

Using *Echinodorus cordifolius* Augmented with *Pseudomonas putida* and *Flavobacterium oryzihabitans* for Domestic Wastewater Treatment

Jirawan Torit and Paitip Thiravetyan

Abstract—Contaminated phosphorus in aquatic environments is a critical problem. This research studied on removal of phosphorus from domestic wastewater by using *Echinodorus cordifolius* augmented with *Pseudomonas putida* and *Flavobacterium oryzihabitans*. Plants treated phosphorus from domestic wastewater for 20 test runs continuously, the effect of phosphorus treatment by microorganisms in domestic wastewater was very high at 58% in the first test run. Therefore, *P. putida* and *F. oryzihabitans* that were dominant microorganisms in domestic wastewater were inoculated in the system to contribute plants for phosphorus treatment. The result found that *E. cordifolius* augmented with *P. putida* and *E. cordifolius* augmented with mixed microorganisms (*P. putida* and *F. oryzihabitans*) could reduce phosphorus in domestic wastewater until passed standard criteria only 14 hrs. Moreover, it was found that the plants were healthy and increased growth rate in a month obviously conform to the increase of plant biomass. This result indicated that *P. putida* and mixed microorganisms (*P. putida* and *F. oryzihabitans*) contributed phosphorus treatment rapidly and supported plants growth that showed sustainable system. Therefore, using *E. cordifolius* augmented with *P. putida* and *F. oryzihabitans* suit to apply for domestic wastewater treatment.

Keywords—phosphorus, domestic wastewater, *Echinodorus cordifolius*, *Pseudomonas putida*, *Flavobacterium oryzihabitans*

I. Introduction

Eutrophication is a worldwide problem which results from the increase of human activities such as fertilizer production for use in agriculture, industrial discharging, and domestic cleaning [1]. The increase of these activities induces nutrient enrichment like phosphorus and nitrogen in aquatic systems, which accelerates the growth rate of algal and aquatic plants [2]. This problem results in the reduction of light penetration, increasing water turbidity, and the reduction of oxygen in aquatic environments, which causes the death of aquatic animals [3]. Phosphorus is the major pollutant that can induce the eutrophication phenomenon in bodies of water [4]. The domestic wastewater in Thailand is an especially major source

of phosphorus [5]. Consequently, this problem should be managed by selecting suitable implementation for treating nutrients. Physical and chemical methods are not suitable due to their costs and less efficient treatments of this pollutant. In contrast, the phytoremediation method is an alternative method for nutrient treatment which is environmentally friendly, has low cost, and has less negative effects [6]. Moreover, there have been investigations of enhancing biological phosphorus removal which contributed to quick phosphorus removal. Cai et al [7] studied the enhanced biological phosphorus removal with *Pseudomonas putida* GM6 from activated sludge. This bacterium is a polyphosphate-accumulating bacterium that accumulates large amounts of polyphosphate. When it was inoculated in synthetic wastewater, the capacity of phosphorus removal increased. Phosphate-solubilizing bacteria inoculations were reported and *Rhizobium leguminosarum* contributed to phosphorus uptake and the growth promotion of lettuce and maize [8, 9]. Therefore, the bioaugmentation or inoculation of microorganisms for phosphorus removal and plant growth promotion was of interest.

Preliminary studies were performed to screen plants in phosphorus treatment. The different plant species used were *Crinum asiaticum*, *Echinodorus cordifolius*, *Spathiphyllum clevelandii*, *Rhizophora apiculata*, *Thalia dealbata*, *Heliconia psittacorum*, and *Sagittaria montevidensis*. *E. cordifolius* showed the best efficiency in phosphorus treatment. This plant is in emergent aquatic plants with rapid growth, has high biomass, is resistant to plant pathogens, and has flowering plants - qualities that are suitable for the treatment of nutrients. In addition, this plant can grow well in water, persists through various environmental conditions, and consumes a large amount of wastewater in a short time. With these special characteristics noted, the objective of this study was to investigate the treatment of phosphorus from domestic wastewater by *E. cordifolius* augmented with *Pseudomonas putida* and *Flavobacterium oryzihabitans*. In addition, the contribution of plants, microorganisms and soil in phosphorus removal was also studied.

II. Materials and Methods

A. Plant Culture Conditions

E. cordifolius was selected at 3 months of plant age and the same fresh weight for use in the experiment. Plants were cleaned with tap and distilled water to disperse soil particles that had adhered to the plant stems and roots. Then plants were

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used in the experiment at the Remediation Laboratory of King Mongkut's University of Technology Thonburi (KMUTT).

