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Effects of different substrate on nitrogen removal in constructed wetlands treating faecal sludge

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ABSTRACT: The faecal sludge disposal situation in urban areas in many developing countries is dramatic. Beside that, faecal sludge management has been received little attention. Being low-cost and low-technology system, eco-technology approaches like “constructed wetland” are now standing as potential alternative for the treatment of wastewater as well as faecal sludge. In a constructed wetland, substrata are the essential components in supporting of growth of emergent plants, attached-growth microorganisms and in the hydraulic conductivity. Expenditure on conventional constructing materials such as conventional ones are soil, sand and gravel contributes as a major part of the total cost for construction of the investment of a constructed wetland. Hence, in order to reduce the cost of the construction, five lab-scale constructed wetlands were installed with different substrates such as gravel, uniform-shape plastic, broken brick, plastic segment and charcoal in this study. The experiments have been conducted by using five constructed wetlands planted with cattail (Typha augustifolia) and operating in a vertical-flow mode. Nitrogen (N) transformation was determine in these 5 units based on measuring TN, TKN, NH3-N, NO2-N, NO3-N in raw faecal sludge and the effluent, nitrification/denitrification rate and N mass balance. Related microbial communities (ammonia-oxidizing bacteria, nitrobacter and denitrifier) were also analysed by using Fluorescence In situ Hybridization (FISH) method. Some biogeochemical parameters such as pH, temperature, oxidation reduction potential (ORP) and dissolved oxygen (DO) in these constructed wetland units suggested the occurrence of anoxic and reduction conditions which were encouraging for the N transformation such as ammonification and nitrification/denitrification. Faecal sludge has been feed with solids loading rate of 250 kg TS/m2.yr and 7-day percolate impoundment. Constructed wetlands were found which not only used low-cost substrate but also could achieve high N removal efficiency.

Keywords: Constructed wetland, low-cost, faecal sludge, N removal, FISH technique.

1. Introduction

1.1 A vertical-subsurface flow constructed wetlands for N removal

A natural treatment system, convenient for treatment of faecal sludge, is the vertical-flow constructed wetlands. This type of operation allows for much more oxygen transfer and thus may be a good option for treatment of wastewaters that have relative high levels of ammonia through nitrification (Watson and Danzig, 1993). Vertical-flow constructed wetlands system not only has a higher hydraulic loading rate (54-64 cm.d^{-1}), but also has a good removal efficiency for organics, ammonia nitrogen (AN) and total phosphorus (TP) (Ying, 2003). Contrary to horizontal-flow CW systems, a vertical flow CW unit forces the flow downwards through the filtering substrate to the drainage system (Koottatep *et al.*, 2005), the oxygenation in the wetland matrix is increased several fold compared to the horizontal ones, which may result in efficient nitrification processes. Laber *et at*, (1996) indicated that a vertical flow subsurface flow CW with 80% effluent recirculation could enhance the nitrification/denitrification reaction, resulting in a TN removal efficiency of 72%. Koottatep *et al*, (2001) summarized that the vertical-flow CW system is a promising technology for FS dewatering with low investment and operation costs.

1.2 Different substrata used in constructed wetlands

Substrata in the CW are the essential components in supporting of growth of emergent plants and attached-growth microorganisms, hydraulic conductivity as well as nutrient adsorption. The role of substrate in wastewater treatment systems is principally to provide sufficient surface area for microbial growth to occur whilst maintaining a satisfactory hydraulic conductivity (Kadlec and Knight, 1996).

The conventional substrate are using now are gravel, sand and soil. However, the expenditure on these conventional constructing materials is a major part of the total cost for construction of the investment of a CW. For instance, Kadlec and Knight, (1996); Crites and Tchobanoglous, (1998) reported that operation and maintenance costs are generally low, but the substrate (usually gravel) required to form the subsurface flow wetland substrate is typically the single most expensive component. Hence, local materials such as char coal, shell sand, coconut char coal and other cheaper materials like peat soil, broken brick, broken plastic bottles, palm shell, slag from blast-furnace can be replaced the usual one in order to reduce the cost of construction.

