

**PHOSPHORUS BINDING TO
MODIFIED WOOD PULP FIBERS
DESIGNED FOR SOILLESS
AGRICULTURE**

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AGRICULTURE**

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Abstract

The long-term goal of this work was to develop a new organic plant substrate from wood pulp that can be used in soilless (hydroponics) agriculture. This work explored the usage of wood pulp as disposable plant substrate that is chemically active and has the capacity to bind and release important plant nutrients such as phosphorus.

The cationic cellulosic pulp fibers had been prepared from softwood bleached kraft pulp according to a water-based modification method involving first the oxidation of pulp with 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO) followed with the adsorption polyvinylamine (PVAm) on pulp surface. The total carboxyl group contents of the TEMPO oxidized pulp fibers were determined via conductometric titration. After treating the oxidized fibers with PVAm, polyelectrolyte titration was used to determine the nitrogen (cationic charges) content on fibers. The fibers' nitrogen content varied with the fibers' carboxyl charge content; 45.5 $\mu\text{mol/g-pulp}$ of carboxyl group content had 18.6 $\mu\text{mol/g-pulp}$ nitrogen content (least observed nitrogen content adsorbed onto pulp), and 207.1 $\mu\text{mol/g-pulp}$ of carboxyl group content had 69.75 $\mu\text{mol/g-pulp}$ nitrogen content (highest observed nitrogen content adsorbed onto pulp).

The adsorption and the release of negatively charged phosphate ions onto/from the treated and untreated pulp were evaluated. A maximum adsorption of $\sim 10 \mu\text{mol/g-pulp}$ of phosphate ions was achieved on oxidized fibers treated with PVAm. Although a layer of adsorbed PVAm greatly increases the anion exchange capacity of cellulose fibers, the

modified pulp showed a very low phosphate binding capacity of 0.16 mol phosphates per mol of nitrogen. Phosphates adsorbed on the PVAm coated fibers release depended on the pH and ionic strength of the surrounding media. Neutral pH conditions (6.0-7.5) released the least amount of phosphates, which was 30%-40% of the phosphates initially retained on pulp fibers. However, high molarity salt solutions (5 mM) and sulfonic groups (in buffer solutions) released 90%-100% of the adsorbed phosphates at all pH ranges.

Cationic pulp fibers matrices with varying phosphate treatment were used to grow Arabidopsis (phosphate sensitive seeds). Arabidopsis grown on cationic pulp fibers displayed the highest total fresh weight, highest leaves weight, and healthiest look. The total fresh weight of the Arabidopsis increased upon growing on cationic pulp fibers by 57% - 67% at the low phosphate treatments. The free phosphates destination in the plant growing media is unpredictable. Free phosphates could end up adsorbing to mineral surfaces or forming secondary compounds with plant nutrients, which make them unavailable for the plants. Even though the PVAm coated fibers had very low phosphate binding capacity, the fibers' cationic surface can bind enough phosphates, at low phosphate treatment, needed for Arabidopsis cultivation. By binding the phosphates on the fibers' surface, less phosphates are available in the plant growing media to react or precipitate with other plant nutrients. However, at intermediate or high phosphate treatment PVAm coated fibers didn't have a great impact on the Arabidopsis plant growth; this is because phosphates available in the plant growing media were relatively greater than the bound phosphates on the PVAm coated fibers.

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Nomenclature

AEC	Anion exchange capacity
CEC	Cation exchange capacity
DAP	Diammonium phosphate
DoI	Degree of ionization
DW	Dry weight
FD	Fractional decomposition
MAP	Monoammonium phosphate
NFT	Nutrient film technique
NMR	Nuclear magnetic resonance
PAA	Polyacrylic acid
PAH	Polyallylamine
PEI	Polyethylenimine
PH45	Lupamin® 4500 Polyvinylamine
PNVF	Poly(N-vinylformamide)
PVAm	Polyvinylamine
P15	Lupamin® 1595 Polyvinylamine
P45	Lupamin® 4595 Polyvinylamine
SSBK	Southern softwood bleached kraft pulp
SSS	Single superphosphate
TEMPO	2,2,6,6-tetramethyl-1-piperidinyoxy radical

TOP	TEMPO oxidized pulp
TOP-P15	TEMPO oxidized pulp treated with Lupamin® 1595 Polyvinylamine
TOP-P45	TEMPO oxidized pulp treated with Lupamin® 4595 Polyvinylamine
TOP-PH45	TEMPO oxidized pulp treated with Lupamin® 4500 Polyvinylamine
TSP	Triple superphosphate
UnP	Unmodified pulp
UnP-P15	Unmodified pulp treated with Lupamin® 1595 Polyvinylamine
UnP-P45	Unmodified pulp treated with Lupamin® 4595 Polyvinylamine
UnP-PH45	Unmodified pulp treated with Lupamin® 4500 Polyvinylamine

Chapter 1 Introduction

Phosphorus has a great impact on the health and productivity of plants. It is an essential component of the organic compound adenosine triphosphate, which drives most of biochemical processes and of ribonucleic acid, which directs protein synthesis in plants. In healthy plants, leaf tissue phosphorus content is usually about 0.2-0.4% of the dry matter. The total quantity of phosphorus is low in most native soils ranging from 500 to 10,000 kg in the upper 50 cm of 1 hectare of soil, and most of it exists in unavailable forms to the plants ¹. Adding far more phosphorus to the soil has been used as a solution to overcompensate this problem. However, the excessive phosphorus has led to phosphorus building up in the agricultural watersheds leading to a serious water pollution and jeopardizing the drinking water supplies ².

Phosphorus used in fertilizers comes from phosphate rocks, which is a finite source formed over millions of years in the earth crust ¹. There have been arguments about whether phosphorus is used up faster than it is economically extracted. The accessibility to available phosphorus rock reserves is declining, and the mining costs are rising. Using excessive phosphorus to overcome phosphorus unavailability to the plants causes eutrophication and water pollution. Phosphorus fertilizers usage increased from 9 Teragram (Tg) to 40 Tg between 1960 and 2000 and is expected to increase to 55-60 Tg by 2030. By some estimates, the inexpensive rock phosphate reserves could be depleted in 60 to 80 years ³.

Soil gives insects a free invitation to munch the plants and is hard to control for weed growth. There have been cleaner substituents for soil in greenhouses to control insect

infestation and retain just enough water and air. Soilless cultivation is also called hydroponics. Hydroponic media such as perlite, rockwool, gravel, or sand are used with plant nutrients to cultivate plants in greenhouses. These cultivation media are inert, chemically inactive, and not biodegradable. The long-term goal of this work was to develop a new organic plant substrate from wood pulp that could be used in the hydroponic applications to grow plants. This work tested the usage of wood pulp as disposable plant substrate that is chemically active and has the capacity to store and release important plant nutrients such as phosphorus.

Chapter 2 Literature Review

2.1 Soil Properties

2.1.1 Interface of Air, Minerals, and Water in Soil

The four main components of soil are: air, water, mineral matter, and organic matter. These components are mixed in complex patterns in soil. Figure 1 shows the components' approximate proportions (by volume) on soil surface. Ideally half of the soil should consist of mineral (derived from the Earth crust's rocks) and organic material, and the other half is air and water in soil pores. The soils with more than 50% of their volume in solids are likely to be too compact for a healthy plant growth ¹.

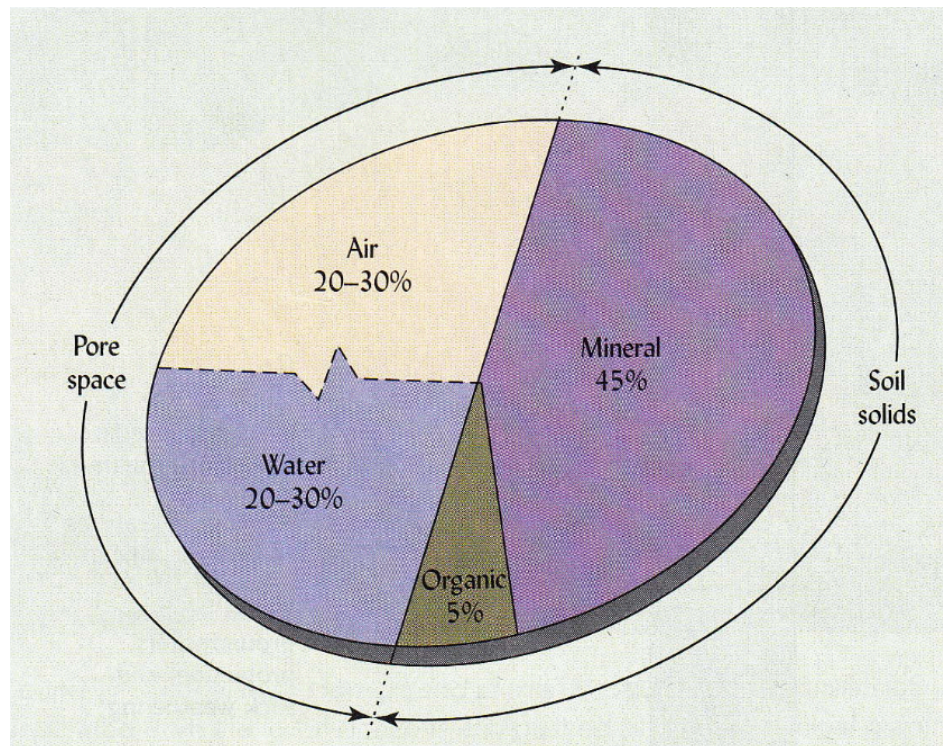


Figure 1. Volume composition of soil texture when conditions are good for plant growth. The broken line between water and air indicates the fluctuation of these two components as soil becomes wetter or drier, adapted from Weil *et al.* ¹

2.1.2 Mineral (Inorganic) Constituents of Soil

The soil's solid framework consists of mineral particles. The large soil particles (stones, gravel, and coarse sands) consist of several different minerals. Sand, silt, and clay particles are the building blocks for the soil construction. The small soil particles tend to be made of a single mineral. Excluding large rock fragments, soil particles range in size from 2 mm (sand) to less than 0.002 mm (clay) in diameter. The clay particles are the smallest mineral particles. The smaller particles (<0.001 mm) of clay have colloidal properties and have a tremendous amount of surface area per unit of mass. The soil colloids exhibit charges that attract positive and negative ions. This fraction of soil is the most chemically and physically active. The proportions of sand, silt, and clay particles in the soil determine the suitability of soil for most uses¹. The different properties of these particles are summarized in Table 1.

Table 1. Some general properties of the three major size classes of inorganic soil particles, adapted from Weil *et al.*¹.

Property	Sand	Silt	Clay
Range of particle diameters in millimeters	2.0-0.05	0.05-0.002	Smaller than 0.002
Means of observation	Naked eye	Microscope	Electron microscope
Attraction of particles for each other	Low	Medium	High
Attraction of particles for water	Low	Medium	High
Cation Exchange Capacity (CEC), number of exchangeable cations on soil particles' surfaces	Very low	Low	High
Consistency when wet	Loose, gritty	smooth	Sticky, malleable
Consistency when dry	Very loose, gritty	Powdery, some clods	Hard clods

2.1.3 Soil Air

Root respiration, which is like the human respiration, produces carbon dioxide (CO₂) and uses oxygen (O₂). Soil ventilation is an important function of soil to maintain the quality and quantity of air by allowing CO₂ to escape and fresh O₂ to enter the root zone. This ventilation is accomplished via the networks of the soil pores. The water content of soil determines the amount and composition of air in soil. The air content is inversely related to the soil's water content. As water enters the soil, water displaces air from some of the soil pores. As the soil drains the irrigation water or the heavy rain, the soil large pores are

first to be drained, followed by the medium-sized soil pores, and finally the small soil pores. This is why soils with tiny pores are poorly aerated ¹.

2.1.4 Soil Water

The soil pores also absorb water and hold it where it can be used by plant roots. The attraction between the surfaces of water and soil particles restricts the ability of water to flow. The soil water is never a pure water and always has a lot of dissolved organic and inorganic nutrients; thus, it's more accurately called **soil solution**. As long as the plants are exposed to sunlight, the plants require a continuous stream of water to use in cooling, nutrient transport, and photosynthesis. At an optimal soil moisture content (Figure 1), the water in large and intermediate-sized pores can move in the soil and can be taken up by the plants. The plant roots remove water from the largest soil pores first followed by the intermediate soil pores. The water in the small soil pores is so close to solid particles that the plant roots cannot pull it away. As a result, not all of the soil water is available to plants.

A fertile soil will provide a continuous supply of dissolved mineral nutrients (in soil solution) in appropriate proportions for an optimal plant growth. Some of the soil solution metallic elements are: potassium, calcium, iron, and copper. The nonmetallic elements include: nitrogen, sulfur, phosphorus, and boron. The roots take up these elements from the soil solution and incorporate them into organic compounds to reconstitute their tissues. The essential elements that are used by the plants in large quantities ($> 0.1\%$ of dry plant tissue) are called macronutrients (calcium, magnesium, nitrogen, potassium, phosphorus, and sulfur). Elements that are used by the plants in small quantities ($< 0.1\%$ of dry plant tissue)

are called micronutrients (copper, cobalt, iron, manganese, nickel, sodium, zinc, boron, chlorine, molybdenum). These elements have shown to be essential for the plants' growth, meaning the plants cannot grow without them ¹.

Soil solution tends to have a buffering capacity which is the capability to resist changes in its composition when compounds are added or removed from the soil. The buffering capacity depends on the chemical reactions in the soil an example would be the attraction and release of substances by the colloidal particles. Figure 2 shows the buffering capacity of soils against changes in pH, in which the well buffered soil (C) is expected to have a higher amount of charged clay material than the moderately buffered soil (B).

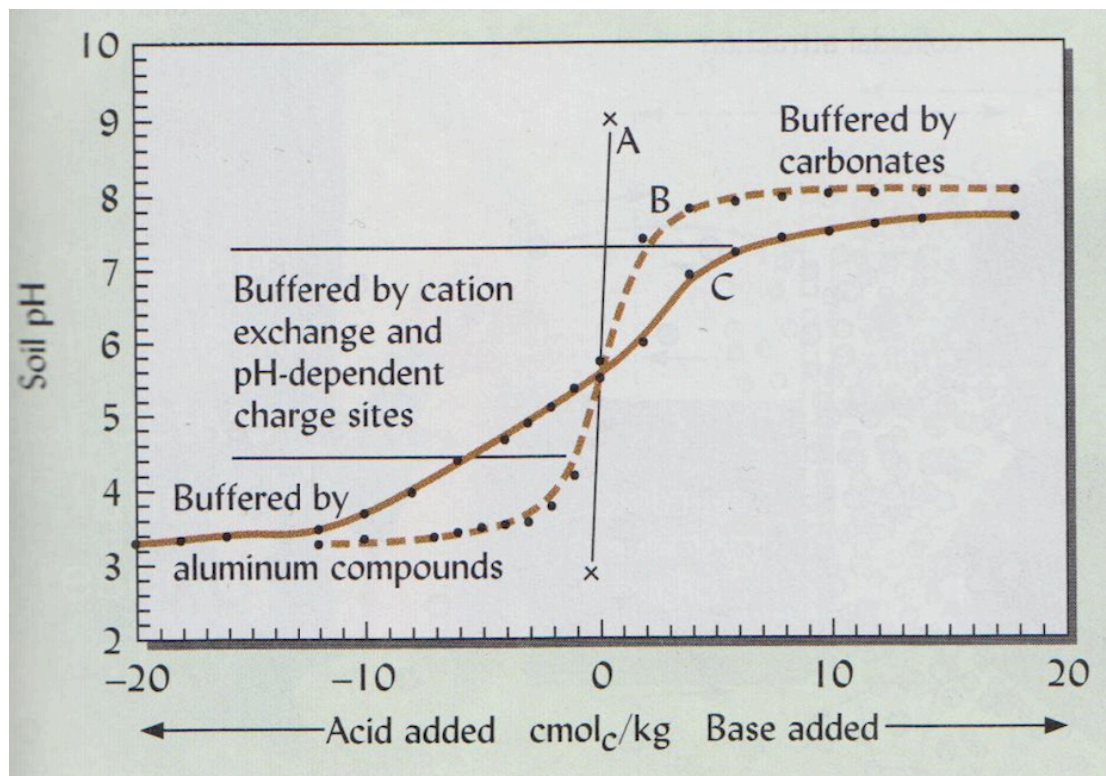


Figure 2. Buffering capacity of soils against changes in pH when sulfuric acid (H_2SO_4) or calcium carbonate base (CaCO_3) is added. A well buffered soil (C) and a moderately buffered soil (B) are compared to unbuffered water (A) adapted from Weil *et al.* ¹

2.2 Review of Phosphorus Chemistry and Soil Culture

2.2.1 Inorganic Phosphate Sources

Plant roots have cross-membrane transporters specific for phosphate ions (HPO_4^{2-} and H_2PO_4^-). The roots absorb the available phosphate ions in the soil or the growing media solution. Inorganic phosphate includes both of orthophosphate and polyphosphate forms. Orthophosphate forms include any salt of phosphoric acid (PO_4^{3-} , HPO_4^{2-} , and H_2PO_4^-); it is the most stable kind of phosphate and the only one taken up by plants. The term orthophosphate is referred to the available phosphorus for the plants. Some of the very common phosphorus fertilizers are: diammonium phosphate (DAP, $(\text{NH}_4)_2\text{HPO}_4$) developing a temporary alkaline pH around the dissolving granule, monoammonium phosphate (MAP, $\text{NH}_4\text{H}_2\text{PO}_4$) developing a temporary acidic pH around the dissolving granule, single superphosphate (SSP, containing both $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and 2CaSO_4), and triple superphosphate (TSP, $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$). DAP and MAP are excellent sources of phosphorus (P) and nitrogen (N). Both of TSP and SSP develop temporary acidic pH around the dissolving granule and are used as an agronomic advantage to supply calcium (Ca) and sulfur (S) where they are deficient ¹.

2.2.2 Phosphorus Forms in Soil

Phosphorus in soils can be thought of existing in three pools: 1- solution phosphorus pool, 2- active phosphorus pool, and 3- fixed phosphorus pool. Solution phosphorus is the pool where plants take up phosphorus. The pool has phosphorus in orthophosphate forms with

small amounts of organic phosphorus⁴. The concentration of phosphorus in soil solution is typically very low; it ranges from 0.001 mg/L in very infertile soil to 1 mg/L in very fertile soils. Some examples of soil solution phosphorus concentration required for 95% maximum growth of some plants are: peanut: 0.01 mg/L, corn: 0.05 mg/L, cabbage: 0.04 mg/L, tomato: 0.2 mg/L, and head lettuce: 0.3 mg/L¹.

Active phosphorus pool, on the other hand, has phosphate ions adsorbed on the surface of soil particles such as calcium (Ca), iron (Fe), aluminum (Al), and magnesium (Mg) forming soluble solids that can release phosphorus to the plants. Gradually the phosphorus soluble solids in the active phosphorus pool form insoluble compounds that could remain in the soil for years without being available to the plant; this form of phosphorus is called the fixed phosphorus⁴.

2.2.3 pH Effect on Phosphorus Fertilizers

When an inorganic phosphate fertilizer comes in contact with the soil, various reactions take place making phosphorus less available to the plants. As the fertilizer granule dissolves in the solution phosphorus pool, phosphate ions slowly move toward the minerals to adsorb to them. In an alkaline soil (pH > 7.3), calcium ions (Ca²⁺) are dominant and will react with phosphate ions forming a sequence of reaction that involves the formation of dibasic calcium phosphate dihydrate, octocalcium phosphate, and hydroxyapatite. With each product formation, the availability of orthophosphates decreases. In an acidic soil (pH

<5.5), aluminum (Al) and iron (Fe) are the dominant ions. Here, the first products formed are amorphous Al and Fe phosphates which gradually change into crystalline variscite (Al phosphate) and strengite (Fe phosphate). Variscite and strengite are very insoluble compounds of phosphate and are not available to plants. Nevertheless, the reactions that decrease P availability to plants can occur in all ranges of soil pH but are mostly very pronounced in soil with $\text{pH} > 7.3$ and $\text{pH} < 5.5$. Maintaining the soil pH at range of 5.5-7.0 (Figure 3) will provide more efficient use of phosphate ions ⁴.

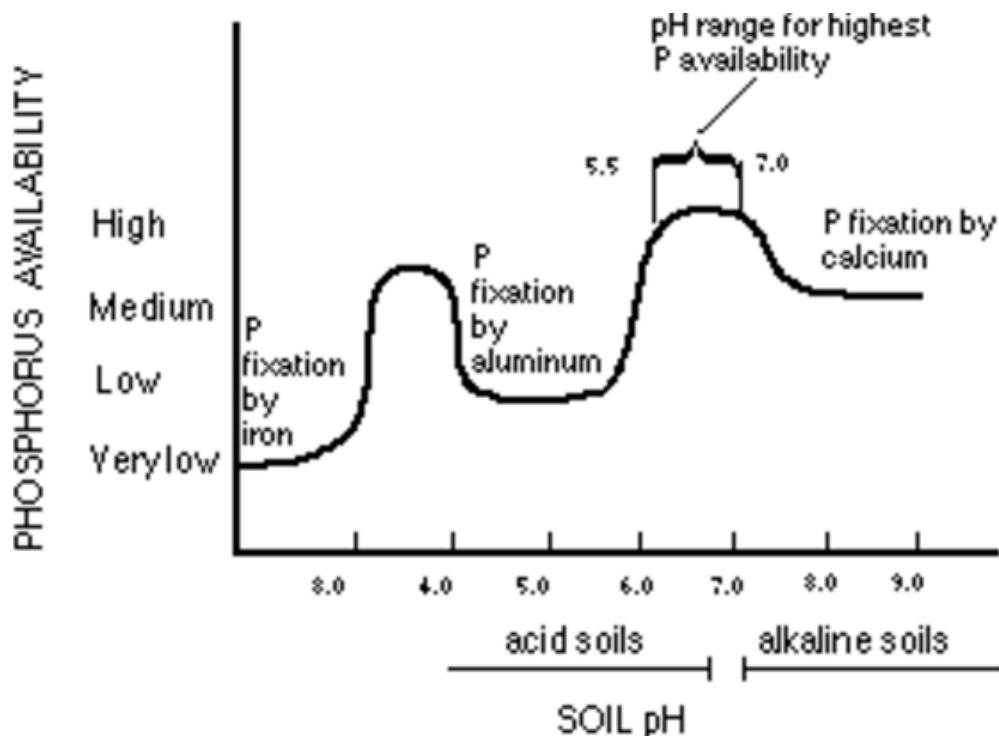


Figure 3. Soil pH effect on the availability of phosphorus, adapted from Busman *et al.* ⁴.

Figure 4 presents the pH effect on the relative concentration of the phosphate ions species in the soil. At lower pH values, more H^+ ions are available in the solution; hence, the phosphate ions with more hydrogen predominate.

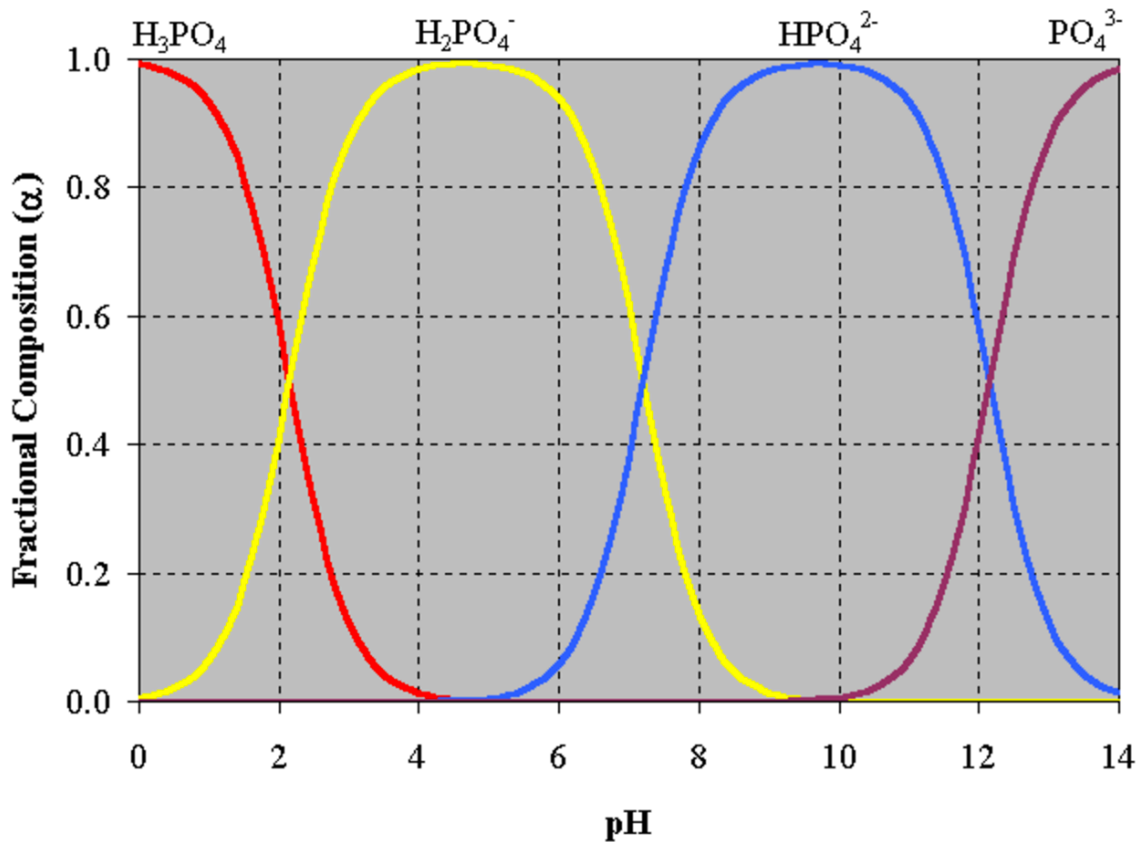


Figure 4. Effect of pH on phosphate ion species composition, adapted from Bialkowski ⁵.

