(\text{OH})_2$ salt (Figure 3.2, 3.3a). This observation can be explained by this reason that calcium was bound in zeolite structure and in order for the phosphate to be removed it had to reach to calcium with diffusion mechanism which was slower. While, in case of adding the only salt, calcium was already in the solution and ready to be precipitated with phosphate. This reason was mentioned by You et al. (You et al., 2017). Figure 3.9 which displays the shaking effect on phosphate removal rate shows more removal for calcium-treated zeolite in a better shaking condition (100%). This results can prove that mass transfer and diffusion can be the reasons for low removal efficiency of calcium-treated zeolite. Since calcium-treated zeolite had a high capacity, better mass transfer condition or more time could increase its efficiency as well.

Based on Figure 3.4a, calcium was always in the solid phase. In all conditions, calcium in zeolite could be exchanged with sodium or ammonium and be released in the solution. However, rapid calcium phosphate precipitation possibly kept calcium in the solid phase. There was still the majority of calcium inside the zeolite which needed to be released or more accessible for phosphate to bound and precipitate. Comparing Figure 3.4b and 3.4c also shows that the amount of calcium in solid phase was much more than phosphate removal requirement.

According to Table 3.1, there was a sufficient amount of calcium in zeolite which showed the high capacity of zeolite for phosphate removal; however, all of this calcium was not accessible for binding with phosphates. This calcium needed to get exchanged and be free to precipitate with phosphate and we cannot use all of its capacity without any pretreatment. So, pretreatment with sodium or having sodium and ammonium in the solution could improve the ion exchanging and release this calcium for phosphate removal. It was thought that a zeolite with a sufficient amount of calcium has high potential for phosphate removal even without pretreatment. To confirm that, two different untreated zeolites were tested for phosphate removal in a solution containing ammonium and sodium and Figure 3.5 shows different efficiency for them. This difference could be explained by calcium concentration of zeolite which was lower for the lower efficient zeolite (Table 3.1).

3.5 Conclusions

Targeted wastewater for this method was an industrial type of wastewater with the objective of reaching more than 90% phosphate removal. Zeolite method was aimed to be applied for relatively small-scale and non-continuous wastewater treatment plants. Based on the results, pretreating the natural zeolite and presence of ammonium in the solution were successful for increasing the phosphate removal efficiency. Calcium phosphate precipitation was identified as the phosphate removal mechanism. Sodium and ammonium were exchanged with calcium in zeolite and then calcium removed the phosphate as calcium phosphate precipitation. The efficiency of this removal depended on the type of zeolite as well. The more calcium in zeolite, the more ion exchanging and calcium phosphate precipitation. Magnesium-pretreated zeolite did not improve the properties of zeolite and no changes happened in phosphate removal by zeolite.

Calcium hydroxide had the ability to remove the whole phosphate without zeolite; however, it increased the final pH and separation of precipitants was another challenge in this case.

Calcium-pretreated zeolite increased the amount of calcium in zeolite which means more phosphate removal capacity. However, the efficiency was low since phosphate had to diffuse into zeolite and reach to calcium. Sodium in the solution increased the removal efficiency by exchanging with calcium. Better shaking condition due to providing a better mass transfer condition also assisted with improving the phosphate removal efficiency.

4 Conclusions

4.1 Bio-electrochemical removal of lead by exoelectrogens

Successful Pb(II) removal was achieved by using only bioanode enriched from an MEC reactor individually without applying any external voltages. More than 90% Pb(II) removal happened in less than 24 hours which was the main goal of using this method as a potential method for industrial wastewater treatment. After each 24 hours, another 1 mg/L of Pb(II) was added and the efficiency remained the same which showed the consistency of the system. According to pH results, this system was able to work in a wide range of pH from 5 to 9. Two main Pb(II) removal mechanisms were identified as direct reduction and biosorption. Also, results showed that exoelectrogenic bacteria play the key role in both mechanisms. Biosorption mechanism provided the higher removal compared to direct reduction.

4.2 Future works for lead removal by exoelectrogens

In order to expand the knowledge in this novel idea, there are some more experiments need to be done. First, high pH values can be investigated and the optimum pH range for this system can be found. Then the highest capacity of the bioanode can be found by increasing the cycles of adding lead to the solution. Higher concentrations of lead can also be examined since the typical concentration of lead in the wastewater of a battery manufacturing industry can go up to 5 mg/L (Petruzzelli, Pagano, Tiravanti, & Passino,

2007) . Finally, the method could be tested on real industrial wastewater as well in order to see the effectiveness of the system in the presence of other elements.

4.3 Phosphate removal using zeolite

Zeolite was considered as a potential material for industrial wastewater treatment containing the phosphate concentration in a range of 20-50 mg/L. Salt pretreatment was done on zeolite in order to improve the phosphate removal efficiency by zeolite. The effectiveness of pretreating zeolite by calcium and sodium for improving the phosphate removal efficiency was proved in this project. However, magnesium pretreatment did not have a significant effect on that. Moreover, ammonium showed a great effect on improving phosphate removal efficiency. Phosphate was identified to be removed as calcium phosphate. In this way, sodium and ammonium were exchanged with calcium in zeolite and then calcium removed the phosphate as calcium phosphate precipitation. Results showed the type of zeolite and the amount of calcium in it had a substantial impact on phosphate removal efficiency.

Calcium- pretreated zeolite had a low efficiency in spite of its high capacity for calcium phosphate removal. However, adding sodium to the solution could improve the ion exchanging and release more calcium for phosphate removal. Better shaking condition could also improve the mass transfer and increase the efficiency of phosphate removal. On the other hand, calcium hydroxide showed high efficiency for phosphate removal even without zeolite. However, it increased the final pH and separation of precipitants could be

another challenge in this case. Using zeolite or only calcium hydroxide can be effective in small-scale and non-continuous plants. However, further improvements may increase their application.

4.4 Future works for phosphate removal by zeolite

In order to confirm the effect of calcium amount of zeolite on phosphate removal, more natural and even synthetic zeolites can be investigated for phosphate removal and compared with each other based on their characteristics and removal efficiencies.

Calcium-pretreated zeolite has a more complicated removal mechanism for phosphate since it showed a low removal efficiency despite its high capacity. More study and analysis can be done in this area to find the removal mechanisms.

Effect of sodium on phosphate removal was an interesting part of this research, different concentrations of sodium and ammonium can be tested to find the maximum effect of sodium and ammonium on phosphate removal. More importantly, zeolite can be added to a real wastewater sample with all different cations that may improve phosphate removal efficiency by exchanging with calcium and releasing it. However, other anions may inhibit phosphate removal as well.

Other effective parameters such as particle size and the optimum amount of zeolite added to the solution can also be investigated.

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