

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characteristic of domestic wastewater

Domestic wastewater obtained from wastewater treatment plant of Tungkru District, Bangkok, Thailand. Characteristic of domestic wastewater was investigated as shown in Table 4.1.

Table 4.1Characteristic of domestic wastewater

Parameters	Value	Standard of domestic wastewater
pH	7.79	5-9 ^a
BOD (mg L ⁻¹)	30	≤ 30
COD (mg L ⁻¹)	123.28	≤ 120 ^a
TSS (mg L ⁻¹)	419	≤ 40 ^a
TKN (mg L ⁻¹)	8.93	≤ 35 ^a
TP (mg L ⁻¹)	2.25	0.1 ^b

^a Notification of the Ministry of Science and Technology (1996), Department of Industrial Works (2007).

^b U.S. EPA (1991).

Domestic wastewater was the major source of phosphorus. Total phosphorus value was excess than standard of domestic wastewater (Table 4.1). This problem induced to the study of nutrient treatment from domestic wastewater by plants.

4.2 Screening plants for phosphorus removal

4.2.1 Screening plants for phosphorus removal under soilless conditions

Crinum asiaticum Linn, *Echinodorus cordifolius*, *Spathiphyllum clevelandii*, *Rhizophora apiculata*, *Thalia dealbata* J. Fraser, and *Heliconia psittacorum* were screened for phosphorus removal under soilless conditions. Plants were cultured in domestic wastewater, each pot used 1.5 L of wastewater and used 600 g of plants, grown in the greenhouse. The result found that at day 7, *C. asiaticum* Linn and *E. cordifolius* could uptake phosphorus almost completely. However, this result showed potential of *E. cordifolius* which uptake phosphorus rapidly during day 0 to day 1, demonstrated efficiency of phosphorus uptake as shown in Figure 4.1. Moreover, characteristic of *S. Clevelandii* Linn and *E. cordifolius* after phosphorus treatment found yellow leaf of plants when compared with control that had green leaves. This characteristic indicated that phosphorus uptake of plants induced to plants senescence rapidly that meant phosphorus accelerated the growth rate of plants.

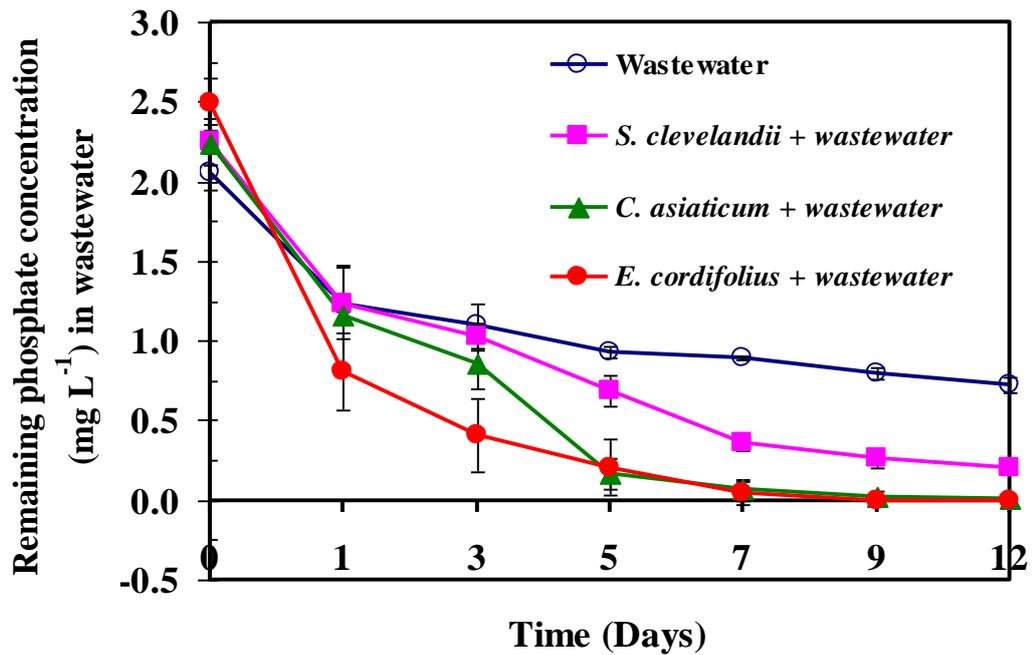


Figure 4.1 Phosphorus removal by *S. Clevelandii*, *C. asiaticum* Linn, and *E. cordifolius* under soilless conditions

R. apiculata, *T. dealbata* J. Fraser and *H. psittacorum* were screened for phosphorus removal under soilless conditions. The result showed that *T. dealbata* J. Fraser could remove phosphorus higher than *Rhizophora apiculata* and *Heliconia psittacorum* as shown in Figure 4.2. However, when compared with *E. cordifolius*, it was found that *T. dealbata* J. Fraser had efficiency of phosphorus removal less than *E. cordifolius* (data not shown). In addition, after phosphorus removal for 7 days, *R. apiculata* died. This demonstrated that *R. apiculata* did not tolerate to domestic wastewater because this plant preferred salt water.

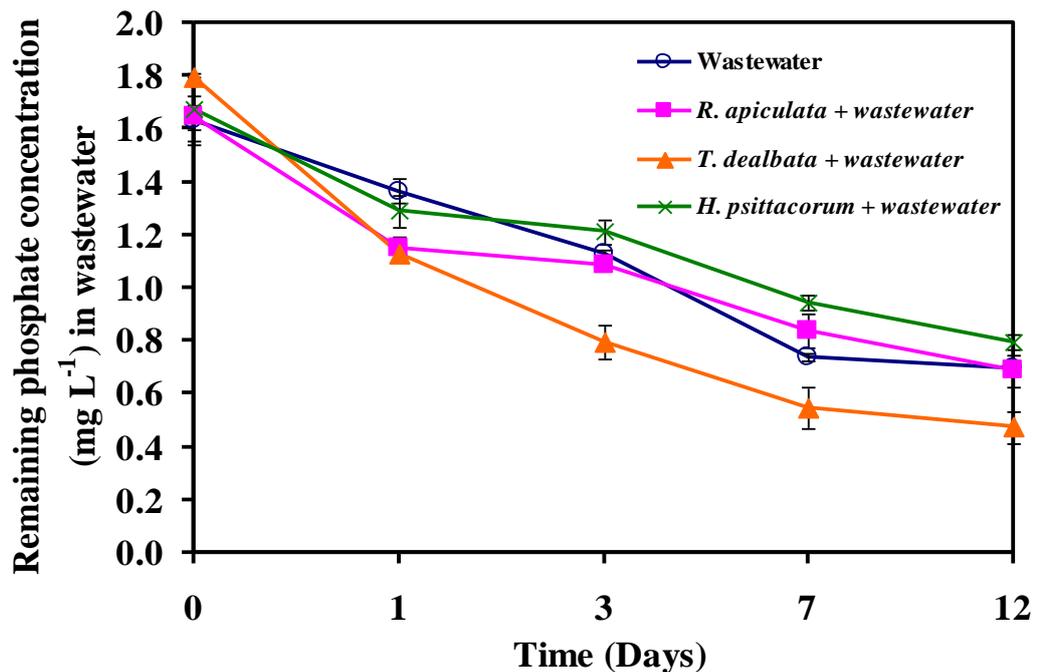


Figure 4.2 Phosphorus removal by *R. apiculata*, *T. dealbata* J. Fraser, and *H. psittacorum* under soilless conditions

In addition, *C. asiaticum* Linn and *E. cordifolius* were studied to phosphorus treatment for 4 cycles. After phosphorus concentration in domestic wastewater was depleted in each cycle, new lot of domestic wastewater was replaced in the pots and continued to phosphorus treatment by plants. The result found that when *C. asiaticum* Linn and *E. cordifolius* treated domestic wastewater for 4 cycles which cycle 1-2 used times for 12 days while cycle 3 took shorter times (only 7 days) as shown in Figure 4.3. This meant that the plants could adapt themselves to uptake phosphorus rapidly and when the time passed a month, the plants remained acquisition phosphorus which plants had efficiency for phosphorus treatment.