2.2.4 Soil Mechanisms in Storing and Releasing Nutrients

The soil colloids and the clay minerals have negatively charged sites on their surfaces. The negatively charged sites can adsorb and hold the positively charged ions (cations) by electrostatic attraction force. Thus, the soil has the capacity to adsorb and store the following nutrients: sodium (Na^+), magnesium (Mg^{2+}), potassium (K^+), calcium (Ca^{2+}), etc.

The adsorbed cations are gradually released in the soil solution in exchange for hydrogen ions obtained from the plants. The total amount of soil retained cations is called cation exchange capacity (CEC) and is expressed per unit mass of soil. In contrast to CEC, the anion exchange capacity (AEC) of the soil is very little. Anions such as orthophosphates, sulphates (SO_4^{2-}), and nitrates (NO_3^-) either adsorb on the solid positively charged sites, which are very scarce, or react with surface oxides forming insoluble complexes. AEC is very important in making anions available for plants and in retarding the leaching of anions from the soil ¹.

2.3 Soilless Culture (Hydroponics)

Soilless culture eliminates a lot of soil culture serious problems like soil born pests, soil and water salinity, chemical residues in soil, soil infertility etc. Soilless culture has an excellent control over the supply of water, nutrients, and pH, increasing the plant productivity. The origins of soilless culture go back to 1666 when Boyle attempted to grow plants in vials containing only water. He then reported that spearmint survived for nine months. However, it wasn't until the 19th century that the systematic study plant nutrients started to take place ⁶.

Soilless culture is called hydroponics. There have been a lot of inorganic media (e.g. rockwool, sand, vermiculite, gravel, peralite, foam mats, hydrogel etc.) used with an irrigation system to grow plants. There have been also liquid cultures used without inorganic media substrates. The irrigation system supplies the media with nutrients

solution, and the excess solution is either run to waste or recirculated⁶.

Figure 5-7 present the different irrigation systems used for hydroponics. Figure 5 is an example of an irrigation system used with porous substrate such as vermiculite, in which the nutrients flow through the grow tray only to flood the plant roots. The roots get access to oxygen after the nutrients drain back to the tank. Figure 6-7 irrigation systems use a nutrient pump to recirculate the nutrient solution back into the substrate media or the grow channel. The nutrient film technique (NFT), Figure 7, is an irrigation method used to eliminate the difficulties of oxygenation by exposing the roots directly to the nutrient solution; it is a liquid culture i.e. no substrate is used. However, the drip system (Figure 6) uses an inert growing medium, such as rockwool, to retain nutrients around the plant roots⁷.

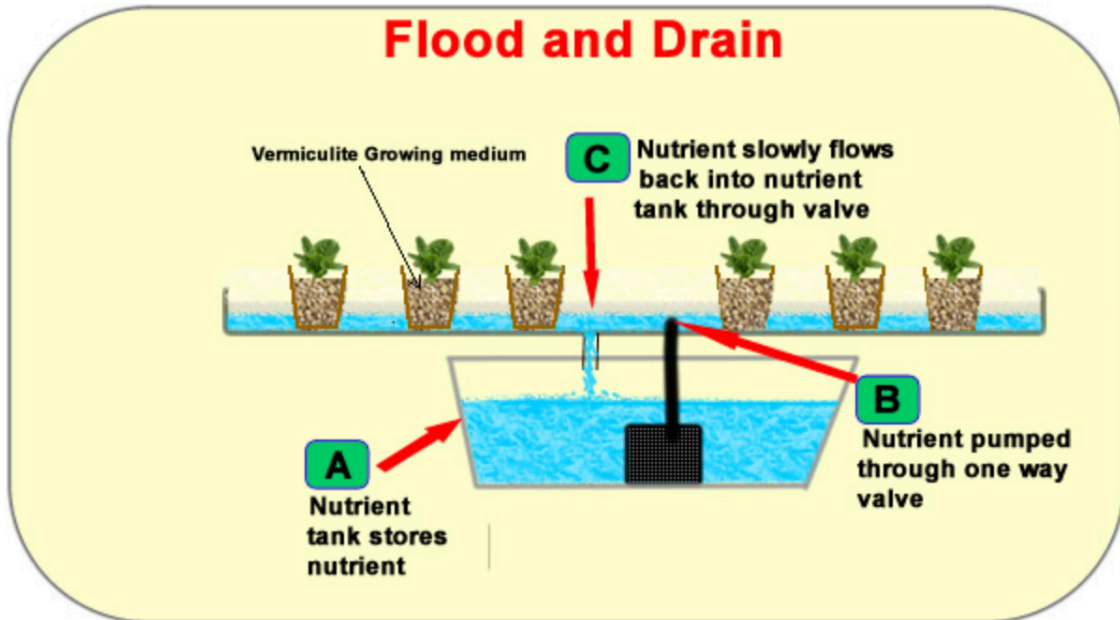


Figure 5. Flood and Drain irrigation method in hydroponic systems, adapted from Hydro-Industries Ltd ⁷.

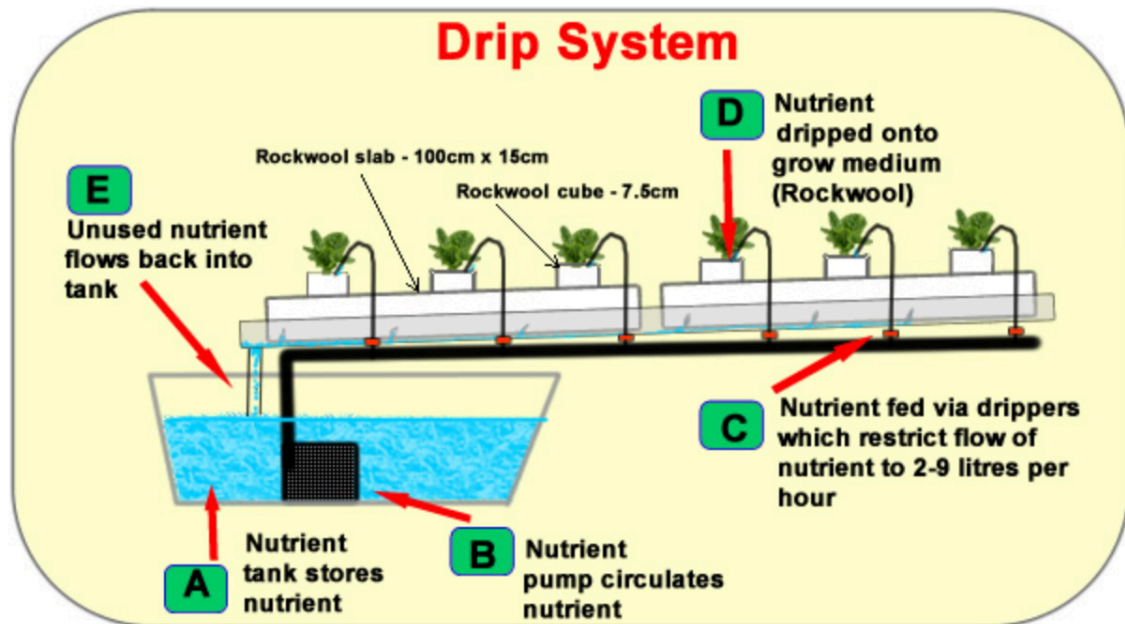


Figure 6. Drip system irrigation method in hydroponic systems, adapted from Hydro-Industries Ltd [7].

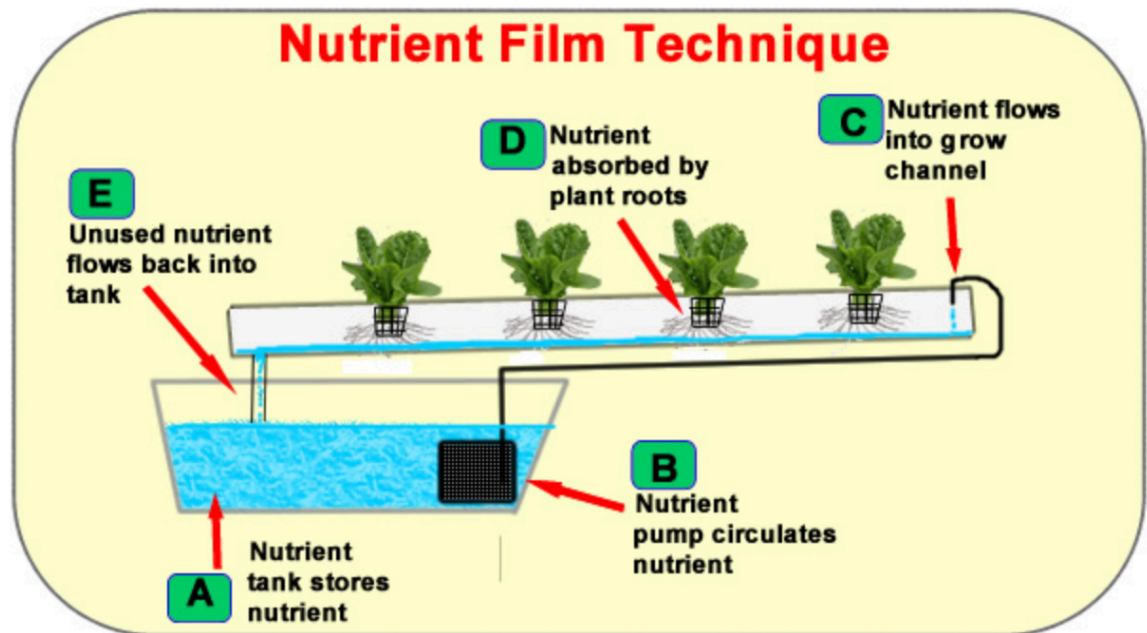


Figure 7. Nutrient Film Technique irrigation method in hydroponic systems, adapted from Hydro-Industries Ltd [7].

The technical specifications for the plant growing medium substrates always include the following properties: - inert (no reaction with the nutrients), pH neutral, porous, low density, hydrophilic, and Free from radioactive pollutants. Rockwool is one of the substrates that meet almost all of these demands, and it has been used widely in many countries like Canada, Holland, France, U.K., Denmark, etc.; however, the inability to recycle rockwool is still an unsolved problem, and its high cost is a limiting factor in some countries ⁶.

This work's incentive was to substitute inert material (like rockwool) with an organic and cost-effective material like wood pulp. This work tested the usage of wood pulp as a chemically active plant substrate that has the capacity to store and release important plant

nutrients such as phosphorus, hence increasing the plant nutrition.

2.4 Review of Wood Pulp Chemistry and Applications

2.4.1 Wood Pulp Production

Cellulose pulp is the main raw material used in the production of paper and paperboard. Pulp is also used in manufacturing absorbent material in diapers and sanitary products. The world's wood pulp production has increased from 120 million tons in 1979 to 180 million tons in 2013. The main producing regions in the world for pulp are North America (38%), Europe (28%), Asia (17%), and South America (13%)⁸.

Wood pulp grades are categorized according to the pulping process: mechanical, semi-chemical, and chemical pulps. The wood chips or logs are ground in the mechanical process. The chemical cooking process involves two main methods: sulphite pulping or sulphate (kraft) pulping. The sulphite pulping process uses aqueous sulphur dioxide and a base of calcium or sodium. Kraft pulping uses a mixture of sodium hydroxide and sodium sulphide at a high pressure and temperature. The wood composition degrades through the cooking process, and brown stock pulp is obtained. Bleaching removes the excess lignin and chromophores to produce white pulp for tissue and printing papers. The usage of bleached kraft cooking process has increased from 43% of chemical pulping processes used worldwide in 1979 to 70% in 2013. Kraft pulping is superior in pulp production because it has low environmental risks in modern mills, more pulp fibre strength, and simple energy recovery. Last category of pulping is the semi-chemical pulping. It combines chemical and

mechanical methods, in which wood chips are softened with chemicals and then mechanically pulped⁸. In this work, unbeaten Southern Softwood Bleached Kraft pulp (SSBK) is used in all of the experiments.

2.4.2 Softwood and Hardwood Pulp

A second classification of wood pulp considers the type of wood: softwood or hardwood. Softwood has long fibers and is produced mainly from pine and spruce. Hardwood has shorter fibers comparatively and produced from birch, poplar, etc. The long fibers of the softwood contribute to the strength of the final product. Softwood has slightly more carboxyl group content than hardwood which contributes to the adsorption of cationic polymers⁹; hence, softwood was used in this work. The adsorption of cationic polymers will be discussed in detail in the following sections.

2.4.3 Chemical Structure of Pulp Fibers

Figure 8 presents the complex chemical structure of the wood biomass. Wood consists of regular arrangements of cells with walls composed of lignin, hemicelluloses, and cellulose. Lignin is what glues the fibers to each other in wood. The kraft pulping dissolves the lignin, and the final bleaching process removes the excess lignin and extractives, separating them from the cellulose of pulp⁹.

Cellulose's basic monomeric unit is D-glucose (anhydroglucose unit, AGU), which links successively through a β -configuration between carbon 1 and carbon 4 of adjacent units. Cellulose is the most abundant and biodegradable polymer. It is a unique and simple

polysaccharide with high crystallinity, rigidity, and insolubility in common organic solvents. These qualities promote cellulose as an ideal structural engineering material for several studies ¹⁰.

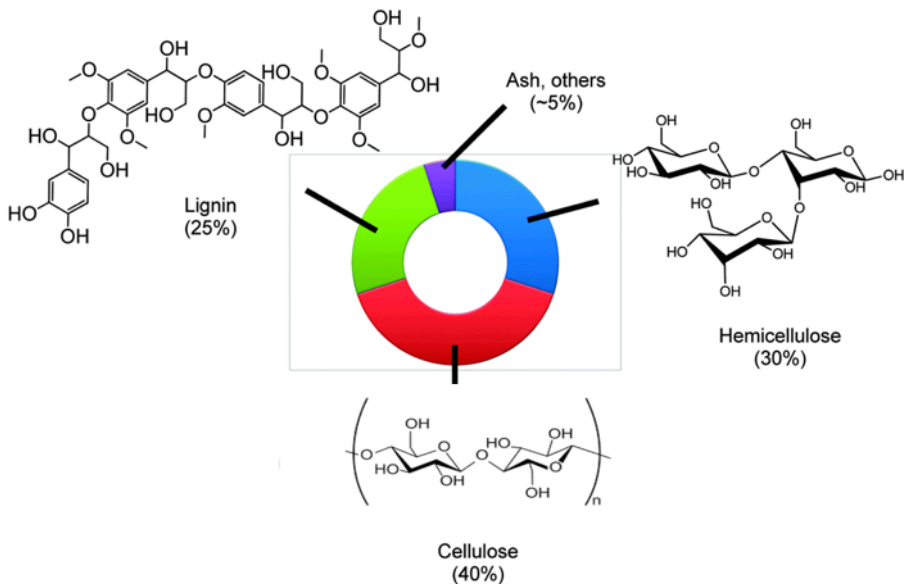


Figure 8. Wood biomasses chemical structure that consists mainly of cellulose, hemicellulose, and lignin.

2.4.4 Chemical Modification of Cellulosic Pulp Fibers by TEMPO Oxidation

The carboxyl groups in chemical pulps play significant roles in papermaking. The carboxyl groups affect the pulp's swelling ability in water, the mechanical properties of dried pulp, degree of formation of inter-fiber bonding during pressing process, and the adsorption capacity of cationic polymers. Some of the carboxyl group content originates from hemicellulose present in native wood, and some are introduced into bleached kraft pulp by oxidative reactions ¹¹.

TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy radical) is a water-soluble and commercially available radical that converts cellulose hydroxyl groups to carboxyl groups under mild conditions. In this system, sodium bromide (NaBr) and TEMPO are used as catalysts to oxidize the primary hydroxyl groups of bleached kraft pulp in water at pH 10-11 and room temperature. The oxidation reaction is started by adding the oxidant, sodium hypochlorite (NaClO). Figure 9 shows the scheme of the oxidation reaction of primary alcohol groups of cellulose. This oxidation also oxidizes part of the aldehyde groups of the reducing ends of cellulose, and it oxidizes the hemicellulose to carboxyl groups. NMR analyses showed that C6 primary hydroxyl groups of cellulose were selectively oxidized to carboxyl groups, giving β -1,4-linked polyglucuronic acid sodium salt ¹¹.

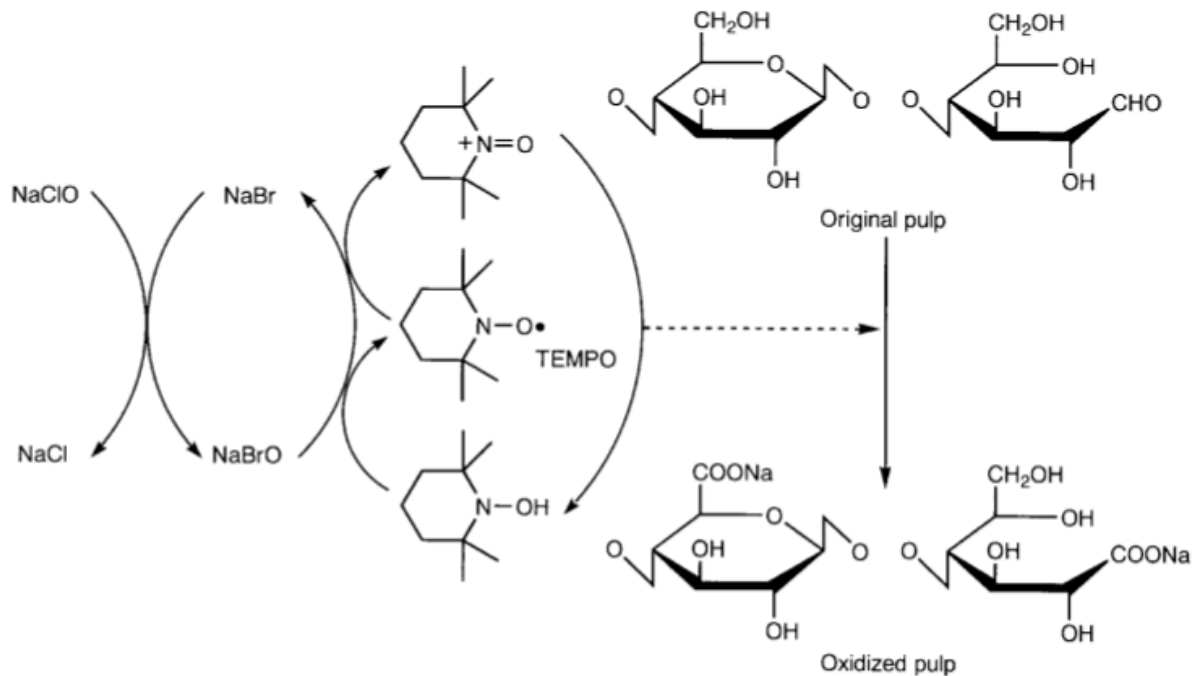


Figure 9. TEMPO-mediated oxidation of hardwood bleached kraft pulp, adapted from Kitaoka *et al.* ¹¹.

2.4.5 Effect of NaClO addition on Carboxyl Group Content of Pulp

Increasing the amounts of NaClO oxidant in TEMPO oxidation reaction increases the carboxyl content of the oxidized pulps. The carboxyl group of the TEMPO oxidized hardwood bleached kraft pulp plateaued at level of about 0.47 mmol/g, as reflected in Figure 10, which is eight times higher than the carboxyl group content of the original pulp. The low accessibility of native cellulose samples to the TEMPO-mediated oxidation may have introduced small amounts of carboxyl group and brought about this limitation of degree of the oxidation ¹¹.

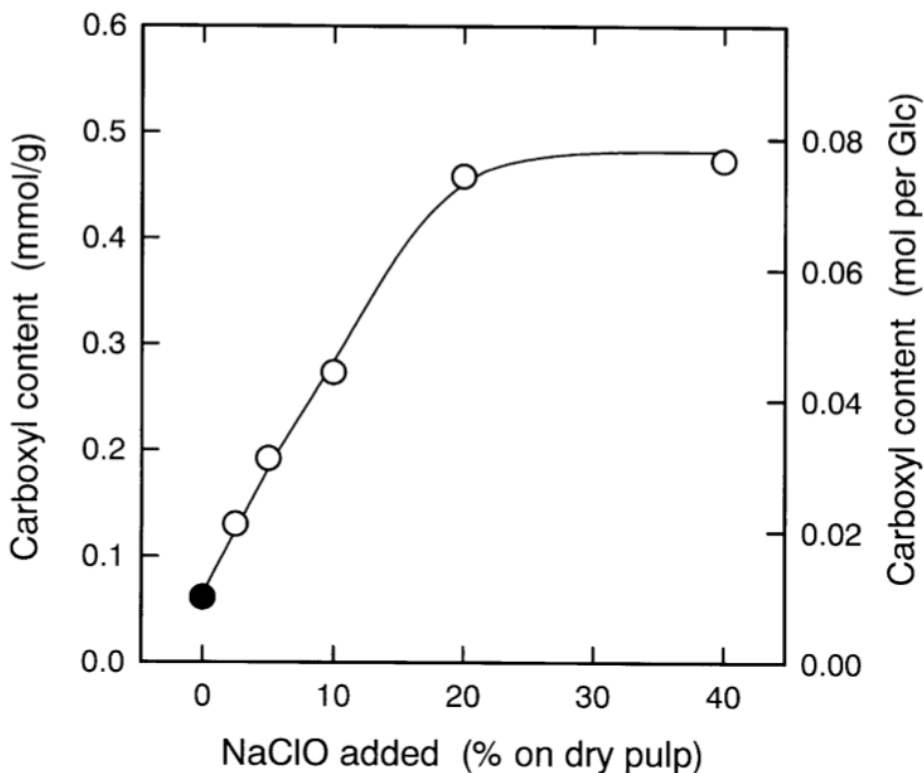


Figure 10. Carboxyl content of the original and oxidized bleached kraft hardwood pulps after 2 hours of TEMPO oxidation reaction at pH 10 - 11 Glc: Glucose residue.

• : Original Pulp, adapted from Kitaoka *et al.* ¹¹.

2.5 Adsorption of Polymeric amines on Pulp

Adsorbing polymers with amine groups on paper surfaces has a big role in paper manufacturing. Polymeric amines introduce cationic surface and can form covalent bonds between fibers, increasing the wet strength of paper. Wet strength polymers are typically cationic polyelectrolytes which adsorb spontaneously to fiber surface in water ¹². However, the purpose of adsorbing amine in this work was to adsorb phosphates groups on pulp and not to enhance the paper wet strength.

2.5.1 Polyvinylamine (PVAm)

Polyvinylamine has the highest content of primary amine functional groups of any polymer. It has the quality of adsorbing irreversibly on most surfaces. Adsorbed PVAm provides cationic surface that can be used to promote adhesion or adsorption¹³. One of the main pathways in making PVAm is by hydrolyzing the polymer poly(N-vinylformamide) (PNVF). As reflected in Figure 11, PNVF has two hydrolysis routes: acidic and basic. Both routes produce primary amino groups with: formic acid in the acidic hydrolysis and sodium formate in the basic hydrolysis. The basic hydrolysis has higher equilibrium amide conversion than acidic hydrolysis. The acidic hydrolysis creates cationic repulsion between charged polymer chains and the approaching acid, converting less amide groups¹⁴.

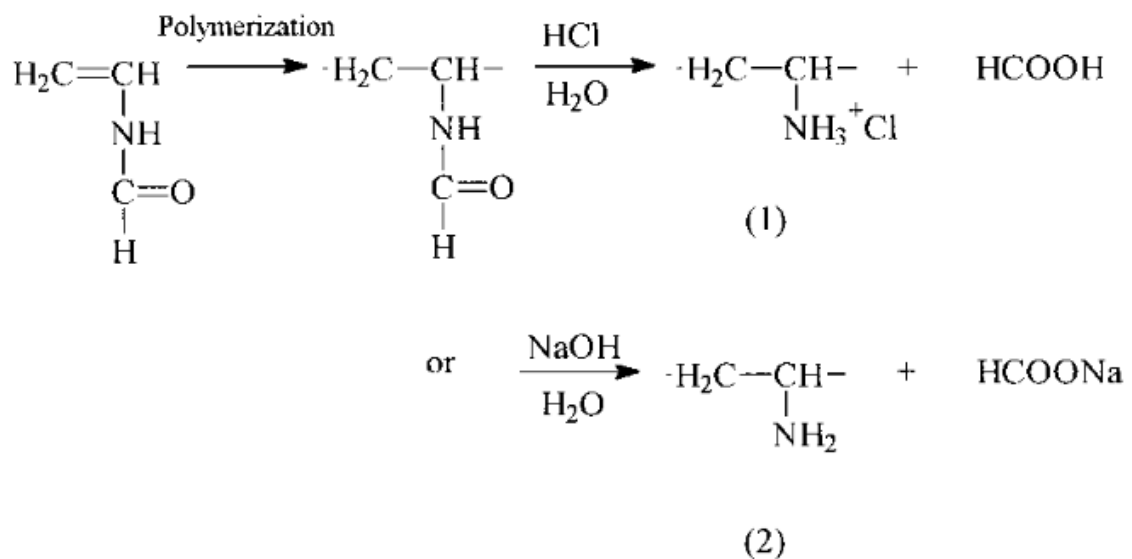


Figure 11. Synthesis route of PVAm via (1) acidic and (2) basic hydrolysis of PNVF, adapted from Gu *et al.*¹⁴.