The successful use of recycled glass fragments in recirculating filters is described by Elliot, (2001); car tyre ‘chip’ have been used in United State as a low-cost alternative to gravel in leachfields (Gimes *et al.*, 2003).
Plastic soft bottles have also been successfully used in unplanted filters for the treatment of domestic greywater in

the West Bank, Palestine (Surani, 2003). Dallas and Ho, (2005) conducted the experiment which using plastic bottle segments as an alternative low-cost substrate for treatment of domestic greywater in Monteverde, Costa Rica, Central America. The performance of the planted reedbeds with plastic bottle segments substrate for BOD and fecal coliform removal was better than that of crushed rock systems (Dallas and Ho, 2005).

Gravel is the most popular form of wetland fill medium, but tire chips provide more porosity, are less dense and, less expensive (Richter and Weaver, 2003).

1.3 Constructed wetlands using different substrata in this study

Five lab-scale constructed wetland units were setup which using small gravel (CW-G), plastic filters (circular shape) (CW-PF), broken brick (CW-B), plastic bottle segments (CW-PB) and charcoal (CW-C). Cattails (*Typha latifolia*), an emergent plant, were planted in these wetland beds.

This study was conducted to investigate the effects of different substrate on nitrogen removal in constructed wetlands treating faecal sludge. The specific objective were : (1) to investigate N removal from faecal sludge by using different types of substrate in constructed wetland units, (2) to determine N mass balance in these constructed wetland units, and (3) to identify microbial communities for Nitrogen transformation in the different substrate types by using fluorescence in-situ hybridization (FISH) method

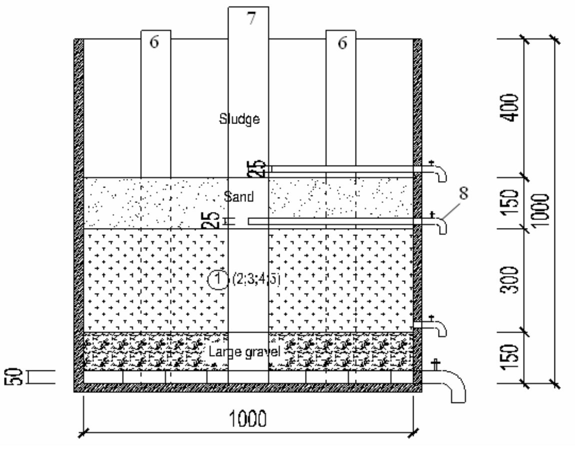
2. Methods

2.1 Experiment setup

Size and configurations. Five vertical-flow lab-scale constructed wetland units, each with a dimension 1×1×1m were employed in at the Environmental Research Station of AIT. The units were white plastic buckets and planted with cattails (*Typha augustifolia*) as shown in Figure 1.

Substrata and vegetation. Each unit was filled with the media consisting of 150-mm layer of large gravel over block layer, 300-mm layer of five different-substrata at the middle and 150-mm sand on the top as the supporting layer. The free board of 400 mm was allowed for dried sludge accumulation. Narrow-leaf cattails (*Typha augustifolia*), an indigenous species in Thailand, were plant at densities of 12 shoots/m^2 by transplanting from natural wetlands at the AIT campus.

Ventilation and drainage systems. To reduce anoxic condition in the root zone area, one vertical pipe, having diameter of 100 mm, was installed in the middle of each constructed wetland units. Hollow concrete blocks, each with dimension 5×20×40 cm (width × length × hollow) were used as the underdrain systems.



1. Small gravel
2. Plastic filter
3. Broken brick
4. Plastic segments
5. Charcoal
6. Microbial sampling tubes
7. Ventilation pipe
8. Percolated pipe

Figure 1. Schematic Drawing of Constructed Wetlands

2.2 System Operations conditions

2.2.1 Acclimatization

The acclimatization was done by feeding continuously with synthetic wastewater at the organic loading rate (OLR) of 50 kg COD/ha.day and hydraulic retention time (HRT) of 7 days until a steady state condition was reached. Afterthat, the experiment was carried out by gradually increase feeding FS with loading rates of 40kg TS/m2.yr, 80 kg TS/m2.yr, and 125 kg TS/m2.yr for one month in order to the plants can adapt to faecal sludge and after that SLR of 250 kg TS/m2.yr was applied into constructed wetland units until the end of the experiment time.

