CHAPTER 2
THEORIES

2.1 Nitrogen [29]

Nitrogen is one of the most widely distributed elements in nature. It is present in the atmosphere, the lithosphere, and the hydrosphere. Nitrogen is a very mobile element circulating between the atmosphere, the soil and living organisms.

2.1.1 Properties of nitrogen [30]

Nitrogen is a chemical element that has the symbol N, atomic number of 7 and atomic mass 14.00674. Elemental nitrogen is a colorless, odorless, tasteless and mostly inert diatomic gas at standard conditions, and constituting 78.08% by volume of Earth's atmosphere.

Nitrogen has several valences (-3, 0, +1, +2, +3, +4, +5) and occur compound in the natural such as ammonia (NH_3), nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO), acid anhydride of nitrous acid (N_2O_3), nitrogen dioxide (NO_2), acid anhydride of nitric acid (N_2O_5). The changing of nitrogen compound mostly occurs by organisms and depends on with or without oxygen condition. Nitrogen compound in the water can transform from organic substance to inorganic substance by nitrification process that show in equation 1.

$$NH_3 \xrightarrow{\text{Nitrosomonas}} NO_2^- \xrightarrow{\text{Nitrobactor}} NO_3^- \qquad (1)$$

This process effects to plants and microorganisms because of they can uptake nitrate (NO_3^-) and ammonia (NH_3) for protein formation that human and animal cannot. In anaerobic condition occurs denitrification process that present in equation 2. However, advantage of this process is nitrogen removal.

$$NO_3^- \longrightarrow NO_2^- \longrightarrow \begin{cases} NH_3 \\ N_2 \end{cases} \qquad (2)$$

2.1.2 Compound of nitrogen [30]

2.1.2.1 Organic nitrogen

The most of wastewater is the form of protein and mainly composition is C H O N and a little of S and P. Organic nitrogen in the wastewater is more than 10 mg/L and in general of raw sewage is 25 mg/L.

2.1.2.2 Ammonia nitrogen

Ammonia nitrogen is ammonium ion (NH_4^+) or ammonia that is nutrient and toxic substance and occurs in surface water, ground water, and sewage water. The ammonia concentration in general surface water varies from less than 10 mg N/L to more than 50 mg N/L, while in wastewater is 5-25 mg N/L.

2.1.2.3 Nitrate nitrogen

The high level of nitrate can occur in some area of ground water and sewage water from biological treatment system. Nitrate is the important nutrient of photosynthetic

autotrophs and growth limiting nutrient. It is cause of eutrophication and the pollution control ministry determines standard of nitrate in water bodies is not more than 5 mg/L.

2.1.2.4 Nitrite nitrogen

Nitrite is not stable substance that can change to nitrate under aerobic condition. In surface water and ground water occur less than 0.1 mg/L. Nitrite is used in the water-supply for corrosion inhibitor.

2.1.3 The occurrence of nitrogen [30]

Nitrogen occurs in all living organisms, and the nitrogen cycle is movement of the element from air into biosphere and organic compounds, then back into the atmosphere. Synthetic nitrate is produced as important ingredients of industrial fertilizers, and also key pollutants in causing the eutrophication of water systems.

2.1.4 The importance of nitrogen [29, 30, 31]

Nitrogen is a constituent element of amino acids, proteins, and nucleic acid (DNA and RNA), and compounds of secondary plant metabolism such as alkaloids. It resides in the chemical structure of almost all neurotransmitters. Higher plants are major contributors to the large amount of nitrogen which is continuously being converted from the inorganic to the organic form. Nitrogen is obtained from the soil environment either as the ammonium (NH_4^+) or nitrate (NO_3^-) ions, with nitrate being chemically reduced within the plant to ammonium prior to incorporation into organic molecules.