2.5.2 Impact of pH on PVAm Ionization Behavior

PVAm is partially ionized over most of the pH scale. The ionization behavior of PVAm cannot be described by a pK_a value. This behavior is called the polyelectrolyte effect and is a consequence of interaction between neighboring amine groups¹³. Figure 12 describes the ionization degree of PVAm comparing it to other cationic polymers like polyethylenimine (PEI), polyallylamine (PAH), and chitosan.

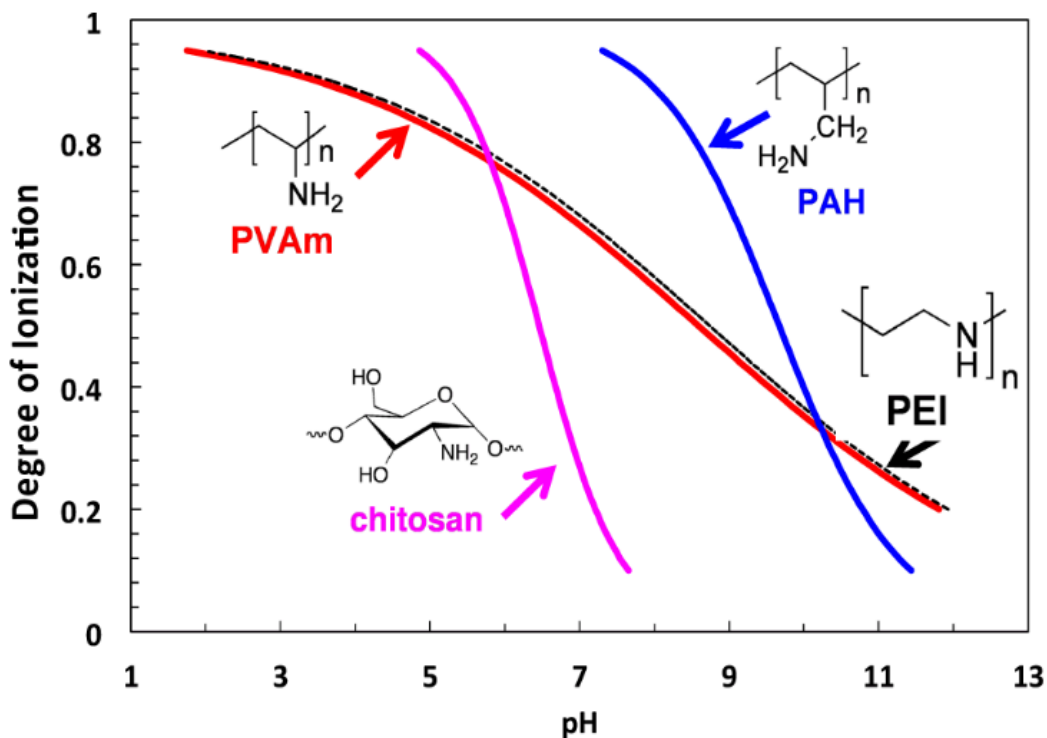


Figure 12. Comparison of the ionization behaviors of PVAm with PEI and PAH based on Henderson–Hasselbalch fits to experimental titration curves in 1 M KCl, adapted from Pelton¹³.

2.5.3 PVAm Anionic Agents Entrapment

In a previous work, PVAm was used to capture anionic agents on a paper based solid phase

biosensors. These biosensors were used to rapidly detect analytes of clinical and environmental interest; an example would be the detection of acetylcholinesterase (AChE) inhibitors such as neurotoxins and organophosphates which have implications for neuroscience and pharmaceutical development. The biosensor utilizes piezoelectric inkjet printing of biocompatible, enzyme-doped, sol-gel-based inks to create colorimetric sensor strips. PVAm was first printed, and then AChE was overprinted by sandwiching the enzyme within two layers of biocompatible sol-gel-derived silica on paper (Figure 13). The biosensor successfully detected the AChE inhibitors by measuring the residual activity of AChE on paper, using Ellman's colorimetric assay, with capture of the 5-thio-2-nitrobenzoate (TNB⁻) product on the PVAm layer. AChE inhibitors detection develops a yellow color on the biosensor which is visible to the eye and can be analyzed using an image analysis software¹⁵.

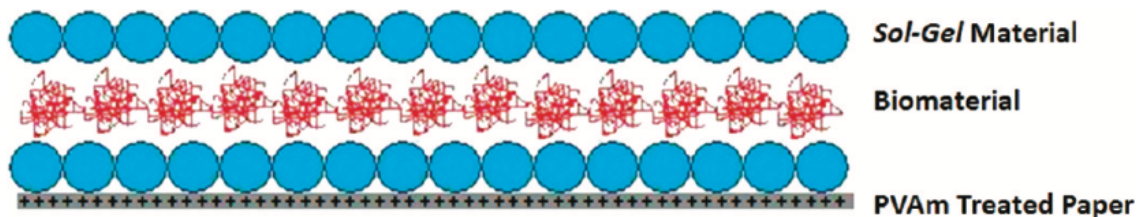


Figure 13. Schematic illustration of inkjet printing sequence of pvam, sodium silicate (ss)-based sol-gel-derived silica matrix, and the tris buffer (100 mm, ph 8.0) containing enzyme acetylcholinesterase (AChE) and dithiobisnitrobenzoate (DTNB) layers on paper for the development of a portable solid-phase biosensor, adapted from Hossain *et al.*¹⁵.

PVAm can entrap and concentrate TNB⁻, on the PVAm treated paper, without diminishing the developed yellow color for at least three weeks; however, the yellow color of the

untreated paper (control) disappeared in 24 hours. From Figure 14, the values of the retardation factor (Rf), which is a measure of the relative mobility of TNB^- as a function of PVAm concentration, decreases with increasing PVAm levels up till 0.5 wt% PVAm.

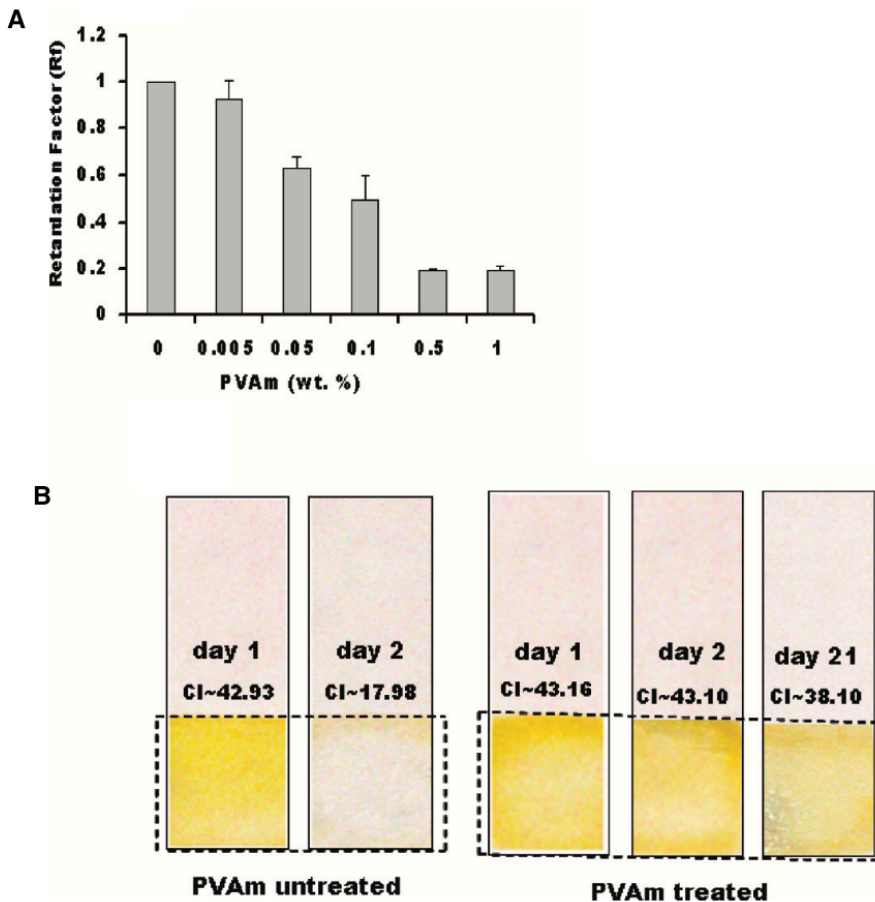


Figure 14. Effects of cationic PVAm on TNB^- entrapment: (A) The values of retardation factor (Rf) for anionic TNB in Milli-Q water in the presence of indicated PVAm levels. (B) Colour Intensity (CI) of Cardboard dipstick with inkjet-printed PVAm or control (no PVAm) and silica/AChE/DTNB/silica layers after being immersed in a ATCh solution, adapted from Hossain *et al.*¹⁵.

This study shows the efficacy of PVAm in adsorbing anions for long periods of time; hence, it can be predicted from this study that PVAm should adsorb phosphate anions on PVAm coated pulp for long periods of time, making phosphates more

available to plant up taking.

2.5.4 PVAm Adsorption on Pulp

In a previous work done by Marais et al., cationic polyelectrolytes with different molecular weight were adsorbed onto lignocellulosic fibers from unbleached and unbeaten spruce chemical fibers to test for the polyelectrolytes effects on the final paper mechanical properties. Adsorption isotherms were established to determine the adsorption capacity of polymer onto the different kinds of fibers¹⁶. Fibers with different total charge density led to different responses in terms of adsorption. The pulp fibers in their proton form were titrated against sodium hydroxide to determine their total charge density¹⁶.

Pulp with high percentage of yield has high content of hemicellulose; thus, it has higher total charge when compared to pulp with lower yield (Table 2). It was observed that the surface charge increased with increasing pulp yield up to 50% and then decreased. This happens because of the competition between two phenomena: the accessibility of charges within the fibers increases with decreasing yield due to the wider pore size of pulp, and further decrease in yield decreases the charge of the fibers more than the accessibility increases¹⁷.

Table 2. Surface charge, total charge, and lignin content of the fibers from three different unbleached pulps, adapted from Marais *et al.* ¹⁶.

Pulp	Yield (%)	Surface charge ^a (μeq/g)	Total charge ^b (μeq/g)	Lignin content ^c (wt%)
K34	49.7	5.6	74	3.3
K75	56.4	9.3	137	10.0
K107	60.4	8.5	177	15.1

^a Determined by polyelectrolyte titration

^b Determined by conductometric titration

^c Determined by chemical analysis

45 kDa (8.0 meq/g) and 340 kDa (8.4 meq/g) PVAm were adsorbed onto the three pulp samples in Table 2 at pH 8 and stirred for 10 minutes in a 0.5 mM sodium bicarbonate (NaHCO₃) solution. Thereafter, the mixture was filtered using a Büchner funnel. The initial pulp suspension solution and the filtrate were analyzed via polyelectrolyte titration and nitrogen analysis to determine the quantity of adsorbed PVAm on fibers. The nitrogen analysis tests were performed on a ANTEK 7000 Model 737 instrument. This measurement determines the difference between the quantity of nitrogen in the initial solution and in the filtrate which corresponds to the amount of polyelectrolyte adsorbed on the fibers ¹⁶.

Table 3 reveals the adsorbed amount of PVAm on fibers obtained for the highest added amounts of PVAm (from adsorption isotherms) on pulps K107, K75, and K34. It also compares the results of nitrogen analysis and polyelectrolyte titration which were relatively very similar; except for high molecular PVAm, they weren't. The high total charge content of pulp K107 contributes to its high PVAm adsorption capacity when compared to other

pulps due to the electrostatic attraction between the cationic amino groups of PVAm and the anionic carboxyl groups on the pulp surface ¹⁶.

Table 3. Adsorbed amount of PVAm (determined by nitrogen analysis) compared with the adsorption saturation levels from the adsorption isotherms, obtained by polyelectrolyte titration, for pulps K107, K75 and K34, adapted from Marais *et al.* ¹⁶.

Pulp	PVAm	Added amount (mg/g pulp)	Adsorbed amount (mg/g pulp) ^a	Adsorption saturation (mg/g pulp) ^b
K107	45 kDa	4.6	3.4	3.2
	340 kDa	3.8	3.3	2.5
K75	45 kDa	4.0	3.2	2.7
	340 kDa	3.8	2.9	2.0
K34	45 kDa	4.1	2.9	2.1
	340 kDa	4.1	2.8	1.6

^a Determined by Nitrogen analysis

^b Determined from adsorption isotherms obtained by polyelectrolyte titration

2.5.5 PVAm Adsorption on TEMPO Oxidized Pulp

Illergard et al. investigated the importance of electrostatic interaction to the antibacterial effect of PVAm coated fibers. Most surfaces in nature are negatively charged or uncharged. On the other hand, positively charged surfaces are an anomaly; thus, most of the bacteria are unprepared to encounter positively charged surfaces ¹⁸.

Disintegrated unbeaten bleached chemical softwood fibers (total net negative charge of 43

$\mu\text{eq/g}$) were TEMPO oxidized at pH 6.8 and 60 °C with constant stirring for 5 hours. Then the fibers were cooled to room temperature and rinsed with deionized water. One and three layers of 340 kDa PVAm were adsorbed onto the unoxidized and oxidized fibers at 0.5% w/w fiber consistency, 0.1 g/L polymer concentration, and 100 mM NaCl background solution under constant stirring. The three layers adsorption scheme was as follows: PVAm (pH 9.5), 240 kDa polyacrylic acid (PAA) (pH 3.5), and PVAm (pH 9.5). The one layer adsorption was done by removing some of the fibers from the sample after the first PVAm adsorption step. Oxidized fibers have high concentration of carboxyl groups that increase the adsorption of PVAm through an electrosorption process as shown in Figure 15¹⁸.

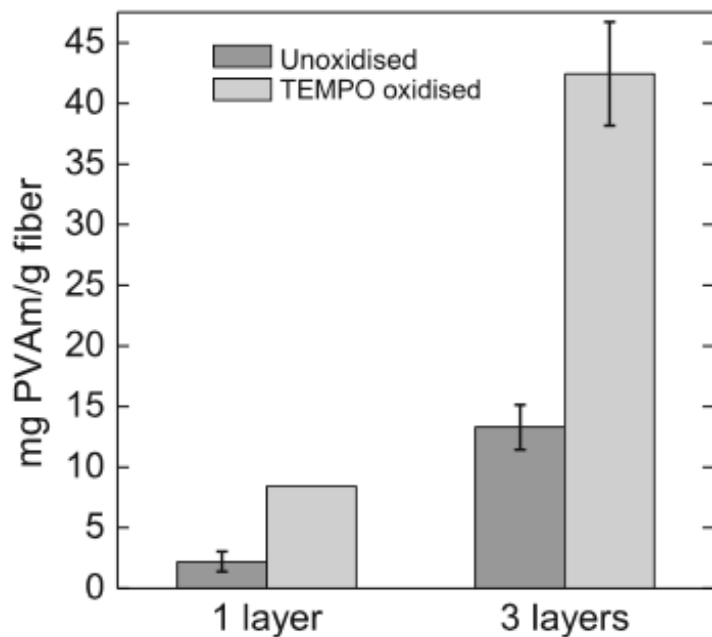


Figure 15. PVAm content after the adsorption step was increased for the TEMPO-oxidized fibers compared with the unoxidized fibers, adapted from Illergard *et al.*¹⁸.

Table 4 reflects the increase in the antibacterial efficacy of PVAm coated fibers against *Escherichia coli* and *Bacillus subtilis* ¹⁸.

Table 4. Summary of bacterial reduction after overnight incubation with fibers modified with 1 and 3 layers of PVAm polymer, adapted from Illergard *et al.* ¹⁸.

	Unoxidised		TEMPO oxidised	
	1L	3L	1L	3L
<i>E. coli</i>	+	+++	++	+++
<i>B. subtilis</i>	+	++	+	+++

+++ corresponds to a $\geq 99.9\%$ reduction, ++ to a 99 % reduction, + to a 90 % reduction and—to no reduction compared with the reference (+)

Consumption of raw food products has increased the exposure of the consumer to foodborne pathogens, like *E. coli* bacteria. *E. coli* has been associated with outbreaks from the consumption of contaminated seed sprouts, radish sprouts, and leafy greens such as lettuce and spinach. Laboratory studies have indicated that the transmission of *E. coli* to the plants could occur when the plants are grown in soils receiving contaminated manure or irrigation water. Whether *E. coli* can move through the plant's rhizosphere via the roots into the aboveground edible part of the plant or move into the inside of the plant tissue are some of the concerns of plant cultivation ¹⁹.

It can be concluded from this work that TEMPO oxidizing the fibers will not only increase the PVAm adsorption, but also will reduce the bacterial inhabitation into the fibers which will be an asset for the current work. PVAm cationic charges on pulp surface are expected

to inhibit bacteria like E. coli, decreasing the bacterial exposure to the plants grown on pulp substrate which will be useful for the end-user's plant intake.

2.6 Phosphate Adsorption on Cationic Cellulose Nanofibers

Cationic cellulose nanofibers (CNF) with quaternary ammonium content in the range of 0.34 –1.2 mmol/g have been prepared by Sehaui et al. to adsorb negatively charged water contaminants (nitrate (NO_3^-), fluoride (F^-), phosphate (PO_4^{3-}), and sulphate (SO_4^{2-})) via electrostatic interactions. To prepare cationic CNF, pulp fibers were mechanically beaten and then mixed overnight with sodium hydroxide (NaOH). Glycidyltrimethylammonium chloride was added to the suspension, and the reaction was carried out at 65 °C for 8 hours under stirring. Hydrochloric acid (HCl) was used to neutralize the filtered mixture, and then mixture was washed thoroughly with deionized water. CNF's high specific area and surface functionalization provide an inexpensive method to remove these contaminants for wastewater treatment. Excess of phosphates, sulphates, and nitrates present in wastewater causes eutrophication problems that affect a lot of water bodies. CNF positive charges showed a promising ability to adsorb negatively charged ions via electrostatic interactions as shown in Figure 16²⁰. CNF displayed higher selectivity of multivalent contaminants; PO_4^{3-} and SO_4^{2-} had better adsorption on CNF than NO_3^- and F^- had.

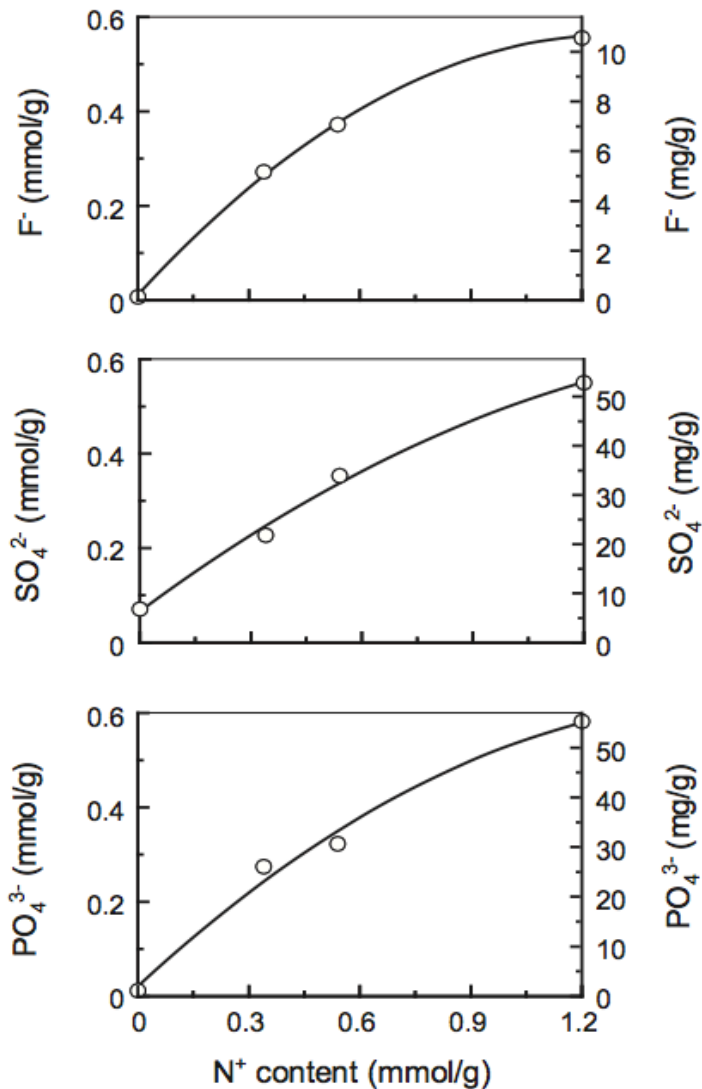


Figure 16. Adsorption capacity of negatively charged contaminants by the CNF, adapted from Sehaqui *et al.*²⁰.

This work investigated the effect of pH on the adsorption of nitrates, by adjusting the pH using dilute NaOH/HCl solutions. The maximum nitrate adsorption took place in the pH range 5.0–7.0 as shown in Figure 17. At low and high pH range, the presence of other ions

like Cl^- and Na^+ could have reduced the adsorption capacity as compared to neutral pH range. At low pH, Cl^- might have been adsorbed on the cationic sites on the nanofibers. At high pH, Na^+ might have had a screening effect on the charges, thus lowering the adsorption onto the nanofibers²⁰.

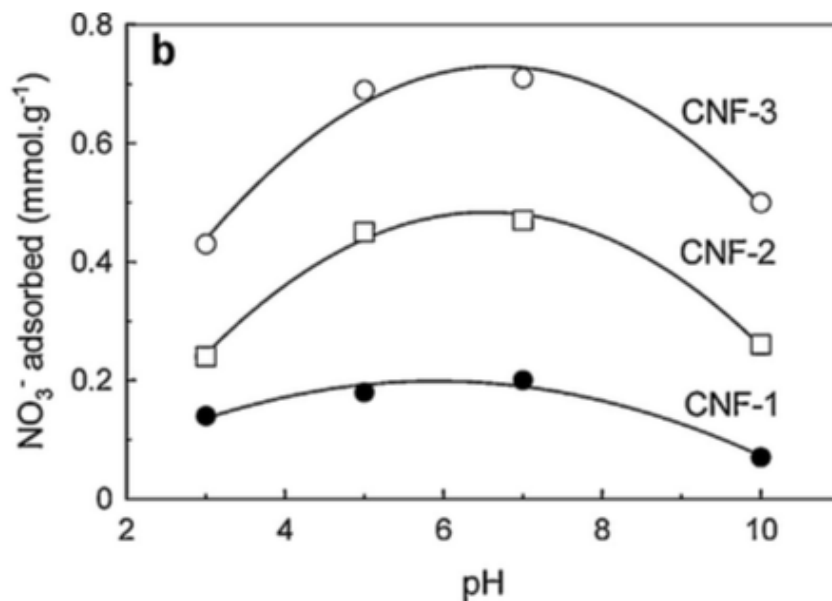


Figure 17. Impact of pH and surface charge content on the nitrate adsorption capacity of CNF. (CNF-1: 0.34 N^+ mmol/g), (CNF-2: 0.54 N^+ mmol/g), and (CNF-3: 1.2 N^+ mmol/g), adapted from Schaqui *et al.*²⁰.

2.7 Wood Pulp Seedling Nursery Sheets for Hydroponic Cultivation

OJI KINOCLOTH is the first company in the world to manufacture pulp air laid nonwoven web sheets, kinocloth sheets, from wood pulp in 1964²¹. Kinocloth sheets were patented on 2010 as a liquid-absorbent sheets that may be used for disposable diapers, sanitary napkins, water-absorbent sheets, and oil-absorbent sheets. The sheets have a basis weight

of 526 g/m² and a thickness of 2.7 mm. The sheets also have a binder on their outer surface that consists of 125 g/m² of SAP (super absorbent polymer manufactured by SUMITOMO SEIKA CHEMICALS CO., LTD, under the trademark of AQUA KEEP SA 60SX). SAP is sprayed onto the rear material tissue using powder feeder ²².

The wood pulp web kinocloth sheets were used in agriculture, livestock farming, and fisheries. The sheets were used as seedbeds for hydroponic cultivation of sprouting vegetables as shown in Figure 18 ²¹. A seedbed or a seedling bed is a controlled environment used to grow seedlings into larger young plants before transplanting them into the field or the garden. A seedling bed is often used to increase the number of seeds germinating.



Figure 18. Seedling Nursery Sheets for Hydroponic Cultivation, adapted from OJI KINOCLOTH LTD. ²¹.

The wood pulp air laid nonwoven sheets can be used as a substitute for urethane seedbeds, which are not environmentally friendly. Their weight lightness also reduces costs during transportation. The web is formed using air laid method, in which pulp is

dispersed in air and not in water. A water-based adhesive (SAP binder) is evenly disturbed on the sheets using original spray technique by OJI KINOCLOTH company. The binder is selected to be suitable to contact with food. The bulky web with the binder is made into sheets via a drying process. Figure 19 shows the cross section view of the kinocloth sheets. The binder and the bulkiness of the sheets make them have great water retention and filtration. The sheets have the capacity to absorb liquids 20 times more than the weight of the sheet, and they also have a very low density of 0.04 g/cm^3 , which makes them very bulky and coarse ²¹.