2.2.2 Operations conditions

Operating conditions in the five constructed wetlands were the same. Faecal sludge (FS) was fed with loading rate of FS was 250 kg TS/m^2.yr (N loading rate was 734 kg N/ha.day) and frequencies of application was once a week.

The N loading rate, based on TKN, was calculated from the faecal sludge loading rate by total solid (TS) concentration in range 1,600 - 26,000 mg/l and TKN concentration in range 450 - 1,350 mg/l and TKN concentration were determined in each feeding time in the raw FS.

FS was applied with loading rate of 250 kg TS/m^2.yr in this study because this loading rate value was found to result in the optimum treatment performances with respect to the TS, total chemical oxygen demand (TCOD) and total Kjeldahl nitrogen (TKN) removal efficiencies as well as less adverse effect on plant growth.

2.2.3 Analytical methods

All parameters were analyzed according to the methods described in “Standard Methods for the Examination of Water and Wastewater” (APHA, AWWA, WPCF, 1992 as shown in the table below:

Parameters	Unit	Analytical methods
pH	-	Measure with meter *in situ*
Temperature	°C	Measure with meter *in situ*
Total Kjeldahl N, TKN	mg/L	Semi Micro Kjeldahl Digestion
Ammonia N, NH_3-N	mg/L	Distillation/titration method
Nitrite N, NO_2-N	mg/L	Colourimetric method
Nitrate N, NO_3-N	mg/L	Sodium salicylate method
Chemical oxygen demand, COD	mg/L	Closed Reflux, Colorimetric method
Dissolved oxygen, DO	mg/L	Measure with DO meter on site
Oxidation-reduction potential, ORP	mV	Measure with ORP meter *in situ*
Total N in plant biomass	kgDW	Micro-Kjeldahl Method
Total N in dry sludge	kgDW	Micro-Kjeldahl Method
Total solid, TS	mg/L	Weight after dried at 103°C
Bacterial analysis	%	Fluorescence in-situ hybridization (FISH)

Table 1. Chemical and Physical Parameters and Methods of Analysis

3. Results and discussions

3.1 Characteristics of Bangkok faecal sludge

The physical-chemical and biological characteristics of the faecal sludge which was collected from Bangkok City, shown in Table 2.

Parameters	**Unit**	**Concentration**	**Mean**
pH	-	6.5 – 8.2	7.4
Temperature	°C	21 – 36	28.5
Oxidation-reduction potential, ORP	mV	-320 to -110	-215
Dissolved oxygen, DO	mg/L	0.0 – 0.7	0.35
Total solid, TS	mg/L	7,200 – 26,000	16,600
Chemical oxygen demand, COD	mg/L	9,000 – 28,000	18,500
Biochemical oxygen demand, BOD	mg/L	6,000 – 22,000	14,000
Total Kjeldahl N, TKN	mg/L	450 – 1,350	900
Ammonia N, NH_3-N	mg/L	180 - 500	340
Nitrite N, NO_2-N	mg/L	0.002 – 0.44	0.221
Nitrate N, NO_3-N	mg/L	0.1 – 2.4	1.25

Table 2. Characteristic of Faecal sludge samples.

This result was in the range of characteristic of 256 raw septage samples in Bangkok taken from April 1997 to May 2003 (Koottatep et al., 2005).

3.2 TN removal in constructed wetland units

The TN removal of 68 - 84% in the five CW units could be due to plant uptake, nitrification-denitrification activities and volatilization. (Table 3)

Concentrations of TKN and NH_3-N in raw FS and percolate varied considerably during the study period. As can be seen from Table 4.4 that the highest TKN and NH_3-N removal efficiency of 91.37% in CW-PB. The higher removal efficiency of TKN and NH_3-N were achieved by CW-PB and CW-PF than other CW units probably due to the higher oxygen availability for nitrifying bacteria convert NH_3-N to NO_3-N or higher capacity of adsorption of plastic substrates. Kootattep, (1999) reported that there were several mechanisms responsible for NH_3-N removal, such as nitrification reaction, adsorption on to substrates, biological uptake by cattail plants or microbial organisms. In general, the removal trends for TKN were similar to removal trends for NH_3-N in all five CW units.

