Removal of Phosphate from Aqueous Solution by Chitosan-Alginate Beads

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ABSTRACT

Phosphorous is an effective and essential mineral nutrient for the growth of biological creatures and it is very necessary for functioning of ecosystems normally. However, the increase in the usage of phosphorous in lots of parts including industry, agricultural and municipalities causes to pollution of water bodies. Additionally, consumption of water which is rich in content of phosphorous can cause to decrease in the levels of calcium amount of blood and that can result in osteoporosis, hyperphosphatemia and hazard public health. For protection of human health and aquatic ecosystems, the amount of phosphorous should be controlled within the limits (<0.5 mg/L as it has been reported by World health organization).

The removal of phosphorous from water bodies relies mostly on the adsorbents usage which are effective. In this study, chitosan-alginate hydrogel beads were prepared by mixing chitosan and sodium alginate solution together and dropping them into calcium chloride solution. Sodium alginate and chitosan mixture causing interpenetrated network improves the resistance of the beads in acidic mediums. For characterization of beads SEM and FTIR analysis were conducted to prove the presence of functional groups and calcium ions which has the main effect in phosphate adsorption. Additionally, UV spectroscopy method was used to study adsorption capacity which illustrated that with increasing adsorbent concentration and time, the amount of adsorbed phosphate increase considerably.

Keywords: Phosphorous, Chitosan-alginate hydrogel beads, SEM, FTIR, UV

ÖZ

Fosfor, biyolojik canlıların büyümesi için etkili ve gerekli bir mineral besindir ve ekosistemlerin normal işleyişi için önemlidir. Ancak fosforun sanayi dahil birçok alanda kullanımının artması, su kütlelerinin kirlenmesine yol açmaktadır. Ayrıca fosfor içeriği zengin su tüketimi, kandaki kalsiyum miktarının azalmasına neden olabilir ve bu da osteoporoz, hiperfosfatemiye yol açar ve halk sağlığını tehlikeye atabilir. İnsan sağlığının ve sucul ekosistemlerin korunması için fosfor miktarı limitler dahilinde kontrol edilmelidir (Dünya sağlık örgütü tarafından bildirildiği gibi <0.5 mg/L).

Fosforun su kütlelerinden uzaklaştırılması, çoğunlukla etkili olan adsorbanların kullanımına bağlıdır. Bu çalışmada, kitosan ve sodyum aljinat çözeltisi karıştırılarak ve kalsiyum klorür çözeltisine damlatılarak kitosan-aljinat hidrojel boncukları hazırlanmıştır. Sodyum aljinat ve kitosan karışımı iç içe geçmiş ağ oluşumuna neden olur ve boncukların asidik ortamlardaki direncini artırır. Boncukların karakterizasyonu için, fosfat adsorpsiyonunda ana etkiye sahip olan fonksiyonel grupların ve kalsiyum iyonlarının varlığını kanıtlamak için SEM ve FTIR analizleri yapıldı. Ek olarak, artan adsorban konsantrasyonu ve süresi ile adsorbe edilen fosfat miktarının önemli ölçüde arttığını gösteren adsorpsiyon kapasitesini incelemek için UV spektroskopi yöntemi kullanıldı.

Anahtar Kelimeler: Fosfor, Kitosan-aljinat hidrojel boncukları, SEM, FTIR, UV

DEDICATION

To My Family

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INTRUDUCTION

1.1 Phosphorus

Phosphorus is considered as a macronutrient that the biota requires, however, it is routinely given in excess to surface waters in many regions of the world, making it a frequent contaminant. Weathering of rock produces phosphorus (P), but from the middle of the 19th century, mining for the mineral has greatly grown. The quantity of P mined by humans for fertilizer (figure 1a) and other purposes is estimated to be 25 Tg annually, which means that in a comparison with natural weathering, mining has increased P flow by about 800%. About 3 teragrams (Tg) of P naturally weather each year. There exists P naturally in a variety of oxidized states that can either dissolve in soil or be adsorbed. It can also be attached to soil particles by clays, iron oxides, calcium compounds, aluminum oxides, or organic particles.

Sims et al. (1998) discovered that particle-bound and dissolved P are equally impacted by precipitation-driven runoff from the environment through water bodies, with approximately 75–90% of the P which does enter the receiving waterways flowing concurrently with the soils which are eroding (figures 1b) In a condition where P, either as a dissolved P or in conjunction with suspended particles, reaches the receiving waters, it becomes a pollutant. The percentage of bioavailable particulate phosphate varies greatly, ranging from 10% to 90%, which depends upon the following items:

the source soils, the characteristics of the water, and the existence of algal-microbial consortia.

Algae and bacteria may easily use inorganic phosphorus that has been dissolved. In the organic form of dissolved phosphorus, dissolved organic phosphorus (DOP) is likely to be bioavailable or not. For instance, upon examining 27 aquatic systems in the Midwest, the authors found bioavailability of DOP in a range of 0-100%, where median value is reported to be 78%.

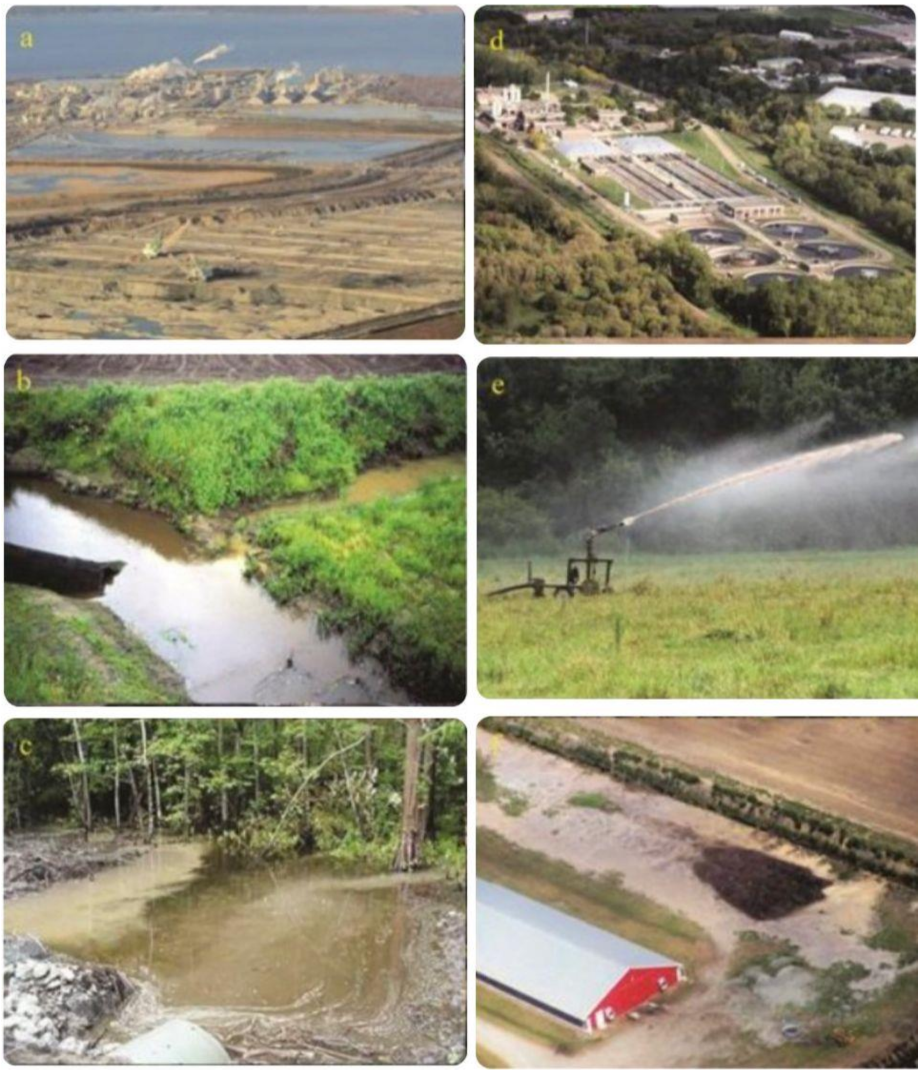


Figure 1: Common sources of phosphate pollution to the landscape and watercourses

The phosphate pollution which typically originates from the environment and waterways include the following:

(a) The annual production of 25 Tg of phosphate has risen.

(b) Suspended sediments are associated with a significant amount of the P delivered to waterways through runoff. The picture shows an Eastern North Carolina's field muddy runoff.

(c) The muddy storm water runoff in Wilmington, North Carolina.

(d) P is released into the environment by wastewater treatment facilities after leaks, spills, and power outages brought on by severe storms.

(e) In Duplin County, North Carolina, liquid pig feces were sprayed on surrounding farms.

(f) Poultry manure before it was distributed as litter on surrounding fields in Duplin County, North Carolina. Michael A. Mallin took all but one of the images, which were provided by Sound Rivers, Inc.

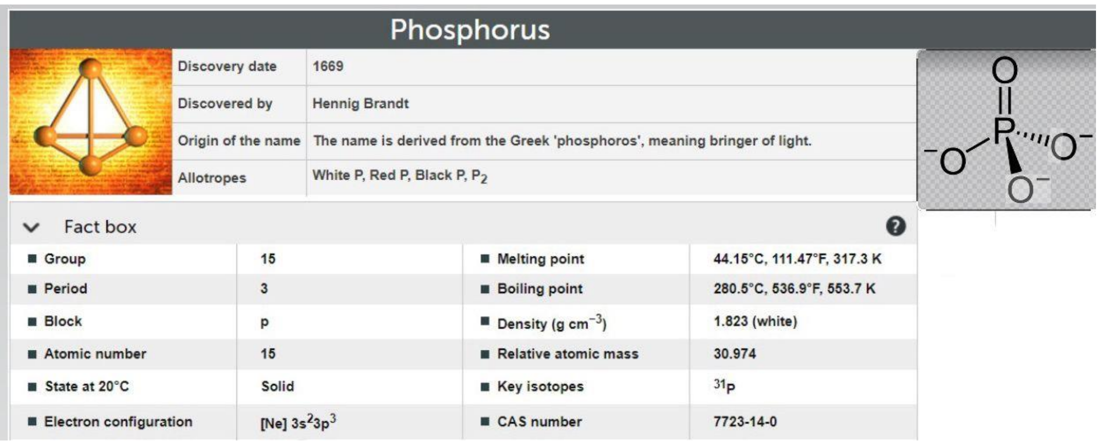
The non-metal phosphorus is located in group 15 of the periodic table, right below nitrogen. There are various variations of this element, but white and red are the most well-known.

