CHAPTER 1
INTRODUCTION

1.1 Rationale/Problem Statement

Nowadays, the increasing of population lead to the washing from house or daily life activity, the using fertilizer in agriculture, animal farm, and industrial effluence are cause of discharge nitrogen and phosphorus in the environment [1]. In Thailand, domestic wastewater is a major source of nitrogen and phosphorus that come from residences, schools, hospitals, and commercial buildings [2]. The high concentration of nitrogen and phosphorus in water bodies induce to eutrophication. Critical levels to occur eutrophication for surface waters of USEPA determine at 1.5 mg/L for nitrogen and 0.1 mg/L for total phosphorus. The eutrophication phenomenon disturbs the balance of natural in aquatic system, poison of “red tides” effected to dead of many macro invertebrates and fish. This phenomenon can occur at rivers, lakes, reservoirs, and coastal waters that is one of the most important problems of worldwide. Several water resources cannot be used for water supply and aquatic life. Moreover, excess nitrate leads to methemoglobinemia (blue baby syndrome) in infants. Characteristic of the disease is the reduction of the ingested nitrate to nitrite in the infant’s acid gut. The nitrite binds with hemoglobin in the bloodstream, poor oxygen transport in the blood. Infants lack the enzymes necessary to reverse the reaction [3, 4]. The detrimental effect of excess nitrogen and phosphorus, it is necessary to remove these nutrients that is usually difficult to remove by physical and chemical method simultaneously owing to their no cost effective, less flexible of system improving, target for remove BOD, and less reduce nitrogen and phosphorus in the system [5]. Phytoremediation is the technology which emerged and has been increasingly attention by using plants clean up contaminated soil and water because of its lower costs, environmentally friendly and fewer negative effects than physical and chemical methods [6, 7, 8]. In addition, the selected plant for removing nutrients is important. The plant should be rapid growth, high biomass, and resistance to plant pathogen. Consequently, after screening plants, selected plant were studied for treatment of domestic wastewater. The plant will use nitrogen and phosphorus for their growth, which these nutrients are component of nucleic acids, phospholipids, ATP, and plant cannot grow without supply of these nutrients [9]. Enhancing phosphorus treatment by plant combined with materials or bioaugmentation was also investigated.

1.2 Literature Review

1.2.1 Nitrogen and phosphorus removal by plants

Nitrogen and phosphorus are the most pollutant in the water bodies and they are present at quite high concentrations in the volumes of water hugely from sewage, agriculture, and urban storm runoff [4]. The excess value of theirs should be removed for improvement water quality. There are several researchers studied nitrogen and phosphorus removal by plants were shown in Table 1.1.

Table 1.1 The study of nitrogen and phosphorus removal by plants

Plants	Details	References
Scirpus validus, Carex lacustris, Phalaris arundinacea, and *Typha latifolia*	Four plant species were tested for reduction total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms. The plants were grown in monoculture and compared the effective of nutrient removal in control. The plants were maintained for one year before the nutrient treatment and monthly water sampling began July 31, 2001 and ended October 23, 2001. Hypothesis of this study was: (1) vegetated microcosms are more effective at reducing concentrations of total N and total P from soil leachate than unvegetated, (2) there is a differential species effect on the potential to reduce N and P, (3) plant mixtures are more effective than monocultures at reducing N and P, and (4) the microcosms will be least effective at reducing N and P concentrations in October compared to August. The result supported for hypotheses 1, 2, and 4. Total N and total P in the soil leachate were higher from unvegetated microcosms compared to vegetate. *Scirpus validus* was most effective and *Phalaris arundinacea* was the least effective at reducing N and P in monocultures.	Fraser, et al. [10]
Free floating plant, Floating leaved plants, Emergent plants, and Submerged plants	Nitrogen and phosphorus removal by constructed wetlands was studied. The result showed that removal of total nitrogen varied between 40 and 50% with removing load range between 250 and 630 g Nm^{-2} yr^{-1}. Removal of total phosphorus varied between 40 and 60% in all types of constructed wetlands with removing load range between 45 and 75 g N m^{-2} yr^{-1} depending on constructed wetlands type and inflow loading.	Vymazal, J. [11]
Kandelia candel, Aegiceras corniculatum, and *Sonneratia caseolaris*	Mangrove species were compared the wastewater treatment efficiencies in constructed wetland. A pilot-scale mangrove wetland was constructed for municipal sewage treatment. Seedlings of mangrove species were transplanted to the belts with one species for each belt.	Yang, et al. [12]