2.1.5 Nitrogen uptake by plants [29]

Plants may be taken up nitrogen in the form of a cation as NH_4^+ or an anion as NO_3^-. Uptake rate of both depends mainly on the availability of these ions in the nutrient medium. It is determined mainly by the physiological need of the plant and the source of cation and anion. The uptake of nitrate increases pH in solution, whereas the uptake of ammonia decreases pH in solution. The uptake of NO_3^- is mainly H^+/NO_3^-cotransport with the H^+ pumped out of the cell by the plasmalemma proton pump being recycled back into the cytosol. Therefore, nitrate uptake is associated with pH increase in the outer medium. NH_4^+ nutrition recycling of H^+ back into the cytosol is restricted and the H^+ pumped out of the cell remain mainly outside and consequently, the pH is decreased.

2.2 Phosphorus [32]

Phosphorus in natural water and wastewater are many forms that may be soluble form or fossiliferous form. Phosphorus is essential element for the growth of plants and animals, it is also growth limiting nutrient. So, the releasing sewage water that has phosphorus effects to stimulation the growth of aquatic plant rapidly and leads to eutrophication problem in lakes and water resources.

2.2.1 Properties of phosphorus [32]

Phosphorus is the chemical element that has the symbol P and atomic number 15. Phosphorus is commonly found in inorganic phosphate rocks. Elemental phosphorus exists in two major forms are white phosphorus and red phosphorus. Due to its high reactivity, phosphorus is never found as a free element in nature on Earth.

2.2.2 Compound of phosphorus [33]

1) Orthophosphate

Orthophosphate or phosphate is the most found form. The compound of this group is soluble inorganic phosphorus such as trisodium phosphate (Na_3PO_4), disodium phosphate (Na_2HPO_4), monosodium phosphate (NaH_2PO_4), and diammonium phosphate ($(NH_4)_2HPO_4$) that the main come from industrial wastewater, detergent. This form is used for the growth of microorganisms and plants.

2) Polyphosphate or dehydrated phosphate

Polyphosphate is phosphorus when occur hydrolysis process, it is transformed to orthophosphate. The compound of this group is sodium hexametaphosphate ($Na_3(PO_4)_6$), sodium tripolyphosphate ($Na_5P_3O_{10}$), and tetrasodium pyrophosphate ($Na_4P_2O_7$).

3) Organic phosphate

The main source come from industrial wastewater and organic phosphate can transform to orthophosphate. The compound of this group is nucleic acids, phospholipids, and sugar phosphate.

2.2.3 The occurrence of phosphorus [33]

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in particles or detritus, or in the bodies of aquatic organisms. These form of phosphate increase from a variety of sources. Small amounts of orthophosphate or certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphate applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primaliry by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of water bodies. For instances, phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macroorganisms in nuisance quantities. Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

2.2.4 The importance of phosphorus [32]

Phosphorus is a structural component of numerous macromolecules, including nucleic acids, phospholipids, certain amino acids, and several coenzymes. It has a significant role in energy transfer via the pyrophosphate bond in ATP, and the attachment of phosphate groups to many different sugars provides metabolic energy in photosynthesis and respiration.

2.2.5 Phosphorus uptake by plants [31, 33]

Phosphorus is absorbed by plants largely as the primary or secondary orthophosphate anions, $H_2PO_4^-$ and HPO_4^{2-}. The ionic form that is predominantly absorbed depends on soil pH. $H_2PO_4^-$ is more readily absorbed in low pH soils whereas HPO_4^{2-} is preferentially absorbed in high pH soils. The best pH range for phosphorus uptake is pH 6.5 to 7.5. Phosphate (HPO_4^{2-}) in the soil solution is readily absorbed by plant roots via an H^+-HPO_4^{2-} symporter and incorporated into a variety of organic compounds, including sugar phosphates, phospholipids, and nucleotides. The main entry point of phosphate into assimilatory pathways occurs during the formation of ATP, the energy of the cell. In the overall reaction for this process, inorganic phosphate is added to the second phosphate group in adenosine diphosphate to form phosphate ester bond. Phosphate is not reduced in plants, but remains in the highly oxidized form of inorganic phosphate, which at the cytoplasmic pH is partly ioned between $H_2PO_4^-$ and HPO_4^{2-}.