Unquestionably, white phosphorus is the most exciting of the two mentioned above. As phosphorus does glow in the dark conditions, is a lethal poison, and may ignite in temperatures above 30 degrees. Unfortunately, red phosphorus lacks all of these fascinating characteristics.

Where did it all start, then? Hennig Brandt created phosphorus for the first time in Hamburg, Germany, in 1669. He heated the urine remnant till it was red hot after evaporating the urine. He exhaled glowing phosphorus vapour, which he condensed in the water. And for more than a century, this was how most phosphorus was produced. Before people realised that bone was an excellent supply of phosphorus, this was the case. To create white phosphorus, phosphoric acid can be heated with charcoal after being dissolved in sulfuric acid with bone.

The elemental form of phosphorus is never found in the natural world. It is only recognized as phosphate, which for a variety of reasons is essential to life. It is a part of DNA and contributes significantly to bone and tooth enamel as calcium phosphate.

Organophosphates are also essential, including the energy molecule ATP and the phospholipids in cell membranes. The phosphate that our bodies require comes from a typical diet. with a ton of tuna, chicken, eggs, and cheese. Even part of it, in the form of phosphoric acid, comes from cola.



Phosphorus

Discovery date	1669
Discovered by	Hennig Brandt
Origin of the name	The name is derived from the Greek 'phosphoros', meaning bringer of light.
Allotropes	White P, Red P, Black P, P_2

Fact box

■ Group	15	■ Melting point	44.15°C, 111.47°F, 317.3 K
■ Period	3	■ Boiling point	280.5°C, 536.9°F, 553.7 K
■ Block	p	■ Density (g cm^{-3})	1.823 (white)
■ Atomic number	15	■ Relative atomic mass	30.974
■ State at 20°C	Solid	■ Key isotopes	^{31}P
■ Electron configuration	[Ne] $3s^2 3p^3$	■ CAS number	7723-14-0

Figure 2: Phosphorous fact box and its details in periodic table

As P readily binds to soil particles, subsurface P migration to streams is often minimal [1]. There are several exceptions, particularly in areas with high water tables, sandy, organic soils, or soils that are saturated with phosphorus from agricultural or urban sources and let phosphorus to be transferred via the soil layer [1,2]. The zones with Saturated-P include places in which P has been considerably joint to the soil through animal dung spread from CAFOs existing on the ground (Figs 1e and 1f;) [4]. More US counties currently contain manure nutrients than their capability for plant absorption and elimination, claim [3]. Soil's p saturation is another possibility in situations when either septic systems are placed so close together or when the earth is too wet to accomplish immobilization appropriate within drain fields.

It has even been reported in some recreational and residential environments [4]. The P concentrations rise in streams and lakes from preindustrial times up to the present and the buildup of P in watershed soils are evidence of the transition from using recycled manure P on a small scale to using mined fossil P on a large scale as commercial fertilizer as well as CAFO wastes disposal.

The orthophosphoric acid (H3PO4) ionized forms that plants and microbes most easily absorb are referred to as "orthophosphate" [1]. After being transported into receiving waters, orthophosphate concentrations are believed to stay within a general range due to adsorption equilibrium, in which dissolved P can either be bound to or leached from particles depending upon the amount of dissolved P in the water.

The amount of P that is particle-bound, however, could significantly rise in response to anthropogenic source inputs. P is commonly desorbed from suspended sediments that are transported down the river continuum to the estuary as a result of competition between other anions for sorption sites and the increase in salinity [3].

Most frequently, orthophosphate P and total P, which combines particle-bound and dissolved organic plus inorganic P, are used to assess P in water, primarily because algae are often luxuriant users of phosphorus, removing more phosphorus than they require from the water, storing it, and then using some of the stored phosphorus for food. As a result, total P is often thought to be the P measurement that is most pertinent for determining the trophic status or water quality. Second, bacteria and algae quickly absorb the majority of the dissolved inorganic P in the water column because it is ephemeral and low in concentration [5].

A measurement of with dissolved inorganic state merely provides a glimpse of the P actually present to the biota as a result [6]. A marker of eutrophic fresh waters are assumed to be total P concentrations in water columns that are higher than the range of 70–84mg of P in each liter [5].

1.1.1 Phosphorus as a resource

Today, phosphate rock is mined globally and converted into phosphoric acid to produce the majority of the phosphorus humans eat. It is produced in 50 million tones annually and has numerous applications. Fertilizers, animal feed, rust removers, corrosion inhibitors, and even dishwasher tablets are made with it.

Coke, sand, and some phosphate rock are burned inside an electric furnace for production of white phosphorus. Next, the white phosphorus is transferred into phosphoric acid and phosphorus trichloride. Accordingly, weed killers, flame retardants and insecticides are made. In a tiny proportion, phosphorus sulphides are also produced, which are added to oil to reduce engine wear.

Flares and other incendiary devices employ white phosphorus. The substance that is adhered to the matchboxes side and employed to strike safety matches for lighting them contains red phosphorous. The majority of phosphorus compounds are used in fertilizers. Phosphate ores are used to make ammonium phosphate. Before being turned into ammonium phosphate, the ores are first transformed into phosphoric acids.

Additionally crucial to the creation of steel is phosphorus. Although phosphoates are a component of several detergents, they are starting to be phased out in some nations. This is so that undesirable algae won't bloom as a result of excessive phosphate levels in natural water supplies. Additionally, phosphorus is employed in the manufacture of exquisite china and specialty glassware.

Furthermore, phosphate is crucial for the ecology. It travels inherently from the soil to rivers, seas, and bottom sediment. Here it builds up until geological uplift transports it to dry land, where the cycle can restart. Numerous plants, microbes, and animals from various eco-systems transport phosphate.

1.1.2 Phosphorus/Phosphate pollution

The roots via which phosphorus does enter the soil and water are phosphorus mining from rock, rain-caused weathering from the rocks, and other abiotic mechanisms such as farming, municipal effluent, industrial processes, etc. After reaching the water and soil, phosphorus can be absorbed for growth by plants and microbes. The collected phosphorus will be released back into soil and water followed by breaking down of such plants and microorganisms. The phosphorus depletion can be taken into consideration as an issue for the next-generation because the above-said natural phosphorus cycle in inorganic form requires millions of years and is not a sustainable supply. In the form of fertilizer, approximately an amount of 27 million tons of phosphorus are applied to farming places annually; however, only 3 million tons of this amount are consumed by humans through food, with the balance being regarded as lost.

High phosphorus concentrations can be caused by ineffective farming methods, leaking septic systems, runoff from cities and lawns, and discharges of sewage treatment plant. The process of eutrophication, also referred to as the proliferation of large aquatic plants and algae, can be triggered by an overabundance of phosphorus. Algal blooms brought on by high phosphorus levels have the potential to produce poisons harmful to either animal or human health.

Figure 3: Eutrophication Process

Chapter 2

LITERATURE REVIEW

2.1 Methodology for phosphate removal from water

It is strongly recommended to remove and recover phosphate using a variety of established and cutting-edge technologies, such as biological reactors, electrochemical processes, adsorption, crystallization and chemical precipitation [7]. In order to achieve phosphorus precipitation by a variety of methods, calcium, iron, and aluminum salts are added as part of the chemical removal of phosphorus. Additionally, essential criteria for phosphorus removal by chemical precipitation include the influence of operating circumstances, particularly waste water characteristics; sludge generation in terms of quality and quantity; and optimization of chemical use.

An alternative to chemical therapy is biological phosphorus removal, which depends on the uptake of phosphorus above usual bacterial metabolic requirements. Adsorption technology, one of the many methods used to reduce water phosphate levels, has proven to be quite effective, even at low phosphate concentrations.

2.1.1 Biological method

To achieve removal of biological phosphorus, numerous biological suspended growth process designs have been during the recent 20 years. The following image displays the most significant ones.

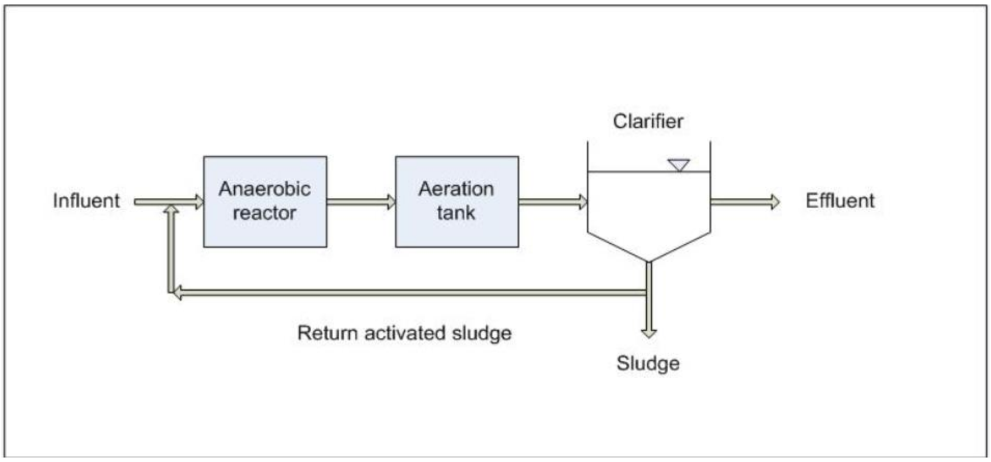


Figure 4: Biological suspended growth process

When compared to chemical precipitation, the main benefits of biological phosphorous removal are lower chemical expenses and less sludge generation.

Phosphorus is absorbed into the cell biomass during biological phosphorus extraction from wastewater and then due to sludge waste, it is removed from the process. The P accumulating organisms (PAOs) have an advantage compared to other bacteria in the reactor design. PAOs are thereby stimulated to consume and proliferate the phosphorus. The configuration and design of reactor consist of a tank of activated sludge and an anaerobic tank. Between 0.50 and 1 hour is the anaerobic tank's retention time, and its contents are mixed to bring influent wastewater and return activated sludge into contact.

Anaerobic zone: In anaerobic environments, PAO does absorb fermentation byproducts (e.g. volatile fatty acids) into cell-based storage products while simultaneously releasing phosphorus from polyphosphates that have been stored. Acetate is produced by fermenting BsCOD, a dissolved degradable organic compound readily ingested via the biomass. The PAO uses energy from the stored polyphosphates to digest acetate and create storage products of intracellular polyhydroxybutyrate (PHB). Concurrent with the acetate ingestion, potassium, orthophosphates, magnesium, and calcium cations are also released. The PAO's PHB concentration increases as the polyphosphate level decreases.