However, phosphate concentration in cycle 4 increased after day 3 (Figure 4.3) due to plant decaying and caused phosphate released from the plant. Therefore, using plant to treat phosphorus should beware of harvesting the plant decaying.

Characteristic of *C. asiaticum* Linn and *E. cordifolius* after treatment for 4 cycles appeared new small leaves when compared with control set that no new small leaves. Characteristic of *E. cordifolius* and *S. Clevelandii* appeared flowering of plants after phosphorus treatment when compared with control set that no appearance of flower from the plants. These results demonstrated role of phosphorus which contributes acceleration of the growth rate of plants. It was found that *E. cordifolius* had the highest efficiency of phosphorus removal because the root systems of *E. cordifolius* have higher ratios of surface area to volume. The root systems of *E. cordifolius* that have root clusters (proteoid roots). These specialized roots might be exude high amounts of organic acids, resulting in the mobilization of phosphorus influx roots zone of plants [9]. Phosphorus is an essential nutrient for plants growth, which is a major building block of DNA molecules. It stored energy in adenosine diphosphate (ADP) and adenosine triphosphate (ATP). The energy stored will be transported across the cell wall and synthesis nucleic acid and proteins [14].

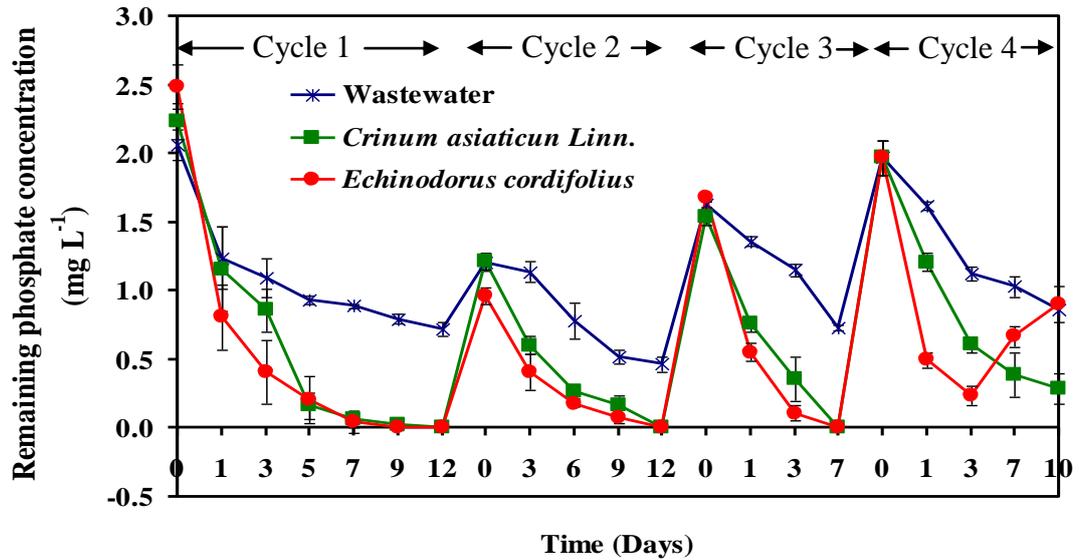


Figure 4.3 Treatment of phosphorus from domestic wastewater by *C. asiaticum* Linn and *E. cordifolius* under soilless conditions for 4 cycles

The result of the control system containing only wastewater found that phosphate concentration was decreased. This was due to microorganisms in wastewater could uptake phosphate for their growth [29] that could be confirmed by the experiment of using sterile wastewater. The result found that the concentration of phosphate was stable because in sterile wastewater had no microorganisms (Figure 4.4).

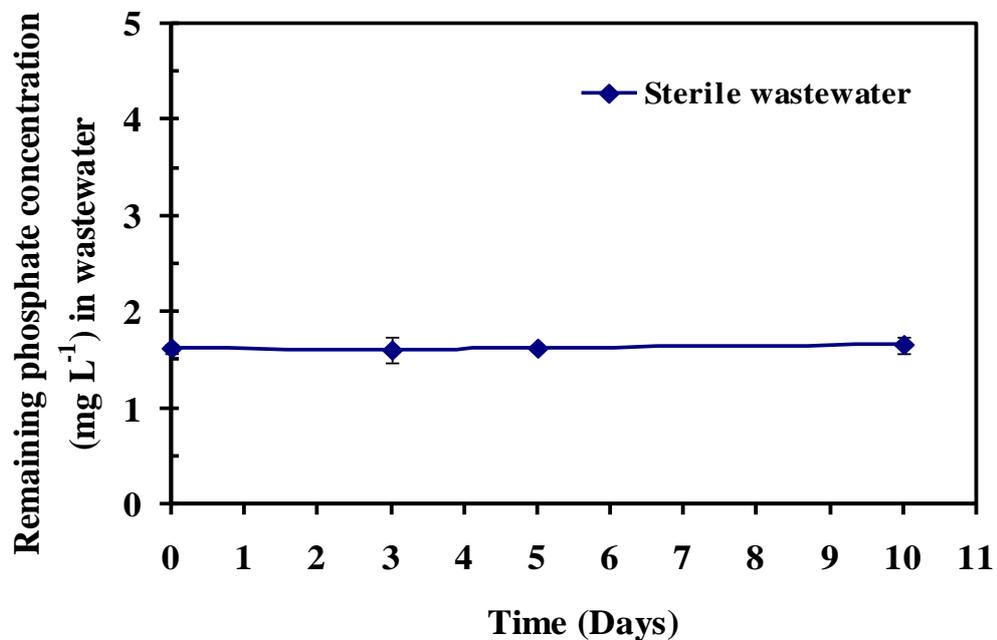


Figure 4.4 Remaining phosphate concentration in sterile domestic wastewater at various times

In addition, the change in pH during phosphorus treatment from domestic wastewater by *E. cordifolius*, *C. asiaticun*, *S. clevelandii*, *R. apiculata*, *T. dealbata*, and *H. psittacorum* under soilless conditions was studied (Figures 4.5 and 4.6). The change in pH during phosphorus treatment from domestic wastewater by *E. cordifolius* and *C. asiaticun* over 4 cycles was also investigated (Figure 4.7). The result showed that the pH of the treatments containing plants were maintained at pH 7-8 because the plants could adjust the system pH to be appropriate pH for their growth. This result indicated that optimum pH for the plants was pH 7-8. However, pH in control system that containing only domestic wastewater had high pH from 7 to 9 (Figures 4.5-4.7). This result might cause from algae growing in control system. Since algae had the photosynthesis, which depleted dissolved CO₂ from the water and increased water pH [52]. At low pH is dangerous to plant growth directly and affect to availability of mineral elements indirectly [53]. In general, plants grow well in the pH range of 5.5-7.0. There was report for optimum pH ranges of water hyacinth were 6.5-7.5 and 5.8-6.0 [54, 55], as well as *Chlorella sorokiniana* which is microalgae grew best at pH 7-8 [56].