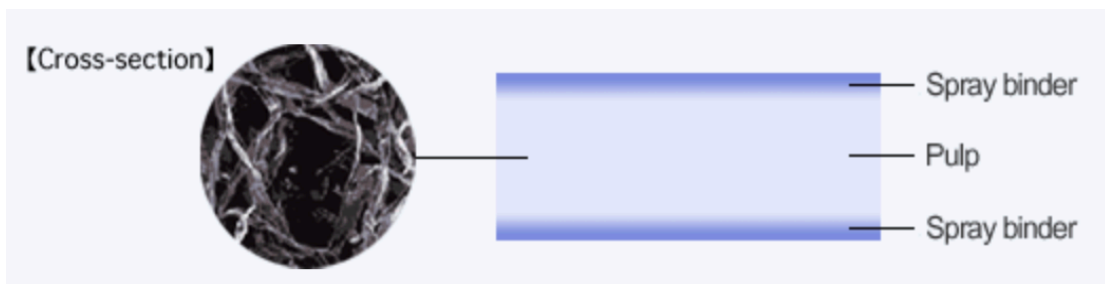


Figure 19. Cross-section view of seedling Nursery Sheets for Hydroponic Cultivation, adapted from OJI KINOCLOTH LTD LTD. ²¹.

2.8 Objectives

Based on the previous work done on cellulose fibers, significant progress has been done on chemically modifying the fibers for paper wet strength or for adsorption of wastewater contaminants. Wood pulp fibers were also mechanically modified to manufacture inert seedling beds in agriculture applications.

The aim of this project was to develop a plant substrate from wood pulp modified fibers that can store and release phosphorus for plant cultivation. This work will be the first to

test the growth of plants on chemically active pulp that has cationic and anionic charges on its surface.

The objectives of this work are listed below:

- 1- Characterize the cellulose surface chemistry before and after being oxidized. TEMPO oxidation reaction has shown great effects on increasing the carboxyl group content of fibers. The effects of reaction conditions, in terms of the reaction time and the amount of oxidant used, on the carboxyl group content of the pulp fibers were examined.
- 2- Correlate the adsorption properties of PVAm to the carboxyl group content of the pulp fibers. PVAm with different molecular weight, charge density, and formulation process has different adsorption on the wood pulp fibers. This study tested PVAm adsorption capacity on pulp with different carboxyl group content to determine the maximum PVAm adsorption on the pulp surface.
- 3- Determine the pulp adsorption capacity of phosphate groups. The role of PVAm and carboxyl groups on adsorbing phosphate groups was tested under various pH conditions; thus, the limitation of phosphate adsorption on pulp was controlled.
- 4- Study the release of phosphate groups from pulp. Ionic strength, pH, and suspension time all had significant impacts on the phosphate release from pulp. This study determined the optimum conditions to release the least amount of phosphates to the surrounding media.
- 5- Test the growing behavior of phosphate sensitive seeds on the modified and unmodified fibers. It is hypothesized that surface of modified fibers will resemble

the surface of the actual soil with extra cationic sites introduced by PVAm. The plant growth allocation, fresh weight, and root growth were examined to correlate the plant growth with the fibers used.

Chapter 3 Experimental Methods

3.1 Introduction

This chapter first describes the preparation of the TEMPO oxidized cellulose fibers and the phosphorus testing method; then, the procedure of adsorbing PVAm and phosphates onto modified and unmodified fibers is described. At the end of the chapter, the method of growing Arabidopsis plant seeds on modified and unmodified is explained.

3.2 Materials

The pulp used in this work is unbeaten southern softwood bleached pulp (Alabama River). Three sources of PVAm, supplied by BASF (Ludwigshafen, Germany), were used in this work: 1- Lupamin® 4595 (**P45**) contains 10-15 wt.% of active substance of about 95% hydrolyzed poly(N-vinylformamide) and has a molecular weight of 45 kDa, 2- Lupamin® 4500 (**PH45**) contains 15-20 wt.% of active substance of unhydrolyzed poly(N-vinylformamide) and has a molecular weight of 45 kDa, and 3- Lupamin® 1595 (**P15**) contains 10-15 wt. % of active substance of about 95% hydrolyzed poly(N-vinylformamide) and has molecular weight of about 15 kDa²³.

TEMPO, sodium bromide (NaBr), sodium hypochlorite (NaClO), phosphoric acid (H₃PO₄), monopotassium phosphate (KH₂PO₄), sodium chloride (NaCl), hydrochloric acid (HCl), sulphuric acid (H₂SO₄), ammonium molybdate (H₂₄Mo₇N₆O₂₄·4H₂O), and ascorbic acid (C₆H₈O₆) were purchased from Sigma-Aldrich and used as received. Potassium

antimonyl tartrate ($C_4H_2KO_6Sb \cdot 1.5H_2O$) was purchased from Acros Organics and used as received.

3.3 Pulp Treatment with TEMPO and PVAm

3.3.1 TEMPO Oxidation of Pulp

TEMPO was used to convert the cellulose hydroxyl groups to carboxyl groups under mild conditions. NaBr and TEMPO were used as catalysts to oxidize the primary hydroxyl groups of SSBK wood pulp. The oxidation reaction was started by adding the oxidant, NaClO. After reaching the desired oxidation time, Ethanol was used to quench the reaction. About 25 g of southern softwood bleached kraft (**SSBK**) dry pulp were soaked overnight in 2 L of DI water and disintegrated the following day using model 500-1 disintegrator (Labtech Instrument Inc.) for 30000 revolutions. The water was filtered off through Büchner funnel using a filter paper. The dry weight content (DW) of the pulp was adjusted to 10 %, i.e. 10 % dry pulp and 90% water. Disintegrated pulp was added to 4 L beaker, and DI water was added to a total volume of 4 L. To start off the catalytic process, 60 mg of TEMPO and 600 mg NaBr were added to the pulp slurry and stirred for 30 minutes until completely dissolved. 0.0175 moles of NaClO was added to the suspension to start the reaction. The basic conditions were provided by maintaining the pH at 10.5 for 30, 60, or 120 minutes using dilute HCl/NaOH solutions. The reaction was quenched by adding 50 mL of 95% Ethanol to the suspension. The oxidized pulp slurry was filtered through a Büchner funnel using filter paper and washed several times until the conductivity

(measured by CDM 83 Conductivity meter) of the filtrate was 10 $\mu\text{S}/\text{cm}$ or lower. The pulp was stored after in sealed plastic bags in refrigerator until further use.

3.3.2 Carboxyl Group Content

The total carboxyl group content of modified fibers was measured by conductometric titration using a Burivar-I2 Burette Module (ManTech Associates) with PC-Titrate software (version 2.0.0.79). Conductometric titration was conducted by dispersing 5 grams of wet TEMPO oxidized pulp (**TOP**) in 250 mL of 5 mM NaCl solution. 0.1 M HCl was used to protonate the TOP and adjust the initial pH to 3. The titrator gradually added 0.1 M NaOH (titrant) with constant 0.1 ΔpH to hold injection control, 0.005 mL minimum single injection, 0.20 maximum single injection, 90 seconds between additions, and 11.2 stopping pH. To determine the carboxyl group content of pulp per pulp weight, the amount of titrant used to titrate the weak acid groups (the plateaued section of titration curve) was multiplied by the molarity of the titrant, and this value was divided by the pulp dry weight (0.5 gm).

3.3.3 PVAm Preparation and Hydrolysis

The degree of hydrolysis of P15 and P45 was used as provided by BASF, 95%. As for PH45, it was not hydrolyzed when purchased, and the hydrolyzed PH45 was synthesized via basic hydrolysis of poly(N-vinylformamide) at 5 v/v% and 90 °C for 5 days; in which 50 mL of PH45 were mixed with 450 mL of 1 M NaOH in a round flask, and the flask was kept in an oil bath on a magnetic stirrer hot plate (RCT basic IKA[®] Werke) at a temperature of 90 °C for 5 days under constant stirring. The degree of hydrolysis was assumed to be

~95%-100%. This assumption was based on a previous work where the basic hydrolysis of poly(N-vinylformamide) at 5 v/v% and at > 80 °C for 12 hours have showed 90% degree of hydrolysis, and the degree of hydrolysis increased with the time and the temperature¹⁴; thus, 5 days and 90 °C were the conditions used in this work for the basic hydrolysis. The PVAm solutions were put in Spectra/Por dialysis molecular porous membranes for purification and freeze dried by Millrock Technology freeze dryer after. The freeze dried PVAm was dissolved in 5 mM NaCl at 0.1% (w/v) for further usage.

3.3.4 PVAm Nitrogen Content

The surface nitrogen content was determined using Mutek PCD T3 Wet End Technology particle charge titrator fitted with Mutek PCD 03 streaming current detector (SCD). In a typical experiment, 1 mL of 0.1% (w/v) PVAm solution was diluted to 10 mL using DI water. The pH was measured before and after the titration to make sure it falls within pH range of 6.5-7.0; in which, ~ 0.70-0.72 of PVAm is ionized (Figure 12). The diluted PVAm is put into titrator's cell and is titrated by 1 mN PVSK (polyvinyl sulfuric acid potassium salt), an anionic polyelectrolyte.

Mutec polyelectrolyte titration gives a measure of the concentration of polymer bound charged groups only. In the PVAm case, the nitrogen (N^+) content is sensitive to the final pH value in the titration cell, and it reflects a fraction of PVAm charged amine groups.

Conductometric titration is different from Mutec polyelectrolyte titration in that conductometric titration gives the total titratable nitrogen content. Pulp treatment with

PVAm was done at the same pH (pH ~7) the polyelectrolyte titration was done at to be consistent with amine charged content of PVAm at the final pH of the titration cell.

3.3.5 PVAm Adsorption onto Pulp

5.0 - 40.0 mg/g-pulp of PVAm/pulp suspensions were tested for maximum PVAm adsorption onto pulp surfaces, and 5 mM NaCl was used as the background electrolyte solution. The pulp samples were kept at 5 g/L pulp density in the PVAm/pulp suspensions. For example: at 20 mg/g-pulp of PVAm/pulp mixture, 10 gm wet pulp (10% DW) was dispersed in 140 mL of 5 mM NaCl. After the pulp slurry evenly mixed, 50 mL of 0.1% (w/v) PVAm solution was added, and the pH was maintained at 7.0 for 15 minutes using dilute HCl/NaOH solutions. The pulp slurry was filtered using Büchner funnel and filter paper, and the filtrate was kept. The nitrogen content difference between the initial PVAm/pulp suspension solution and the filtrate was used to calculate the amount of PVAm charges adsorbed onto the pulp. A sample calculation can be found in Appendix A.1.

The filtered PVAm treated pulp was rinsed four times with 50 mL of DI water (same total volume as the initial suspension volume). The last rinse filtrate was tested for its conductivity and PVAm concentration, and it had shown 10-15 $\mu\text{S}/\text{cm}$ conductivity and ~ 0 PVAm content. After the final rinse, the PVAm treated pulp is kept in a sealed plastic bag in the refrigerator until further use.

3.4 Phosphorus Assay and Adsorption onto Pulp

3.4.1 Phosphorus Testing Method

To determine the phosphorus (P) concentration, ascorbic acid colorimetric standardized method 4500-P E. APHA was used. The following chemicals were used for this test: A- 5N H_2SO_4 , B- 0.34% (w/v) Potassium antimonyl tartrate, C- 4% (w/v) Ammonium molybdate, and D- 0.1 M Ascorbic acid. The combined reagent consists of 50 mL of A, 5 mL of B, 15 mL C, and 30 mL of C. The 5 N H_2SO_4 has the role of dissolving the soluble and insoluble inorganic forms of phosphorus i.e. this method determines the content of orthophosphate forms only of phosphorus. Ammonium molybdate reacts in the acid medium with orthophosphate to form phosphomolybdic acid that is reduced to colored molybdenum blue by ascorbic acid. Potassium antimonyl tartrate is used as a catalyst. The reagent was added to standardized phosphate solutions at 13.8% (v/v), and the absorbance was measured after different periods of reduction time through a wavelength range of 600-1000 nm using UV/VIS light spectrophotometer (DU 800 Beckman Coulter). 219.5 mg of anhydrous KH_2PO_4 were dissolved in 1000 mL of DI water and used as the stock standard phosphate solution for the calibration curve, in which 1.00 mL = 50 μg P.

3.4.2 Phosphate Adsorption onto Oxidized and Unoxidized Pulp

Unoxidized and TEMPO oxidized samples were first treated with 20 mg/g-pulp PVAm, as described in section 3.3.5. H_3PO_4 was the phosphate source used to adsorb phosphates on cationic pulp surface. H_3PO_4 was used because it is an acid that can protonate the adsorbed PVAm on cationic pulp fibers resulting in more electrostatic attraction force between the

amine and phosphate groups. The following is an example of adsorbing phosphates onto one of the pulp samples: 1 gram of wet TOP with P45 adsorbed onto its surface (TOP-P45) was dispersed in 19 mL of 2 mg P/L solution in centrifuge tubes. Dilute HCl/NaOH solutions were used to control the pH of the pulp slurry before agitating the samples with the tube rotator. pH was measured once again after one hour of agitation to make sure it fell within the required pH range. Thereafter, the mixture was filtered using a Büchner funnel, and the filtrate was kept for further tests. The difference between the initial suspension solution P concentration (2 mg/L) and the filtrate P concentration was used to determine the phosphate amount retained on the pulp surface. The filtered pulp was not rinsed and was kept in sealed plastic bags in the refrigerator for further studies. A sample calculation for phosphate retention on pulp can be found in Appendix A.2.

3.5 Phosphate Release from Phosphate Loaded Fibers

TOP-PH45 samples loaded with phosphates were suspended in different background media with pH controlled to test for the effect of pH and competitive ions on the release of phosphates from PVAm coated pulp fibers.

Three different background media were used: 1- 5 mM NaCl, 2- Plant nutrients media, and 3- Plant nutrients media with a buffering agent. The plant nutrients media had the following components: 0.5 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 mM KNO_3 , 100 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 100 μM H_3BO_3 , 20 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1 μM CuSO_4 , 3 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 μM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 0.01 μM CoCl_2 . The recipe of the growing media was adapted from the Biology Department at McMaster University, and it was used to grow plants at their

labs. The buffering agent, 2-(N-morpholino) ethanesulfonic acid (MES), was used at 0.5 mg/L with the plant nutrients. MES has been used in a previous work as a buffering agent to test the effect of plant growing medium pH on the plant root development²⁴; thus, it was used in this work.

TOP-PH45 loaded with phosphates were dispersed in each background media at 5 g/L pulp density in centrifuge tubes for several hours (2-120 hrs) under constant agitation provided by the tube rotator. Dilute HCl/NaOH solutions were used to control the pH of the pulp samples. The pH of the samples was checked before and after the agitation to make sure it fell within the required ranges. The filtrate solutions of the samples were collected and tested for the phosphate released. The phosphates released presented the release of phosphates adsorbed onto the fibers and **not** the entrained phosphates within the fibers. The amount of phosphates entrained within the fibers was deduced from the pulp consistency when it was filtered after the phosphate treatment. A sample calculation for phosphate release from pulp can be found in Appendix A.3.

3.6 pH Titration Curve of TEMPO Oxidized Pulp Treated with PVAm

pH titration curve was performed to determine the buffering capacity of the treated fibers. TEMPO oxidized pulp treated with PVAm was dispersed in plant nutrients media at pulp density of 5 g/L, and the pH was lowered to 3 using HCl acid. 0.05 mM NaOH base was the titrant used at 0.05 mL single injections and 60 seconds between additions. The titration was done manually using 100 μ L pipette.

3.7 Arabidopsis Plant Study

Arabidopsis seeds were used in this work to be grown on modified and unmodified pulp matrices to test for the impact of pulp matrices on the phenotype of cultivated Arabidopsis plants. The pulp matrices were prepared by mixing nutrients media with pulp in petri dishes; then the seeds were surface sterilized and plated on the matrices for stratification and cultivation.

3.7.1 Preparation of Nutrients Media

Defined nutrients medium was composed of: variable phosphate content, 0.5 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 mM KNO_3 , 100 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 100 μM H_3BO_3 , 20 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1 μM CuSO_4 , 3 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 μM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 0.01 μM CoCl_2 (same plant nutrients solution used in the phosphate release study), 2 % (w/v) sucrose, and 0.8% (w/v) phytigel. The phosphate solution (P_i) was used as a buffer and phosphate source, and it was made by using about 75% of 0.5 M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ stock solution and adding 0.5 M Na_2HPO_4 stock solution until desired pH is reached (pH 5.6). The nutrients media was autoclaved by benchtop autoclave (Model 3850) at cycle 3 for liquids.

3.7.2 Preparation of Nutrient Media/Pulp Matrices

The P_i content of the nutrient media was :0, 0.05, and 0.25 mM. The nutrient medium was mixed with modified and unmodified pulp samples at 0.5 % (w/v). The pulp samples used

were: UnP, UnP-PH45, TOP, and TOP-PH45. The nutrient media and the pulp samples were autoclaved separately before use by benchtop autoclave (Model 3850) at cycle 3 for liquids. The nutrient media/pulp mixtures were then transferred to petri dishes in which each dish contained 20 mL nutrients solution and 100 mg pulp (or no pulp for control).

3.7.3 Arabidopsis Seed Sterilization and Plating

Before the seeds were plated on the matrices, they were surface sterilized. Procedure for surface sterilization:

- 1- Place seeds in Eppendorf tube.
- 2- Add 1 mL of 70% ethanol and shake the content for 1 minutes, then remove the ethanol.
- 3- Add 1 mL of 50% bleach and shake the contents for 1 minute, then remove the bleach.
- 4- Add 1 mL of Autoclaved DI water and shake the contents for 1 minutes, then remove the water.
- 5- Repeat step 4, 6 times.

Following the surface sterilization, 5 seeds were plated on the nutrients/pulp matrices on each plate and stratified in the dark for 72 hours at 4 °C. 3 plates, with 5 seeds each, were prepared for every matrix. Thereafter, the plates were vertically placed in greenhouse kit

(provided by Home Hardware) under a 24 hours photoperiod at room temperature and 95.5-101.7 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 10 days.

3.7.4 Fresh Weight Measurements of Arabidopsis Seedlings

After growing the seedlings for 10 days on matrices with Pi content of :0, 0.05, and 0.25 mM and with modified/unmodified pulp fibers, the seedlings fresh weight was measured by gently removing the seedling with its roots from the matrix and rinsing off all matrix remnants on the roots then gently dabbing the plants with a tissue to remove the excess water. To avoid drying out the seedlings while weighing them, the clean extracted seedlings were placed on a weigh boat filled with some DI water on the analytical balance (AX204 Mettler Toledo).

3.7.5 Primary Root Length Measurements of Arabidopsis Seedlings

After gently extracting the seedlings from the nutrients/pulp matrices, the primary root length of the seedlings for each seedling was measured using a ruler.

3.7.6 Leaves Fresh Weight Measurements of Arabidopsis Seedlings

The roots' weight contributed to the total weight of the seedlings; thus, the roots were separated from the leaves and weighed separately to be equal to 1.44 ± 0.12 mg per plate. This value was subtracted from the total fresh weight of the seedlings to reflect the fresh weight of the leaves only.

Chapter 4 Results and Discussion

4.1 Carboxyl Group Content of Modified and Unmodified Pulp Fibers

The pulp carboxyl group content has a key functionality of adsorbing cationic polymers. Conductometric titration was performed (section 3.3.1 procedure) to quantify the total carboxyl group content in oxidized and unoxidized fibers as shown in Figure 20. The best fit lines for the excess acid and base were used to determine the intersection points (V1 & V2) with the plateau curve. The plateau curve data points were selected based on the lowest absolute value of the curve's slope (typically close to 0.0-1.0). To calculate the carboxyl group (COOH) content, the difference between (V2 and V1) was multiplied by the molarity of the titrant, and this value was divided by the pulp dry weight (0.5 gm).

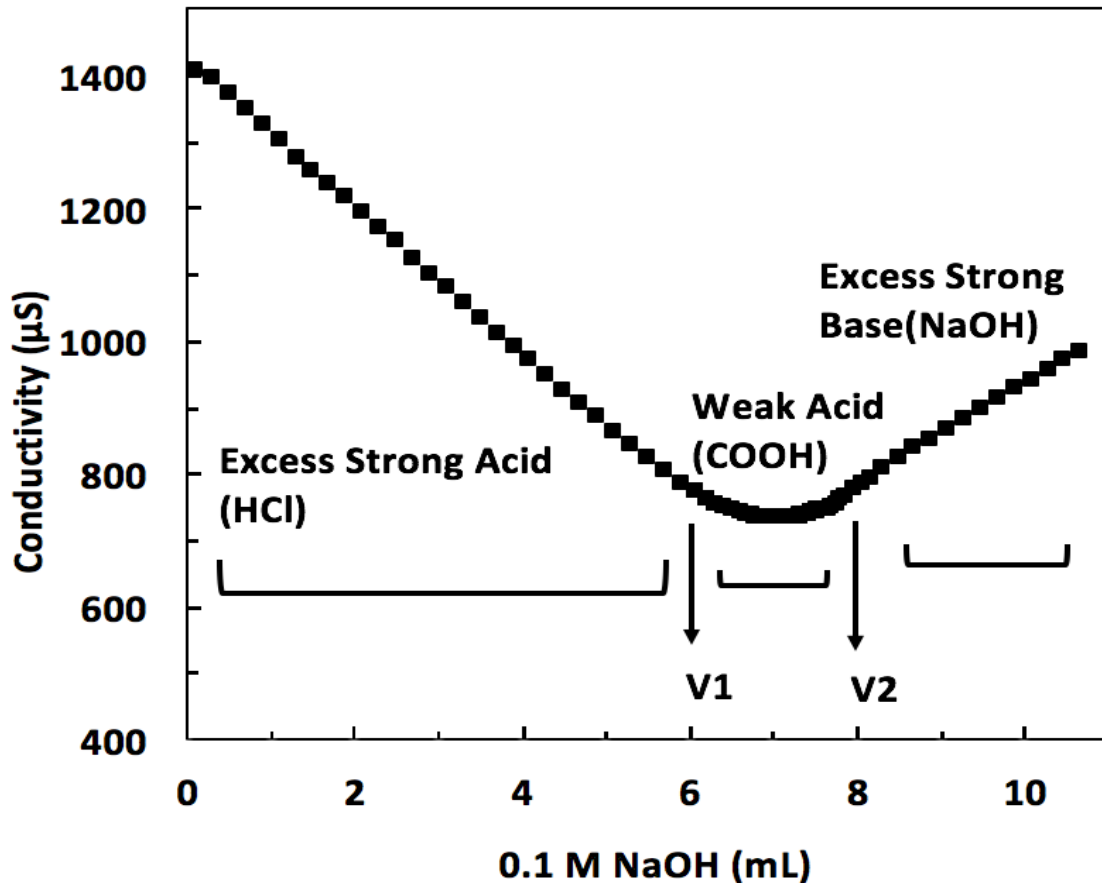


Figure 20. Conductometric titration of carboxyl groups on TOP at 2 g/L pulp density. Weak acid group content in this example = 273.5 $\mu\text{mol/g}$.

An additional amount of oxidant and extended oxidation time are required for a complete TEMPO oxidation, thus increasing the carboxyl group content²⁵. In Table 5, the carboxyl group content of the TOP increased from 207.1 $\mu\text{mol/g}$ to 273.5 $\mu\text{mol/g}$ after increasing the amount of NaClO oxidant from 0.70 mmol/g-pulp to 2.68 mmol/g-pulp. In Figure 10 from the literature, 0% added NaClO on dry pulp corresponds to the carboxyl group content of unmodified pulp fibers (**UnP**), which is 50 $\mu\text{mol/g}$. In Figure 10, 5.2% (0.70 mmol/g-pulp of NaClO) and 20% (2.68 mmol/g-pulp of NaClO) NaClO on dry pulp corresponds to

the carboxyl group content of 150 and 450 $\mu\text{mol/g-pulp}$ respectively. The values in Table 5 can be compared to the values in the literature from Figure 10. However, in Figure 10 the reaction took two hours for completion as opposed to one hour (maximum) in Table 5; thus, the carboxyl group content at 20% NaClO in Figure 10 (450 $\mu\text{mol/g-pulp}$) is higher than the value reflected in Table 5 (273.4 $\mu\text{mol/g-pulp}$). Hence, it was concluded that different reaction times and oxidant amount combinations will deliver different carboxyl group contents.

Table 5. Carboxyl group content (determined using conductometric titration) of UnP and TOP pulp.

Pulp	Carboxyl Group Content ($\mu\text{mol/g-pulp}$)
UnP	45.5
30 minutes TOP with 0.70 mmol/g-pulp of NaClO	152.7
60 minutes TOP with 0.70 mmol/g-pulp of NaClO	207.1
60 minutes TOP with 2.68 mmol/g-pulp of NaClO	273.4

4.2 Impact of PVAm Molecular Weight and Nitrogen Content on PVAm Adsorption onto Pulp

Table 6 summarizes the nitrogen content of the PVAm solutions used in this work at pH 7 and pH 2 (procedure in section 3.3.4). From Figure 12, PVAm is $\sim 95\%$ ionized at pH 2 and

is ~75% ionized at pH 7; hence, the nitrogen content at pH 2 is higher. The three PVAm solutions had relatively close nitrogen content values at pH 7. In a previous work, the charge density of Lupamin® 4595 was detected to be 8.0 mmol/g using conductometric titration ¹⁶, which is relatively close to the values presented in Table 6.