The amount of polyphosphate stored by the cell increases in the zone of aerobic, and energy will be generated through storage byproducts oxidation. PHB that has been stored is digested, producing carbon for new cell development as well as energy from oxidation. PHB metabolism results in the production of some glycogen. In order to create polyphosphate linkages in cell storage, PHB oxidation releases energy. Within the bacterial cell, the soluble orthophosphate is taken out of solution and combined with other phosphates to form polyphosphates. The use of PHB promotes celldevelopment while also removing phosphorus, and this new biomass has a high polyphosphate storage capacity. The stored phosphorous is taken out of the bio treatment reactor since some of the biomass is wasted and will ultimately be disposed of with the waste sludge.

The amount of bsCOD present in the wastewater influent can be used to determine the amount of phosphorous recovered by biological storage. When bsCOD acetate is offered at a consistent rate, BPR systems operate better [8].

2.1.2 Adsorption process

Among the methods available for treating water, one of the most effective processes for the treatment and eradication of organic contaminants in wastewater treatment is the adsorption process using solid adsorbents. Adsorption offers advantages over other technologies due to its straightforward design and potential for modest initial investment as well as minimal acreage requirements. The adsorption technique receives a lot of attention from researchers and is frequently used to clean industrial waste water of organic and inorganic impurities. The hunt for inexpensive adsorbents with pollutant-binding abilities has intensified recently. Natural resources, industrial wastes, and agricultural waste are all readily available local resources that can be employed as affordable adsorbents. These materials can be used to create activated carbon, which are also usable for water/wastewater treatment as an adsorbent [9].

The phenomenon of adsorption, which takes place on surfaces, is a common technique for eliminating organic/inorganic pollutants. When a solution touches a solid with a structure which is highly porous, some of the solute molecules from the solution areconcentrated or deposited at the solid surface due to the intermolecular forces of attraction between the liquid and solid. Adsorbent, as opposed to adsorbate, refers to the solid on which the solute is held (onto the solid-type surface). Adsorbate builds up on an adsorbent's surface, which is referred to as adsorption. The core of separation by adsorption technology is the production of an adsorbed phase with a distinct composition from the bulk fluid phase. All of the individual atoms' bonding requirements, whether they be ionic, covalent, or metallic, are supplied by other atoms in the bulk substance. Because they are partially surrounded by other adsorbent atoms, adsorbate may be drawn to the atoms on the surface of the adsorbent. However, Physicsorption, which is characterized by weak Van Der Waals interactions, and chemisorption are two common classifications for the adsorption process. The sort of bonding will be determined by the exact species involved (characteristic of covalent bonding). Another potential culprit is electrostatic attraction.

A balance between the solution and the adsorbent is reached as the adsorption process proceeds for the solute (in which the solute adsorption from the bulk through the adsorbent is minimum). Equation was used to determine the amount of molecules adsorbing (qe, mmol g^{-1}) at the equilibrium step:

$$qe = V\,(Co-Ce)\,/M \qquad (1)$$

In which V and M indicate the solution volume (L) and the monolithic adsorbents mass (g), respectively. In addition, Co and Ce express the adsorbate's starting and equilibrium concentrations, respectively. The process through which a substance is transferred from the liquid phase through a solid’s surface and then joined by physical and/or chemical interactions is known as adsorption. Surface reactivity and high capacity of adsorption is correlated with large surface area [10].

2.1.2.1 Isotherms and models of adsorption

The display of the solute adsorbed amount in each unit weight of adsorbent in relation to equilibrium concentration in the bulk solution at a fixed temperature is known as an adsorption isotherm. In order to express adsorption data, adsorption isotherms developed by Langmuir and Freundlich are frequently utilized:

$$Ce\,/\,qe = 1\,/\,bXm + Ce\,/\,Xm, \qquad (2)$$

In which Xm expresses the adsorption capacity (mmol g^{-1}), or monolayer capacity, Ce and qe indicate the solute’s equilibrium concentration (mmol L^{-1}) and the quantity of the solute which is adsorbed in each unit weight of adsorbent (mmol g^{-1} of clay), respectively, and b is a constant (L $mmol^{-1}$). Heterogeneous surface adsorption is described by the Freundlich isotherm. Adsorptive sites' energy distribution (in the Freundlich isotherm) closely reflects the real world and is modeled by an exponential type function. The rate of desorption and adsorption is influenced by the energy intensity in the adsorptive sites. This is how the Freundlich equation can be read:

$$\log qe = \log k + 1\,/\,n \log Ce, \qquad (3)$$

In which 1/n and k (mmol g^{-1}) indicate the system's constant properties [11].

2.1.2.2 Advantages and disadvantages

Table 1: Advantages and disadvantages of different waste water treatment methods

Process	Advantages	Disadvantages
Adsorption	• Easy process operation. • Effective at low phosphate concentrations. • Low initial cost. • Low amount of by- products. • Fast process. • Adsorbed phosphate could be recovered (Ghorbani et al., 2020; Lin et al., 2016; Siyal et al., 2020).	• Adsorbent regeneration requires additional cost. • The effect of co-existing ions, selectivity against other contaminants.
Biological processes such as the conventional activated sludge treatment	• Can achieve complete removal of phosphate. • Low operation cost. • Low amount of by- products (Huang et al., 2017).	• Strict control of operational parameters is required (Loganathan et al., 2014). • Not effective in the removal of trace amounts of phosphate (Bacelo et al., 2020; Huang et al., 2017).
Chemical precipitation using lime, calcium, magnesium, aluminum and iron salts	• Well established process. • Reliable process, • Can achieve high phosphate removal (Kyong et al., 2014).	• Requires continuous effluent neutralization. • High chemical consumption. • Sludge disposal. • Not effective at low phosphate concentrations (Loganathan et al., 2014).

2.1.2.3 Kinds of adsorbents

Different adsorbent kinds are divided into two categories: synthetic and natural. Examples of the latter include clays, ores, zeolites, clay minerals, charcoal, and clays. In many cases, these natural materials are inexpensive, plentiful, and have a lot of room for modification, which could eventually enhance their capacities for adsorption. The former are made from waste materials such as sewage sludge, household waste, industrial waste, and waste from the agricultural industry. Each adsorbent has unique qualities including porosity, pore structure and the surfaces types it may adsorb on. A few examples of the numerous used waste materials encompass the followings: coconut shells, rice husk, bark and additional tannin-rich materials, fruit wastes, clays, chitosan, scrap tires, fly ash, ore minerals, zeolites, sawdust, petroleum/fertiliser wastes, wastes in sugar industry, seaweed and algae, blast furnace slag, seafood processing wastes, peat moss, red mud, sediment and soil and so on.

2.1.2.4 Adsorption by biomass

Wastewater treatment using biosorption, or the adsorption of biomass, is efficient, economical and environmentally friendly [12]. Since they require the least amount of processing, farming products and byproducts (biomass) are effective for biosorption of contaminants including colors and heavy metals in waste treatment [13,14]. Hence, they can be employed in biofiltration systems and other things [12,15]. Pyrolysis, also known as biomass thermal decomposition (acquired by plants), takes place at a range of T from 300-1000°C in the either partial or total deprivation of oxygen and generates a number of combustible gases, including tarry vapours, non-combustible CO2 gas, flammable oils, and char, a solid product with a high organic carbon concentration. A variety of organic materials can be used to create biochar, including organic kitchen waste, sewage sludge, agricultural waste, biomass energy crops, manure, and more [16,17]. Biochar provides a wide range of uses, such as the elimination of heavy dyes and metals, the elimination of pollutants, the generation of power and so on. Because of its value economically and sustainably, it can be used for industrial production operations in a large scale [18,19]. A new area of research in the realm of pollution prevention is pyrolysis technology, which creates biochar from lignocellulosic biomass. The production of biochar through the pyrolysis process results in the release of gases and vapors. Considerations for pyrolysis include choosing the feedstock based on the shape of the particle size, biomass, pre-treatment and reaction conditions. This illustrates the production of biochar from various lignocellulosic biomass kinds and its application in industries including farming and wastewater treatment. Due to the fact that many components, such as hemicellulose, cellulose and lignin connect with one another throughout the breakdown process, it is hard to predict the features of biomass pyrolysis simply on the thermal behavior of the components present in thebiomass [15,20]. Biochar has a lot of great physical and chemical properties. The kind of feedstock used and how it is handled beforehand, the production process and its limitations, and the way biochar is handled after it has been produced may have an impact on these [17,20].

There are numerous ways to vary the chemical and physical properties of biochar, which assist in assessment of the use of biochar for a number of objectives, including the elimination of impurities (heavy metals, organic pollutants, dyes and so on) [21,22]. The purpose of biochar modification is to increase raw biochar's effectiveness at removing pollutants at higher concentrations. The benefits of biochar generated through biomass, including its high calorific value and high accessibility, have sparked a lot of attention [23,24]. As a result, there are fewer atmospheric emissions of greenhouse gases in general and of carbon dioxide in particular. It also assists to store carbon in a relatively stable condition. Biochar is a fantastic instrument for lessening the consequences of climate change because it can generate renewable energy and help the environment [16]. Since it utilises all of the agricultural waste materials profitably and effectively, creating biochar from lignocellulosic biomass is an environmentally friendly approach that lowers the number of contaminants in the environment [25]. They are effective at eliminating pollutants from aquatic environments, including contaminated water, soil, and waste effluents.

They function as a greener substitute for fossil fuels for the production of cleaner power, thefiltration of water to remove metals, additional contaminants and so on [17].

2.1.2.5 Lignocellulosic biomass

Fuels can be produced from biomass using a variety of conversion techniques (physical, chemical, and biological). The solid carbonaceous material transformation is done through biomass conversion. Industries that use biomass produce a lot of trash, which is unwanted. The pyrolysis process offers a remedy for this since it transforms biomass and biomass waste into solid products like biochar, which have uses in a variety of industries [20].