Table 1.1 The study of nitrogen and phosphorus removal by plants (cont.)

Plants	Details	References
	The hydraulic loading was5 m^3 d^{-1} and hydraulic retention time 3 d. Water qualities were detected and high levels of TN, NH_3-N, and TP were removed. The removal efficiency of TN andNH_3-N was 46% and 50%, respectively with higher removal of TN by *Sonneratia caseolaris*. For NH_3-N, *Kandelia candel* had lower removal efficiency than the other two mangroves. The removal efficiency of TP was 60% with higher in *Sonneratia caseolaris* and *Aegiceras corniculatum* than in *Kandelia candel.* This study indicated that mangroves could be used in a constructed wetland for effective municipal sewage treatment.	
Ryegrass	Reactive substrates were Filtra P, Polonite, and wollastonite, were used for the removal of phosphorus from wastewater on the fertility of acid soils. The reactive substrates were used as amendments to an acid soil in a pot experiment. They saturated with P and can act as a source of P for plants (ryegrass) and improve nutrients availability in acid soils, whereas also increasing soil pH and acting as soil conditioners. Polonite produced the highest yield/amendment ratio, while Filtra P and Polonite increased the concentrations of P and Ca in the ryegrass. All three substrates applied to an acid soil at a ratio of 1:200 (w/w) presented to increase the yield of ryegrass and improved soil fertility by increasing soil pH, availability of nutrients and cation exchange capacity.	Cucarella, et al. [13]
Ipomoea aquatic Forsskal	An aquatic macrophyte was studied to treat eutrophic water for nutrient reduction in a horizontal-flow, Deep Flow Technique (DFT) system.The removal of total nitrogen and total phosphorus were 41.5 and 75.5%, respectively.	Hu, et al. [14]

Table 1.1 The study of nitrogen and phosphorus removal by plants (cont.)

Plants	Details	References
Duo grass	Duo grass was studied the capability to metabolize P from various P-substrates in the growth media and the P uptake capacity of Duo grass grown in the presence of varying concentrations of added P in the soil in the form of KH_2PO_4. The Duo grass seedlings were planted in soil amended with KH_2PO_4 at the rate of 2.5, 5 and 7.5 g/kg soil and grown for 5 weeks and control that no KH_2PO_4 treatment. The highest fresh weight in grass seedlings was measured in plants that grown in P-enriched soil containing 7.5 g KH_2PO_4. The grass seedling grown in the presence of KH_2PO_4 in the soil also accumulated higher amount of P in their tissues compared to the control plants. Duo grass plants grown in soil amended with 2.5, 5, and 7.5 g KH_2PO_4 /kg soil presented P uptake of 0.82, 1.14, and 1.23% (dry weight), respectively.	Priya, P. and Sahi, S.V. [15]
Pistiastratiotes L.	The plant was used to remove nutrients and improve eutrophic stormwaters. The experiment was performed as the treatment plots of two stormwater detention ponds (East and West Ponds) in 2005-2007 and water sample from both treatment and control plots were weekly collected and analyzed for water quality properties including pH, turbidity, and nutrients (N and P). Plant samples were collected monthly and analyzed for nutrient contents. Water quality in both ponds was improved which the decrease of water turbidity and nutrients concentrations. Inorganic N (NH_4^+ and NO_3^-) and PO_4^{3-}, total P concentrations in treatment plots were more than 50% and 14-31%, respectively lower than those in control plots (without plant). In addition, water lettuce contained average N and P concentrations of 17 and 3 g/kg, respectively.	Lu, et al. [5]

Table 1.1 The study of nitrogen and phosphorus removal by plants (cont.)