Sample	Unit No	Parameter, mg/l					
		TKN		NH_3-N		TN	
		Concentration (mg/l)	Removal %	Concentration (mg/l)	Removal %	Concentration (mg/l)	Removal %
Raw FS		957		400		961	
Perco-late	CW-G	134	84	106	74	229	73
	CW-PF	105	88	70	82	194	77
	CW-B	140	85	85	79	215	75
	CW-PB	801	91	77	81	145	84
	CW-C	167	80	88	78	274	68

Note: Based on 30 percolated samples taken from January to April 2007 per CW unit

Table 3. Average TKN, NH_3 and TN contents in raw FS, CW percolate

Influent and effluent concentrations of NO_3-N also varied considerable during the operation time**.** The CW units treating FS produce percolates with considerable NO_3-N concentrations (Koottatep *et al*., 2001). For instance, percolate NO_3 concentrations of up to 186 mg/l were observed in CW-C (charcoal), whereas the lowest concentration value, found in CW-B amounted to 11 mg/l.

NO_3-N removal rates were greater in CW-PB unit than CW-G and CW-C units in spite of greater oxygen concentration in CW-PB. The very high NO_3-N concentration may come from the very high nitrification and less denitrification in those CW units. This result was unexpected because it was guessed that CW-G and CW-

C units provided a better anaerobic environment for denitrification based on the lower void space inside these units than others. Hence, the presence of O_2 did not appear to be the limiting factor in NO_3-N removal in these units. Organic carbon could have been the limiting factor for denitrification (Sarah *et al.*, 2000). Vegetation was considering that the main source of organic carbon for denitrification. At the beginning, CW-C unit produced the most vegetation which was gradually grown afterward. CW-PB and CW-PF were produce greater vegetation than other CW units.

The removal trends for TN were similar to removal trends for TKN and NH_3-N in all five CW units. Most NH_3-N removal was accompanied by TN removal, which means that if nitrification was the major mechanism responsible for NH_3-N removal, denitrification of the nitrate produced must have been complete.

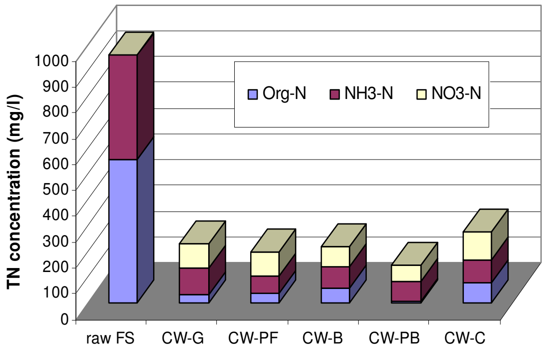


Figure 2 Influent and Effluent concentrations of Total nitrogen in 5 CW units

3.3 N mass balance in constructed wetland units

3.3.1N accumulation in plants

Percentage of N in dried weight and N accumulation of the cattail plants after 3 months of operation were calculated for N uptake as present in Table 4.5.

The total N uptake rate of the cattail plants of five CW units were in the range of 2.0 - 7.3 kg N/ha day. CW-G and CW-B had the lowest N accumulation of litter over 2.0 kg/ha day but higher than 0.29 kg N/ha day which reported by Harbert and Perfler (1991). These low N uptake values could be cause of lower cattail plants growth in CW-G and CW-B than other CW units as shown in the Table 4.2. The highest N accumulation was achieved by CW-PB of 7.3 kg N/ha day. This might be the reason why CW-PB's cattail plants were 16.7% taller than those in CW-G. The N uptake of 5.7 and 6.5 kg N/ha day of CW-PF and CW-C, respectively were still within the range of 1.64 - 7.21 kg N/ha day for cattails as reported by Reddy and Debusk (1987). cattails required larger amount of N to be support growth of their roots than leaves. During 3 months operation, cattails required larger amount of N to be support growth of their roots than leaves.