Lignocellulosic biomass typically contains 15–30% hemicellulose, 40–50% cellulose, and 15–30% lignin. The structure of lignocellulose cell walls is composed of cellulose which is a linear-type polysaccharide polymer of glucose tightly bound by a -1, 4-glycoside linkage. Numerous hydroxyl groups found in cellulose chains cause hydrogen bonding to form. In cellulose, there exist regions that are crystalline (having high crystallinity) and amorphous (having low crystallinity). Hemicellulose and/or pectin also connect cellulose microfibrils, but lignin protects them. Hemicellulose is more variable, amorphous, and varied than cellulose. Hemicellulose consists such hexoses as galactose, rhamnose, glucose, mannose, and fucose as well as such pentoses as xylose and arabinose, and uronic acids, such as methyl glucuronic acid, glucuronic acid, and galacturonic acid [26]. The matrix lignocellulose has a particular rigid structure as a result of the branched and short hemicellulose chains which assist lignin and cellulose microfibrils in forming networks. Lignin is considered as a plentiful organic substance that is both optically and water-insoluble.

In order to cross-link cellulose and hemicellulose and produce a rigid, three-dimensional cell wall structure; lignin is also required. Additionally, because of high amount of carbon and biomass in lignin, it stores about forty percent of the energy in lignocellulosic materials. generally, lignin proportions in softwood and the majority of agricultural waste products are higher compared to those in hardwood [27] (See Fig. 5 and Table 2). For instance, grassy plants will contain less lignin compared to woody ones [23,28].

Additionally contained in trace levels are ash (inorganic material), extractives including gums, proteins, glycosides, pectins, saponins, essential oils, terpenes, simple sugars, alkaloids, mucilage, lipids, phenolics, resins, starches, and waxes (Fig. 2) [28]. Cellulose, a polysaccharide having a high level of polymerization made up of D-glucose units, makes up the majority of LB. Due to its crystalline structure, it has unique qualities like mechanical strength and chemical stability. This material is composed of insoluble microfibrils. The component that encircles cellulose microfibrils and joins cellulose and lignin is called hemicellulose.

Xylose, mannose, glucose, and arabinose are all present, albeit in various degrees, in this polysaccharide. It is less polymerized than cellulose, and due to its noncrystalline form, it has also lower stability.

Lignin which is an organic polymer, aids in making plants more rigid and robust. It provides the most abundant aromatic moiety of any naturally occurring chemical. The empty spaces in the cellulose microfibrils covered with hemicellulose are filled with lignin. Lignin acts as a binding agent for cellulose and hemicellulose inside the cell wall. Because it contains phenylpropane units, breaking down lignin is a difficult task [28,29].

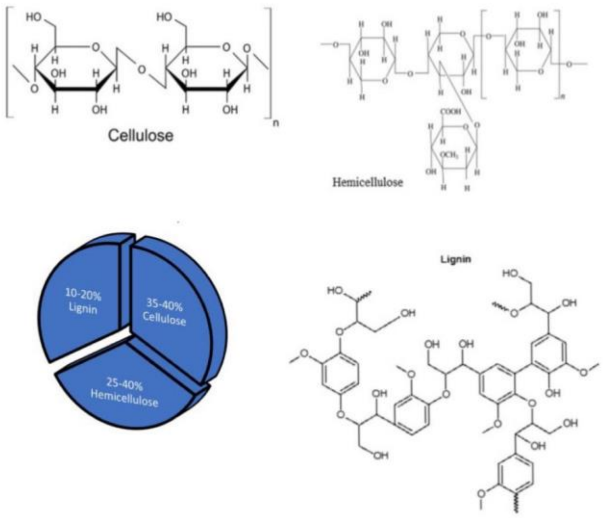


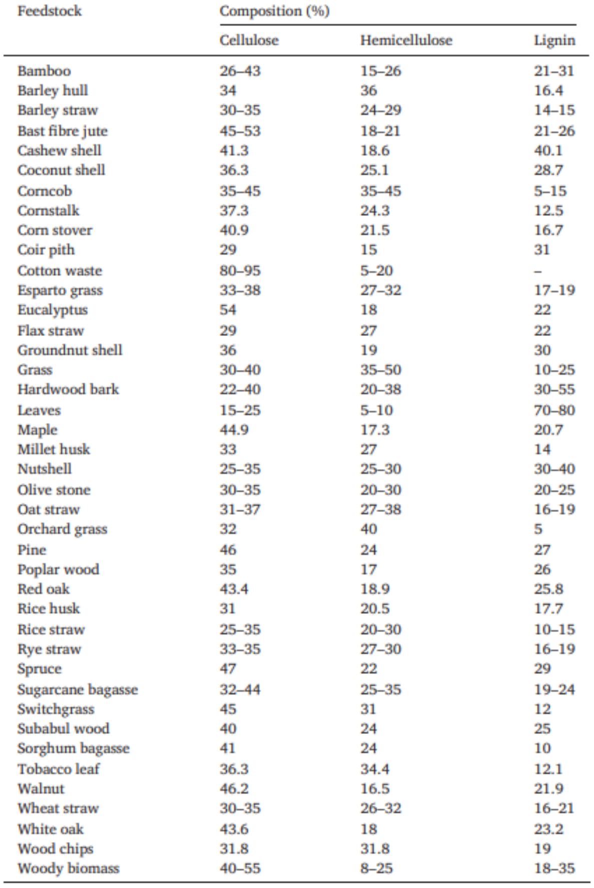
Figure 5: lignocellulosic biomass Components and composition

2.1.2.6 Feedstock of lignocellulosic biomass

Agricultural waste, industrial biomass waste utilized as raw materials, and materials from other sources including household garbage, sewage sludge, animal manure, etc. are examples of lignocellulosic biomass feedstock. (Fig. 3) have additionally been found [23,28]. They can be classified as softwood, hardwood, grasses, and agricultural waste which depend upon their kind and source [15,25]. The biochar yield is influenced by the type of used feedstock. Although different feedstocks can be used in pyrolysis procedures, the feedstock's variability is what causes the final product's inconsistent qualities. In order to prevent issues, feedstock quality criteria should be established. Another problem is the biomass's level of ash.

The inorganic components of ash, especially potassium and calcium, catalyse the breakdown of biomass and the creation of char [28]. An essential factor affecting the unique nature, molecular make-up, and kind of feedstock employed in biochar formation is their type. The energy content of the produced biochar is also influenced by the feedstock. Sometimes, the pre-treatment techniques selected for the feedstock prior to pyrolysis may have an effect on the characteristics of biochar.

Table 2: Different lignocellulosic feedstocks and their chemical compositions



Feedstock	Composition (%)		
	Cellulose	Hemicellulose	Lignin
Bamboo	26–43	15–26	21–31
Barley hull	34	36	16.4
Barley straw	30–35	24–29	14–15
Bast fibre jute	45–53	18–21	21–26
Cashew shell	41.3	18.6	40.1
Coconut shell	36.3	25.1	28.7
Corncob	35–45	35–45	5–15
Cornstalk	37.3	24.3	12.5
Corn stover	40.9	21.5	16.7
Coir pith	29	15	31
Cotton waste	80–95	5–20	–
Esparto grass	33–38	27–32	17–19
Eucalyptus	54	18	22
Flax straw	29	27	22
Groundnut shell	36	19	30
Grass	30–40	35–50	10–25
Hardwood bark	22–40	20–38	30–55
Leaves	15–25	5–10	70–80
Maple	44.9	17.3	20.7
Millet husk	33	27	14
Nutshell	25–35	25–30	30–40
Olive stone	30–35	20–30	20–25
Oat straw	31–37	27–38	16–19
Orchard grass	32	40	5
Pine	46	24	27
Poplar wood	35	17	26
Red oak	43.4	18.9	25.8
Rice husk	31	20.5	17.7
Rice straw	25–35	20–30	10–15
Rye straw	33–35	27–30	16–19
Spruce	47	22	29
Sugarcane bagasse	32–44	25–35	19–24
Switchgrass	45	31	12
Subabul wood	40	24	25
Sorghum bagasse	41	24	10
Tobacco leaf	36.3	34.4	12.1
Walnut	46.2	16.5	21.9
Wheat straw	30–35	26–32	16–21
White oak	43.6	18	23.2
Wood chips	31.8	31.8	19
Woody biomass	40–55	8–25	18–35

Therefore, the produced biochar`s quality during pyrolysis can be enhanced with effective feedstock pretreatment, which can also reduce negative consequences [28]. Chemical (the load of precursors and functional agents, treat with chemicals or functional materials and so on), physical (crush, wash, dry, sift and so on) as well as biological (e.g. bacterial treatment) pre-treatment procedures are employed depending on the feedstock and the goals to apply biochar. The dimensions, critical components of the lignocellulosic feedstock as well as high-temperature decomposition all affect the carbonaceous structure of biochar.

The lignocellulosic components reactivity varies, which causes their thermal degradation to occur during pyrolysis within a certain temperature range and at different heating rates. First to decay are cellulose and hemicellulose, then lignin comes in.

The production of biochar from hemicellulose, cellulose, and lignin depends upon both the experimental characteristics of the process as well as pyrolysis temperature and the feedstock. It is crucial to choose the optimum feedstock and production technique for biochar in order to produce it with the desired qualities for a particular operation [18].

2.1.2.7 Phosphate removal by biomass, biochar

Ca's ability to modify biochar is especially intriguing because it produces no harmful waste or byproducts and is simple to carry out. According to reports, calcium-modified biochar has a high adsorption capacity for phosphate in water, with values at the range 96.56-197 mg/g. Common biochar Ca-modification techniques involve applying chemicals that contain Ca that raises the overall manufacturing cost and makes scaling up challenging. Researchers subsequently began seeking for affordable raw materials. Natural materials, which are frequently thrown away, are rich in calcium, which is frequently bonded with carbon and its derivatives. Oyster shells are one illustration. Calcium carbonate is the primary ingredient in oyster shells ($CaCO_3$). The findings of China's 2016 national pollution report showed that oyster cultures had grown to 4.83 million tonnes, translating into tonnes of shell waste that was consuming significant amounts of land and potentially endangering both human health and the environment.

These discarded oyster shells can be used as a source of calcium to change biochar, which is one approach to make use of them. Therefore, our effort created high surface area biochar using sheep manure known as a Ca source. Then, Ca from the used oyster shells was added to the biochar. The materials that were produced were used to take phosphate out of the wastewater.