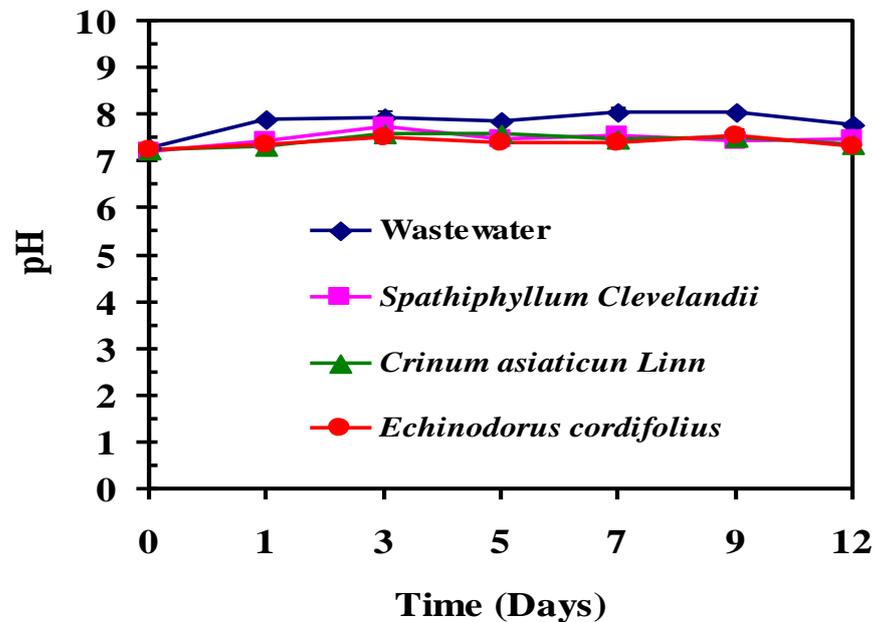


Figure 4.5 Change in pH during phosphorus treatment from domestic wastewater by *E. cordifolius*, *C. asiaticun*, and *S. clevelandii* under soilless conditions

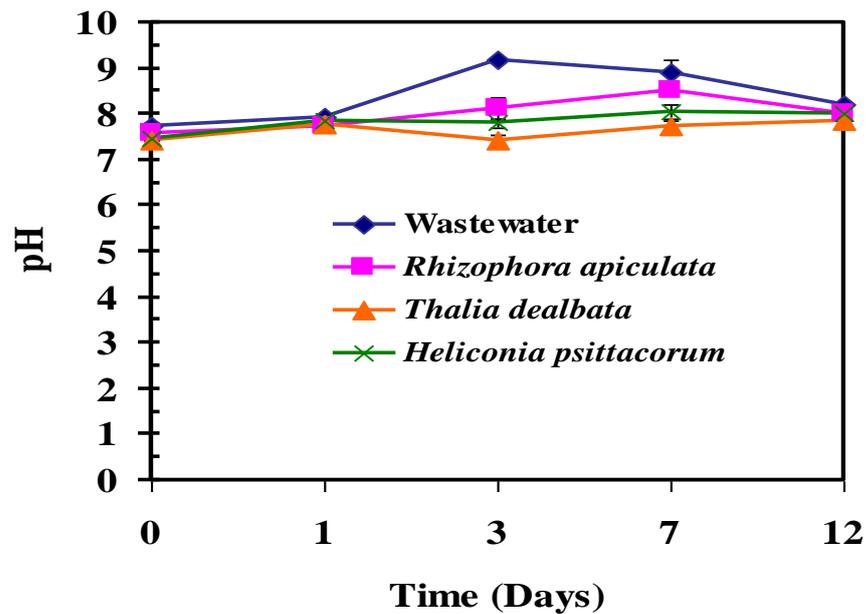


Figure 4.6 Change in pH during phosphorus treatment from domestic wastewater by *R. apiculata*, *T. dealbata*, and *H. psittacorum* under soilless conditions

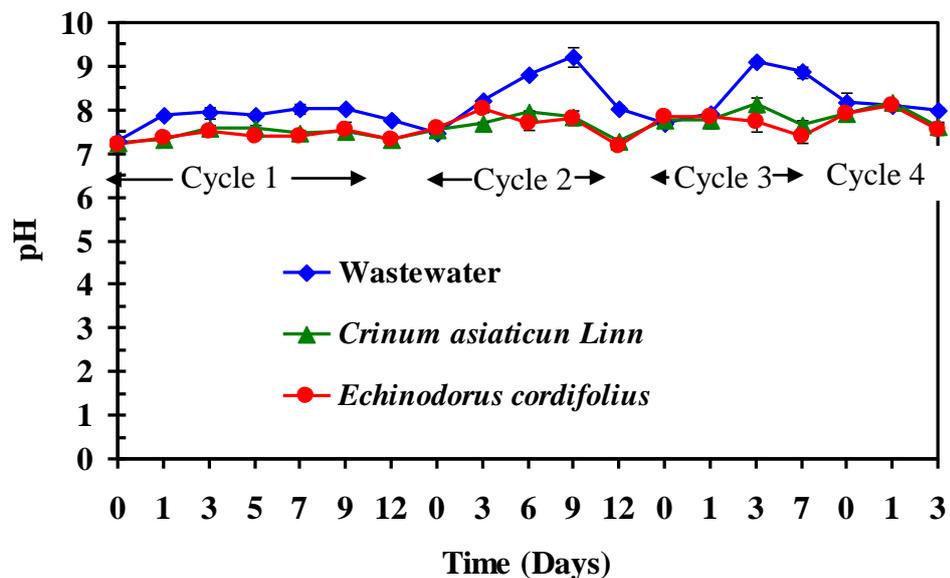


Figure 4.7 Change in pH during phosphorus treatment from domestic wastewater by *E. cordifolius* and *C. asiaticum* over 4 cycles

4.2.2 Screening plants for phosphorus removal under soil conditions

For screening the plants for phosphorus removal under soil conditions, *Echinodorus cordifolius*, *Sonnertia ovata* and *Sagittaria montevidensis* were used. The result showed that *S. montevidensis* or Arrowhead had efficiency of phosphorus removal better than *S. ovata* and had nearly efficient with *E. cordifolius* because it is healthy, fast growth and also beautiful flowering plant (Figure 4.8). Therefore, *S. montevidensis* was selected for phosphorus removal compared to *E. cordifolius* in next section. This screening plants for phosphorus removal under soil conditions showed different efficiency in

phosphorus removal by different plants that plants can take up phosphorus from wastewater through root to shoot. Moreover, the major of phosphorus uptake by plants from soil with high-affinity phosphorus transporters will occur via roots and transport to the root and the shoot [57, 58, 11].

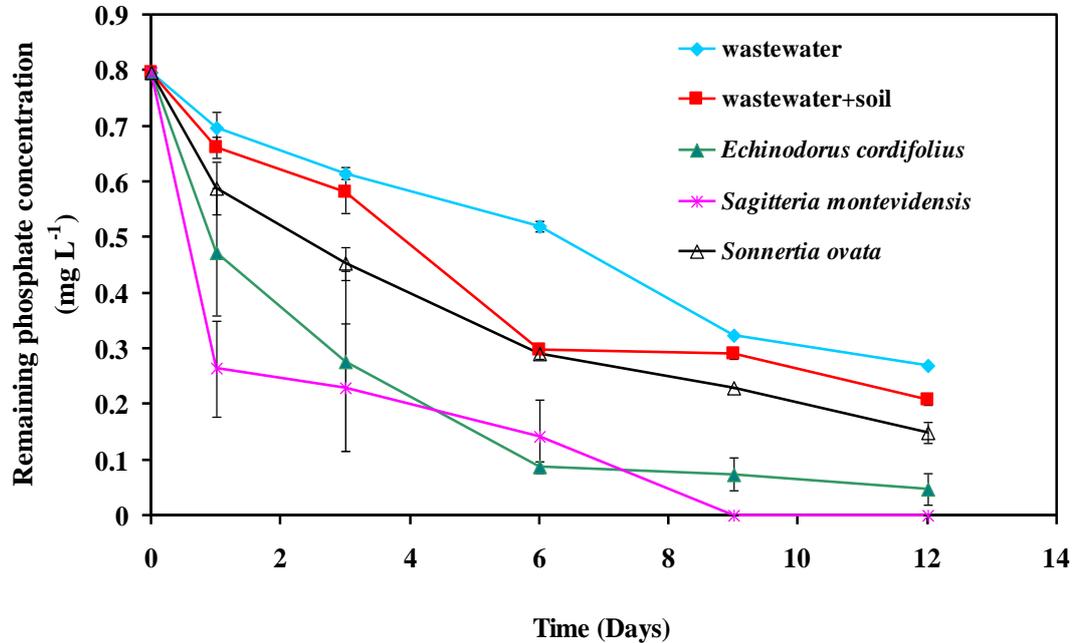


Figure 4.8 Treatment of phosphorus from domestic wastewater by *E. cordifolius*, *S. ovata* and *S. montevidensis* under soil conditions