Plants	Details	References
Rosegold pussy willow, giant pussy willow, Korean willow, and bald cypress	The plants were examined the removal potentials of nitrogen and phosphorus from eutrophic aquatic environment. Water of different concentration levels in nitrate were 5, 10, and 20 ppm and phosphate were 0.5, 1, and 2 ppm, they were funneled into the pots. The residence time of inflow was controlled ranging from 1 to 4 h. The rosegold pussy willow pots presented percentage of nitrate removal higher than in those of the other two willow species, and bald cypress showed the lowest nitrate abatement. The giant pussy willow pots presented the highest of phosphate removal and rosegold pussy willow pots showed the lowest of phosphate removal.	Seo, et al. [16]
Polygonum hydropiper	The mining ecotype and non-mining ecotype of *Polygonum hydropiper* were studied in nitrogen and phosphorus removal from livestock wastewater. The efficiency in TN and TP removal of the mining ecotype were 87.47% and 97.63%, respectively. Moreover, the shoot biomass of two ecotypes of plant increased.	Zheng, et al. [17]
Nelumbo nucifera Gaertn. and *Cyperus alternifolius* L.	The plants were used for phosphorus removal from domestic wastewater. *N. nucifera* and *C. alternifolius* could remove phosphorus from 1.038±0.001 mg $L^{-1}$to 0.094±0.001 mg L^{-1} and 0.048±0.004 mg L^{-1}, respectively, within 5 days. After phosphorus treatment, the weight of *N. nucifera* and *C. alternifolius* increased from 4060±0.05 g to 4820±0.17 g and from 4000±0.00 g to 4600±0.14 g.	Thongtha, et al. [18]

1.2.2 Nitrogen and phosphorus removal by microorganisms

There were several researchers studied nitrogen and phosphorus removal by microorganisms were shown in Table 1.2.

Table 1.2 The study of nitrogen and phosphorus removal by microorganisms

Microorganisms	Details	References
P. laminosum	The thermophilic cyanobacterium was immobilized on hollow cellulose fiber and used for nutrients elimination. Phosphate concentration was reduced from 6.62 to 0.02 mgP L^{-1}.	Sawayama, et al. [19]
P. tenue and *Oscillatoria*	Filamentous, mat-forming cyanobacteria were isolated from Arctic and Antarctic environments for nutrients removal. They could remove phosphorus to 0.6 mgP L^{-1} day^{-1}.	Chevalier, et al. [20]
Rh. sphaeroides, Rb. sphaeroides, and *Rhodopseudomonas palustris*	The photosynthetic bacteria were immobilized in porous ceramic under aerobic conditions to treat phosphate from synthetic sewage wastewater. The efficiency of phosphate removal was 77%, within 48 h.	Nagadomi, et al. [21]
Staphylococcus auricularis	Microorganism grew in sequencing batch reactors in wastewater under anaerobic and aerobic conditions. The ability of microorganism for phosphorus removal between 5 and 50 mgP $L^{-1}$was more than 90%.	Choi and Yoo [22]
Bacillus sp, *Pseudomonas* sp, and *Enterobacter* sp	Microorganisms were used for phosphate removal from synthetic medium by batch scale process. The maximum phosphate removal of the consortium was 92.5%. *Pseudomonas* sp could remove maximum phosphate to 68.2%.	Krishnaswamy, et al. [23]
Filamentous bacteria	Filamentous bacteria were evaluated for biological nutrient removal processes by batch tests. The result found that nitrate from 18.46 mg/L was reduced to nitrogen gas. Phosphate was accumulated up to 17.12 mgP/L	Ramothokang, et al. [24]
Algal-bacterial	Algal-bacterial was cultivated in a stirred tank photo bioreactor, was used for nitrogen and phosphorus removal. The algal-bacterial could remove total kjeldahl nitrogen and phosphate were 88.3±1.6% and 64.8±1.0%, respectively, within 8 day. Total nitrogen and total phosphorus were accumulated into biomass as 44.9±0.4% and 61.6±0.5%, respectively.	Su, et al. [25]