2.3 Burhead (*Echinodorus cordifolius* (L.) Griseb) [34, 35]

Common name of *Echinodorus cordifolius* (L.) Griseb is burhead. Original source of burhead is southern North America. Burhead is freshwater plants and invasive. The plant has a creeping stem and large, ovate leaves about 5 cm long. It grows in neutral to soft water at sub-tropical temperatures easily and can grow in tropical countries. The plant can be grown submersed and produce flowers and seeds.

Figure 2.1 Characterictic of *Echinodorus cordifolius*

2.3.1 Scientific classification [36]

Kingdom:	Plantae
Order:	Alismatales
Family:	Alismataceae
Genus:	*Echinodorus* Rich. Ex Engelm.
Species:	*Echinodorus cordifolius* (L.) Griseb.

2.4 Arrowhead (*Sagittaria montevidensis*) [37]

Sagittaria montevidensis (Figure 1.1) is a species of flowering plant in the water-plantain family that is native to North and South America. Common names include Giant Arrowhead and California Arrowhead. It is an aquatic plant that is found in shallow waters. The leaves are sagittate and glabrous. Its terete, spongy petioles may reach a length of more than 0.75 m (2.5 ft). Inflorescences are typically shorter than the leaves and decumbent. Flowers are in whorls or pairs at nodes. They have three petals, each of which is white with a yellow base, and three green sepals. The thick pedicels are as long 5 cm (2 in). Flowering occurs from June to September.

Figure 2.2 Characterictic of *Sagittaria montevidensis*

2.4.1 Scientific classification [37]

Kingdom:	Plantae
Order:	Alismatales
Family:	Alismataceae
Genus:	*Sagittaria*
Species:	*S. montevidensis*
Binomial name:	*Sagittariamontevidensis*

2.5 Domestic wastewater [38]

Domestic wastewater is wastewater that occurs from daily life activity of population who live in community, from cooking and washing in household and buildings. It is discharged from community involve sewage water of house, market, and hospital. Generally, characteristic of domestic wastewater has neutral pH, dirty water that is organic and inorganic substance, germ, and heavy metal contaminated. Characteristic of domestic wastewater in general was shown in Table 2.1.

Table 2.1 Characteristic of domestic wastewater [39]

Composition	mg L^{-1}
Total solids	350 – 1200
Total suspended solids	100 – 350
Total dissolved solids	100 – 300
BOD_5	110 – 440
COD	1.75 × BOD_5
Total nitrogen, as N	20 – 85
Organic nitrogen	0.4 × total-N
Ammonia nitrogen	0.6 × total-N
Nitrate nitrogen	(0.0-0.05) × total-N
Total phosphorus, as P	4 – 15
Organic phosphorus	0.3 × total-P
Inorganic phosphorus (Ortho-P and Poly-P)	0.7 × total-P

2.6 Nutrient removal [38]

Using of nutrient element by plants is the important role in nitrogen and phosphorus removal. The use of nutrient element rate is limited by the growth rate and the concentration of nutrient in plant tissues. When plant is less old, nutrient element is the high concentration in plant. While plant is old, nutrient element in plant is decreased. Wastewater treatment of plant depends on the nutrient absorption ability of plant roots and biochemical process in plant. Plant roots contribute for attach area increasing to provide microorganisms and vary gas translocation, involve oxygen from top to roots result in microorganisms can transform non-available of nutrient element to available, the quality of water is better.

2.6.1 Mechanism of nitrogen removal [38]

1) Ammonifacation

Ammonification is a biological process that transforms organic nitrogen to ammonia and releases energy for new cell formation by bacteria following equation 3;

$$\text{Amino acids} \longrightarrow \text{imino acids} \longrightarrow \text{keto acids} \longrightarrow NH_3 \qquad (3)$$

2) Nitrification

Nitrification is biological oxidation process that transforms ammonium ions to nitrate with nitrite is mediate of reaction. Oxidation occur 2 steps;