4.3 Comparison of the abilities between *E. cordifolius* and *S. montevidensis* in phosphorus, nitrogen, and COD treatment from domestic wastewater under soil conditions

4.3.1 Treatment of phosphorus by *E. cordifolius* and *S. montevidensis*

E. cordifolius was used for 2 days for treatment in the first cycle. Cycles 2, 3, 4, and 5 used 1 day, 1 day, 21 hrs, and 20 hrs, respectively, for treatment of phosphorus (Figure 4.9) and passed standard criterion of the U.S. EPA (0.1 mg L^{-1}) [3]. This result demonstrated that the plants adapted themselves to take up phosphorus faster than the original plant and revealed the potential for a sustainable system. Meanwhile, *S. montevidensis* was used for 2 days to pass standard criterion for all cycles of phosphorus treatment. In cycle 2 to cycle 5, remaining phosphate concentration in the system with *S. montevidensis* was higher than the system of soil and wastewater. The result showed that *S. montevidensis* appeared wilt and was obviously illustrated in cycle 5, which led to phosphate being released into the system by the decomposition process [59]. The results showed that the efficiency of phosphorus treatment by *E. cordifolius* was better than *S. montevidensis*.

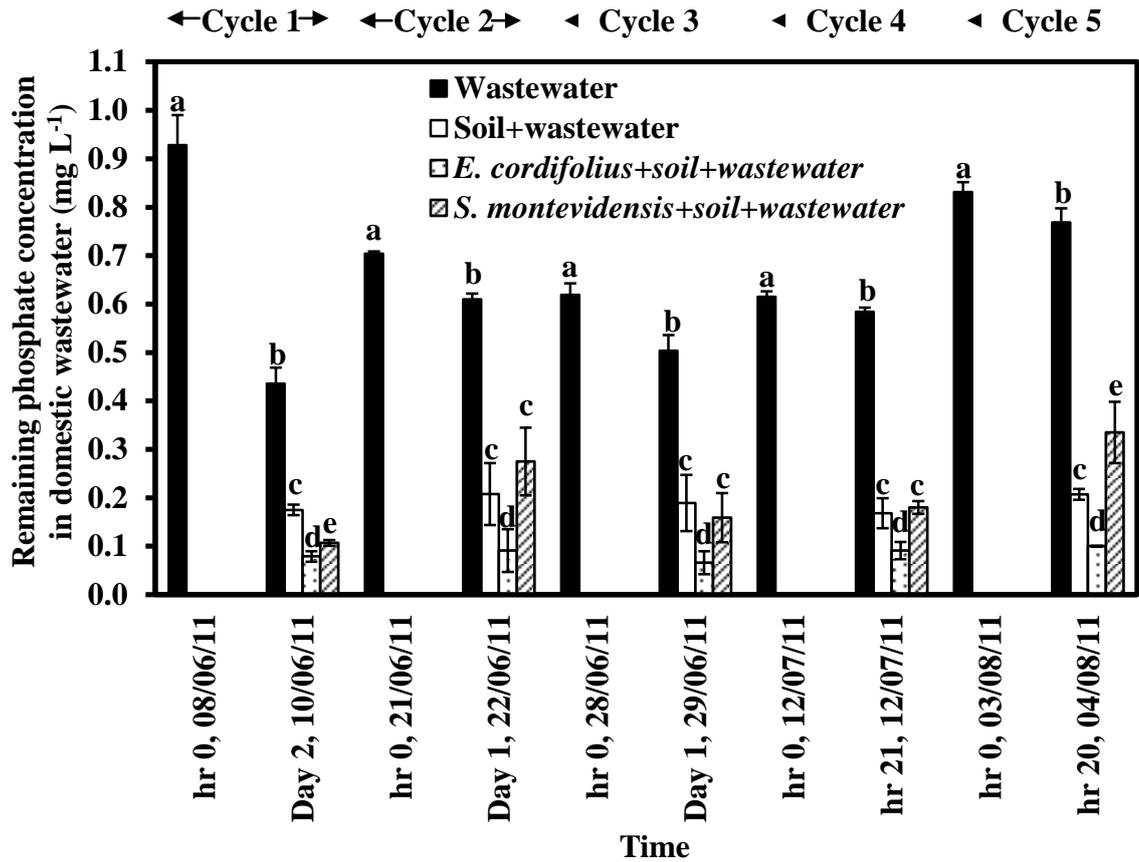


Figure 4.9 Remaining phosphate concentration in domestic wastewater treated with *E. cordifolius* and *S. montevideensis* after adding a new amount of domestic wastewater for five cycles under soil conditions

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

4.3.2 Treatment of nitrogen by *E. cordifolius* and *S. montevideensis*

Treatment of nitrogen by *E. cordifolius* and *S. montevideensis* was investigated in cycle 5. With ammonia-nitrogen and nitrate-nitrogen, the treatment ability of *E. cordifolius* was significantly higher than *S. montevideensis* (Tables 4.2), and it was found that the system with plants could remove ammonia-nitrogen and nitrate-nitrogen in higher amounts than the system without plants. This result was due to the fact that the plants take up nitrogen in ammonia and nitrate forms available for their growth [60]. However, nitrate-nitrogen concentration in the *S. montevideensis* + soil + wastewater system was higher than the wastewater and soil + wastewater system. The reason because of *S. montevideensis* decayed and released nitrate-nitrogen into the system. This result indicated that should beware decaying of selected plant for nitrogen treatment affect to release nitrate back to the system. Moreover, the reduction of ammonia-nitrogen in the system might be due to the nitrification process, in which microorganisms in the system transform ammonia-nitrogen to nitrite and nitrate-nitrogen [16]. This result obviously showed the efficiency in nitrogen treatment by *E. cordifolius* was better than *S. montevideensis*.

Table 4.2 Treatment of ammonia-nitrogen and nitrate-nitrogen by *E. cordifolius* and *S. montevidensis* in cycle 5

Samples	NH ₃ ⁺ -N concentration (mg L ⁻¹)		NO ₃ ⁻ -N concentration (mg L ⁻¹)	
	Hr 0	Hr 20	Hr 0	Hr 20
Wastewater	6.17±0.00 ^a	5.59±0.00 ^a	1.7±0.0 ^a	0.5±0.2 ^a
Soil+wastewater	6.17±0.00 ^a	3.97±0.21 ^b	1.7±0.0 ^a	0.5±0.1 ^a
<i>E.cordifolius</i> +soil+wastewater	6.17±0.00 ^a	0.74±0.21 ^c	1.7±0.0 ^a	0.4±0.1 ^a
<i>S. montevidensis</i> +soil+wastewater	6.17±0.00 ^a	1.32±0.21 ^d	1.7±0.0 ^a	2.3±1.2 ^b

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

4.3.3 Treatment of COD by *E. cordifolius* and *S. montevidensis*

Treatment of COD by *E. cordifolius* and *S. montevidensis* was studied for 5 cycles by investigation of the initial and final COD concentration in each cycle of the system. The results found that the reduction of COD levels in a system with plants at day 2 of each cycle performed better than the system without plants (Figure 4.10). The decrease in COD level may be due to the filtration of suspended solids and particulates by the extensive root system of plants and also microbial degradation in the system [61]. However, in cycles 4 and 5, remaining COD in the system with *S. montevidensis* was higher than the system without plants. This result was due to *S. montevidensis* becoming decayed, which affected organic matter contaminants in the wastewater. From this result, it can be seen that the efficiency of *E. cordifolius* in COD treatment was better than *S. montevidensis*.