Table 6. PVAm nitrogen content from polyelectrolyte titration. The error reported on the figures and tables in this thesis presents the standard deviation of the average of 2-4 replicates at identical conditions to represent the variability between the samples.

PVAm	Nitrogen Content at pH 7 (mmol/g)	Nitrogen Content at pH 2 (mmol/g)
P15	8.95 ± 0.01	10.62 ± 0.02
P45	10.03 ± 0.05	12.17 ± 0.02
PH45	9.54 ± 0.19	15.82 ± 0.03

In Figure 21, P15, P45, and PH45 PVAm adsorption isotherms were performed on oxidized (carboxyl group content of 207.1 $\mu\text{mol/g-pulp}$) and unoxidized fibers (procedure in section 3.3.5). A sample calculation of PVAm loading on pulps can be found in Appendix A.1. P15 showed the highest adsorption on UnP and TOP fibers. The nitrogen content and the molecular weight of PVAm influence the adsorption on pulps. From Table 6, P15 nitrogen content was less than P45 and PH45 nitrogen content, and PH45 nitrogen content was less than P45 nitrogen content. Thus, P15 and P45 had slightly higher adsorption onto the pulp surface than PH45 had. Also, the low molecular weight of P15 (15 kDa) influences the

adsorption on the modified fibers by accessing the small pores that high molecular weight polymers cannot access.

Low charge density cationic polyacrylamides have also shown more adsorption onto the cellulose fibers than high charge density polyacrylamides. Low charge density molecules have extended configuration, and high charge density have flat configuration. Low charged density molecules are coiled in solution, and when adsorbed on the surface, some parts will remain in loops and tails. Thus, low charge density molecules have more tendency to be adsorbed onto cellulose surfaces²⁶.

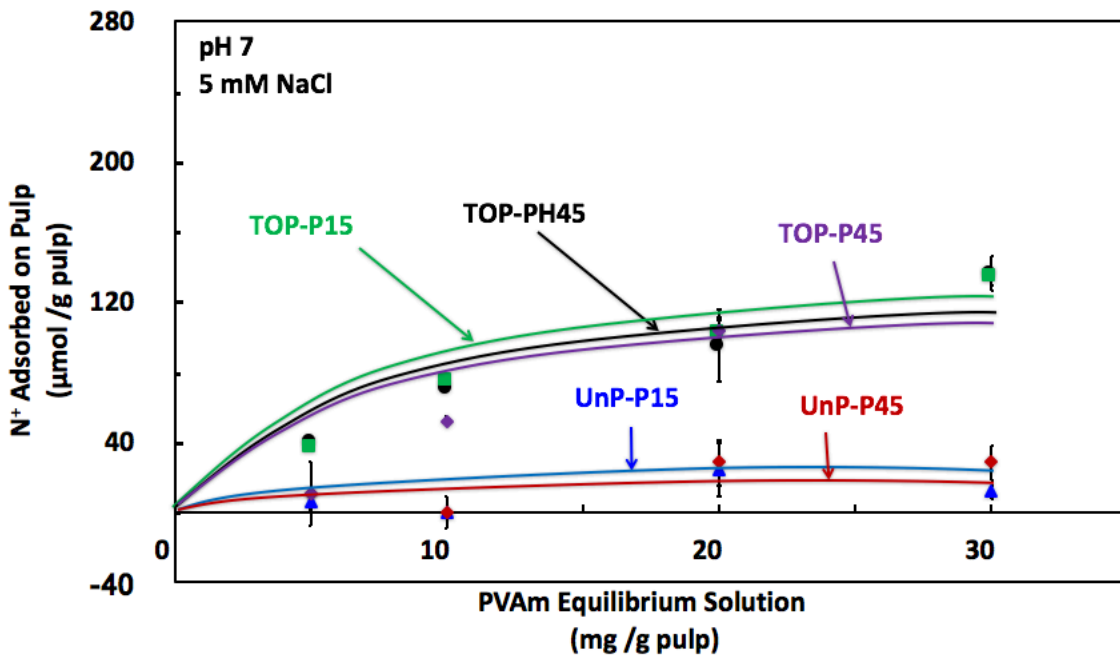


Figure 21. TOP pulp (207.1 $\mu\text{mol/g}$ of carboxyl group content) and UnP pulp (45.5 $\mu\text{mol/g}$ of carboxyl group content) adsorption capacity of P15, P45, and PH45 PVAm.

4.3 Impact of Carboxyl Group Content on PVAm Adsorption onto Pulp

PVAm adsorption onto pulp fibers increases with the carboxyl group content of pulp fibers¹⁸. The PVAm adsorption curves plateaued at 15-20 mg/g-pulp of PVAm content (Figure 21); hence, 20 mg/g-pulp of PVAm content was used to treat UnP and TOP for this study to test for the impact of carboxyl group content on fibers' PVAm adsorption (Figure 22). Once again, the PVAm treatment was done at 5 g/L pulp density, pH 7, and under 15 minutes of constant stirring.

The carboxyl groups on pulp fibers provide an anionic surface that can adsorb amine cationic groups of PVAm. PH45 adsorption didn't increase with increasing the carboxyl content of TOP from 152.7 to 273.4 $\mu\text{mol/g-pulp}$. Only a small fraction of the carboxyl groups is on the exterior surface of the fibers and is accessible to the polymers. In a previous work done by a colleague in our labs, the surface charge density (determined by Mutek PCD T3 titrator) of TEMPO oxidized SSBK pulp for 60 minutes and 0.70 mmol/g-pulp of NaClO was equal to 40 $\mu\text{mol/g-pulp}$ ²⁷.

The PH45 adsorption isotherm plateaued at 59.0 - 69.0 $\mu\text{mol/g-pulp}$ of PH45 content. UnP pulp had ~ 6 folds less carboxyl group content than TOP pulp; thus, UnP pulp had shown less PVAm adsorption in Figure 22. PVAm retention ratio increased from 20.0 $\mu\text{mol/g-pulp}$ to ~ 69.0 $\mu\text{mol/g-pulp}$ by TEMPO oxidizing the original fibers.

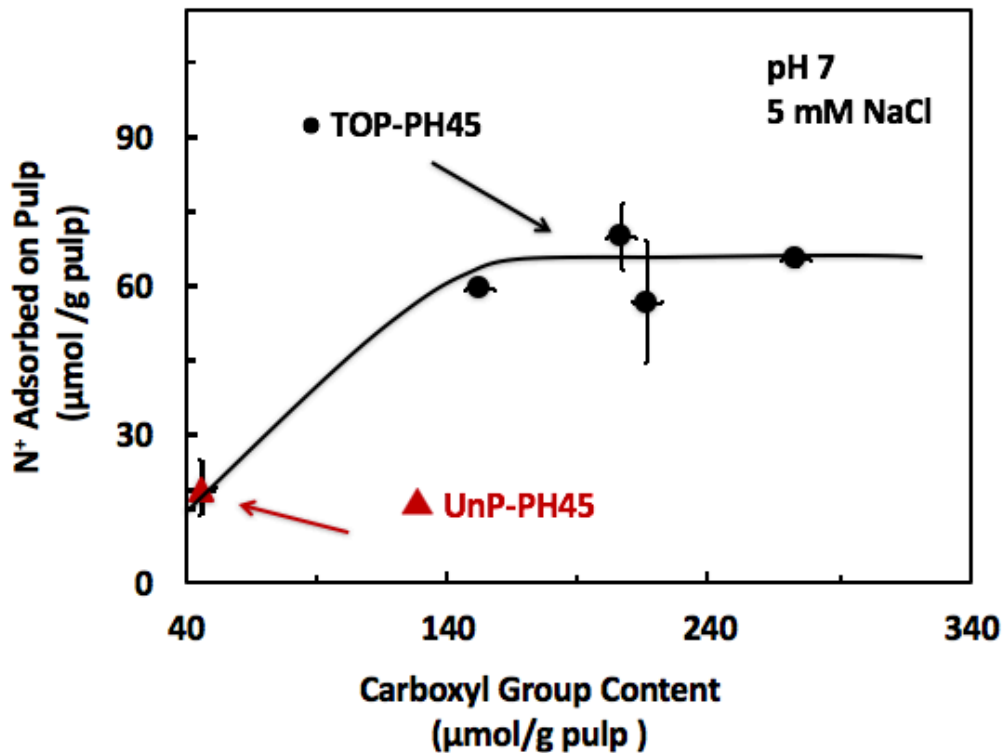


Figure 22. Adsorption capacity of PH45 on UnP and on TOP (with different carboxyl group content).

These results can be compared to the results in Figure 15 in the literature review chapter. In Figure 15, unoxidized and TEMPO oxidized softwood bleached pulp corresponded to PVAm adsorption of 2.5 and 8.0 mg PVAm/g fibers, respectively¹⁸. From Figure 22, 20.0 µmol/g-pulp and 65.0 µmol/g-pulp (unoxidized and TEMPO oxidized PVAm adsorption capacity, respectively) corresponded to 2.0 and 7.2 mg/g-pulp of PVAm content.

4.4 Phosphorus Absorbance and Calibration Curves

Spectrophotometric method (procedure in section 3.4.1) was used to determine the concentration of phosphorus (P) solutions and of pulp suspension filtrates after dispersing the pulp in P solutions. By determining the initial and final concentration of P suspension solutions, the difference was used to quantify the amount of adsorbed P on pulp.

The spectrophotometer absorbance curves of KH_2PO_4 standardized P solutions appeared to have two peaks; however, the (880-900 nm) peaks shifted to the left with more concentrated phosphate solutions (Figure 23). Thus, the (700-715 nm) peaks were of interest because they were stable at all of the P concentrations tested. The calibration curves were done for 700, 710, and 715 nm peaks for 10, 20, 30, 40, 50, and 60 minutes of colorimetric reagent reduction time. 715 nm peak at 50 minutes of reagent reduction time showed the best linearity and was used for the calibration curve (Figure 24).

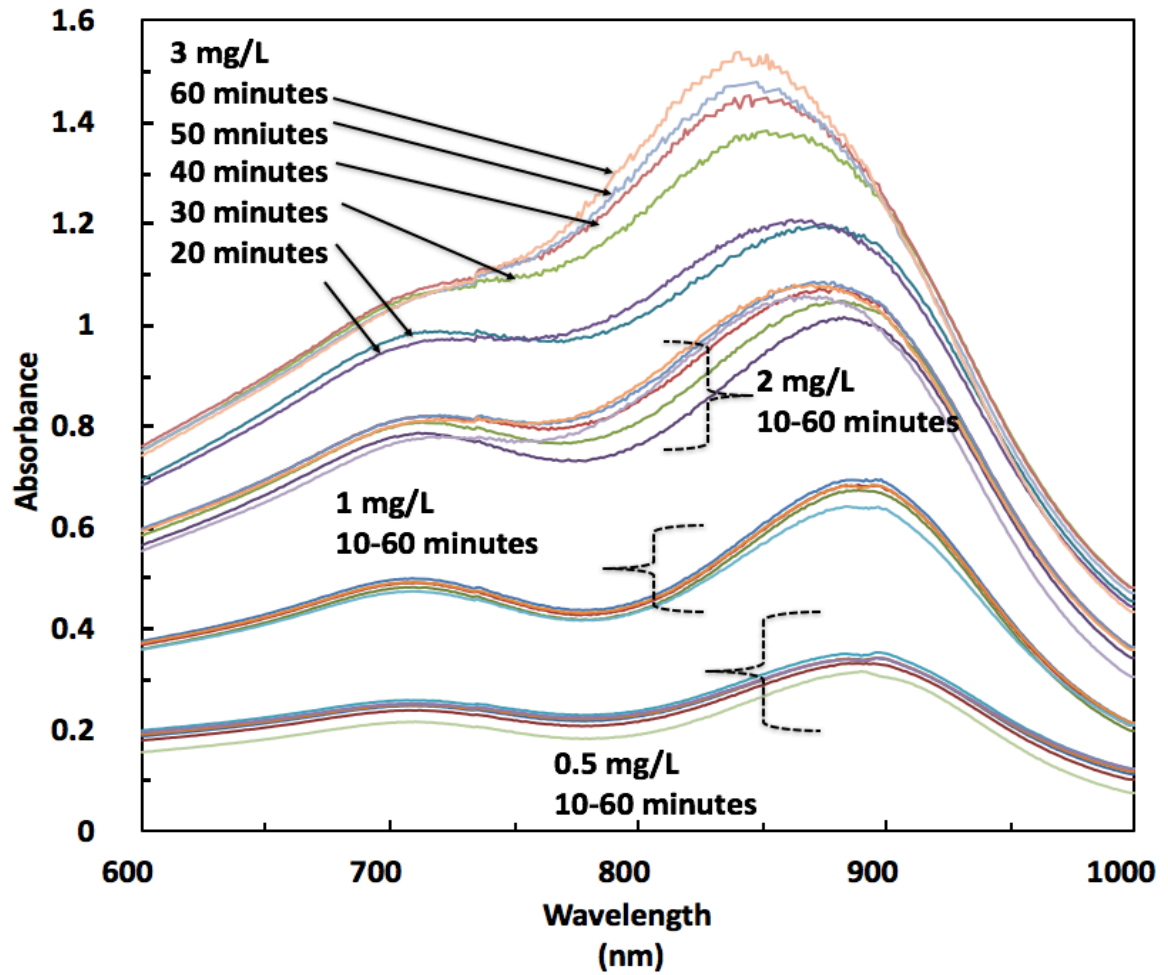


Figure 23. Spectrophotometer absorbance curves for standardized phosphorus solutions after mixing with colometric test method reagent for different periods of reduction time.

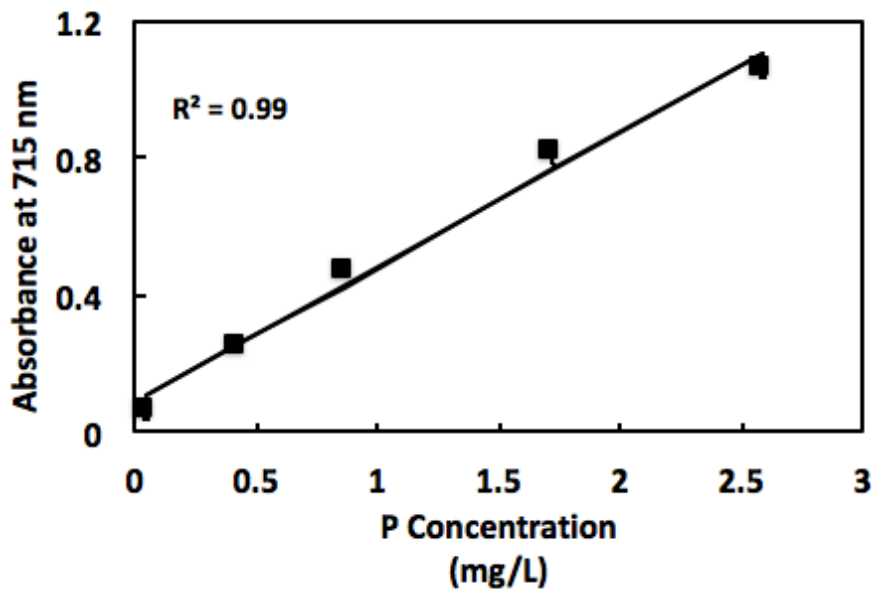


Figure 24. Phosphorus calibration curve. Absorbance measured at 715 nm fixed wavelength after 50 minutes of reduction time with the colorimetric test method reagent.

4.5 Phosphate Adsorption onto Pulp

4.5.1 Impact of Suspension Time on Phosphate Adsorption onto Pulp

In this section, oxidized pulp samples treated with PVAm were dispersed in 2 mg P/L solutions for different periods of agitation time (1 - 4 hours). This experiment was done to determine whether increasing the suspension time will increase phosphate adsorption or not. From Figure 25, one hour suspension time provided same phosphate adsorption as 2, 3, and 4 hours, and hence one hour was used in further experiments in this work.

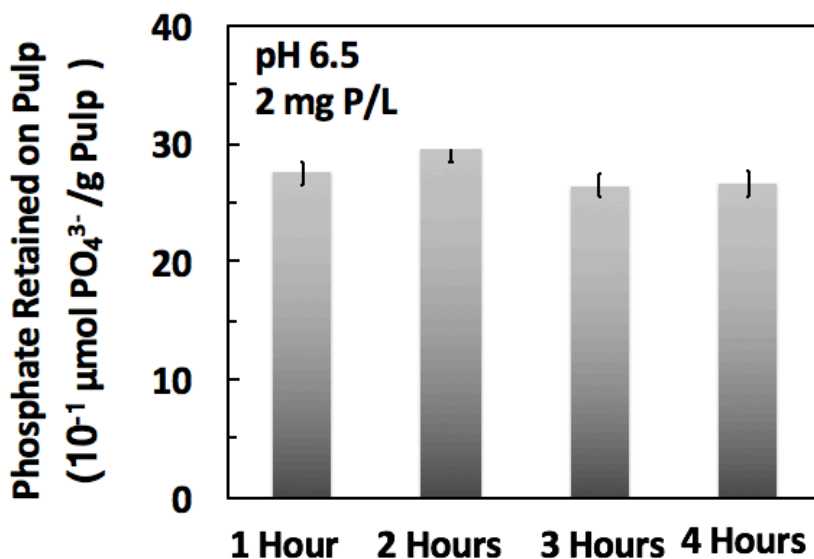


Figure 25. Effect of suspension time on phosphate adsorption. TOP-P45 pulp samples are suspended in 2 mg P/L solution.

4.5.2 Impact of pH on Phosphate Adsorption onto Pulp

The ionization behaviour of PVAm and H_3PO_4 changes with the pH (Figure 4 & 12), which affects the phosphate adsorption onto PVAm amine groups on pulp fibers. Thus, phosphate adsorption onto cationic pulp fibers was tested out under different pH ranges (Figure 26) to determine the optimum pH for phosphate adsorption.

TOP pulp samples were first treated with PVAm. H_3PO_4 was the phosphate source used to adsorb phosphates on cationic pulp surface (procedure in section 3.4.2). The optimum pH range for maximum phosphorus adsorption was found to be 5.0-6.5.

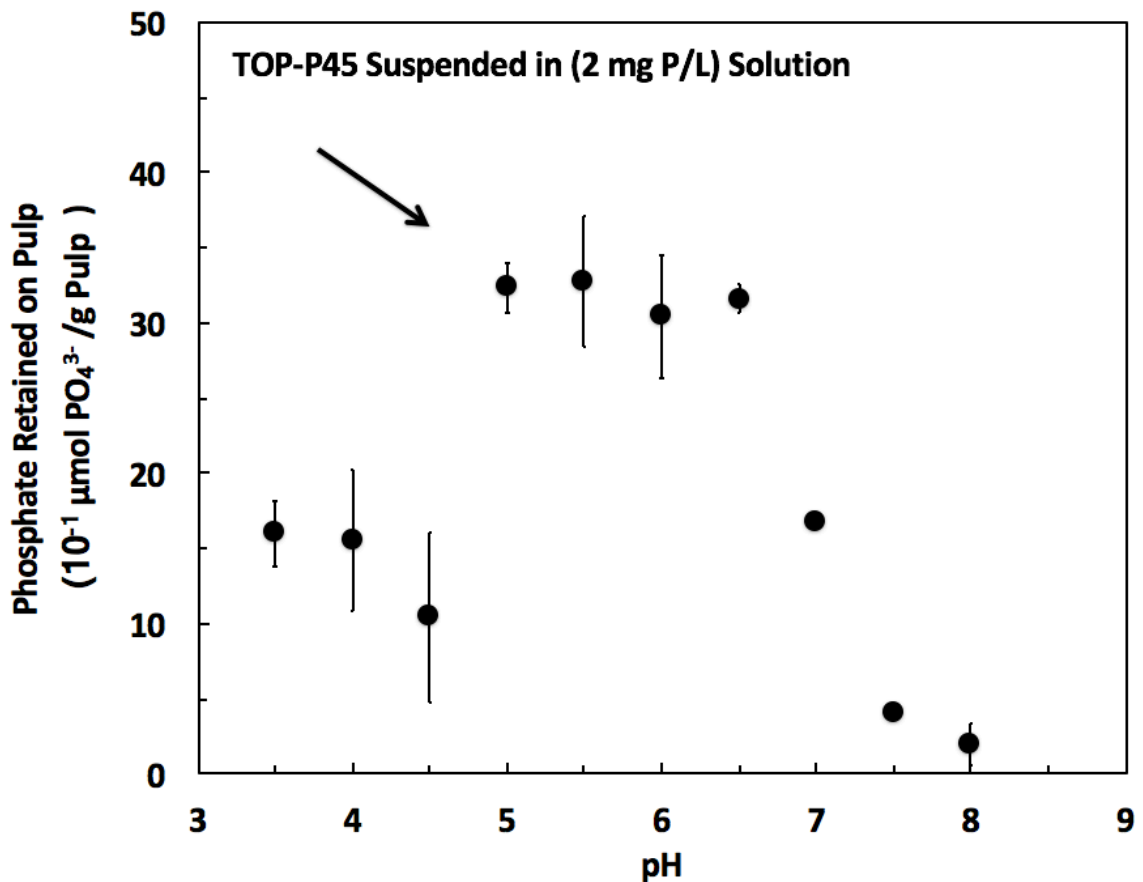


Figure 26. Effect of pH on phosphate adsorption on TOP-P45 suspended in 2 mg P/L solution.

It was concluded from the experiments performed that phosphate adsorption on pulp depends on : the degree of ionization of PVAm, the present phosphate group in the solution, and the solution ionic strength. At pH range of 3.5 - 4.0, small fraction of phosphoric acid is not dissociated leading to less adsorption on the amine groups that are almost completely ionized at this pH range; see Table 7. At pH range of 7.0 - 8.0, all of the phosphoric acid is dissociated, but some of the amine groups are deprotonated i.e. less positive charge on pulp surface to capture the phosphate groups. At the intermediate pH range of 5.0 - 6.5, the

phosphoric acid is fully dissociated and the amine groups are relatively more ionized than in pH range of 7.0 - 8.0. This makes pH range of 5.0 - 6.5 the best for phosphates adsorption on PVAm coated pulp.

The highest phosphorus availability for plants occur at pH 5.5 - 7.0 (Figure 3)⁴; thus, the adsorption of phosphates on pulp was done under pH of 5.6 - 6.5 for the rest of the experiments.

Table 7. Correlation among PVAm degree of ionization (DoI), phosphoric acid fractional composition (FC), and phosphate adsorption on pulp. DoI and FC values are obtained from Figure 2 and 23^{5 13}.

pH	PVAm Degree of Ionization	Phosphoric Acid Fractional Composition	Phosphate Adsorption (10^{-1} $\mu\text{mol/g-pulp}$)
3.5	0.95	0.9 H_2PO_4^- , 0.1 H_3PO_4	16.0
4	0.90	1 H_2PO_4^- , 0.0 H_3PO_4	9.6
5	0.80	1 H_2PO_4^- , 0.0 H_3PO_4	32.0
6.5	0.75	0.9 H_2PO_4^- , 0.1 HPO_4^{2-}	35.2
7	0.7	0.5 H_2PO_4^- , 0.5 HPO_4^{2-}	16.0
8.0	0.55	0.1 H_2PO_4^- , 0.9 HPO_4^{2-}	3.2

Similar trend was shown in a previous work to adsorb nitrates on cationic cellulose nanofibers; in which, the highest adsorption of nitrates took place at pH 5.0 – 7.0 (Figure 17). The trend in Figure 17 was justified by presuming the adsorption of chloride anions at low pH on the nanofibers cationic sites and the screening effect of sodium cations at high pH²⁰.

4.5.3 Phosphate Loading on Unoxidized and Oxidized Fibers

In Figure 27, Phosphate adsorption isotherms were performed on UnP and TOP (treated and untreated with PVAm) with pH controlled at 5.6, optimum pH for plant to uptake phosphorus from soil (Figure 3) and maximum phosphate adsorption on PVAm treated fibers (Figure 26).