The first step is ammonium ions oxidation to nitrite by *Nitrosomonas* sp. bacteria following equation 4;

$$NH_4^+ + 1.5O_2 \longrightarrow 2H^+ + H_2O + NO_2^- \qquad (4)$$

The second step is the oxidation of nitrite to nitrate by *Nitrobacter* sp. bacteria following equation 5;

$$NO_2^- + 0.5O_2 \longrightarrow NO_3^- \quad (5)$$

3) Denitrification

Denitrification is process that transforms nitrite and nitrate to nitrogen gas. This process often occurs under anaerobic condition by *Pseudomonas* sp. and *Micrococcus* sp. following equation 6;

$$2NO_3^- \longrightarrow 2NO_2^- \longrightarrow 2NO \longrightarrow N_2O \longrightarrow N_2 \quad (6)$$

4) Ammonia volatilization

This process is ammonia that transformed to gas and released to the atmosphere by water permeation and translation from water surface to the atmosphere.

5) Plant uptake

Plant uptake is a biological process that transforms inorganic nitrogen to organic nitrogen for building block function of cell and plant tissue and 2 forms of nitrogen are ammonium ions and nitrate. Normally plant absorbs ammonium ions better than nitrate because ammonium ions are more reduce condition than nitrate, except the high concentration of nitrate than ammonium ions, plant absorbs nitrate is better.

2.6.2 Mechanism of phosphorus removal [38]

1) Plant uptake

Plant absorbs phosphorus by roots and translocated to plant tissues for cell formation. When plant dies, some part of phosphorus is released and residual phosphorus is attached with detritus. Plant roots are an important phosphorus accumulation source of plants and plant available phosphorus is dihydrogen phosphate ($H_2PO_4^-$), monohydrogen phosphate (HPO_4^{2-}), and phosphate (PO_4^{3-}).

2) Adsorption by soil

Adsorption mechanism of soil depends on;

1) The amount of iron, aluminium, calcium, and pH of soil are the high amount of iron and aluminium in the soil involved the low pH lead to phosphorus is adsorbed with iron and aluminium. Contrastably, the high amount of calcium in the soil involve the high pH lead to phosphorus is adsorbed with calcium.
2) Redox potential, in case of water overflow condition effect to low oxygen in the soil, low redox potential, also low pH
3) Type of soil, in case of media constitutes the most of clay soil lead to the high phosphorus adsorption of the soil because clay soil has cation exchange capacity (C.E.C.) and high clay mineral.

2.7 Phytoremediation

Phytoremediation is a new tool for pollutant remediation in the environment. This method is using plants to clean nature that can absorb, accumulate and detoxify pollutants [40].

2.7.1 Categories of phytoremediation [41]

1) Phytoextraction is pollutants removal from soil and water by absorption, pollutant translation entry to the plant and storage in plant tissue.
2) Phytotransformation is pollutants absorption, entry to the plant and pollutants can be transformed or degraded by plants.
3) Phytovolatilization is using plants for condition changing of some pollutants to gas and volatile into the atmosphere.
4) Phytostabilization is pollutants transformation to less soluble form that pollutant is stable.
5) Plant-assisted bioremediation is pollutants degradation with microorganisms that plant is the important role for stimulate degradation.

2.8 Plant-microbe interaction [42]

Plant-microbe interaction can be classified as positive interactions and negative interactions. Positive interactions, during plant growth, roots release organic compounds such as carbohydrates, carboxylic acids and amino acids that serve as nutrients for microbes in the rhizosphere. Microbes often confer beneficial effects such as promoting plant growth and reducing susceptibility to diseases caused by plant pathogens. Microbial plant growth-promoting mechanisms include the fixation of atmosphere nitrogen and production of siderophores. Conversely, negative interactions, rhizosphere microbes have detrimental effects on plant health and survival by increasing the risk for infection with plant pathogens or parasites. Root exudates containing toxic substances, such as antimicrobial and phytotoxins might inhibit the growth.

2.8.1 Phosphate-solubilizing bacteria as plant growth promoters [43, 44]

Phosphorus in soil is generally not sufficient for increase plant growth. Therefore, inoculation of plants by microorganisms is necessary. There were reports about plant growth promotion bacteria. The role of plant growth promotion bacteria was inorganic or organic phosphorus solubilization from soil. These microorganisms produced phytohormones, antibiotics, or siderophores that had beneficial to the plant growth and stimulated yield.