B. The Sustainability of the System in Phosphorus Treatment by *Echinodorus cordifolius* in Domestic Wastewater

The plants were cultured in pots (12 inches in diameter); each pot used 600 g of plants, 300 g of soil and 3 liters of domestic wastewater which was obtained from the wastewater treatment plant of Tungkrui District, Bangkok, Thailand.

The system was comprised of only domestic wastewater, plant + soil + domestic wastewater, and soil + domestic wastewater. Phosphorus treatment in domestic wastewater by *E. cordifolius* was studied continuously for 20 test runs to determine the sustainability of the system. A new lot of domestic wastewater was replaced in the system after phosphate concentration in the previous test run had passed the standard criteria. The remaining phosphate concentration in the systems was determined by the ascorbic acid colorimetric method [10].

C. The Relationship between Plants, Microorganisms, and Soil in Phosphorus Treatment

The relationship between plants, microorganisms, and soil in phosphorus treatment was investigated during the study of the sustainability in phosphorus treatment by *E. cordifolius* in domestic wastewater for 20 test runs. The systems were performed as plant + soil + domestic wastewater and soil + domestic wastewater. Only domestic wastewater and sterile domestic wastewater were used to study the effect of phosphorus uptake by microorganisms in domestic wastewater. In addition, sterile soil + sterile domestic wastewater was also studied in order to determine the effect of phosphorus adsorption by soil. The percentage of phosphorus uptake by plants, phosphorus adsorption by soil, and phosphorus uptake by microorganisms of each test run in the system was determined by the following equations [11].

D. Percentage of Carbon, Nitrogen, and Phosphorus in Plants after Phosphorus Treatment

E. cordifolius was harvested after phosphorus treatment for 20 test runs from the systems of the sustainability in phosphorus treatment from domestic wastewater. Plants were washed with distilled water, oven dried (60-65°C) and then ground into a fine powder for determination of the percentage of carbon, nitrogen, and phosphorus in the plant. The percentage of carbon and nitrogen were determined by using an elemental analyzer (Leco CHN-2000 model FN24B3). The percentage of phosphorus was determined by digesting plant samples with sulphuric-nitric acid followed by the vanadomolybdo-phosphoric acid colorimetric method [12].

The percentage of phosphorus in the plant was calculated as follows:

$$\% \text{ Phosphorus} = \frac{\text{P concentration (mg L}^{-1}) \times 100}{\text{The weight of plant samples (mg)}}$$

E. Biomass of Plants after Phosphorus Treatment in Domestic Wastewater for 20 Test Runs

E. cordifolius in the control and treatment systems of phosphorus treatment were harvested after treatment for 20 test runs to determine the biomass. Harvested plants were washed with distilled water, the fresh weight was recorded, and the samples were oven dried (60-65 °C) to a constant weight. The plant samples were cooled in desiccators and the dry weight was measured [13].

F. Microorganism Preparation

The study of microorganisms in domestic wastewater found that *P. putida* and *F. oryzihabitans* were dominant microorganisms [14]. Therefore, enhancement of phosphorus treatment by *E. cordifolius* augmented with microorganisms was studied.

P. putida and *F. oryzihabitans* were obtained from BIOTEC Culture Collection (BCC) and Department of Medical Sciences (DMST Culture Collection), respectively. Microorganisms were picked up with 2 loops of each plate, inoculated in a flask of 500 mL LB medium, incubated for 24 hrs at room temperature, and then shaken at 200 rpm. The cells were recovered by centrifugation (4,000 rpm for 15 min) and then transferred in sterile distilled water. The cell concentration of each strain and mixed strains were measured by a spectrophotometer to an optical density at 560 nm (OD₅₆₀) of 0.4 and used as an inoculum.

G. Bioaugmentation of Phosphorus Treatment by *Echinodorus cordifolius* with Microorganisms (*Pseudomonas putida* and *Flavobacterium oryzihabitans*)

In this experiment, 300 g of plants, 150 g of soil, 1.5 liters of domestic wastewater, and 10% of each strain and mixed strains were inoculated in domestic wastewater. The experiment was performed in triplicate which was comprised of the systems of wastewater including plants + soil + domestic wastewater, plants + soil + domestic wastewater + *P. putida*, plants + soil + domestic wastewater + *F. oryzihabitans* and plants + soil + domestic wastewater + *P. putida* + *F. oryzihabitans*. The water samples were collected at 0, 1, 6, 12, 14, and 17 hr for analysis of the remaining phosphate concentration in the system.