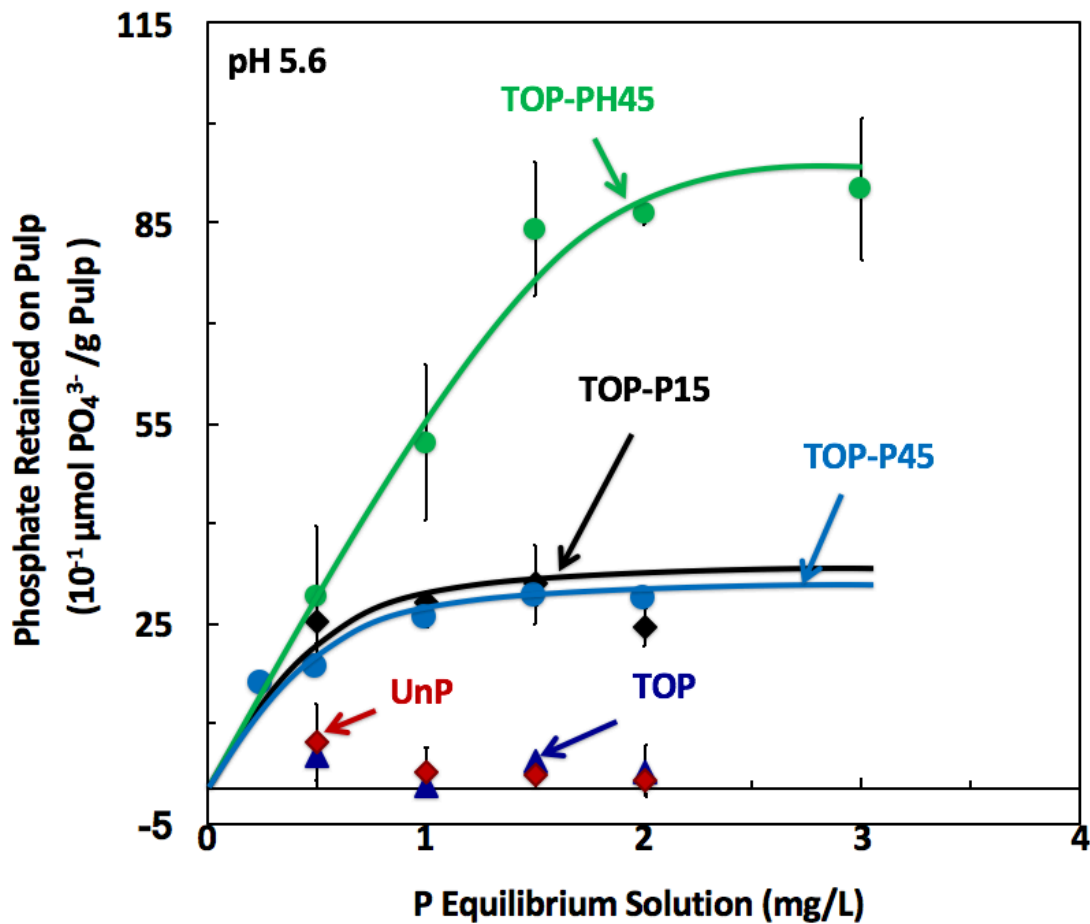


Figure 27. Phosphate adsorption isotherms of UnP, TOP, TOP-P15, TOP-P45, and TOP-PH45.

From Figure 27, pulps untreated with PVAm (UnP and TOP) showed no phosphate adsorption at all on pulp surface. The three PVAm used in Figure 27 showed different phosphate adsorption. From Table 6, P15 and P45 had relatively lower charge density than PH45 had. Low charge density molecules are coiled in solution, and when adsorbed on the pulp surface, some parts will remain in loops and tails. On the other hand, higher charge density molecules have a flat configuration²⁶. The flat configuration of PVAm adsorbed on pulp surface is expected to increase the phosphate adsorption by exposing more cationic groups to the phosphates, thus increasing the adsorption of the negatively charged phosphates.

4.5.4 Correlation among PVAm Content, Carboxyl Group Content, and Phosphate Adsorption

The Oxidized pulp (with different carboxyl group content) and unoxidized pulp both treated with PVAm were tested out for phosphate adsorption to determine the effect of carboxyl groups on phosphate adsorption (Figure 28). Even though increasing the carboxyl group content of the TOP increased the PVAm adsorption onto the pulp surface by inducing electrostatic attraction force, high carboxyl group content ($> 250 \mu\text{mol/g-pulp}$) decreased the phosphate adsorption onto PVAm cationic sites available on the TOP. The negative charge of the carboxyl groups induced repulsion force against the phosphate groups leading to less phosphate adsorption. The phosphate retained on the PVAm cationic sites, adsorbed on the pulp, decreased linearly with the pulp carboxyl group content. This linear relationship is expressed on Figure 28.

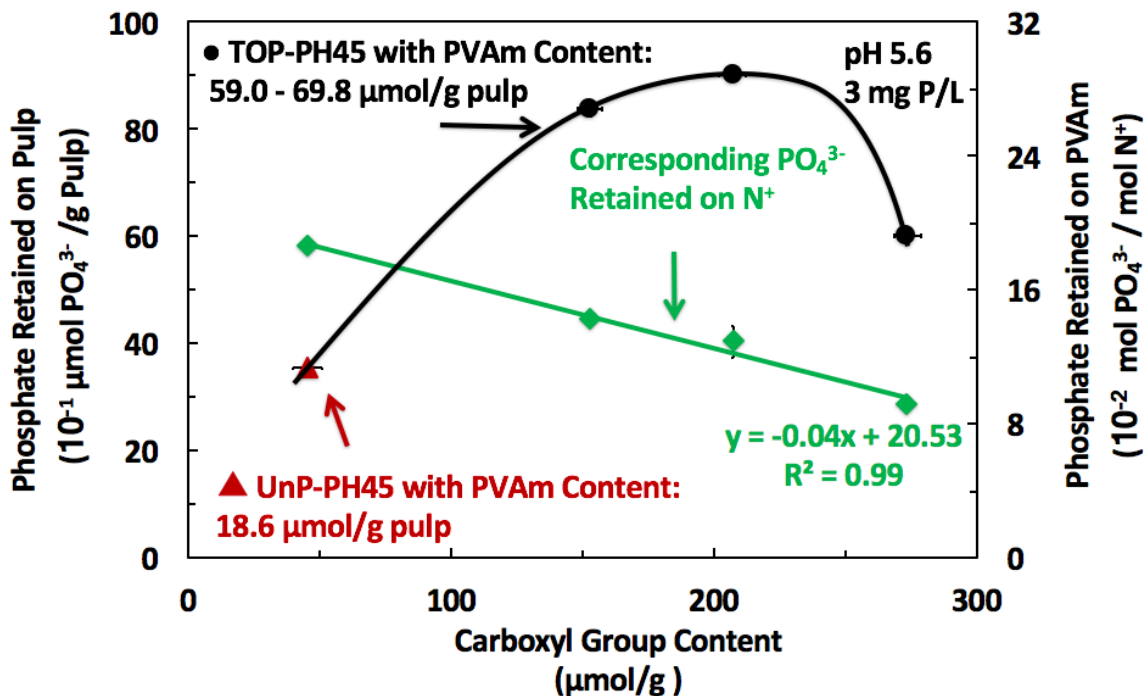


Figure 28. Adsorption of phosphates onto PH45 cationic sites adsorbed on UnP and on TOP. Pulp samples were treated with 3 mg P/L solution.

Even though the oxidized pulp was coated with one layer of PVAm, it still had very low phosphate binding capacity of 0.08 – 0.20 mol PO₄³⁻/mol N⁺ which corresponds to 1.36 mmol PO₄³⁻/g-PVAm – 1.78 mmol PO₄³⁻/g-PVAm.

In a previous patented work, anion exchange polymers were used to improve phosphate binding properties. The invention features a method of removing phosphate from the patients' stomachs by ion exchange. The invention provides an effective treatment for decreasing the serum level of phosphate by binding phosphate in the gastrointestinal tract without increasing the absorption of clinically undesirable materials. PVAm anion exchange resin was stirred in 10-20 mM phosphate solution, 80 mM NaCl, 30 mM Na₂CO₃

solution for three hours at pH 7. These conditions mimic the small intestine conditions. The resulting binding capacity of PVAm was 0.4 mmol PO_4^{3-} /g-PVAm²⁸. These results confirm that PVAm has low phosphate binding capacity.

4.6 Phosphorus Release Study

4.6.1 Phosphates' Release from Modified Pulp

After determining the optimum carboxyl group content and maximum PVAm adsorption of TOP pulp for phosphate adsorption, TOP-PH45 samples loaded with phosphates were suspended in different background media with the pH controlled to test for the effect of pH and competitive ions on the release of phosphates from PVAm coated pulp fibers.

TOP-PH45 loaded with phosphates were dispersed in each background media at 5 g/L pulp density in centrifuge tubes for ~ 2 hours under constant agitation. Diluted HCl/NaOH solutions were used to control the pH of the pulp samples.

Figure 29 presents the release behaviour of the pulp samples in the background media. All of the phosphate groups were released when the pulp samples were suspended in a 5 mM NaCl solution and in a 0.05% (w/v) MES buffer (combined with plant nutrients mentioned in section 3.5) under all of the pH ranges.

In a previous work, amine anion-exchange column (in its chloride form) showed the following sequence of relative affinities for some anions : hydroxide > sulfate > phosphate > chloride²⁹. For an efficient ion exchange, the affinity of the ions need to be removed from the solution should be higher than the ions in the resin. In another study, NaCl salt

was used to leach phosphates from soil samples, and the results showed that NaCl compared to other salts (like potassium chloride (KCl) and calcium chloride (CaCl₂)) leached the least amount of phosphates. The results showed that for chloride salts the effect of leaching phosphates into the soil solutions follows this sequence $\text{Ca} > \text{K} > \text{Na}$ ³⁰.

From these two studies, it can be concluded that the phosphates released in the MES buffer was because of MES's sulfonic groups replacing all of the adsorbed phosphates on the PVAm coated pulp since sulfonic groups have higher affinity towards amines than phosphate groups have²⁹. Also, 5 mM NaCl salt background media is capable of leaching phosphates off the pulp loaded with phosphates especially that the amount of phosphates bound to the pulp was very small.

The plant nutrients media without buffer background had shown the least phosphate release around pH range 5.5 – 8.0. Since dilute HCl/NaOH solutions were used to control the pH, the presence of other ions like Cl⁻, OH⁻, and Na⁺ could have desorbed the bound phosphates from the pulp or replaced it at acidic and basic pH ranges.

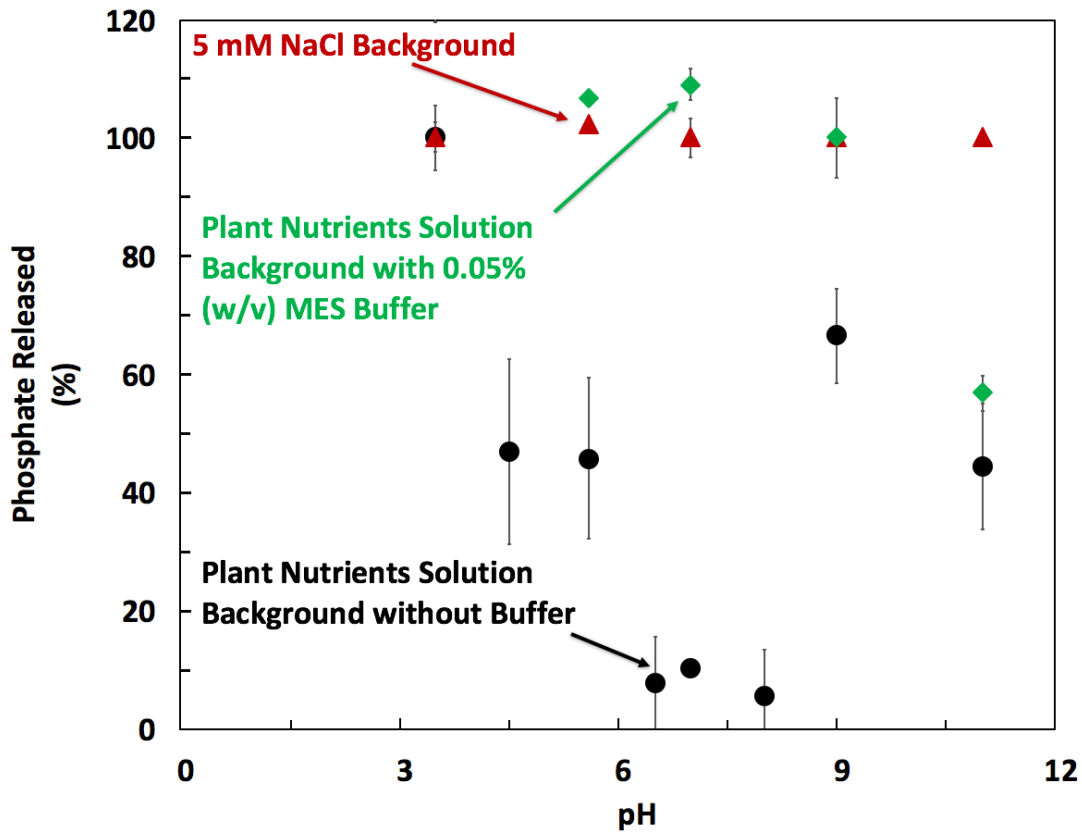


Figure 29. Release of phosphates adsorbed on TOP-PH45. Each sample had $70.2 \times 10^{-1} \mu\text{mol/g-pulp}$ phosphates.

4.6.2 Effect of Phosphate Loaded Amount on Phosphate Released Amount

The next part of the release study was testing the phosphates release from pulp samples initially loaded with different amounts of phosphates. Table 8 compares the phosphate released amount from pulp loaded with $70.2 \times 10^{-1} \mu\text{mol/g-pulp}$ and with $13.1 \mu\text{mol/g-pulp}$. The pulp samples loaded with more phosphates released more phosphates. It can be concluded from Table 9 that the initial phosphates loaded on pulp, the pH of the suspension solution, and the ionic strength of the background media are important factors in determining how much phosphates will be released from the fibers.

Table 8. Comparison between the fraction of phosphates released from TOP-PH45 loaded with different amount of phosphates. Pulp samples were mixed with plant nutrients solution at pulp density of 5 g/L for 1.5-2.0 hours.

pH	PO ₄ ³⁻ released from pulp loaded with 70.2 x 10 ¹ μmol PO ₄ ³⁻ /g-pulp	PO ₄ ³⁻ released from pulp loaded with 13.1 μmol PO ₄ ³⁻ /g-pulp
3.5	100.00% ± 2.51	98.00% ± 3.46
5.6	43.93% ± 16.27	63.47% ± 6.33
6.5	7.59% ± 8.03	30.41% ± 0.760
7	10.11% ± 0.11	33.13% ± 4.40
11	44.25% ± 10.71	81.58% ± 0.81

4.6.3 Nutrients Interference with Phosphate Spectrophotometric Assay

Having determined the pH range and the suitable background for the least release of phosphates, it was important to examine two things: 1- interference of other ions with phosphate assay 2- the buffering capacity of the pulp samples.

To determine the interference of other ions in the plant nutrients media with the phosphate assay, three phosphates media with the same phosphate concentration were prepared at acidic, neutral, and basic conditions and tested for the phosphate concentration using the spectrophotometric phosphate assay method. Dilute HCl/NaOH solutions were used to control the pH. Table 9 presents the absorbance of the three phosphate solutions and their corresponding concentration.

The results showed that the three solutions had the same concentration ~ 3 mg/L i.e. none of the ions in the plant nutrients solution interfered with the phosphate assay. The results

in Table are within $\pm 7\%$ percent error, and this source of error could be due to the usage of dilute HCl/NaOH solutions to control the pH which slightly affected the phosphate concentration.

Table 9. Absorbance of 3 mg P/L solution combined with plant nutrients at different pH ranges.

pH	Absorbance	Corresponding Concentration (mg P/L)
3.8	1.031 \pm 0.003	2.785 \pm 0.010
7	1.028 \pm 0.001	2.776 \pm 0.002
10	1.026 \pm 0.005	2.757 \pm 0.013

4.6.4 Buffering Capacity of TEMPO Oxidized Pulp Treated with PVAm

Maintaining the pH of TOP samples treated with PVAm at pH range 5.6 - 7.0 (best pH range for plants to uptake phosphates) using MES buffering agent (pKa 6.16) released all of the phosphate groups (Figure 29). However, TOP-PVAm samples showed buffering capacity around different pH regions (Figure 30).

In Figure 30, the carboxyl group content of TOP is 200 $\mu\text{mol/g-pulp}$, and the amine content of PVAm adsorbed on the pulp is 70 $\mu\text{mol/g-pulp}$. Inside the fiber wall pores, there will be little PVAm, and the charged groups will be carboxyl groups. On the fibers' exterior surfaces, underneath adsorbed PVAm, the net charge is positive. Figure 30 showed two pKa values of 3.3 and 9. These pKa values can be compared to amino acids pKa values. Amino acids are organic compounds containing amine and carboxyl functional groups, along with a side chain group specific to each amino acid. Amino acids pKa charts always reflect two pKa values that fall within these ranges: 1.8-2.35 and 8.9-10.64. It can be

concluded from Figure 30 that TOP coated with PVAm has three buffering regions; however, the region of the isoelectric point (pH range of 5.0-6.5) where the net charge is zero is expected to have the least buffering capacity.

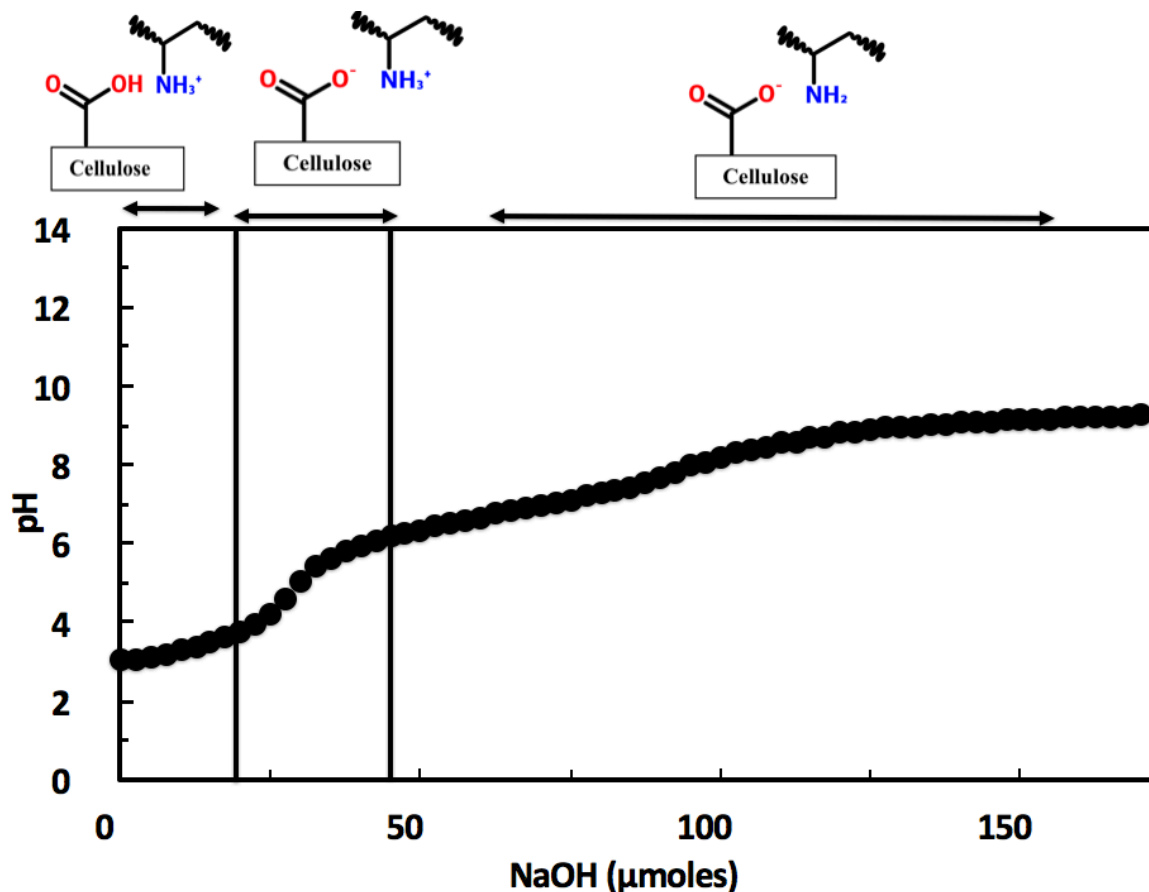


Figure 30. Buffering capacity of TOP coated with PVAm.

4.6.5 Effect of Suspension Time on Phosphate Released Amount

TOP pulp samples treated with PVAm and phosphates were suspended in plant nutrients solution with and without MES buffer for longer periods of time (1-5 days) with constant agitation as shown in Figure 31. The pH of pulp samples was adjusted before the agitation started and was tested after the suspension time. Pulp samples, without MES buffer,

maintained the pH around 6.0 - 6.5, for the five days. The pH was kept at this range because TOP coated with PVAm had good buffering capacity at pH range of 6.0 - 7.5 (Figure 30). The pH of pulp samples with MES/plant nutrients solution was adjusted to pH 5.6 - 6.0, suitable pH for plant phosphate uptake.

The results in Figure 31 showed that 1- MES buffer once again released all of the phosphates, and 2- the amount of phosphates released in plant nutrients only solution plateaued at 40% after 2 days of suspension time. Since the results started plateauing after two days, two days of suspension time at pH 6.0 - 6.5 in plant nutrients solution were sufficient to predict the exact amount of phosphates released from pulp fibers loaded with phosphates.

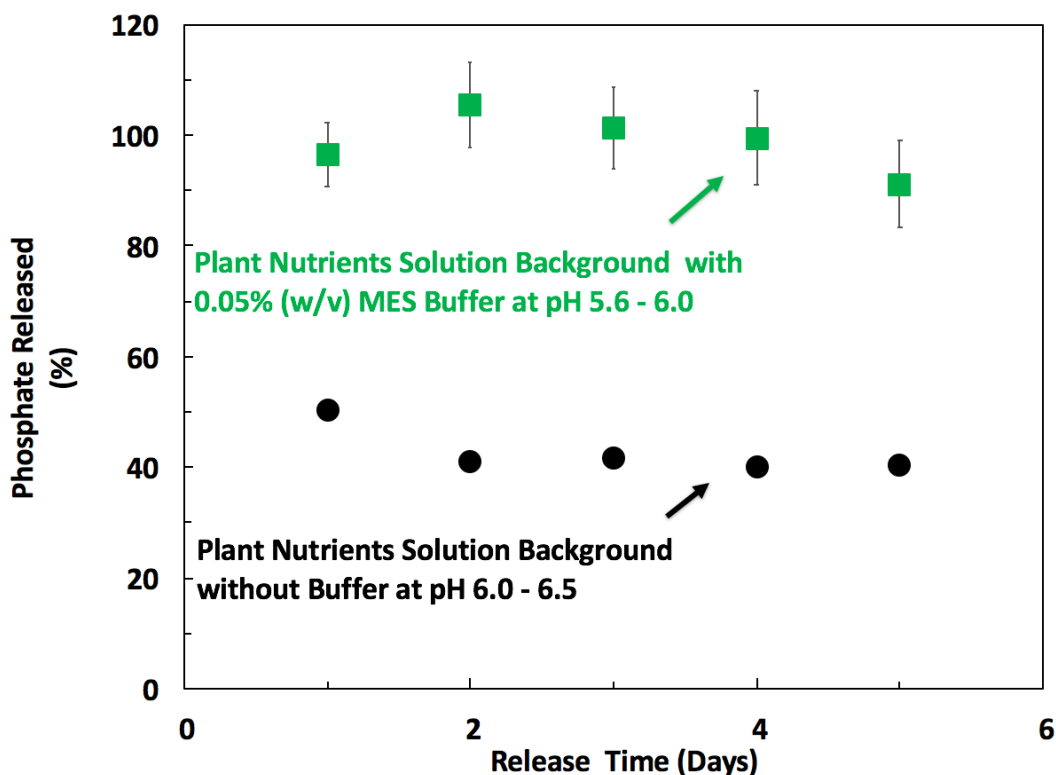


Figure 31. Release of phosphate adsorbed on TOP-PH45. Each sample had $96.9 \times 10^{-1} \mu\text{mol/g-pulp}$ phosphate.

4.7 Arabidopsis Plant Study

Arabidopsis is currently the most studied plant species in plant biology³¹. Its short life cycle and small genome size have rendered it one of the principal model systems. Columbia (Col) species is one of the most popular Arabidopsis accessions. Col has a lot of both molecular and genetic studies and is chosen for the genetic background for the majority of Arabidopsis plants³¹. Col Arabidopsis seedlings have shown classical phenotype of shortened primary roots, proliferation of lateral roots, and noticeable reduced shoot biomass in response to phosphates deficient growing media³². Col Arabidopsis seeds were used in this work to be grown on the modified and unmodified pulp matrices to test for the impact of pulp matrices on the phenotype of cultivated Arabidopsis plants (procedures in section 3.7). Figure 32 is a simple example of Arabidopsis seedlings grown on plant nutrients/pulp matrix.

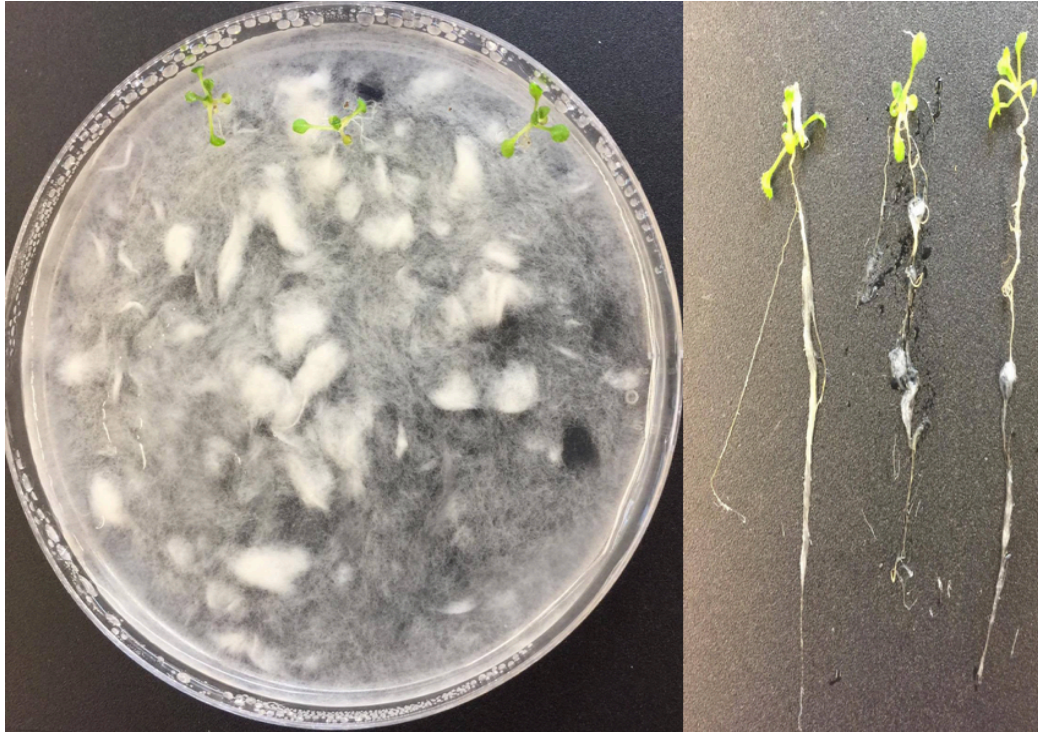


Figure 32. A generic example of Arabidopsis seeds grown on pulp w/ plant nutrients matrix.