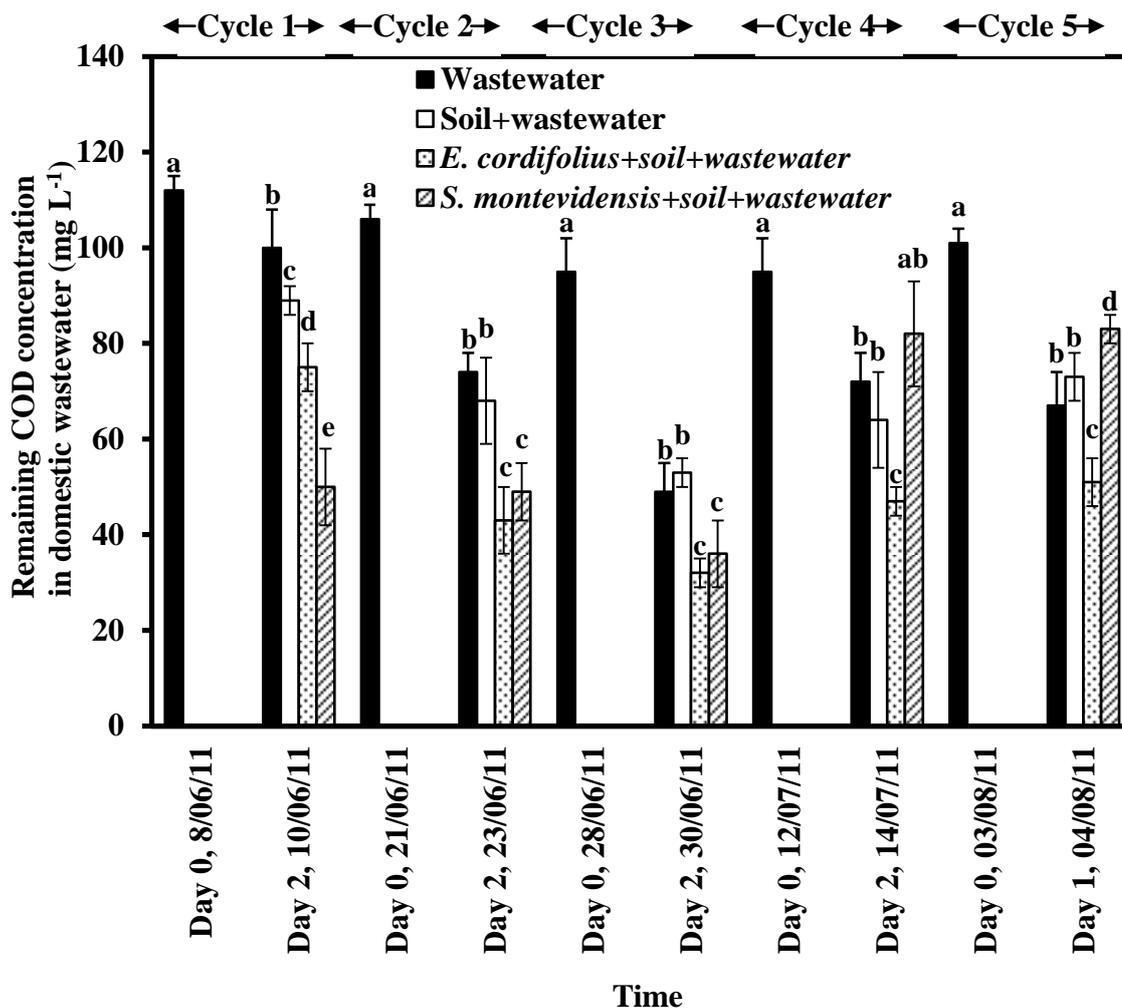


Figure 4.10 Remaining COD concentration in domestic wastewater treated with *E. cordifolius* and *S. montevidensis* after adding a new amount of domestic wastewater for five cycles under soil conditions

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

4.4 Enhancing phosphorus removal of plants by using sawdust bottom ash

4.4.1 Enhancing phosphorus removal of *E. cordifolius* by using sawdust bottom ash

The increasing of phosphorus removal efficiency by soil amendment is now an emerging and promising trend [62]. There was several researchers studied phosphorus adsorption by using media containing calcium, magnesium or iron composition such as sand, limestone, and fly ash [63]. Therefore, sawdust bottom ash was selected for enhancing phosphorus removal. In treatment pots, 1% and 5% (w/w) sawdust bottom ash was added in 300 g of soil and used the same weight of plants (*E. cordifolius*) and used 2.5 L of wastewater. The result showed that concentration of phosphate decreased in treatment set with plants and sawdust bottom ash, demonstrated that sawdust bottom ash could increase efficiency of phosphorus removal due to calcium, magnesium, and potassium are composition of sawdust bottom ash might be affected to phosphorus uptake by plants (Figure 4.11). In addition, sawdust bottom ash might be affected to plants. However, the result of treatment set with plants but without sawdust and

treatment of sawdust bottom ash 1% and 5% with plants were not significantly different ($p < 0.05$) of phosphate removal. The ability of phosphorus removal in the treatment set with soil and 1%, 5% of sawdust bottom ash was lower than the treatment set with soil and domestic wastewater. This was due to sawdust bottom ash containing phosphorus. This was confirmed by the measurement of phosphate concentration in the pot containing sawdust bottom ash and distilled water (data not shown). Therefore, adding soil amendment with plant for phosphorus removal should beware of phosphorus from materials releasing into the system.

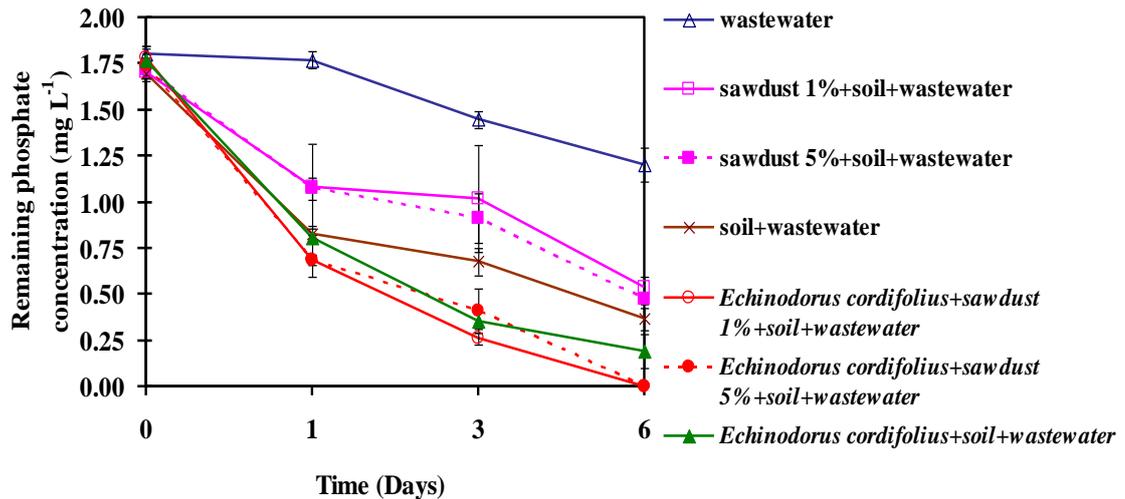


Figure 4.11 Effect of sawdust bottom ash to phosphorus removal in domestic wastewater by *Echinodorus cordifolius*

4.4.2 Enhancing phosphorus removal of *S. montevidensis* by using sawdust bottom ash

In treatment pots were put 1% of sawdust bottom ash in 300 g of soil and used the same weight of plants (*S. montevidensis*) and used 2.5 L of wastewater. The result was shown in (Figure 4.12) and found that treatment set with plant was not significantly different of phosphorus removal from treatment set with 1% sawdust bottom ash and plant ($p < 0.05$). This result suggested that only treatment set with plant is adequate potential for phosphorus removal from domestic wastewater because its ability reduced phosphate concentration passed standard criterion (0.1 mg L^{-1}) [3] quickly when compared with other treatment. In addition, this result was consistent with the experiment of phosphorus removal enhancing by using sawdust bottom ash with *E. cordifolius*.