1.2.3 Nitrogen and phosphorus removal by plant-microbe

Bioaugmentation is interested alternative for nitrogen and phosphorus removal from wastewater. This method contributes to enhance nutrients treatment. There were researchers studied nitrogen and phosphorus removal by plant-microbe was shown in Table 1.3.

Table 1.3 The study of nitrogen and phosphorus removal by plant-microbe

Plant-microbe	Details	References
Eichhornia crassipes, Elodea nuttallii-nitrogen cycling bacteria including ammonifying, nitrosating, nitrifying and denitrifying bacteria	Interaction between aquatic macrophytes and immobilized nitrogen cycling bacteria was assessed in nitrogen removal from the eutrophic waterbody. *Eichhornia crassipes*+bacteria showed the best of the efficiency in nitrogen removal. Total nitrogen was removed to 70.2%, nitrite and ammonium were removed to 92.2% and 50.9%, respectively.	Chang, et al. [26]
Festuca arundinacea-denitrifying polyphosphate accumulating microorganisms (DPAOs)	Floating beds of tall fescue plants (*Festuca arundinacea*) inoculated with denitrifying polyphosphate accumulating microorganisms (DPAOs) was used for nitrogen and phosphorus removal from eutrophic water. The result found that the efficiency of NH_4^+-N, NO_3^--N, TN, TP, and ortho-P removal by tall fescue plants inoculated with DPAOs were 86.32%, 93.60%, 90.12%, 72.09%, and 84.29%, respectively.	Zhao, et al. [27]
Lolium perenne var. Top One (LPT) and *Lolium perenne var.* respect (LPR)-*Bacillus* sp. and *Microbacterium* sp.	Two species of perennial grasses and two denitrifying polyphosphate-accumulating organisms (DPAOs) were used for nutrients removal from eutrophic water. The efficiency of LPT in total nitrogen and total phosphorus was 40% and 87%, respectively within 20 days. After combining DPAOs with LPT, the efficiency of total nitrogen and total phosphorus was increased.	Li, et al. [28]

1.3 Research Objectives

1.3.1 To screen the plants for phosphorus treatment from domestic wastewater

1.3.2 To study the efficiency of *Echinodorus cordifolius* in nitrogen and phosphorus treatment from domestic wastewater

1.3.3 To study the bioaugmentation of phosphorus treatment by *Echinodorus cordifolius* with microorganisms (*Pseudomonas putida* and *Flavobacterium oryzihabitans*)

1.4 Scopes of research work

1.4.1 To screened the plants such as *Crinum asiaticun* Linn, *Spathiphyllum Clevelandii*, *Rhizophora apiculata*, *Thalia dealbata* J. Fraser, *Heliconia psittacorum*, *Sonnertia ovat*a, *Sagittaria montevidensis*, and *Echinodorus cordifolius* for phosphorus treatment in domestic wastewater

1.4.2 To study the efficiency of *Echinodorus cordifolius* for nitrogen treatment (in ammonia, nitrite and nitrate-nitrogen forms) and phosphorus treatment from domestic wastewater

1.4.3 To study the relation among plants, microorganisms and soil in the system of phosphorus treatment by investigation relation of plants, microorganisms and soil in phosphorus treatment for 20 cycles which assured the sustainability of the system in phosphorus treatment

1.4.4 To study the bioaugmentation of phosphorus treatment by *Echinodorus cordifolius* with microorganisms (*Pseudomonas putida* and *Flavobacterium oryzihabitans*) which was inoculated in the system