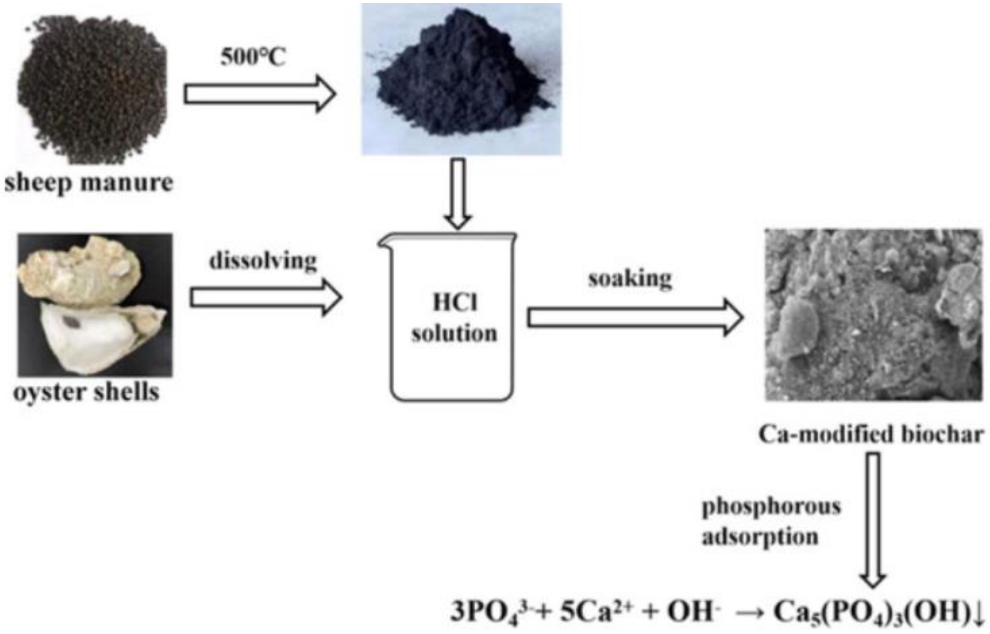


Figure 6: Phosphorous adsorption by Ca-modified biochar prepared from sheep manure and oyster shells

2.1.2.8 Adsorption by polymeric adsorbents

Polymeric adsorbents are synthetic polymers that are spherical in shape, have a defined pore structure, and have a large surface area. They are typically used in aqueous applications to remove organic molecules effectively and selectively. Depending on the environment, such as heat, pH, competing molecules, solvents, etc., adsorbentsinteract with molecules in various ways.

Polymeric adsorbents are generally more expensive than other types of materials, however, they offer benefits such great design flexibility, physical stability, porosity, homogeneous pore size distribution, large surface area, chemical stability toward acids and bases, and thermal durability [30]. An exciting and urgent research requirement is the creation of an adsorbent that can purify wastewater with high efficiency, low cost, and aesthetic appeal [31].

Both types of natural and synthetic polymers can be used as polymeric adsorbents. The fact is, in chemistry, polymers can be classified into two groups, which are as follows:

- Natural polymers
- Synthetic polymers

Natural polymers are those that have arisen due to either biological or natural processes and we find them everywhere in nature, including in not only plants, animals, but also the human body in the form of fats, proteins, amino acids and so on. Natural polymers are different from synthetic due to the ability to be distinguished from them by a single term. Simply explained, synthetics are generated in labs through artificial inputs and tools and do not occur naturally or through a natural process.

Both natural and artificial polymers have the ability to function as polymeric adsorbents, but artificial polymers may result in secondary pollution, which is why many studies on natural polymers are now being conducted.

Numerous important natural polymers are used in waste water treatment, including chitosan, alginate, cellulose, and others.

Table 3: Advantages and disadvantage of natural and synthetic polymers

Natural polymers		Synthetic polymers

. Less toxic . Biocompatibility . Biodegradability . Easy available	**Advantages**	. Biocompatibility
. High degree of variability in natural materials derived from animal sources . Structurally more complex . Extraction process is very complicated and high cost	**Disadvantages**	. Toxic . Non degradable . synthetic process is very complicated and high cost

2.2 Chitosan

A series of linear polysaccharides comprised of different ratios of N-acetyl-2 amino-2-deoxy-D-glucose (glucosamine, GlcN) as well as 2-amino-2-deoxy-D-glucose (N-acetyl-glucosamine, GlcNAc) residues is known as chitin and its deacetylated derivative, chitosan. Chitosan disintegrates in an acidic aqueous environment by basic protonation of amine. Chitin, on the other hand, contains an excessive number of acetylated residues that prevent the polymer from dissolving in an acidic aqueous solution.

Chitin is a widely prevalent biopolymer that is present in the cell walls of fungus, algae, insect cuticles, and the exoskeletons of crustaceans. Less frequently seen in nature, chitosan is found in several fungus (Mucoraceae). In the past, most commercially available chitosan samples were created by chemically deacetylating chitin obtained from sources such as crustaceans. Recently, market interest in chitosan made from fungi has surged due to demand from vegans. Additionally, these samples have lower viscosities that are well regulated and have a very high deacetylation level [32]. The rising interest in producing protein from these sources has led to a rise in interest in production from insect cuticles. The wide range of biological and technical features demonstrated by chitin and chitosan are what generate interest in these polymers.

However, these characteristics are closely linked to the physical characteristics of the polymers, particularly their molecular weight and degree of acetylation [33]. Therefore, in case of applying chitin and chitosan, a thorough and accurate polymer characterization is required.

The only polycation in nature is chitosan, and depending on the media's pH and acetylation level, several factors affect its charge density. The polymer's solubility is dependent on its molecular weight and level of acetylation. At a variety of pH values, including both acidic and basic ones, chitosan oligomers are soluble (namely, physiological pH of 7.4). Besides, even at high levels of deacetylation, chitosan samples having greater Mw can be just solved in acidic aqueous conditions. Since it is difficult to dissolve at basic and neutral pH levels, there are fewer physiological uses for chitosan (namely, pH of 7.4). This is why chitosan compounds with better solubility have undergone substantial development.

In 2019, the market for selling chitosan was forecasted to provide a value of 6.8 billion USD. Accordingly, for the years 2020-27, this value is predicted to increase at a CAGR of 24.7%, measured in terms of revenue. The primary factors driving market expansion include the expanding usage of the polymer in water treatment and numerous industries with a high-value, such as biomedical, foodstuff, pharmaceutical and cosmetics [34]. The modification of polymers to increase their applications, understanding the biological mechanisms underlying the biological activity of chitosan, chitosan derivatives, and chitooligosaccharides, and a thorough investigation of the chitosanolytic and chitinolytic enzymes present in various microorganisms are a few of the areas of interest that have been taken into consideration [35].

2.2.1 Chemistry of chitosan

Chitosan has a number of reactive groups, including primary and secondary hydroxyl groups, a primary amino group (C2), and more, as can be seen in Figure 1. (C6, C3). Functional groups can also include acetamide and glycosidic linkages. Numerous alterations can be made to these functional groups, resulting in polymers with novel characteristics and behaviours.

Chitosan derivatives have been created in an effort to enhance either the biodegradability or solubility or of chitosan or to add new features/functions. As anexample, deacetylation, depolymerization, or quaternization among other procedures have enhanced solubility in water-based aqueous systems [36].

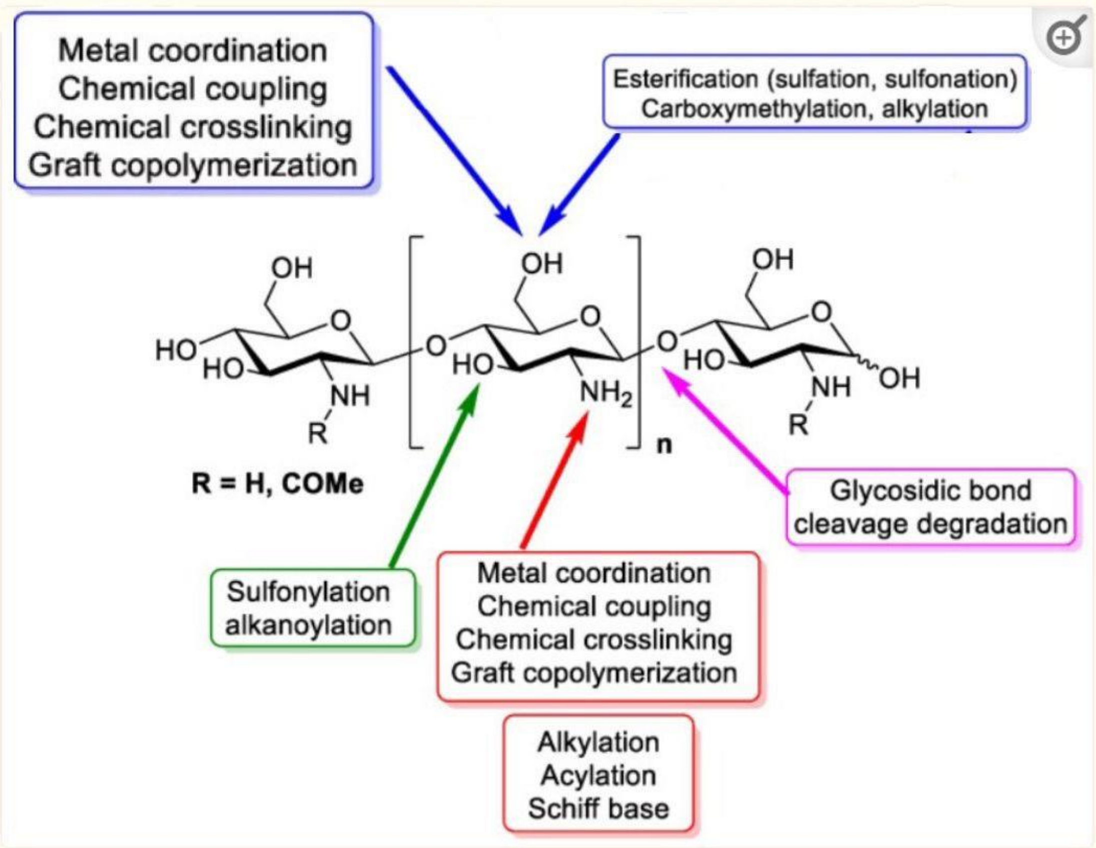


Figure 7: Functional groups in chitosan`s structure that are able to be chemically modified

2.2.2 Adsorption property of chitosan

Appealing qualities make Chitosan a useful adsorbent to eliminate the pollutants from wastewater because of its biodegradability, biocompatibility, hydrophobicity, non-toxicity as well as highly reactive amino (-NH2) and hydroxyl (-OH) categories in the backbone of Chitosan.

It provides a number of advantages over other polysaccharides, such as cellulose and starch, including the ability to be modified specifically to create polymers for particular uses. One benefit of using these materials is that their reactive groups can be combined with other materials to make composites that have been demonstrated to have a higher capacity to tolerate acidic environments and absorb contaminants of wastewater.