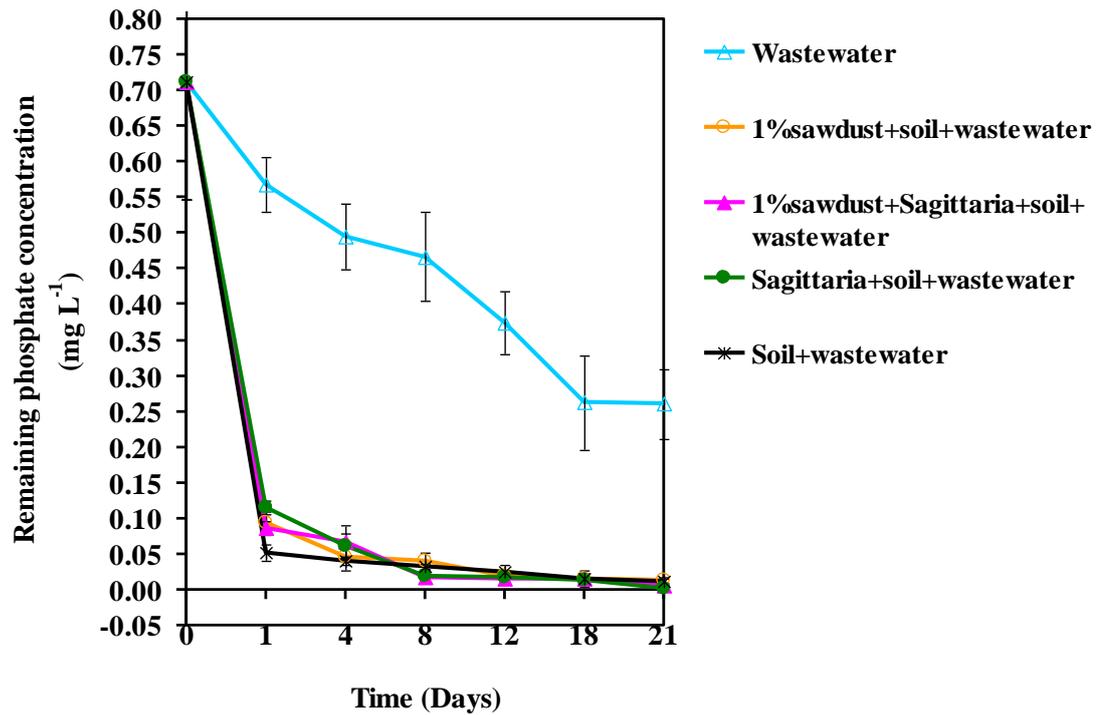


Figure 4.12 Effect of sawdust bottom ash to phosphorus removal in domestic wastewater by *S. montevidensis*

4.5 Phosphorus removal from domestic wastewater by *E. cordifolius* under soil conditions for 4 cycles

Phosphorus removal in domestic wastewater by *E. cordifolius* under soil conditions was studied. The result showed phosphorus removal ability of system for 4 cycles (Figure 4.13) which could remove phosphorus rapidly and passed standard criterion [3] within 3 days. Phosphorus treatment by *E. cordifolius* under soil conditions was faster than under soilless conditions due to the effect of phosphorus adsorption by soil. Charging minerals in soil can be attached to phosphorus from domestic wastewater [64] including microorganisms in soil and wastewater can be used phosphorus for their growth.

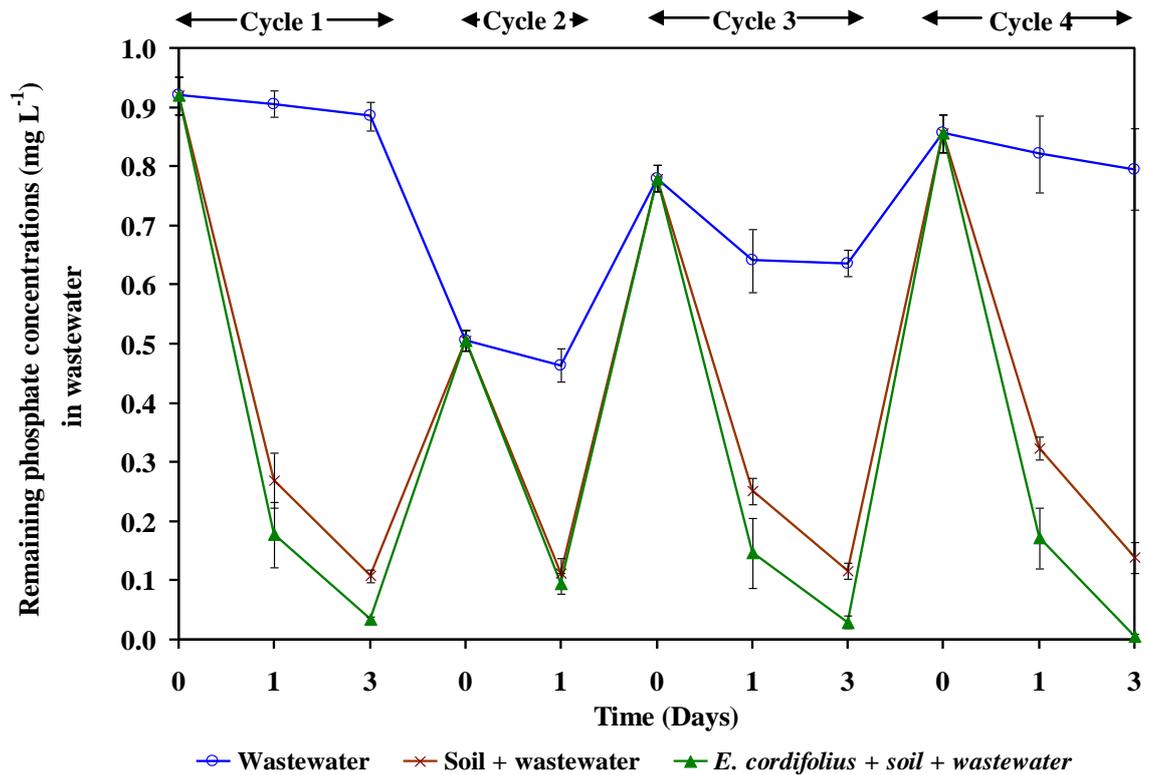


Figure 4.13 Remaining phosphate concentration in domestic wastewater of *E. cordifolius* after adding new domestic wastewater for 4 cycles under soil conditions

4.6 The relationship among plants, microorganisms, and soil in phosphorus removal of cycle 4

The relationship among plants, microorganisms, and soil in phosphorus removal was investigated in cycle 4. This result indicated that the system was not only plant role for phosphorus removal but also depended on soil and microorganisms in the system (Figure 4.14). The efficiency of phosphorus uptake by plant, phosphorus uptake by microorganisms in soil, phosphorus uptake by microorganisms in domestic wastewater, and phosphorus adsorption by soil was 16%, 6%, 7%, and 71%, respectively. Plant revealed a role for phosphorus removal more than microorganisms in the system. This study, phosphorus was removed by plant to 16% which differed from other researcher who found that soil adsorption was main factor and phosphorus was removed by plants only less than 1% [65]. Moreover, the result illustrated that when soil was saturated with phosphorus, plant might be play the major role for phosphorus removal in the system because of phosphorus requirement for plant growth continuously.