H. Biomass of Plants after Bioaugmentation of Phosphorus Treatment by *Echinodorus cordifolius* with Microorganisms (*Pseudomonas putida* and *Flavobacterium oryzihabitans*)

E. cordifolius in the system of phosphorus treatments (plants + soil + domestic wastewater, plants + soil + domestic wastewater + *P. putida*, plants + soil + domestic wastewater + *F. oryzihabitans*, and plants + soil + domestic wastewater + *P. putida* + *F. oryzihabitans*) were harvested after treatment for one month in order to determine the biomass which was the same method in previous section.

III. Results and Discussion

A. The Sustainability of the System in Phosphorus Treatment by *Echinodorus cordifolius* in Domestic Wastewater

The sustainability of the system of phosphorus treatment in domestic wastewater was continuously investigated for 20 test runs. The results showed that phosphorus uptake by the plant at the first run was about 11% and gradually increased to 81% at the last test run (Table 1). During phosphorus uptake by *E. cordifolius*, the prolongation of shoots and roots occurred and flowers came out. This result confirmed that phosphorus accelerated the growth rate of plants [15]. In addition, the plant was also healthy in all test runs and produced new leaves and roots of the plants. This result demonstrated a sustainable system of phosphorus treatment by *E. cordifolius*.

B. The Relationship between Plants, Microorganisms, and Soil in Phosphorus Treatment

The relationship between plants, microorganisms, and soil in phosphorus treatment was studied for 20 test runs until soil was saturated. The results showed that the percentage of phosphorus uptake by microorganisms in domestic wastewater was higher than microorganisms in soil (Table 1). This result revealed that microorganisms in domestic wastewater attributed to phosphorus treatment [16, 17]. This result implied that bioaugmentation with microorganisms might increase the efficiency of phosphorus removal. Therefore, the study of bioaugmentation of microorganisms was also investigated (see next section). However, in the first test run, the percentage of phosphorus uptake by microorganisms in domestic wastewater was very high at about 58%. After that, the percentage

decreased due to dead microorganisms after being kept the wastewater in the refrigerator 4 °C before running the experiments. The percentage of phosphorus adsorption by soil gradually increased from test run no. 1 to test run no. 3 and then gradually decreased to 1% at test run no. 20. This meant that the soil started to saturate at test run no. 20. After that the plant played a major role in phosphorus removal which was about 81% at test run no. 20 which was superior to microorganisms in the system (Table 1). This result illustrated the sustainability of plant in phosphorus treatment.

C. Percentage of Carbon, Nitrogen, and Phosphorus in Plants after Phosphorus Treatment

Arnon and Stout [18] proposed that carbon, nitrogen, and phosphorus were essential elements for the growth of higher plants. Carbon and nitrogen are major components of organic material and are involved in enzymatic processes. Phosphorus uptake by plants is in phosphate form which is involved in energy transfer reactions [15]. The percentage of these elements may be different in each plant due to plant age, growth rate, species, and environmental condition [19]. This study found that there was a significant difference in the percentage of nitrogen and phosphorus in the control and domestic wastewater treatment system after running the test experiments 20 times. The percentage of nitrogen and phosphorus in the treatment system was higher than the control system (Table 2). From Torit et al. study [11], there was not a significant difference in the percentage of nitrogen and phosphorus under the control and treatment systems after running the test experiments only four times. These results suggested that nitrogen and phosphorus were increasingly accumulated in plant tissues when the time increased. In addition, the results also found that the percentage of nitrogen in the control and treatment system was higher than the percentage of phosphorus. It demonstrated that plants used nitrogen for growth at a higher rate which was similar to the study of nitrogen and phosphorus concentration in the tissues of canna, iris, and pickerel weed [20].

D. Biomass of Plants in Domestic Wastewater after 20 Test Runs

Plants grown in tap water (control) and domestic wastewater (treatment) were determined for biomass after running the experiment 20 times. The results showed that the biomass in the treatment system increased, whereas in the control system, it was relatively constant (Table 3). This result was due to phosphorus and other nutrients from domestic wastewater which accelerated the growth of plants [15]. The plant characteristic appeared to extend the shoots and roots including the production of new leaves.