Besides, the cationic charge of the anionic suspended colloidal particles (chitosan is a biopolymer with a single cation) allows them to be neutralized and flocculate in a successful manner, which also reduces the wastewaters turbidity, demand of chemical oxygen and chlorides [37,38].

Figure 7 displays the chitosan and chitin infrared (IR) absorption spectra. It can be noticed that there are 3 three types of absorption bands: (a) the amide II bands of chitin at around 1560 cm^{-1}, (b) the amide I bands of chitosan typified by absorption at roughly 1655-1630 cm^{-1}, and (c) the absorption bands for –OH groups at 3450 cm^{-1} [39].

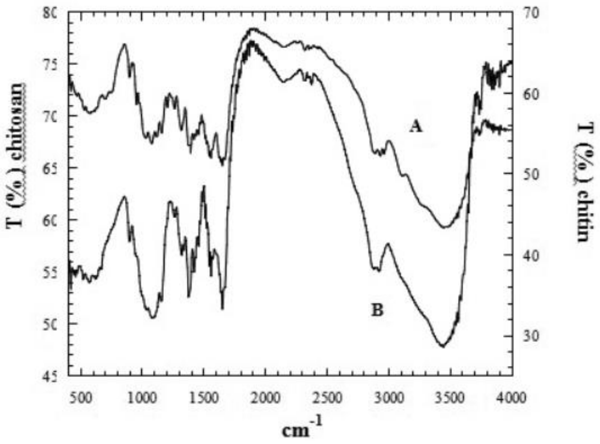


Figure 8: Infrared spectra of Chitin (A) and Chitosan (B)

2.2.3 Chitosan composites in wastewater treatment

Chitosan is a very versatile adsorbent that shows great promise for producing composites. (i.e. forming composites through cross-linking, grafting, functionalization, etc.). Because chitosan is extremely sensitive to pH and either forms a gel or dissolves depending on pH levels, some cross-linking reagents, including glutaraldehyde, formaldehyde, glyoxal, isocyanates, ethylene glycon diglycidyl ether and epichlorohydrin have been employed to enhance chitosan's performance as an adsorbent [38]. Chitosan becomes insoluble when exposed to acidic liquids, and thecross-linking process enhances its mechanical properties.

2.3 Sodium alginate

The sodium alginate is a flexible functional biomaterial used in transdermal and transmucosal drug delivery as a bioadhesive, film-forming agent, matrixing agent, and viscosity enhancer. E.C.C. Stanford, a British pharmacist, created the alginates; commercial manufacture began in 1929. Around 30,000 tonnes of alginates are produced worldwide each year; 30% of them are used in the food business, with the remaining amounts going to industrial, medicinal, and dental uses [40,41].

2.3.1 Chemical structure of alginate

Alginates are considered as monomers of a-l guluronic acid (G) residues and their C5 epimer, b-D mannuronic acid (M), linked by (1-4) glycoside linkages. They are linear, unbranched polysaccharides (Fig.1). The residues are often organised along the chain in a block-like arrangement and vary greatly in composition and order. There are interspersed sections of alternate structure throughout these homopolymeric regions of blocks of b-D mannuronic acid and blocks of a-L guluronic acid. The kind, size, and molecular weight of the sequences, as well as their molecular weight specify the alginates' physical properties. The molecular heterogeneity depends upon the tissueand organism from which the alginates are extracted. Alginates produced from aged L. Stipes for example, a-L-guluronic acid residues are most abundant in hyperborea kelp, whereas alginates produced from A. L. and Nodosum The lowest concentration is in Japonica. Alginates have no regular repeating unit; hence Bernoulli statistics cannot explain how the monomers are distributed along the polymer chain (12).

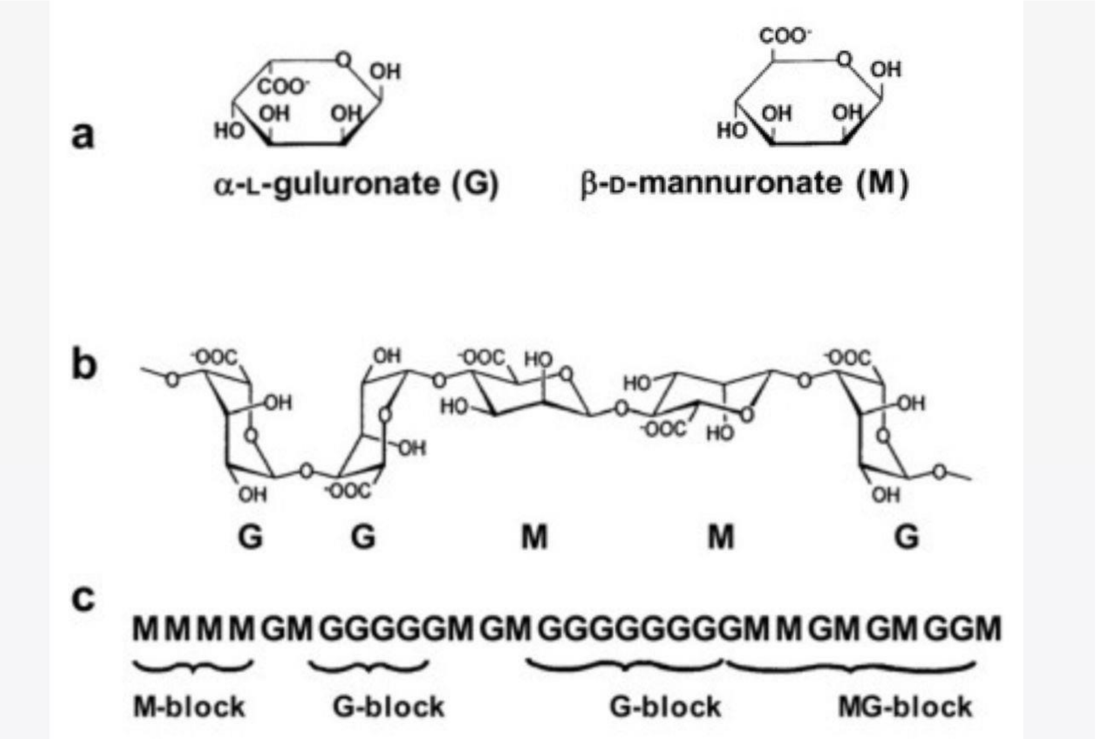


Figure 9: Structural characteristics of alginate (a) alginate monomers, (b) chain conformation, (c) block distribution.

2.4 Advantages and disadvantages of both chitosan and alginate

There are benefits and drawbacks with utilising adsorbents such chitosan, alginate, and related materials for the removal of metal ions. Recent study has proven the potential of biosorbents derived from natural and agricultural resources for the sorption of different contaminants. In this context, commercial applications of chitosan and alginate have expanded, because they derive from natural, renewable sources and are economically inexpensive compared to standard synthetic resins. Both biological and synthetic materials trap metal ions with identical functional groups [43]. Biopolymers, which are adaptable, biodegradable, and less toxic than their synthetic counterparts, have gained popularity as a result of the emphasis on environmentally friendly technology. These biomaterials were chosen due to their adaptability and strong metal ion adsorption capability, both with and without modification (natural or grafted; ionic, chemical, or covalent crosslinking). Chitosan and alginate biosorbents already have a number of qualities, or you can easily add more to their surface.

One advantage of crosslinking is that it provides additional sorption mechanism options. Comparing the two polysaccharides reveals that chitosan has more effective fixing abilities because of its abundance in amine functional groups, which enables the formation of complexes with the majority of harmful metal ions. The most significant reactive group toward metal ions in amino sites is nitrogen, which also contributes to the formation of bonds through chelation processes.

For the treatment of solutions containing toxic metal ions and pollutants, alginate has demonstrated its applicability and potential as a highly effective adsorptive material. Alginate, like chitosan, is a naturally anionic polymer that is commonly extracted from brown seaweed. Because it is biodegradable, biocompatible, affordable, environmentally benign, and harmless to microorganisms and the environment, alginate plays a crucial role in wastewater treatment to remove heavy metal ions [44]. Alginate and chitosan demand particular attention due to their structural characteristics [45,46,47]]. These materials' proteins, polysaccharides, and phenolic compounds have frequently been directly linked to their adsorption properties. These biopolymers have active groups that can fix metal ions, including carboxylic, sulphate, phosphate, amine, and hydroxyl groups. According to research, both the kind of ion and the functional arrangements of the active sites have an impact on the binding mechanisms [45]. The presence of chemically reactive functionalities (hydroxyl, carboxyl, acetamide, oramine) in the chains of the biopolymers justifies their application in the field of biosorption and the treatment of industrial wastewater. The advantages of utilizing alginate and chitosan as bio-adsorbents include the potential of chemical changes, convenience of usage at laboratory scale, diverse formatting, biodegradability, biocompatibility, and use at many pH intervals. Chitosan and alginate are adaptable and can be adapted to many shapes and sizes such as powder, threads, membranes, films, nanoparticles, beads, fibres, hollow fibres, grains, resins, flakes, and sponges.

Alginate and chitosan both have a variety of special benefits and traits, including their abundance, non-toxicity, biocompatibility, reactivity, biodegradability, and efficiency in the treatment of metallic pollutants. A polymer called chitosan has a variety of functional amino groups [48]. Chitosan is the most popular biosorbent, according to Bailey et al. (1999), because it has free amino groups that increase its ability for adsorption [50]. It also has a greater capacity for adsorption than commercial activated carbon [49]. Compared to other adsorbents, such as commercial activated carbon, polysaccharide materials, etc., which are often employed for pollution management,alginate and chitosan have a number of benefits. Alginate and chitosan adsorbents, however, also have a number of drawbacks.