Compare with N plant uptake rate1.5 - 2.0 kg N/ha day cited by Gersberg *et al*, (1986), the N uptake by cattail plants obtained from this study was relatively higher because the first 3 month-old cattail plants normally required greater amount of N for there growth.

3.3.2 Analysis of N mass balance

Analysis of TN mass balance was done after finishing experimental time. As can be seen from the Table 4.5 that the major N content which analyzed, was in dried sludge (39 -47%), followed by the percolate (10-19%), while plant uptake played a very minor role (3-10%). For example, N contents in the tropical cattails were in the range of 1-2% of dry plant biomass (Kadlec and Knight, 1995). The unaccounted for N (25-42%) included adsorption, retention, volatilization, nitrification/denitrification and some unknown factors.

It can be seen from the calculation that CW-PB is the CW which using plastic bottle segments has the lowest percentage of N in the percolate (10%) and highest percentage of N uptake by cattail plants (10%).

These results suggest that about one second of the input N would be retained in the dried sludge on the wetland substrate surface which would be eventually be mineralized and uptake by the cattail plants.

4. Microbial community analysis in CW units by using FISH method

The most important goal of this work was to gain understanding of the microbiological mechanisms underlying erratic and irregular N-removing performance of the CW.

In situ study of microbial community was followed a top-to-bottom approach. First the samples were hybridized with EUB338, designed to target almost all bacteria, then group specific bacteria such as ammonia-oxidizers (NSO1225), *Nitrobacter* species specific nitrite-oxidiziers (NIT3), denitrifiers (RRP1088) were identified.

It is thought that very diverse microorganisms were present in CW units; because autotrophic and heterotrophic reactions were carried out in the CW beds (influent-effluent nitrogen species forms/concentration change and COD reduction also support autotrophic and heterotrophic reaction respectively). The composition and distribution of microbial populations in five CW units under same operating conditions were not found at 20th day operation (before loading with SLR 250 kg TS/ha.yr), however they were detected after that and presented in figure 4.4, (a) at 42nd day (b) at 56th day and (c) 77th day sample. The signals obtained with oligonucleotide probes were in the range from 17 - 54% (as shown in appendix F) of DAPI stained cells were detected by probe EUB338, which was designed to target almost all bacteria.

Ammonia oxidizing bacterial were detected by the probe NSO1225 which was specific for β-proteobacterial ammonia-oxidizing bacteria (*AOB*). Genus *Nitrosomonas*, *Nitrosospira, Nitrosovibrio, Nitrosolobus* including 14 species are belonging to β-*Proteobacteria* which are ammonia-oxidizers.