4.7.1 Fresh Weight Measurements of Arabidopsis Seedlings

Figures 33-35 present pictures of 10-days old Arabidopsis grown on matrices with Pi (phosphate treatment solution, section 3.7.1) content of: 0, 0.05, and 0.25 mM and with modified/unmodified pulp fibers. Zero Pi content plates, Figure 33, had small and unhealthy-looking leaves when compared to 0.05 and 0.25 mM Pi content plates in Figure 34 & 35.

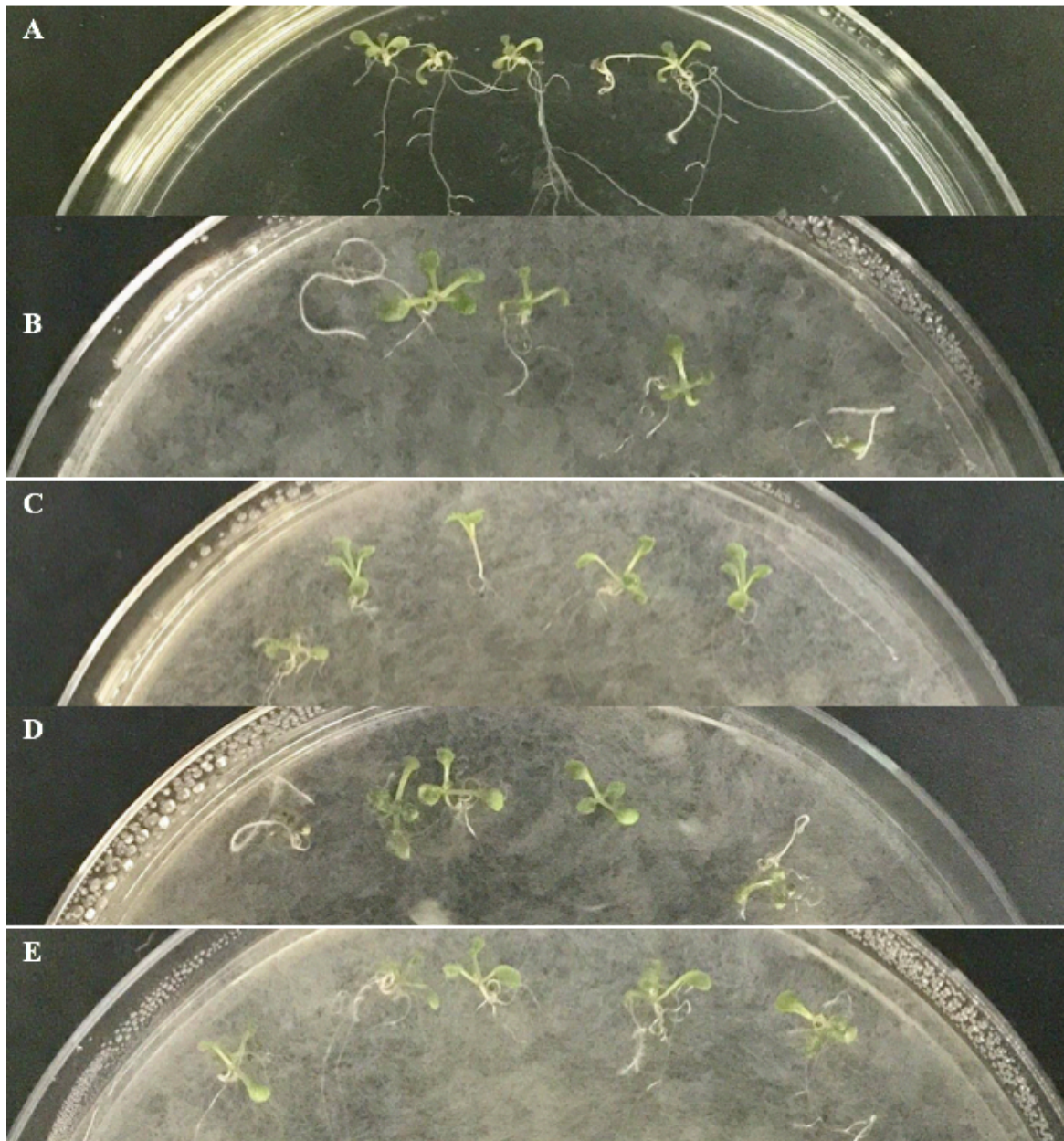


Figure 33. Representative seedlings after 10 days of germination on nutrients media supplemented with 0 mM Pi and:
A- No pulp, B- 0.5% (w/v) UnP, C- 0.5% (w/v) TOP, D- 0.5% (w/v) TOP-PH45, and E- 0.5% (w/v) UnP-PH45.

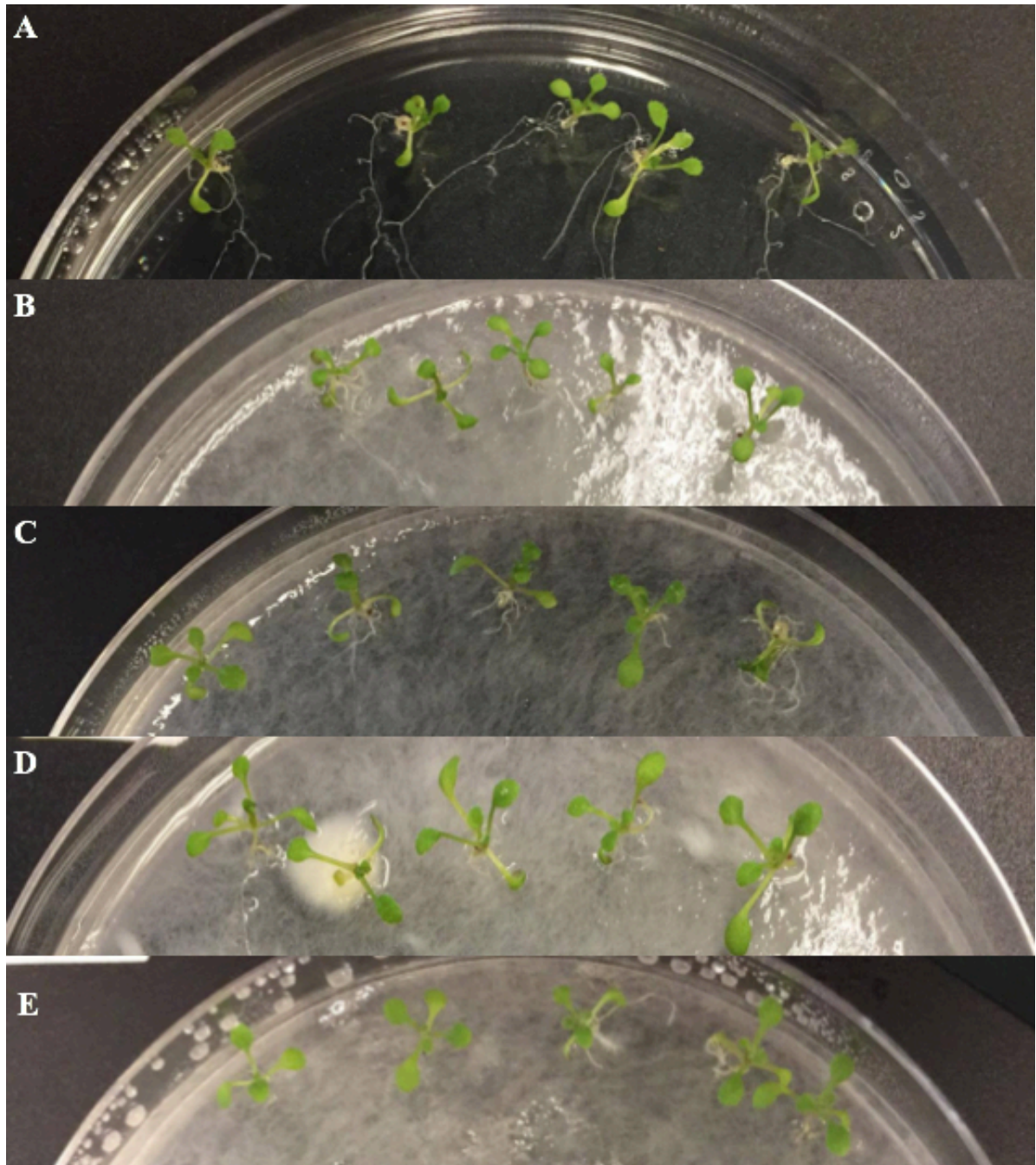


Figure 34. Representative seedlings after 10 days of germination on nutrients media supplemented with 0.05 mM Pi and:
A- No pulp, B- 0.5% (w/v) UnP, C- 0.5% (w/v) TOP, D- 0.5% (w/v) TOP-PH45, and E- 0.5% (w/v) UnP-PH45.

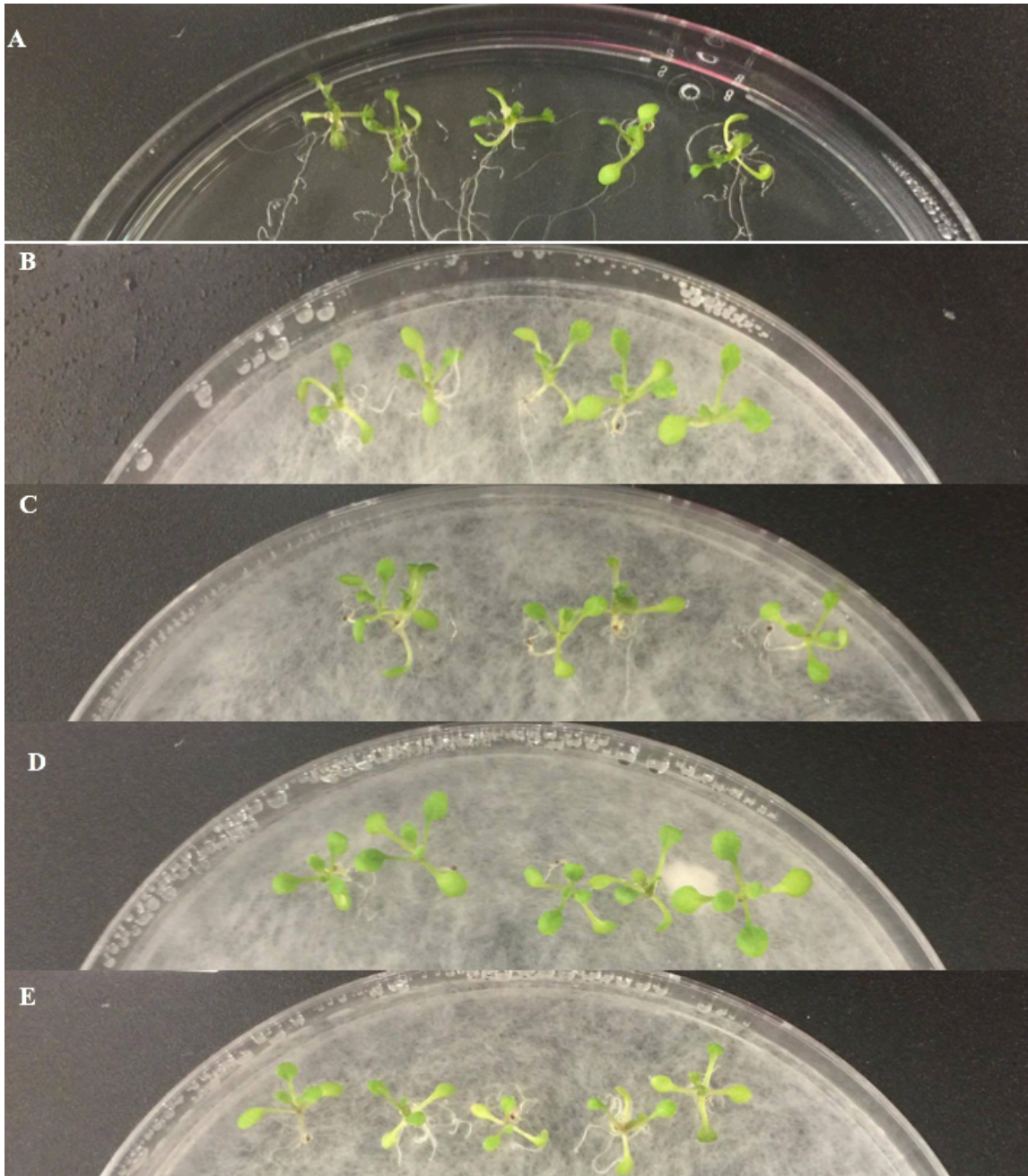


Figure 35. Representative seedlings after 10 days of germination on nutrients media supplemented with 0.25 mM Pi and:
A- No pulp, B- 0.5% (w/v) UnP, C- 0.5% (w/v) TOP, D- 0.5% (w/v) TOP-PH45, and E- 0.5% (w/v) UnP-PH45.

The fresh weight of the 5 seedlings per plate was measured to compare the fresh weight of seedlings cultivated in different matrices as shown in Figure 36. Error bars in Figure 36 represent the standard deviation of the measurements gathered from three plates per matrix; in which each plate had 5 seedlings. TOP-PH45 mixed with plant nutrients matrices had shown the greatest weight per plate. This can also be observed by looking at the D pictures in Figure 33-35.

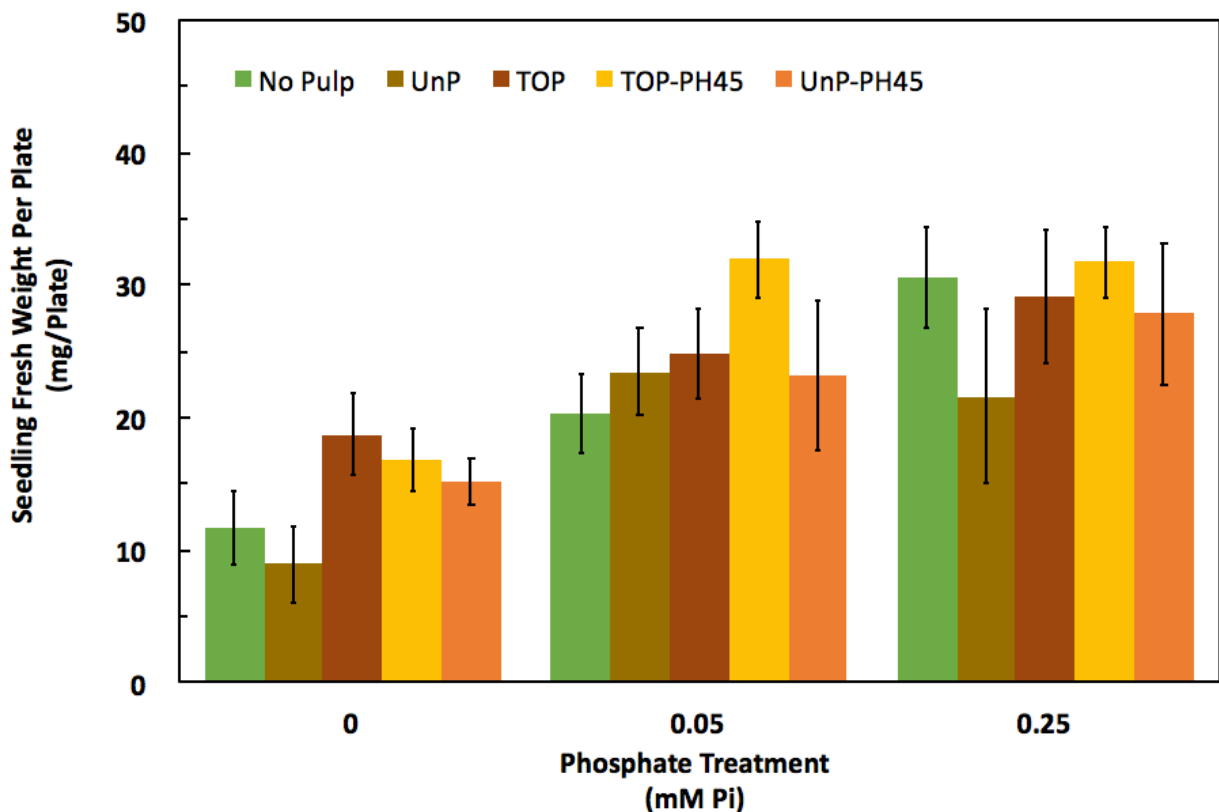


Figure 36. Seedlings fresh weight after 10 days of germination on nutrients media supplemented with 0, 0.05, and 0.25 mM Pi and:
 • No pulp, • 0.5% (w/v) UnP, • 0.5% (w/v) TOP, • 0.5% (w/v) TOP-PH45, and • 0.5% (w/v) UnP-PH45.

Even though at 0 mM Pi content matrices no phosphates were used, phytigel could have had some phosphorus contamination, and this could be why TOP-PH45 with 0 mM Pi

content matrices showed a better growing behavior than the rest of the plates. Also, this could be because the cationic and anionic sites on the treated pulp provided an efficient anion or cation exchange capacity for the other ions in the plant media.

The no pulp and UnP matrices had the least seedling fresh weight per plate, and this could be due to the inert state of the no pulp matrix and of the unmodified fibers and their incapability in storing any of the nutrients. TOP-PH45 had the highest content of cationic sites that were expected to store the phosphates for long periods of time around the roots; thus, plants would be up-taking more phosphates and looking healthier. It is speculated that TOP-PH45 enhances the growth of Arabidopsis plants by storing phosphates on the pulp's surface, exchanging phosphates for ions provided by the roots, and not making them readily available to react or precipitate with other ions in the nutrient solution media. The fresh weights of the plants grown in 0.25 mM matrices were almost the same, and this is because there were plenty of phosphates readily available to the plants.

4.7.2 Primary Root Length of Arabidopsis Seedlings

The primary root length of the seedlings for each plate was measured as shown in Figure 37. Error bars in Figure 37 represent standard deviation of the average length of the 5 seedlings primary roots per 3 plates. All of the pulp matrices showed the same primary root length. The seedlings growing in the no pulp matrices showed a gradual increase in the primary root length with Pi content. It can be concluded that modified and unmodified pulp

matrices created a hindrance to the growth of the roots, and that's why the primary root lengths were almost the same for all of the pulp matrices.

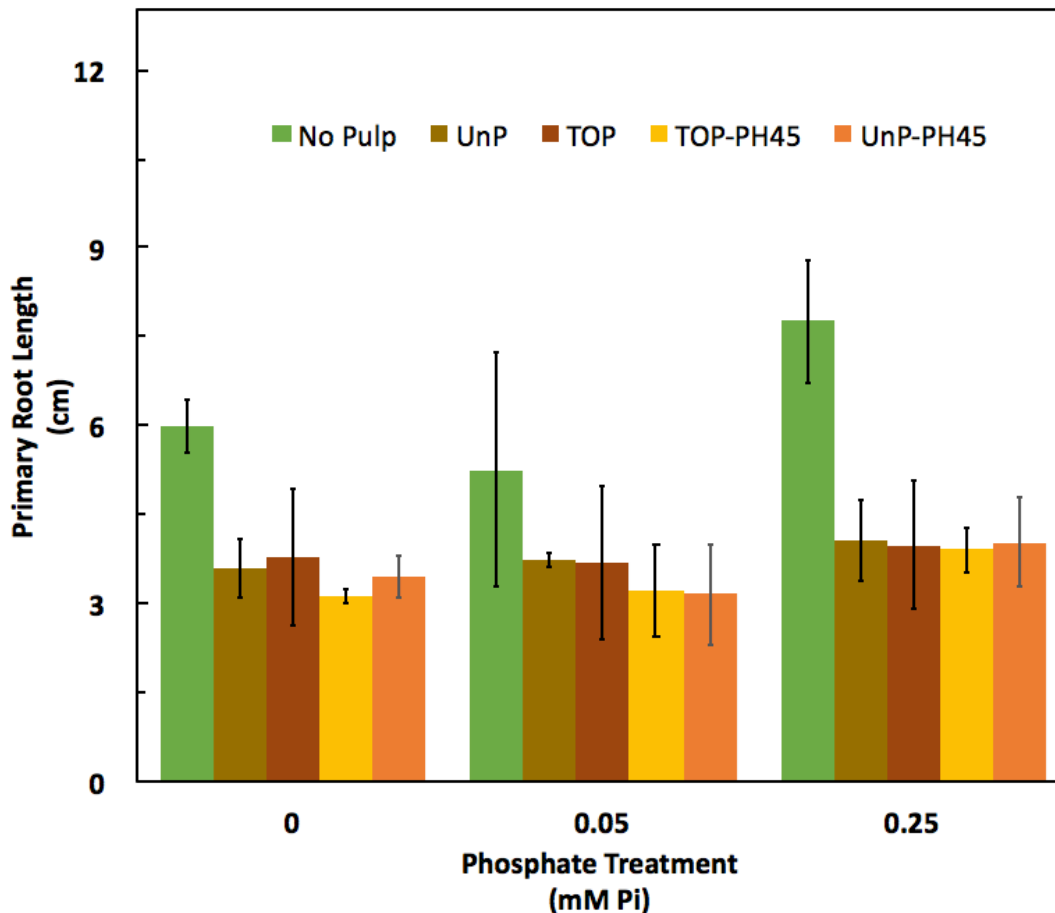


Figure 37. Seedlings primary root length after 10 days of germination on nutrients media supplemented with 0, 0.05, and 0.25 mM Pi and:
 • No pulp, • 0.5% (w/v) UnP, • 0.5% (w/v) TOP, • 0.5% (w/v) TOP-PH45, and • 0.5% (w/v) UnP-PH45.

4.7.3 Leaves Fresh Weight of Arabidopsis Seedlings

In Figure 38, the fresh weight of the leaves showed the same total fresh weight increasing trend in Figure 36; however, it was more obvious in Figure 38 that TOP-PH45 plates had the healthiest leaves in 0, 0.05, 0.25 mM Pi content plates.

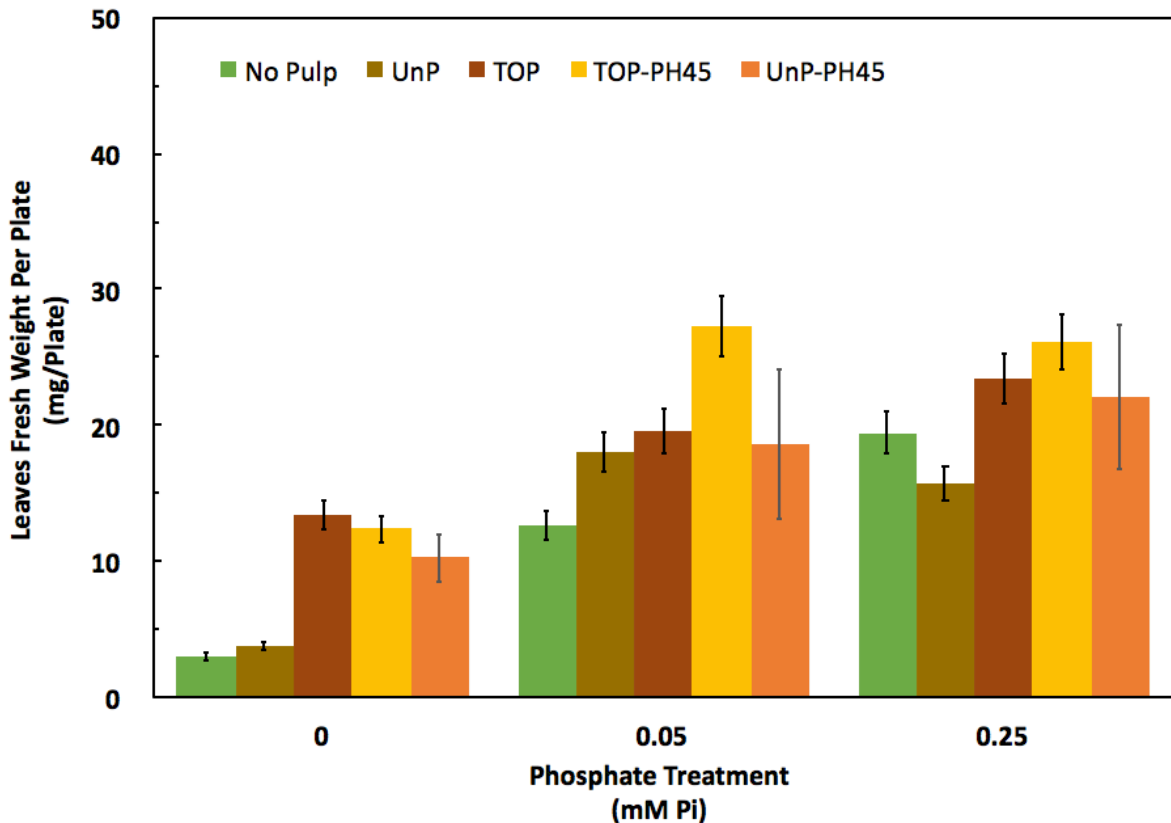


Figure 38. Seedlings leaves fresh weight after 10 days of germination on nutrients media supplemented with 0, 0.05, and 0.25 mM Pi and:

- No pulp,
- 0.5% (w/v) UnP,
- 0.5% (w/v) TOP,
- 0.5% (w/v) TOP-PH45, and
- 0.5% (w/v) UnP-PH45.