Table 4: summarizes the advantages and disadvantages of applying bio-adsorbents based on chitosan and alginate adsorbents

Advantages	Disadvantages

• Renewable and versatile resource • Several methods for the manufacture of chitosan and alginate (national waste recovery) • Low cost hydrophilic biopolymer • Low toxicity • Abundant material in many countries, profitable • Cationic (chitosan) and anionic (alginate) polysaccharide are able to change charge at different pH values • Environmentally friendly and publicly acceptable • Remarkable binding capacity for a wide range of pollutants, selective for some pollutants in their natural forms • Reusability and extended life cycle • Fast kinetics • Possibility of modification using different reactions • Ease of obtaining different physical forms • Easy ionic gelation protocol and possibility of obtaining beads by other gelation process • Possibility of the intervention of several sorption mechanisms (mixed and complex mechanisms)	• Chitosan is expensive compared to other biomaterials, reducing profitability • Variability of polymer characteristics, which become more complex after modification • Performance dependent on origin and treatment of polymer, as well as degree of N-acetylation for chitosan, spherification process and junction points for alginate hydrogel • Non-porous adsorbent; modification is necessary to improve the porosity • pH sensitivity and need for chemical bypass to increase adsorption performance • Not effective for cationic dyes and some metals (except after modification) • Limited use in sorption columns in its initial form (hydrodynamic limits and fouling of columns) • Natural chitosan limited by being highly soluble in most dilute mineral and organic acid solutions by protonation of amine groups • For alginate, high solubility in water • Low chemical, mechanical, and thermal resistance • Mixed and complex mechanisms

2.5 Chitosan and alginate hydrogels

Two incredibly promising precursors, chitosan and alginate, are capable of producing gels in straightforward, risk-free processes like at room temperature and without the need of toxic solvents [51,52]. For the most part, hydrophilic macromolecular chains are employed to produce hydrogels, which are produced immediately through a colloidal aqueous solution/sol at low temperature and organised in a polymeric 3D network (or gel) with visco-elastic properties [51]. For the hydrogels to form a stable three-dimensional structure (avoiding partial/complete dissolution of the 3D network or its collapse), crosslinks between the polymeric chains/segments are necessary [51].

Hydrogels can be classed as chemical or physical gels, depending upon the type of crosslinks utilized. Physical gels are networks that, among other secondary factors, are held together by hydrophobic interactions, ionic and H-bonds and/or molecule entanglements. In contrast, covalent linkages between the various polymer chains that make up the network are present in chemically cross-linked gels (linked by addition or condensation processes, chemical responses with aldehydes, or by high irradiation of energy) [53, 51, 54]. Due to the cooperative electrostatic contact between the poly-cationic chains of chitosan and the poly-anionic chains of alginate, gels created by combining alginate and chitosan have undergone extensive research [55].

Additionally, the evaporation of the swelling ingredient makes it simple to transform hydrogels into their dry shapes (xerogels) [56,57]. They are able to enhance their capacity for absorption in this way, which can result in an up to three-order increase in dry weight when they are re-soaked in water. The gels which are based on either chitosan or alginate have already been considerably employed in the form of adsorbents in water remediation due to the existence of amine and carboxylic acids groups, which can serve as coordination/chelation sites to eliminate dyes, heavy metals as well as additional contaminants [58,59].

Chapter 3

EXPERIMENTAL

3.1 Preparation of alginate-chitosan double-gel interpenetrated porous beads (ACBs)

First of all, alginate and chitosan solutions were prepared. 0.25 g sodium alginate as dissolved in 25 ml distilled water and left for stirring for 20 minutes at normal room temperature.

Also, for preparing chitosan solution, 0.25 g of chitosan was dissolved in 25 ml of 2% glacial acetic acid solution and left for stirring for 20 at minutes at room temperature.

Both solutions kept aside for 1 h to obtain a bubble free solution. Then, 5% calcium chloride solution was prepared to use as our precipitation bath. For the purpose of making spherical beads, both chitosan and alginate solutions mixed together at room temperature and dropped into CaCl2 solution. Due to highly gelling property of (CS/Al) solution, drop-wise addition step should be done immediately after mixing solutions. Otherwise, the mixture will form gelatin (hard gel form) which would not drop from our dropper.

the beads were kept inside CaCl2 solution for 24 h due to chemically modification and reaction of calcium units with beads. After 24 h, the spherical beads are taken from solution by filtration and washed with distilled water several times to remove unreacted units like excess calcium. Then, the beads left for 24 h in room temperature to dry. The prepared biosorbent beads is kept in sealed glass tube to protect from contamination and for later use.

Alginate polymer includes L-guluronic acid and D-mannuronic acid. The conformation of D-mannuronic acid is 1C which is connected in β-configuration through the positions of 1- and 4- and L-guluronic acid has the 1C conformation but it is α-1,4-linked configuration. Due to the special shapes of the monomers and their different types of linkages (α and β) in the polymer the geometric of G-block and geometric of M-block regions are significantly different. Considerably, M-blocks have a similar form of an extended ribbon while G-blocks are buckled. If two G-blocks regions arrange in a straight line side by side; it causes forming a diamond form hole.

The resulted hole has special sites on its dimensions which are so unique for binding with Ca ions. When Ca ions diffuse into a sodium alginate solution, look like an arranging in a straight line of the G-blocks occurs and the Ca ions are binding between the two chains of polymer which this configuration is called egg box model.

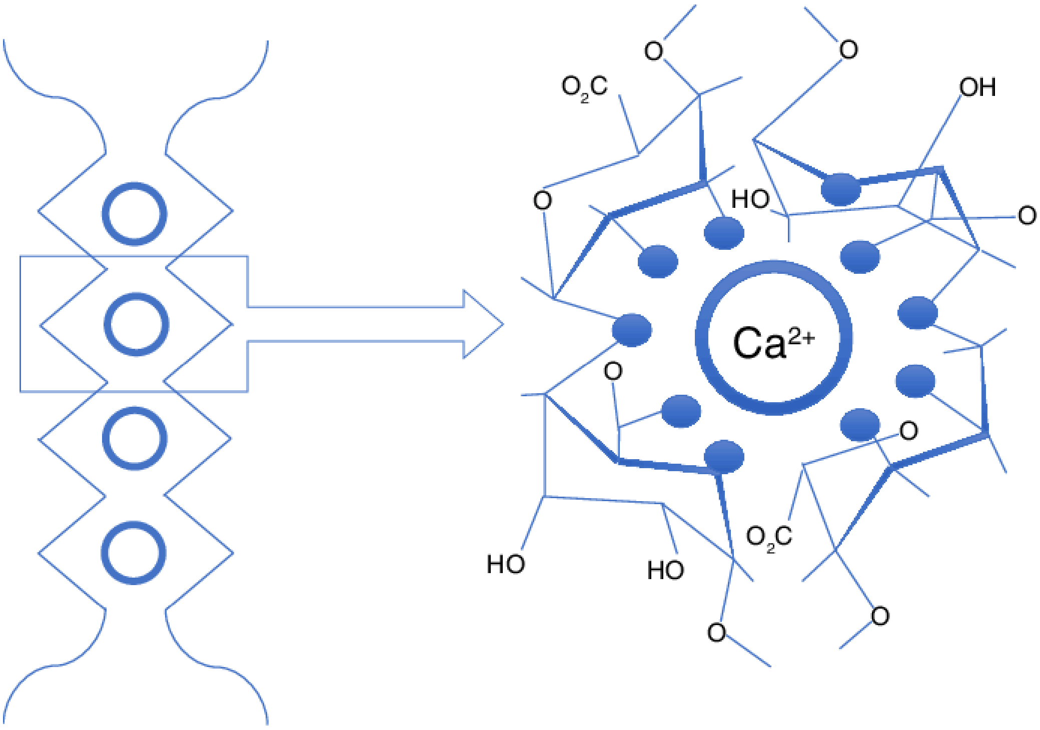


Figure 10: Egg box model configuration

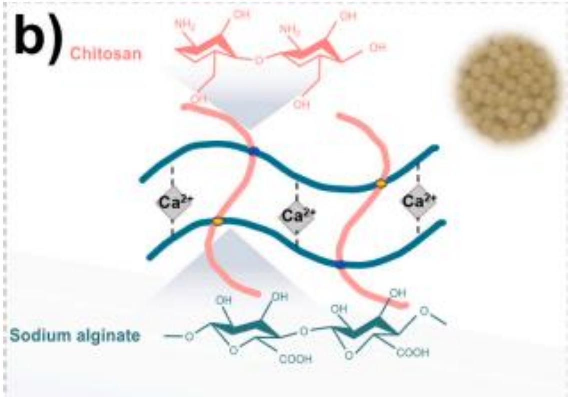


Figure 11: Configuration of chitosan, alginate and calcium molecules in chitosan-alginate beads

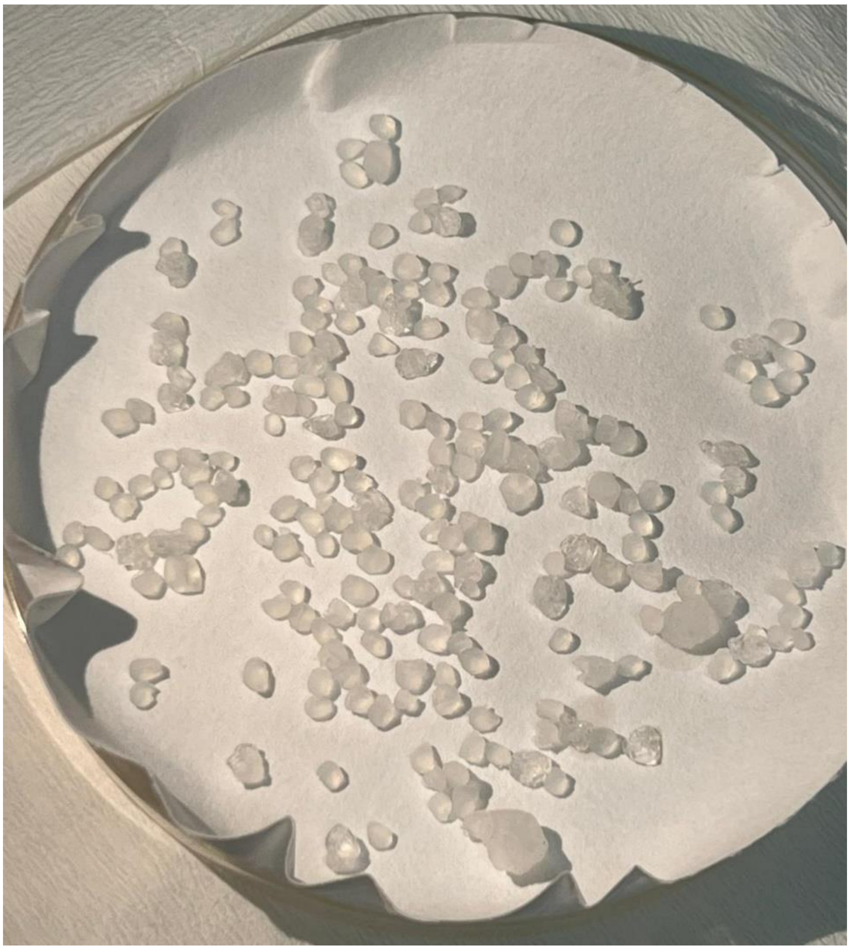


Figure 12: The prepared Chitosan-Alginate beads in the laboratory

3.2 Phosphate determination materials

A UV spectroscopic method was used. The method is easy, sensitive, economical, and applicable for estimation of phosphate concentration in aqueous solutions.