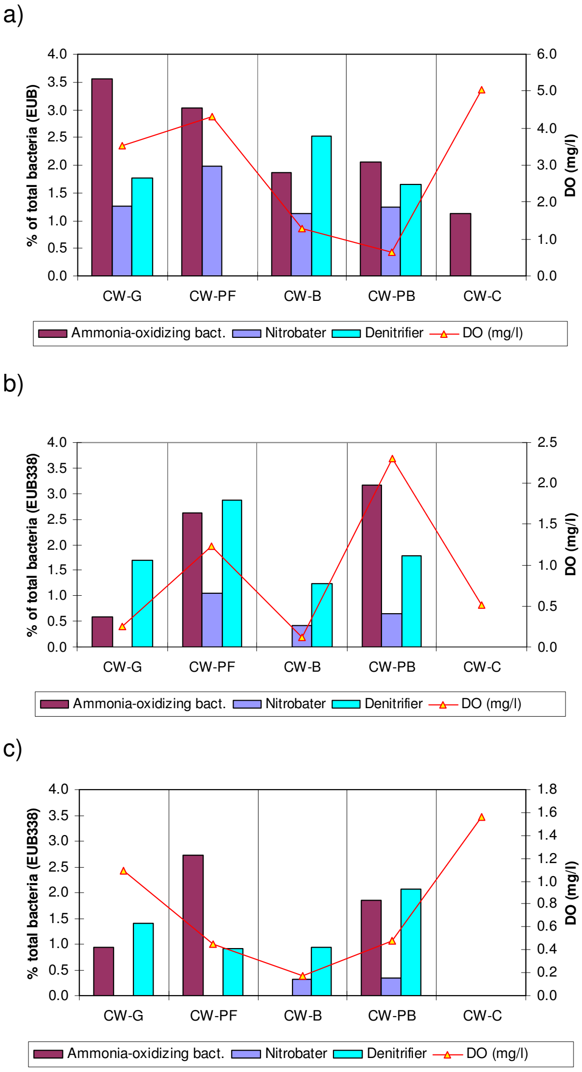


Figure 3 Quantitative FISH analysis of microbial community in CW units. (a) at 42nd day, (b) at 56th day, (c) at 77th day.

Other ammonia-oxidizing bacteria that are not belong to β-*Proteobacteria* are *Nitrosococus oceani* and *Nitrosococus halophilus* which belong to γ subclass of the *Proteobacteria*. β-*Proteobacteria* were detected in all CW units in which over 3% (in CW-G, CW-PF), about 2% (in CW-B, CW-PB) and 1% in CW-C of total bacteria at 42nd day sample. However, these bacterial species were not found in CW-B and CW-C at 56th and 77th day sample. The absence of nitrifying bacteria probably because the DO concentration was less than 0.2 mg/l in CW-B and CW-C, hence these bacteria could not grow. The negative range of ORP values found in both CW-B & CW-C at that time would not be favorable for the nitrification process. Only CW-PF had stable amount of β-*Proteobacteria* of around 3% at 42nd day, 56th and 77th day sample.

Genus *Nitrobacter* (α-*Proteobacteria*) were detected by probe NIT3. *Nitrobater* were present in 4 CW units (such as CW-G, CW-PF, CW-B and CW-PB), in 3 CW units (CW-PF, CW-B and CW-PB) and in 2 CW units (CW-B and CW-PB) at 42nd day, 56th and 77th day sample, respectively. There was no *Nitrobacter* species in CW-C at all day sample or may be they were out of detection limit. The percentage of *Nitrobacter* had decreased day by day from around 3% to only 0.3% in the 42nd day to 77th day sample respectively. At 77th day sample, *Nitrobacteria* were found only in CW-B and CW-PB in the range of 0.31 - 0.33% of total bacteria.

Another genus which was detected by probe RRP1088 was α-Proteobacterial denitrifiers. This probe was specific to genera *Rhodovulum, Roseobacter, Paracocus* and *Rhodobacter*. These denitrifiers were present in all four CW units which are CW-G, CW-PF, CW-B and CW-PB at the 56th day and 77th day sample which were in the range of 0.9 - 2.8% of total bacteria. This might due to limited DO (0.2-1.2 mg/l) concentration at that time in these four CW units. There was also no denitrifiers species in CW-C at all day sample. Because of NO_3-N concentrations in the percolate of CW-C were still quite high, this might due to denitrification processes were not occurred well. Hence the growth of denitrifier species in the CW units should be supported.

5. Cattail growth in the five constructed wetlands

The synthetic wastewater was initially fed to all of the units. After three to four weeks, young roots and stems began to grow. After that, the faecal sludge was pumped to five CW units; almost cattail plants could grow even thought some of them were died. When the dewatered sludge was accumulated on the surface of the CW units, most of the plants was adapted to FS and exhibited a healthy growth pattern because of some moisture was retained in the sludge to be used by the plants for growing and supplied enough nutrients as well.

The growth of cattail plants in the CW units was determined by monthly measuring height and density of the cattails and observing changes of the cattail leaves and color

From Table 3 it can be seen that CW-PF, CW-PB and CW-C showed an average wet mass increase of 2257%, 2109% and 2059%, respectively in total plant biomass over the study period compared to only 1139% for the conventional unit - CW-G. The majority of this biomass increase can be attributed to root growth, these three CW units increased root mass 15 - 32 times greater than the CW-G. Especially, the additional root growth in CW-PF, CW-PB and CW-C provides significantly greater surface area for the biofilms, which are responsible for microbial processing.