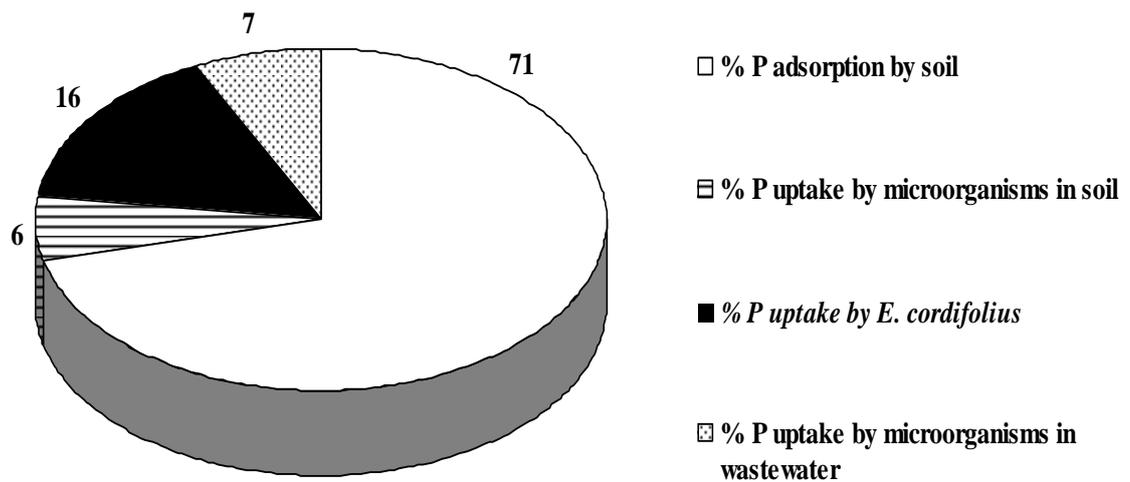


Figure 4.14 Effects of plant, soil and microorganisms in phosphorus removal from domestic wastewater of cycle 4

4.7 Relation between photosynthesis and biomass

The relation between photosynthesis and biomass of phosphorus removal system was studied. Chlorophyll fluorescence was measured by the ratio of variable fluorescence to maximal fluorescence after dark-adaptation (F_v/F_m). This measurement revealed plant photosynthesis performance. The result found that chlorophyll fluorescence of treatment (*E. cordifolius* + soil + wastewater) was higher than the control (*E. cordifolius* + soil + tap water). Chlorophyll fluorescence related with photosynthesis performance, when high chlorophyll fluorescence affected to good photosynthesis capability [66]. Chlorophyll fluorescence of both treatment and control were significantly different after phosphorus treatment in cycle 1, 2, 3, and 4 (Table 4.3) because plant in treatment system got nutrients from domestic wastewater which enhanced the plant growth well and also higher photosynthesis capability [67]. The control system was deficient nutrients affected to limit the photosynthesis capability. This result also enhanced the biomass of plant (Table 4.4) which biomass increased after phosphorus treatment for 4 cycles and positive correlations between photosynthesis and biomass [68]. Moreover, this result revealed advantage of phosphorus treatment by *E. cordifolius* that nutrients in wastewater supported the growth of plant and induced plant photosynthesis increasing.

Table 4.3 Photosynthesis of *E. cordifolius* in control and treatment systems after each treatment cycles

Systems	Chlorophyll fluorescence (Fv/Fm)				
	Before treatment (day 0)	After treatment cycle 1	After treatment cycle 2	After treatment cycle 3	After treatment cycle 4
<i>E. cordifolius</i> +soil+ tap water (control)	0.743±0.008 ^a	0.680±0.030 ^a	0.676±0.049 ^a	0.676±0.023 ^a	0.699±0.044 ^a
<i>E. cordifolius</i> +soil+ wastewater (treatment)	0.783±0.043 ^a	0.791±0.012 ^b	0.783±0.014 ^b	0.791±0.003 ^b	0.793±0.007 ^b

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

Table 4.4 Biomass of *E. cordifolius* before and after four cycles of phosphorus treatment

Systems	Biomass (g dry weight plant ⁻¹)	
	Before treatment	After treatment
<i>E. cordifolius</i> +soil+tap water (control)	46.60±0.00 ^a	29.51±7.77 ^a
<i>E. cordifolius</i> +soil+wastewater (treatment)	46.60±0.00 ^a	52.81±2.69 ^b

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

4.8 Percentage of carbon, nitrogen, and phosphorus in plant after four treatment cycles

Carbon, nitrogen, and phosphorus are essential elements for plants. The ratio of carbon, nitrogen, and phosphorus each plant was different which depended on environmental conditioning, species, growth rate, and plant age [69]. There was study in the ratio of carbon, nitrogen, and phosphorus for freshwater angiosperms plants and *Phragmite communis* were 500:24:1 and 46:3:0.02, respectively [70, 71]. In this study, the percentage of carbon, nitrogen, and phosphorus in plant after four treatment cycles was determined. The result found that the ratio of C:N:P in control system (*E. cordifolius*+soil+tap water) and treatment system (*E. cordifolius*+soil+wastewater) were not significantly different (Table 4.5). Percentage of phosphorus in both control system and treatment system was lower than percentage of nitrogen. This result suggested that plant might be took up nitrogen better than phosphorus and effected higher nitrogen concentration in plants, which conformed to general pattern of higher plants [29].

Table 4.5 Percentage of carbon, nitrogen, and phosphorus in *E. cordifolius* in control and domestic wastewater after four treatment cycles

Systems	% Carbon	% Nitrogen	% Phosphorus
<i>E. cordifolius</i> +soil+ tap water (control)	48.06±0.56 ^a	5.40±0.36 ^a	0.04±0.00 ^a
<i>E. cordifolius</i> +soil+ Wastewater (treatment)	48.77±1.26 ^a	6.66±0.76 ^a	0.04±0.00 ^a

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

4.9 The sustainability of the system in phosphorus treatment from domestic wastewater by *E. cordifolius* for 20 cycles

The sustainability of the system in phosphorus treatment from domestic wastewater was continuously investigated for 20 cycles. The results showed that at the first cycle, phosphorus uptake by plant was about 11% while the last cycle, phosphorus uptake was gradually increased to 81% (Table 4.6). During phosphorus treatment 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 [29]. In addition, plant was also healthy in all cycles and produced new leaves and roots of the plants (Figure 4.15). This result demonstrated a sustainable system of phosphorus treatment by *E. cordifolius*.



Figure 4.15 Characteristic of *E. cordifolius* after phosphorus treatment for 20 cycles

4.10 The relationship among plants, microorganisms, and soil in phosphorus treatment for 20 cycles

The relationship between plants, microorganisms, and soil in phosphorus treatment was studied for 20 cycles until soil was not adsorbed phosphorus or saturated. The results illustrated that the percentage of phosphorus uptake by microorganisms in domestic wastewater was higher than microorganisms in soil (Table 4.6). This result revealed that microorganisms in domestic wastewater attributed to phosphorus treatment [72, 73]. 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 cycle, 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. The percentage of phosphorus adsorption by soil gradually increased from cycle 1 to cycle 3 and then gradually decreased to 1% at the last cycle. This meant that the soil started to saturate at cycle 20 that soil was saturated with phosphorus by the process of the soil phosphorus cycle [74]. After that the plant played a major role in phosphorus removal which was about 81% at cycle 20 which was superior to microorganisms in the system (Table 4.6). This result illustrated the sustainability of plant in phosphorus treatment.