Accordingly, 100 mg of KH2PO4 (Monobasic potassium phosphate) was dissolved in 100 mL of distilled water. 1 mL of stock solution was taken and diluted to 100 mL with distilled water.

3.2.1 Preparation of reagents

Reagent 1: 600mM Ascorbic Acid (1L);

First of all, 60.0g ascorbic acid was dissolved in 600ml of distilled water. Then, 1.5g sodium dodecyl sulfate added to the solution and mixed until dissolved. (it was mixed so slowly to prevent foaming). Afterwards, deionized water added to a total volume of

1L. Finally, the prepared solution transferred to a dark glass bottle for later use.

Reagent 2: 6mM Ammonium Molybdate (1L);

Firstly, 8.52g of ammonium molybdate tetrahydrate and 0.225ml hydrazine monohydrate added to 800ml of distilled water and mixed until dissolved completely. Then, 40ml of sulfuric acid mixed into the solution and left for stirring. After complete dissolving, allowed to be cool at room temperature. Finally, deionized water added to a total volume of 1L and the prepared solution transferred to sealed glass.

Chapter 4

RESULTS

4.1 Phosphate determination

The absorbance values of the solutions containing 1-10 g of phosphate at 10 different concentrations at 820 nm were read and the equation of the curve obtained was bobtained by plotting the absorbance graph against the phosphate concentration. The phosphate adsorbing capacity of the adsorbent was determined by calculating the phosphate content of the samples taken at different concentrations at the end of one-hour incubation. It was clearly demonstrated that the adsorbent has no solubility in the aqueous prepared.

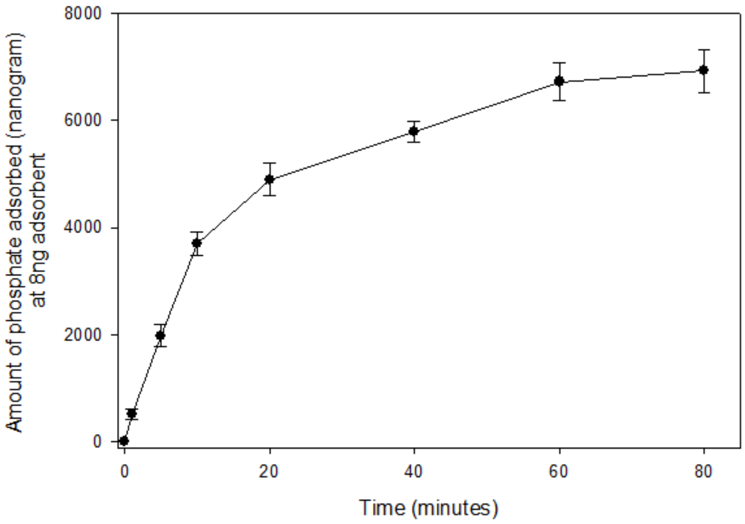


Figure 13: Phosphate adsorption with changing time at 8 ng chitosan-alginate beads

4.2 The PH point zero charge (PHzpc)

For understanding the mechanism of adsorption in different PHs, the PH zero-point charge (PHzpc) of adsorbent was studied using a drift method as follows: initial PH (2-11) was prepared by 0.1 mol/L HCL and 0.1 mol/L NaOH. 0.2g of Chitosan-Alginate hydrogel beads was added individually for each PH and kept at room temperature for 24 hours. At the end, the final PH of the all solutions were measured and plotted vs initial PH. PHzpc determined as 4.0 as illustrated in Fig13.

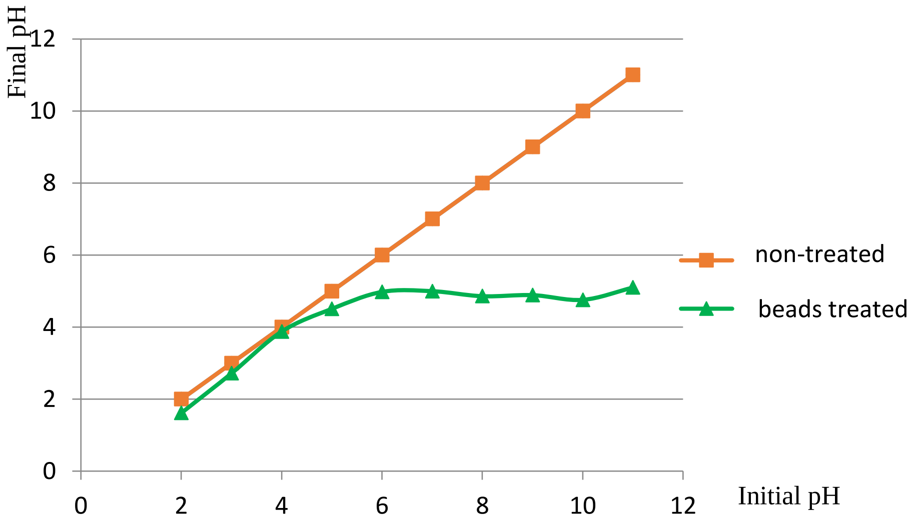


Figure 14: The PH point zero charge (PHzpc)

Table 5: Numerical data obtained in ZPH analysis due to solutions with pH changes from 2 to 11 and solutions which include samples inside of them

pH initial	pH final	pH initial	pH final
2	2	2	1.62
3	3	3	2.72
4	4	4	3.88
5	5	5	4.51
6	6	6	4.98
7	7	7	5
8	8	8	4.86

9	9	9	4.89
10	10	10	4.76
11	11	11	5.1
2	2	2	1.62

4.3 Characterization of beads

4.3.1 Fourier transform infrared spectroscopy (FTIR)

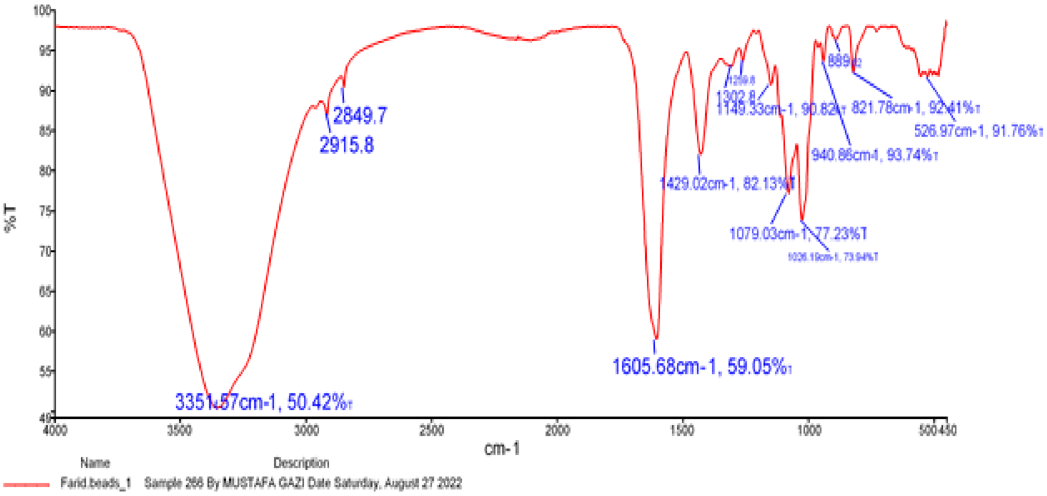


Figure 14: FTIR analysis of chitosan-alginate sample

4.4 Scanning electron microscopy (SEM)

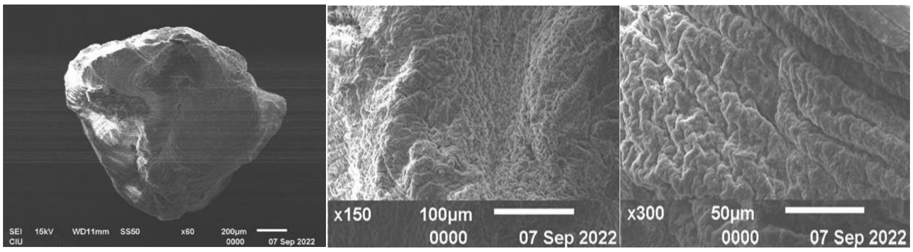


Figure 15: SEM analysis of Chitosan Alginate beads

Chapter 5

DISCUSSION

Among the methods available for treating water, one of the most effective processes for the treatment and eradication of organic contaminants in wastewater treatment is the adsorption process using solid adsorbents. Adsorption offers advantages over other technologies due to its straightforward design and potential for modest initial investment as well as minimal acreage requirements. The adsorption technique receives a lot of attention from researchers and is frequently used to clean industrial waste water of organic and inorganic impurities [9].

Within this study, two different natural polymers were mixed together to study adsorption of phosphate by polymers. The main activities that were under investigation are adsorption of phosphate in different concentrations and with the changes of time. A complex of chitosan and sodium alginate polymers were prepared and chemically interact with calcium and prepared beads form of polymers.

According to removal of phosphate assay results, it was found that by increasing the concentration of chitosan-alginate hydrogel beads in phosphate solution the amount of adsorbed phosphate increased and due to the changes of time, with passing time the amount of adsorbed phosphate increased too. By double raising of adsorbent concentration, it was shown nearly 1.5times increasing in adsorption amount in average. According to the time, an increase nearly to2.92 times was shown in phosphate adsorption with passing time from 5 minutes to 40 minutes.

Chapter 6

CONCLUSION

Chitosan- alginate beads adsorption capacity was studied in different PH values. Due to the PH point zero charge (PHzpc) analysis, it was illustrated that PHs less than 4.0 are suitable conditions for adsorbent to have a better capacity for phosphate adsorption. because in acidic mediums the positive charges of H+ increases and causes producing more NH3+ ions for binding to PO4- ions. Furthermore, as we know chitosan beads lonely are not stable in acidic medium while in our sample, mixing chitosan with sodium alginate causes formation of network polymer due to the presence of carboxylic groups in alginate which act as crosslinker.

So, the produced beads form more stable and the resistance to acidic conditions increases. Generally, these chitosan-alginate beads can have better phosphate adsorption capacity than simple chitosan beads due to this fact that acidic medium and passing time are important factors in phosphate removal process. This feature was studied by FTIR analysis which completely shows us the presence of COO- and NH2 groups.

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