Unit	Growth of cattails			
	Nov '06	Dec '06	Jan '07 & Feb '07	Mar '07
CW-G	Green Gradually grew Height 30cm Wet wt. 505g*	Light green, brown Gradually grew Height 50-70cm	Green, dark green Grew very well Height 100-175cm	Green, some turned to Yellow, thin leaves Height 150-180cm Wet wt. 5750g* (1139)
CW-PF	Green Gradually grew Height 30cm Wet wt. 505g*	Green Significant grew Height 65-85cm	Dark green Significant grew Height 100-200cm	Dark green, grew well, thick leaves Height 200-240mm Wet wt. 11400g* (2257)
CW-B	Green Gradually grew Height 30cm Wet wt. 505g*	Light green, brown Gradually grew Height 50-65cm	Green, dark green Grew well Height 100-175cm	Green, some turned to Yellow, thin leaves Height 150-180cm Wet wt. 4625g*(916)
CW-PB	Green Gradually grew Height 30cm Wet wt. 505g*	Light green, brown Gradually grew Height 45-70cm	Dark green Considerable grew Height 100-180cm	Dark green, grew well, thick leaves Height 200 – 230mm Wet wt. 10650g*(2109)
CW-C	Green Gradually grew Height 30cm Wet wt. 505g*	Green Considerable grew Height 70-90cm	Dark green Grew very well Height 100-200cm	Green, some turned to yellow, thick leaves, some became curly Height 180-200cm Wet wt. 10400g*(2059)

**Wet wt. = Total wet weight of cattail plants in CW*

Table 3 Growth of cattails during experiment period

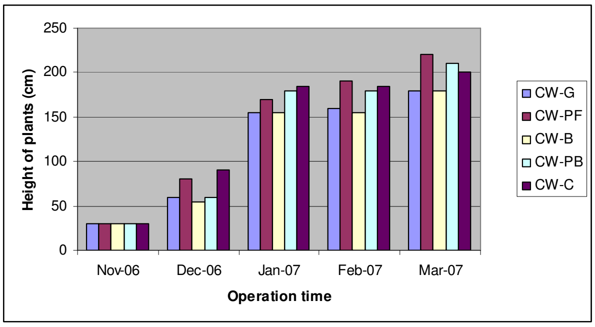


Figure 4 Height of plants during the operation time

Plants were monitored for rates of growth over a period of 160 days averaging of 0.93-1.31 cm/day in CW units. The plants of CW-PF and CW-PB were approximately 16.7% taller than those in comparable CW-G throughout the study period.

6. Conclusion

Based on the lab-scale results obtained during 3-months of operation, the conclusions can be drawn as follow:

1. The overall efficiencies of CW-PB were higher than those of the CW units in term of TKN removal but lower NO_3-N concentration in the percolate. Conversely, in CW-C the lower TKN removal efficiency was achieved but contained the higher NO_3-N concentration in the percolate. In regard to TN removal efficiency, CW-PF, CW-B and CW-PB obtained higher were higher TN removal efficiencies than those of CW-G.
2. Based on the N mass balance in all CW units, about 10-19% of the raw FS were found in the percolate, 39-47% in the dried sludge, only 3-10% by plant uptake, and the remaining of 25-42% as adsorption, retention, volatilization and nitrification/denitrification. As can be seen that CW-PB is the CW which using plastic bottle segments, has the lowest percentage of N in the percolate (10%) and highest percentage of N uptake by cattail plants (10%).
3. FISH analysis of each substrate in showed the increasing trend in the growth of most bacteria (EUB338) during the operation time. Nitrifier species (NSO1225 and NIT3) and denitrifiers (RRP1088) were occurring simultaneously in only CW-PB. There was no *Nitrobacter* species (NIT3) and denitrifiers (RRP1088) in CW-C or may be they were out of detection limit.
4. During the period of 160 days operation, the rates of growth of plants in all CW units were in the range of 0.93-1.31 cm/day. The plants of CW-PF, CW-PB and CW-C were approximately 16.7% taller than those in comparable CW-G. CW-B had the similar rate of growth to CW-G.

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