Table 4.6 The relationship between plants, microorganisms and soil in phosphorus treatment (Cycle 1-20).

The relationship between plants, microorganisms, and soil in phosphorus treatment	No. of cycle																			
	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

4.11 Percentage of carbon, nitrogen, and phosphorus in plants after phosphorus treatment for 20 cycles

Carbon, nitrogen, and phosphorus were essential elements for the growth of higher plants [75]. 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 [29]. The percentage of these elements may be different in each plant because of plant age, growth rate, species, and environmental condition [69]. This study found that the percentage of nitrogen and phosphorus in the control and domestic wastewater treatment system after running the cycle experiments 20 times was significantly different. The percentage of nitrogen and phosphorus in the treatment system was higher than the control system (Table 4.7). This result differed from the study of percentage of carbon, nitrogen, and phosphorus in plant after four treatment cycles (Section 4.8), found that the percentage of nitrogen and phosphorus under the control and treatment systems after running the cycle experiments for four times was not significantly different. These results indicated that when the time increased, nitrogen and phosphorus were increasingly accumulated in plant tissues. 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 their 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 [76].

Table 4.7 Percentage of carbon, nitrogen, and phosphorus in *E. cordifolius* in the control and treatment (domestic wastewater) after the 20 cycles.

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. ($\alpha = 0.05$)

4.12 Biomass of plants before and after phosphorus treatment from domestic wastewater for 20 cycles

Biomass of plants was determined in the control system which was grown in tap water and the treatment system which was grown in domestic wastewater after running 20 times. The results showed that the biomass in the treatment system increased, whereas in the control system, it was relatively constant (Table 4.8). This result was due to phosphorus and other nutrients from domestic wastewater could accelerate the growth of plants [29]. The plant characteristic appeared to extend the shoots and roots including the production of new leaves. In addition, plants produced flowers and appeared healthy in domestic wastewater. The result also showed the increase in the biomass of plants, which resulted from the resource of bio energy production [77].

Table 4.8 Biomass of *E. cordifolius* before and after phosphorus treatment from domestic wastewater for 20 cycles.

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. ($\alpha = 0.05$)

4.13 Bioaugmentation of phosphorus treatment by *Echinodorus cordifolius* with microorganisms (*Pseudomonas putida* and *Flavobacterium oryzihabitans*)

Since the result of relationship among plants, microorganisms, and soil in phosphorus treatment for 20 cycles showed that microorganisms in domestic wastewater attributed to phosphorus treatment. Thongtha et al. [18] studied microorganism communities in wetland system of phosphorus treatment from domestic wastewater. This study found that *Pseudomonas putida* and *Flavobacterium oryzihabitans* were dominant microorganisms in domestic wastewater. Both microorganisms were free living in domestic wastewater. Therefore, bioaugmentation of phosphorus treatment by *E. cordifolius* and microorganisms (*P. putida* and *F. oryzihabitans*) was investigated. The result showed phosphorus treatment efficiency by *E. cordifolius* and microorganisms was higher than the treatment without microorganisms. The efficiency of phosphorus treatment in the system augmented with microorganisms was about 60% at hour 6 while in the system without augmented with microorganisms was only about 40% (Figure 4.16). 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]. 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% at hour 12 (Figure 4.16). This result might be due to *P. putida* can degrade organic phosphorus to be available form for plant uptake [78] and can both produce and release acid phosphatase to increase phosphorus solubilization [79, 80]. In addition, acid-producing bacteria associated with *E. cordifolius* roots might enhance phosphorus removal. Sriprapat et al. found that bacteria associated with *E. cordifolius* roots produced organic acids [81]. So these acids can degrade non-available phosphorus to be available phosphorus for plant. However, the efficiency of phosphorus treatment in the system augmented with *P. putida* was not significantly different from the system augmented with mixed microorganisms that was 90% at hour 14 and passed the standard criterion of the U.S.EPA (0.1 mg L⁻¹) [3] rapidly in this hour. This result indicated that the efficiency of *P. putida* and mixed microorganisms (*P. putida* and *F. oryzihabitans*) contributed plants for enhancing phosphorus treatment. The system of *E. cordifolius* augmented with *P. putida* appeared to increase the plant growth rate obviously after 2 weeks. The plant was very healthy and had extended shoots and leaves. After phosphorus treatment for 3 weeks, the plant also had a flowering stage (Figure 4.17). Moreover, the result of growth rate was consistent with the result of plant increasing biomass after treatment for one month (Table 4.9). The systems of phosphorus treatment by *E. cordifolius* with microorganisms and the system of phosphorus treatment by the plant alone were significantly different. 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) [82]. There have been several researches that investigated the solubilization of inorganic phosphate by microorganisms. For example, Illmer and Schinner [83] found that *Pseudomonas* sp. produced organic acid for mineral phosphate solubilization in soil. There was the study of *Pseudomonas putida* could promote poplar trees growth and increased shoot and root extension of tomato, canola, and lettuce [80, 84, 85]. From this study revealed that *Pseudomonas* and *Flavobacterium* suit to phosphorus removal with plant because of phosphorus uptake increasing and plant growth stimulation. These microorganisms were confirmed as phosphate solubilizing bacteria and plant growth promoting bacteria. Therefore, the role of these bacteria contributed to organic phosphorus solubilization in domestic wastewater that enhanced available phosphorus uptake by plants.

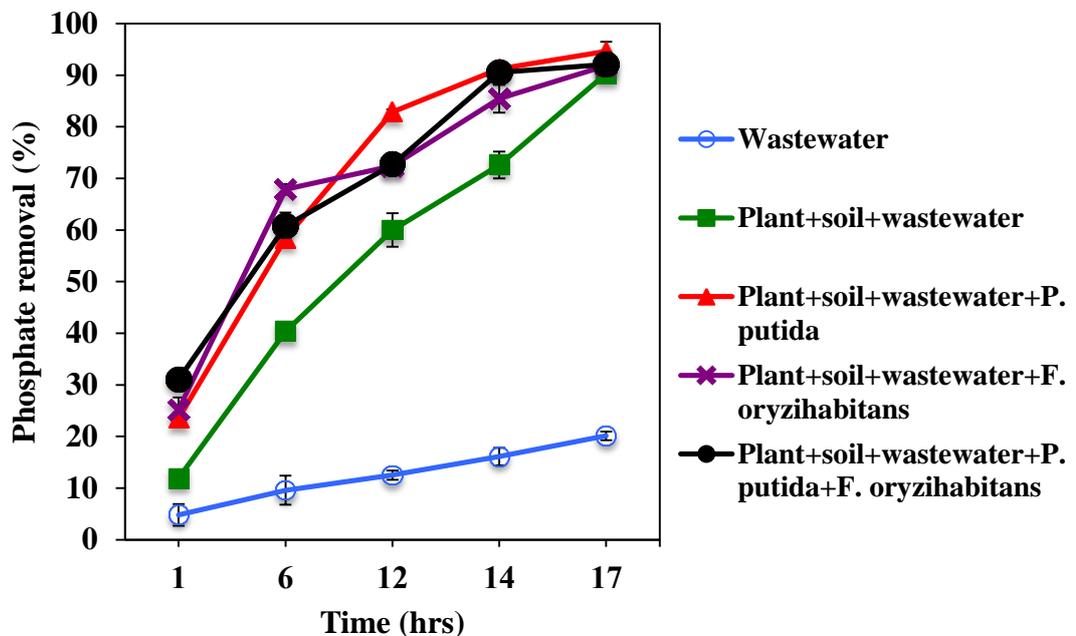


Figure 4.16 Efficiency of phosphate removal in domestic wastewater by *E. cordifolius* augmented with microorganisms.

Table 4.9 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. ($\alpha = 0.05$)



Figure 4.17 Characteristic of *E. cordifolius* before (A) and after phosphorus treatment for 3 weeks (B)