TABLE I. THE RELATIONSHIP BETWEEN PLANTS, MICROORGANISMS AND SOIL IN PHOSPHORUS TREATMENT (NO. OF TEST RUN 1-20)

The Relationship between Plants, Microorganisms, and Soil in Phosphorus Treatment	No. of Test Run																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
% P uptake by microorganisms in wastewater	58	17	10	10	9	8	7	12	9	5	7	12	7	4	20	9	8	11	12	14
% P uptake by microorganisms in soil	3	2	2	1	2	4	2	1	1	2	2	1	2	1	1	1	1	1	5	4
% P uptake by <i>E. cordifolius</i>	11	22	12	14	15	17	19	20	25	29	56	50	57	57	47	67	67	71	74	81
% P adsorption by soil	28	60	77	75	74	71	72	67	65	64	36	37	33	38	32	23	24	17	9	1

TABLE II. PERCENTAGE OF CARBON, NITROGEN, AND PHOSPHORUS IN *ECHINODORUS CORDIFOLIUS* IN THE CONTROL AND TREATMENT (DOMESTIC WASTEWATER) AFTER THE 20 TEST RUNS

Systems	% Carbon	% Nitrogen	% Phosphorus
<i>E. cordifolius</i> +soil+tap water (control)	35.17±1.46 ^a	1.24±0.02 ^a	0.71±0.04 ^a
<i>E. cordifolius</i> +soil+wastewater (treatment)	35.90±1.35 ^a	2.03±0.59 ^b	1.29±0.06 ^b

*Values in the same column with the same letter are not significantly different. ($\alpha = 0.05$)

TABLE III. BIOMASS OF *ECHINODORUS CORDIFOLIUS* BEFORE AND AFTER THE 20 TEST RUNS

Systems	Biomass (g dry weight plant ⁻¹)	
	Before treatment	After treatment
<i>E. cordifolius</i> +soil+tap water (control)	110.16±1.08 ^a	111.73±2.06 ^a
<i>E. cordifolius</i> +soil+wastewater (treatment)	110.16±1.08 ^a	142.74±3.89 ^b

*Values in the same column with the same letter are not significantly different. ($\alpha = 0.05$)

In addition, plants produced flowers and appeared healthy in domestic wastewater [21]. The result also showed the increase in the biomass of plants which resulted from the resource of bioenergy production [22].

E. Bioaugmentation of Phosphorus Treatment by Echinodorus cordifolius with Microorganisms (Pseudomonas putida and Flavobacterium oryzihabitans)

Bioaugmentation of phosphorus treatment by *E. cordifolius* with microorganisms (*P. putida* and *F. oryzihabitans*) was

investigated. The result showed the efficiency of phosphorus treatment of *E. cordifolius* with microorganisms was higher than the treatment without microorganisms. At hr 6, the efficiency of phosphorus treatment in the system augmented with microorganisms was about 60% while in the system without augmented with microorganisms was only about 40% (Fig. 1). This result suggested that microorganisms used phosphorus for their cellular maintenance, synthesis of nucleic acid, construction of cell membranes as phospholipids, and energy transfer within the cells [23]. At hr 12, the efficiency of phosphorus treatment in the system augmented with *P. putida* was 83% that differed from the system augmented with *F. oryzihabitans* and mixed microorganisms system obviously, which was 73% (Fig. 1). However, the efficiency of phosphorus treatment in the system augmented with *P. putida* was not significant difference from the system augmented with mixed microorganisms that was 90% at hr 14 and passed the standard criteria of the U.S.EPA (0.1 mg L⁻¹) [6] rapidly in this hour. This result indicated that the efficiency of *P. putida* and mixed microorganisms (*P. putida* and *F. oryzihabitans*) contributed plants for enhanced phosphorus treatment. The system of *E. cordifolius* augmented with *P. putida* appeared to increase the plant growth rate after 2 weeks obviously. The plant was very healthy and had extended shoots and leaves. After phosphorus treatment for 3 weeks, the plant also had a flowering stage. Moreover, the result of this plant growth rate

was consistent with the result of plant increased biomass after treatment for one month (Table 4). The systems of phosphorus treatment by *E. cordifolius* with microorganisms and the system of phosphorus treatment by the plant alone were significantly different.