4.7.4 Growth Promotion of Arabidopsis by TOP-PH45 Loaded with Phosphate Masses

100 mg TOP-PH45 samples loaded with $96.9 \times 10^{-1} \mu\text{mol/g-pulp}$ phosphates were placed in random places on plates that contained 20 mL of 0 mM Pi content of plant nutrients solution. The pulp samples were placed in the center, top, and bottom of the 0 mM Pi content plates to compare the growth of these plants to the plants grown in 0 mM plates with no pulp. The results obtained showed the efficacy of pulp samples in enhancing the

growth of Arabidopsis as shown in Figure 39 and Table 11. These results confirmed the previous conclusion that TOP-PH45 enhances Arabidopsis growth. The results also showed how the roots didn't grow further than where the pulp was located even though the roots were growing through the pulp and some roots were just adhering to the pulp. This is could be due to the effectiveness of Arabidopsis roots in reaching the phosphate potential available to them.

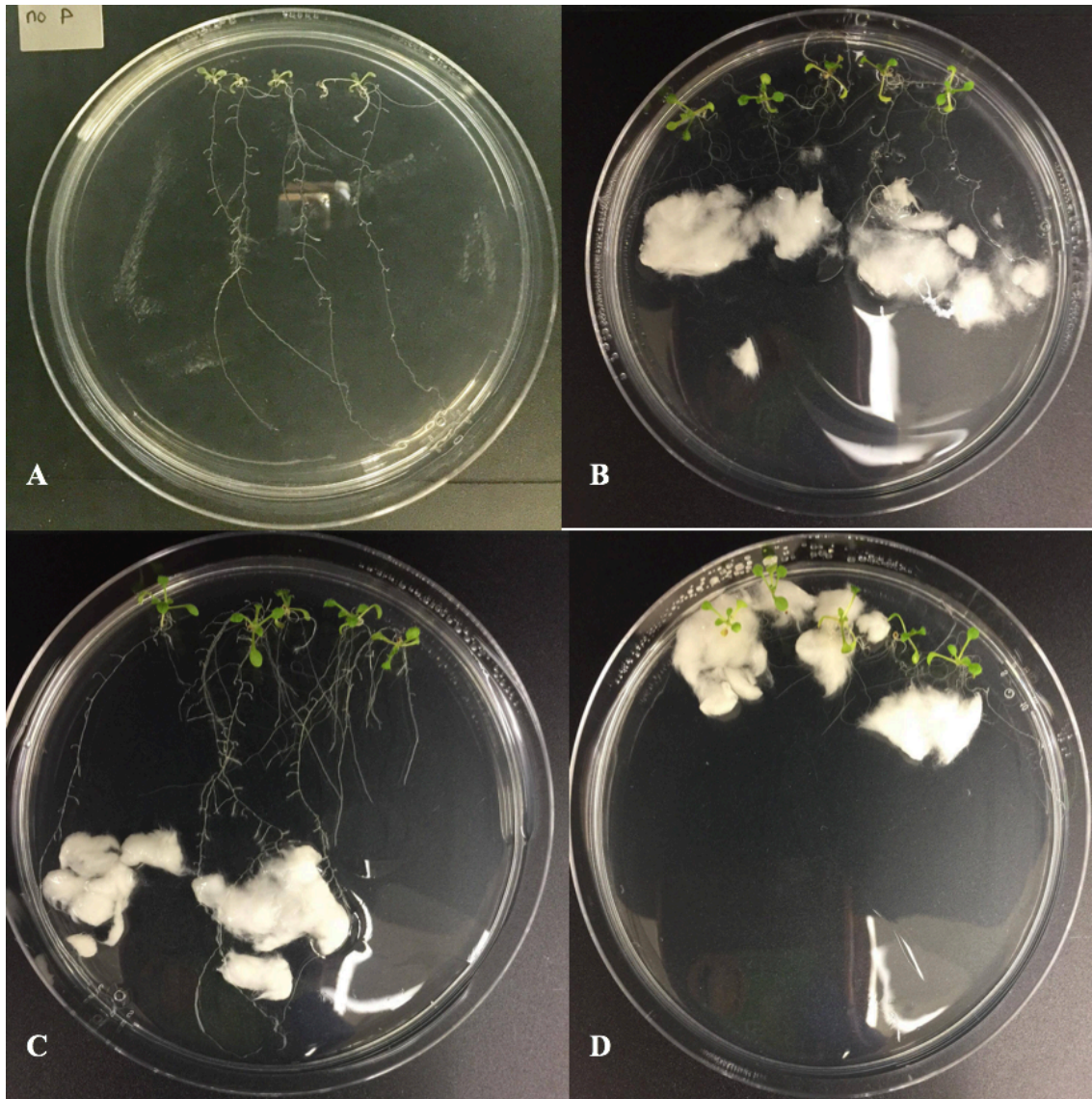


Figure 39. Representative seedlings after 10 days of germination on nutrients media supplemented with 0 mM Pi and A- No pulp, 100 mg of TOP-PH45 with $96.9 \times 10^{-1} \mu\text{mol/g-pulp}$ loaded phosphate located at B- center of plate, C- bottom of plate, and D- top of plate.

Table 10 summarizes the fresh weight of the seedlings in Figure 39, and it is also including the fresh weight of seedlings grown in 0.05 mM and 0.25 mM Pi content plates with no pulp. The 100 mg pulp samples were loaded with $96.9 \times 10^{-1} \mu\text{mol/g-pulp}$; thus, the

phosphate concentration in these plates can be calculated as 0.001 μmol of phosphates per 20 mL of plant solutions which corresponds to 0.05 mM phosphate concentration.

Table 10. Summary of Seedlings fresh weight and leaves only fresh weight for different growing media matrices.

Plate Matrix	Seedlings Fresh Weight per Plate (mg/plate)	Leaves Fresh Weight per Plate (mg/plate)
0 mM Pi content w/o pulp	11.67 \pm 2.74	3.05 \pm 2.69
0.05 mM Pi content w/o pulp	20.23 \pm 2.97	12.69 \pm 2.74
0.25 mM Pi content w/o pulp	30.63 \pm 3.78	19.47 \pm 3.66
TOP-PH45 w/ PO_4^{3-} content of $96.9 \times 10^{-1} \mu\text{mol/g-pulp}$ placed in 0 mM Pi plant media at:		
Center of the plate	27.30 \pm 3.82	20.62 \pm 3.81
Bottom of the plate	22.90 \pm 9.19	12.93 \pm 9.04
Top of the plate	28.50 \pm 0.28	23.21 \pm 0.24

4.7.5 Growing Arabidopsis Seeds on Pulp Only Matrix w/o Phytagel

Gellan is marketed under phytagel tradename. Phytagel is a polysaccharide produced by the bacterium *Sphingomonas elodea*. Unlike cellulose which consists of only one identical building block, phytagel consists of several different building blocks. It consists of a rhamnose, a glucuronic acid, and two glucose basic units. Phytagel serves as a gelling and thickening agent in the food industry.

Arabidopsis seeds are small and cannot be used with hydroponic set-ups easily. Phytagel provides a suitable aeration for small-seeded species like Arabidopsis; otherwise, an aquarium pump would be needed if grown in liquid media. Another advantage of phytagel

is that the roots in the phytigel media have limited access to water which provides nutrients gradient at the root zone; thus, the roots architecture can be examined for specific nutrients response. However, growing *Arabidopsis* seeds in liquid media, provides unlimited access to water and no nutrient gradient for the roots. Hence, big plants can have small roots in liquid media.

Arabidopsis seeds were grown on pulp matrices with plant nutrients solution not including phytigel to test if the pulp matrices will provide the appropriate growing conditions as phytigel did. 1 gm of TOP-PH45 was evenly placed on the petri dishes, and enough plant nutrients solution (w/o phytigel & w/ Pi content of 0.25 mM) was added to the pulp in the plates till the pulp was completely soaked with the nutrient solution (Figure 40). 14 mL of nutrients solution were sufficient to soak the pulp and were added to the 1 gm TOP-PH45 pulp in the plates, and plates were placed vertically in greenhouse kit with 5 seeds plated on each for 10 days.



Figure 40. Representative seedlings after 10 days of germination on TOP-PH45 w/o phytigel & w/ Pi content of 0.25 mM.

The seedlings grown on pulp matrices w/o phytigel showed poor growth of 13.9 ± 1.98 gm per plate and 1.44 ± 0.10 cm primary root length. Also, the leaves were dark green with some noticeable brown spots. There are some issues associated with growing *Arabidopsis* on TOP-PH45 w/o phytigel matrices; the texture of the modified pulp fibers could have led to lack of aeration and overwatering of the seeds. In section 2.7 of the literature review chapter, kinocloth sheets (Figure 18) worked successfully to provide seedling nursery sheets for plant cultivation. The fiber layer (web) structure of kinocloth sheets is coarse, providing filtration and ventilation characteristics to the sheets²¹.

TOP-PH45 w/o phytigel matrix is lacking filtration characteristics and is expected to suffocate Arabidopsis by overwatering them, especially that Arabidopsis seeds are very small. This plant study proved that TOP-PH45 can assist the plant growth as a chemically active substance that can bind and release phosphates, but further modifications and studies should be done on the pulp texture to cultivate various kinds of plants.

Chapter 5 Conclusions and Recommendations

5.1 Conclusions

The following conclusions can be drawn from this study:

- 1- The configuration of the PVAm adsorbed on southern softwood bleached kraft pulp affected the adsorption of phosphate groups. The flat configuration of high charge density PVAm adsorbed on the pulp surface increased the phosphate adsorption by exposing more cationic groups to the phosphates, thus binding the negatively charged phosphates on the cationic sites. On the other hand, the coiled configuration of low charge density PVAm on the pulp surface had relatively lower phosphate adsorption than the flat configuration had.
- 2- Although a layer of PVAm adsorbed on TEMPO oxidized pulp increase the anion exchange capacity of pulp, it had a very low phosphate binding capacity, a maximum of $0.16 \text{ mol PO}_4^{3-}/\text{mol N}^+$.
- 3- Unmodified pulp and TEMPO oxidized pulp have absolutely no capacity to bind phosphates on their surfaces if not treated with PVAm. The electrostatic attraction between the amine groups and the phosphates is the only way to retain phosphates on pulp surface.
- 4- The TEMPO oxidized fibers coated with PVAm showed better phosphate adsorption than unmodified fibers coated with PVAm. However, anionic charge density (carboxyl group content) of oxidized fibers influences the phosphate

adsorption by creating an electrostatic repulsion force against the phosphates (negatively charged), decreasing the phosphate adsorption.

- 5- 5.0 - 6.5 pH range provides the optimum ionization behavior of PVAm and phosphoric acid to adsorb the highest content of phosphates on TEMPO oxidized fibers coated with PVAm.
- 6- The TEMPO oxidized fibers coated with PVAm and loaded with phosphates will release the loaded phosphates depending on the presence of competitive ions and the pH of the surrounding media. The very acidic and basic media will always release all of the loaded phosphates. Maintaining the media's pH at 5.5 - 7.0 releases the least amount of phosphates.
- 7- The PVAm coated fibers have a positive effect on growing Arabidopsis phosphate sensitive seeds. They increased the Arabidopsis seedling fresh weight growth by 67% at low phosphate treatment. Even though PVAm coated fibers have low phosphate binding capacity, it is hypothesised that at low phosphate treatment they adsorb phosphates and make them not readily available to react or precipitate with other ions in the nutrient solution media, thus increasing the seedling fresh weight by providing more phosphates to the plants.
- 8- The soil anion exchange capacity falls within this range: 0.05-0.15 $\mu\text{mol/g-soil}^1$. PVAm coated TEMPO oxidized pulp had a nitrogen content of 70 $\mu\text{mol/g-pulp}$, in which 9% - 16% of these cationic sites adsorbed phosphates and released them depending on the pH and the surrounding competitive ions. It can be concluded here that even though the PVAm cationic sites on the modified pulp that adsorbed

phosphates were very low, the anion exchange capacity of the modified pulp is 42 - 220% higher than that of the soil.

5.2 Recommendations

The pulp texture, w/o phytagel, used in this work didn't let the Arabidopsis plant roots to penetrate through it. The problem could be because either Arabidopsis roots are weak in nature and need a very porous media to push through their roots or the pulp texture was too compact and needed holes or less water content to grow plants. Different plants should be grown on the pulp to completely understand the mechanisms of plant roots growing on pulp, and also further studies should be done on the pulp texture that can cultivate various kinds of plants. In the work of OJI KINOCLOTH company, Air Laid technology was successfully applied to wood pulp to make seedling nursery sheets ²¹. Air laid sheets, as described in section 2.7, are dispersed in air and not in water Air, producing very light and highly absorbent sheets. Air Laid technology could be applied on the TEMPO oxidized fibers coated with PVAm to produce low density pulp sheets that can be used to replace other inorganic hydroponic substrates used in greenhouses like rockwool, gravel, or mulch.

References

- (1) Weil, R.; Brady, N., *The Nature and Properties of Soil*. Fifteenth ed.; Pearson: 2016.
- (2) Jj, E. Phosphorus: A Limiting Nutrient for Humanity? *Current Opinion in Biotechnology* **2012**, *23*, 833-838.
- (3) Vance, C. P. Symbiotic Nitrogen Fixation and Phosphorus Acquisition. Plant Nutrition in a World of Declining Renewable Resources. *Plant Physiol* **2001**, *127*, 390-397.
- (4) Busman, L.; Lamb, J.; Randall, G.; Rehm, G.; Schmitt, M. The Nature of Phosphorus in Soils. <https://www.extension.umn.edu/agriculture/nutrient-management/phosphorus/the-nature-of-phosphorus/> (accessed September 19th).
- (5) Bialkowski, S. Use of Acid/Base Distributions in Ph Problems. <http://ion.chem.usu.edu/~sbialkow/Classes/3600/alpha/alpha2.html> (accessed September 26th).
- (6) Olympios, C. M. Overview of Soilless Culture: Advantages, Constraints and Perspectives for Its Use in Mediterranean Countries. *Cahiers Options Méditerranéenn* **1999**, *31*, 307-324.
- (7) Hydro-Industries. Hydroponic Systems. <http://www.hydro-industries.com/hydroponic-systems/-water> (accessed October 25th).
- (8) Cabrera, M., In *Biological Wastewater Treatment and Resource Recovery*, Chapter 7, INTECH: 2017.
- (9) Brodin, F. W.; Theliander, H. A Comparison of Softwood and Birch Kraft Pulp Fibers as Raw Materials for Production of Tempo-Oxidized Pulp, Mfc and Superabsorbent Foam. *Cellulose* **2013**, *20*, 2825-2838.
- (10) Varshney, V.; Naithani, S., *Chemical Functionalization of Cellulose Derived from Nonconventional Sources*. Springer: 2011.
- (11) Kitaoka, T.; Isogai, A.; Onabe, F. Chemical Modification of Pulp Fibers by Tempo-Mediated Oxidation. *Nordic Pulp & Paper Research Journal* **1999**, *14*, 279-284.
- (12) Flavio, J. L. D. The Wet Adhesion of Polyvinylamine to Cellulose. McMaster University, Canada, 2013.

- (13) Pelton, R. Polyvinylamine: A Tool for Engineering Interfaces. *Langmuir* **2014**, *30*, 15373-82.
- (14) Gu, L.; Zhu, S.; Hrymak, A. N. Acidic and Basic Hydrolysis of Poly(N-Vinylformamide). *Journal of Applied Polymer Science* **2002**, *86*, 3412-3419.
- (15) Hossain, S. M. Z.; Luckham, R. E.; Smith, A. M.; Lebert, J. M.; Davies, L. M.; Pelton, R. H.; Filipe, C. D. M.; Brennan, J. D. Development of a Bioactive Paper Sensor for Detection of Neurotoxins Using Piezoelectric Inkjet Printing of Sol-Gel-Derived Bioinks. *Analytical Chemistry* **2009**, *81*, 5474-5483.
- (16) Marais, A.; Wagberg, L. The Use of Polymeric Amines to Enhance the Mechanical Properties of Lignocellulosic Fibrous Networks. *Cellulose* **2012**, *19*, 1437-1447.
- (17) Andreasson, B.; Forsstrom, J.; Wagberg, L. The Porous Structure of Pulp Fibres with Different Yields and Its Influence on Paper Strength. *Cellulose* **2003**, *10*, 111-123.
- (18) Illergard, J.; Wagberg, L.; Ek, M. Contact-Active Antibacterial Multilayers on Fibres: A Step Towards Understanding the Antibacterial Mechanism by Increasing the Fibre Charge. *Cellulose* **2015**, *22*, 2023-2034.
- (19) Habteselassie, M. Y.; Bischoff, M.; Applegate, B.; Reuhs, B.; Turco, R. F. Understanding the Role of Agricultural Practices in the Potential Colonization and Contamination by Escherichia Coli in the Rhizospheres of Fresh Produce. *J Food Prot* **2010**, *73*, 2001-9.
- (20) Sehaquia, H.; Mautnerb, A.; Larrayad, U. P. D.; Pfenningere, N.; Tingauta, P.; Zimmermann, T. Cationic Cellulose Nanofibers from Waste Pulp Residues and Their Nitrate, Fluoride, Sulphate and Phosphate Adsorption Properties. *Carbohydrate Polymers* **2016**, *135*, 334-340.
- (21) Oji-Kinocloth. Agriculture, Livestock Farming and Fisheries. http://www.kinocloth.co.jp/english/purposes/agricultural/water_culture/index.html (accessed October 24th).
- (22) Ikeda, H.; Okada, M. Liquid-Absorbent Sheet and Method for Producing the Same. Pat. US7717894B2, 2010.
- (23) Höffkes, H.; Moch, M.; Mucha, T.; Oberkobusch, D. Composition for Dyeing Keratin Fibers, Especially Human Hair, Includes a Polymer Substituted by Amino or Ammonium Groups, to Improve Color Intensity. Pat. DE102005050913A1, 2007.

- (24) Klerk, G.-J. D.; Hanecakova, J.; Sik, J. J. Effect of Medium-Ph and Mes on Adventitious Root Formation from Stem Disks of Apple. *Plant Cell Tiss Organ Cult* **2008**, *95*, 285–292.
- (25) Isogai, A.; Saito, T.; Fukuzumi, H. Tempo-Oxidized Cellulose Nanofibers. *Nanoscale* **2011**, *3*, 71-85.
- (26) Tanaka, H.; Odber, L.; Wagberg, L.; Lindstrom, T. Adsorption of Cationic Polyacrylamides onto Monodisperse Polystyrene Latices and Cellulose Fiber: Effect of Molecular Weight and Charge Density of Cationic Polyacrylamides. *Journal of Colloid and Interface Science* **1990**, *134*, 219-228.
- (27) Gustafsson, E.; Wang, Z.; Polat, O.; Ostendorf, W. W.; Sheehan, J. G.; Pelton, R., Towards Wet Resilient Paper – Fiber Modifications and Test Method Development In *16th Fundamental Research Symposium*, Oxford, 2017.
- (28) Holmes-Farley, S. R.; Mandeville, W. B.; Whitesides, G. M. Phosphate-Binding Polymers for Oral Administration
. Pat. US005667775A, 1997.
- (29) Armarego, W. L. F.; Chai, C., *Purification of Laboratory Chemicals, Seventh Edition*. Butterworth-Heinemann: 2012.
- (30) Ernani, P.; Barber, S. Phosphorus Availability in a Low Ph Highly Weathered Soil as Affected by Added Salts
. *Cienc. Rural* **1995**, *25*, 219-222.
- (31) Passardi, F.; Dobias, J.; Valerio, L.; Guimil, S.; Penel, C.; Dunand, C. Morphological and Physiological Traits of Three Major Arabidopsis Thaliana Accessions. *J Plant Physiol* **2007**, *164*, 980-92.
- (32) Velasco, V. M. E.; Mansbridge, J.; Bremner, S.; Carruthers, K.; Summers, P. S.; Sung, W. W. L.; Champigny, M. J.; Weretilnyk, E. A. Acclimation of the Crucifer *Eutrema Salsugineum* to Phosphate Limitation Is Associated with Constitutively High Expression of Phosphate-Starvation Genes. *Plant Cell and Environment* **2016**, *39*, 1818-1834.

Appendix A : Sample Calculations

A.1 PVAm Loading on TEMPO Oxidized Pulp

$$m_{wp} = 10.0 \text{ gm} \quad \text{Mass wet pulp}$$

$$c_{wp} = 0.1 \quad \text{Wet pulp consistency}$$

$$m_{dp} = c_{wp} \times m_{wp} = 1.0 \text{ gm} \quad \text{Mass of dry pulp}$$

At 20.0 mg PVAm/g-pulp (15 kDa PVAm Equilibrium solution)

$$V_S = 140.0 \text{ mL} \quad 5 \text{ mM NaCl Solution volume during adsorption for } \mathbf{1 \text{ gm}} \text{ of dry pulp}$$

$$V_{PVAm} = 50.0 \text{ mL} \quad 0.1\% \text{ (w/v) PVAm solution volume during adsorption for } \mathbf{1 \text{ gm}} \text{ of dry pulp}$$

$$V_T = V_S + V_{PVAm} + m_{wp} - m_{dp} = 140.0 + 50.0 + 10.0 - 1.0 = 199.0 \text{ mL} \quad \text{Total suspension solution during adsorption for } \mathbf{1 \text{ gm}} \text{ of dry pulp}$$

$$15 \text{ kDa PVAm charge density} = 8,945.5 \text{ } \mu\text{mol/g}$$

$$C_{iPVAm} = \frac{20 \frac{\text{mg}}{\text{g-pulp}} \times m_{dp}}{V_T} = 100.5 \text{ mg/L} = 899.0 \text{ } \mu\text{mol/L} \quad \text{Initial concentration of PVAm in suspension solution}$$

$c_{fPVAm} = 496.9 \mu\text{mol/L}$ Filtrate PVAm concentration after adsorption (determined by nitrogen polyelectrolyte titration)

$c_{rPVAm} = (c_{ip} \times V_p - c_{fp} \times V_p) / 1 \text{ gm} = 80.0 \mu\text{mol/g pulp}$ Adsorbed PVAm per 1 gm of dry pulp

A.2 Phosphate Loading on TEMPO Oxidized Pulp Coated with PVAm

$m_{wp} = 100 \text{ gm}$ Mass wet pulp

$c_{wp} = 0.1$ Wet pulp consistency

$m_{dp} = c_{wp} \times m_{wp} = 1 \text{ gm}$ Mass of dry pulp

H₃PO₄ acid has: 31.6% P content, 1.685 g/mL density, 85% wt, and 98.0 g/mol molecular weight.

1 mL of H₃PO₄ acid = $0.85 \times 31.6\% \times 1.685 = 0.452 \text{ gm of P}$
 = 14.6 mmol of H₃PO₄

$c_{ips} = 25 \text{ mg P/L}$ Concentration of stock H₃PO₄ acid solution
 = 0.8 mM

$c_{ip} = 3 \text{ mg P/L}$ Initial concentration of P solution

$c_{fp} = 1.9 \text{ mg P/L}$ Filtrate P concentration after adsorption (determined by spectrophotometer)

$V_p = 199 \text{ mL}$ Liquid volume during adsorption for **1 gm** of dry pulp

$c_{rp} = (c_{ip} \times V_p - c_{fp} \times V_p) / \mathbf{1 \text{ gm}} = 21.9 \times 10^{-2} \text{ mg P/g-pulp} = 7.0 \text{ } \mu\text{mol/g-pulp}$ Adsorbed phosphorus per 1 gm of dry pulp

A.3 Phosphate Release from TEMPO Oxidized Pulp Coated with PVAm and Loaded with Phosphates

$m_{ad} = \mathbf{0.1} \times 21.9 \times 10^{-2} = 21.9 \times 10^{-3} \text{ mg P}$ Mass of adsorbed phosphate for **0.1 gm** of dry pulp

$c_{wfp} = 20\%$ Consistency of filtered pulp

$c_{fpr} = 0.43 \text{ mg P/L}$ Filtrate P concentration after release

$V_a = 19.9 \text{ mL}$ Liquid volume during adsorption for **0.1 gm** of dry pulp

$V_{ep} = \mathbf{0.1} / c_{wfp} - \mathbf{0.1} = 0.4 \text{ gm} = 0.4 \text{ mL}$ Volume of entrained P filtrate

$m_{ep} = V_{ep} \times c_{fp} = 0.76 \text{ } \mu\text{g}$ Mass of entrained P

$m_{rp} = m_{ad} - (c_{fpr} \times V_a + m_{ep}) = 8.05 \times 10^{-2} \text{ mg}$ Mass of released adsorbed P

$P_r = m_{rp} / m_{ad} = 36.6\%$ Fraction of released adsorbed P, i.e., not including the entrained P