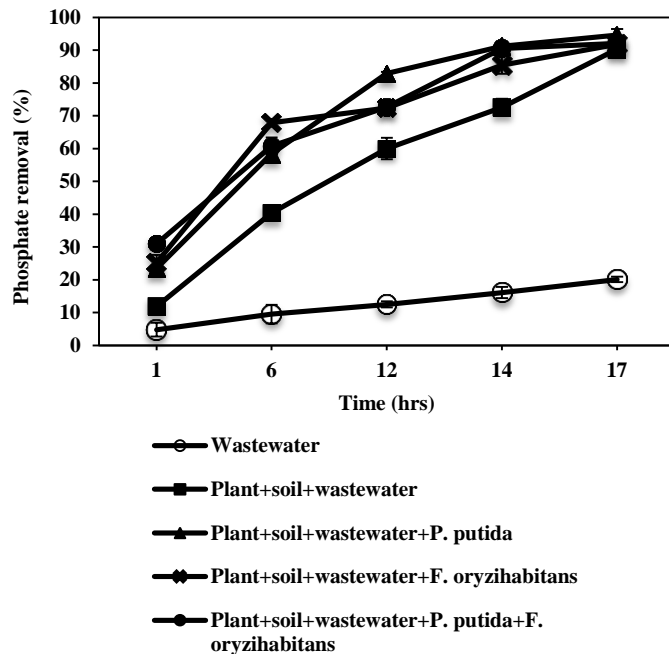


Figure 1. Efficiency of phosphate removal in domestic wastewater under various conditions

TABLE IV. BIOMASS OF PLANTS BEFORE AND AFTER PHOSPHORUS TREATMENT BY *ECHINODORUS CORDIFOLIUS* AUGMENTED WITH MICROORGANISMS (*PSEUDOMONAS PUTIDA* AND *FLAVOBACTERIUM ORYZIHABITANS*)

Systems	Biomass (g dry weight plant ⁻¹)	
	Before treatment	After treatment
<i>E. cordifolius</i> +soil+wastewater	37.20±1.36 ^a	39.30±0.67 ^a
<i>E. cordifolius</i> +soil+wastewater+ <i>P. putida</i>	37.20±1.36 ^a	46.17±4.74 ^b
<i>E. cordifolius</i> +soil+wastewater+ <i>F. oryzihabitans</i>	37.20±1.36 ^a	42.88±2.81 ^b
<i>E. cordifolius</i> +soil+wastewater+ <i>P. putida</i> + <i>F. oryzihabitans</i>	37.20±1.36 ^a	41.46±0.80 ^b

*Values in the same column with the same letter are not significantly different. ($\alpha = 0.05$)

The available phosphorus analysis compared with the total phosphorus in domestic wastewater (data not shown) suggested that phosphorus uptake and the growth rate of plants in the system increased due to the contribution of organic phosphorus solubilization by microorganisms. The roles of microorganisms to the solubilization of organic phosphorus may release proton, CO₂, and secondary organic metabolites (sugars, organic acid anions, amino acids, siderophores, enzymes, and phenols) [24]. There have been several

researches that investigated the solubilization of inorganic phosphate by microorganisms. For example, Illmer and Schinner [25] found that *Pseudomonas* sp. produced organic acid for mineral phosphate solubilization in soil. There was the study of *Pseudomonas putida* can promote poplar trees growth [26]. From this study, *Pseudomonas* and *Flavobacterium* were used as phosphate solubilizing bacteria and plant growth promotion [27]. Therefore, the role of these bacteria contributed to organic phosphorus solubilization in domestic wastewater that enhanced available phosphorus uptake by plants.

IV. Conclusions

The study of the sustainable system of phosphorus treatment, plants can continuously take up phosphorus in domestic wastewater though 20 test runs. The plants were healthy and had prolonged shoots, roots, and new leaves. This result was consistent with the increasing plant biomass. Plants also appeared to have a flowering stage that resulted from phosphorus uptake in domestic wastewater. The percentage of phosphorus and nitrogen increased in plants treated with domestic wastewater for 20 test runs. These results showed a sustainable system in phosphorus treatment by *E. cordifolius*. In the first test run, microorganisms in domestic wastewater had the best efficiency in phosphorus treatment. After the soil was saturated in phosphorus adsorption, the *E. cordifolius* plant played a major role in phosphorus treatment. In addition, bioaugmentation of phosphorus treatment by *E. cordifolius* with *P. putida* and *F. oryzihabitans* showed higher phosphorus treatment than the system without microorganisms. The result indicated that microorganisms enhanced the plant in phosphorus treatment by solubilization of organic phosphorus in domestic wastewater. This affected the plant growth rate promotion which was consistent with the increasing plant biomass in the system with microorganisms. Therefore, *E. cordifolius* augmented with *P. putida* and *F. oryzihabitans* enhanced better in phosphorus treatment.

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This paper introduces the plant growth promoting bacteria for domestic wastewater treatment. Using plant with microorganisms could be enhanced phosphorus removal and suit to apply for domestic wastewater treatment.