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Interactive effects of NH_4^+ concentration and O_2 availability on growth, morphology, and mineral allocation of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1)

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ABSTRACT

Biogas production was of increasing interest as it could not only produce biogas, but also treated manure that can be used as fertilizers. However, increasing level of NH_4^+ in the effluent after anaerobic digestion needed to be considered. Using forage crops with high biomass production for bio-remediation of contaminated wastewater was an interesting alternative for wastewater treatment, but responses of the plants to such wastewater with nitrogen mainly in the form of NH_4^+ associated with low O_2 availability were little known. Hence, growth, morphology, and minerals in plant tissues of hybrid Napier grass (*Pennisetum purpureum* Schumach × *Pennisetum americanum* (L.) Leeke cv. Pakchong1) were determined under different combinations of NH_4^+ (0.5, 1, 5, 10, 15 mM) and O_2 level (anoxia, hypoxia and normoxia). The plant growth decreased with O_2 depletion and strong effects were found in the plants grown in the anoxic condition, particularly, at the high NH_4^+ concentrations (10, 15 mM). Similar results were found for number of roots, root length, and number of new shoots. Mineral concentrations in the plant tissues were affected by the O_2 depletion, especially N and P in the roots that decreased by 26% and 50%, respectively, at the absolute anoxic conditions as compared with the normoxic conditions. However, high Fe accumulation was found in the plants grown under the anoxic conditions. The hybrid Napier grass was able to tolerate the high NH_4^+ up to 5 mM and could grow under the hypoxic condition with the total N slightly decreased. This species may have a potential for treating the wastewater discharged from the biogas production tank and at the same time could be used for animal feed or green manure after being harvested.

1. Introduction

High nutrient loading from agricultural activities such as fertilizer use and animal farming caused degradation of the environment. Water bodies were the main targets for disposing pollutants both directly and indirectly. The excess nitrogen led to widespread eutrophication that organisms in aquatic ecosystems might suffer from low dissolved oxygen. Growth of aquatic macrophytes was also affected, particularly under anoxic condition where nitrogen most commonly exists in reduced organic and ammonium forms (organic-N and NH_4^+-N, respectively) (Davison et al., 2006). The high concentrations of NH_4^+ (>0.5 mM) were toxic to plants even though most aquatic macrophytes prefer NH_4^+ as an inorganic nitrogen source (Jampeetong and Brix, 2009a; Britto and Kronzucker, 2002). At the high NH_4^+ concentrations, the plants showed toxicity symptoms such as slow growth rate, rotted roots or submerged stems, and chlorosis of leaves (Britto and Kronzucker, 2002; Tylova et al., 2008; Jampeetong and Brix, 2009b).

Nowadays, biogas production was an option to reduce the waste from animal farms. Nitrogen entered into biogas production tank was either ammonium (NH_4^+) or organic nitrogen. Due to anaerobic digestion process, organic nitrogen was transformed from organic forms to inorganic forms resulted in higher ammonium concentration coming out of the anaerobic digester (Field et al., 1984). Approximately 60% of the total nitrogen in effluent was ammonium (Kirchmann and Witter, 1992). To avoid a contamination of natural aquatic and terrestrial ecosystems with nutrients, an improvement of the effluent quality was suggested (Weiland, 2010).

Using aquatic macrophytes for wastewater treatments was eco-friendly and cheap alternative ecological methods for treating wastewater (Schierup and Brix, 1989). Additionally, biomass rich in N was generated making many aquatic macrophytes good post-harvest fertilizer or animal feed (Leterme et al., 2009). In recent times, increasing use of perennial C_4 grasses for bio-remediation as well as bio-energy crops or animal feed has been documented,

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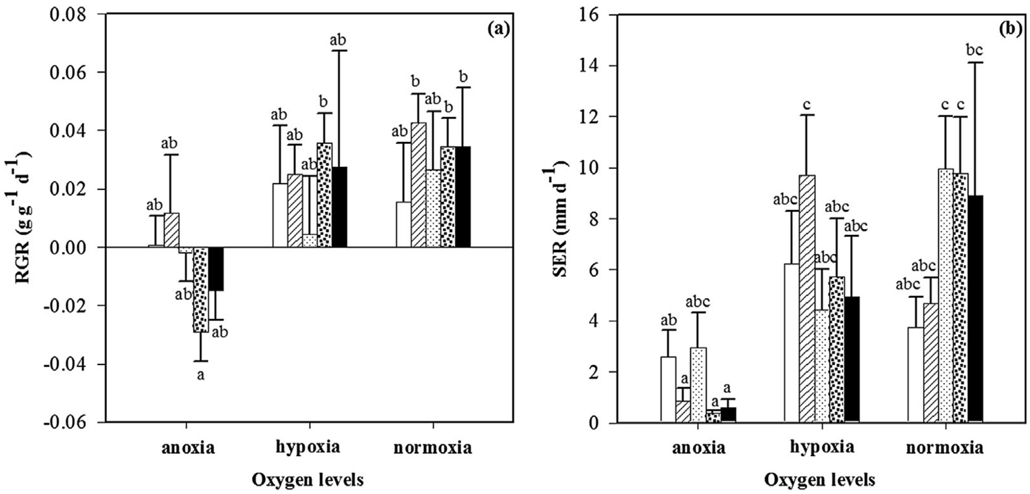

Fig. 1. Relative growth rate, RGR (a) and shoot elongation rate, SER (b) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) (mean ± SD) grown on different NH_4^+ concentrations (□ 0.5, ▨ 1, ⬚ 5, ▩ 10, ■ 15 mM) and O_2 levels (anoxia, hypoxia, normoxia). Different letters above columns indicate significant differences between treatments.

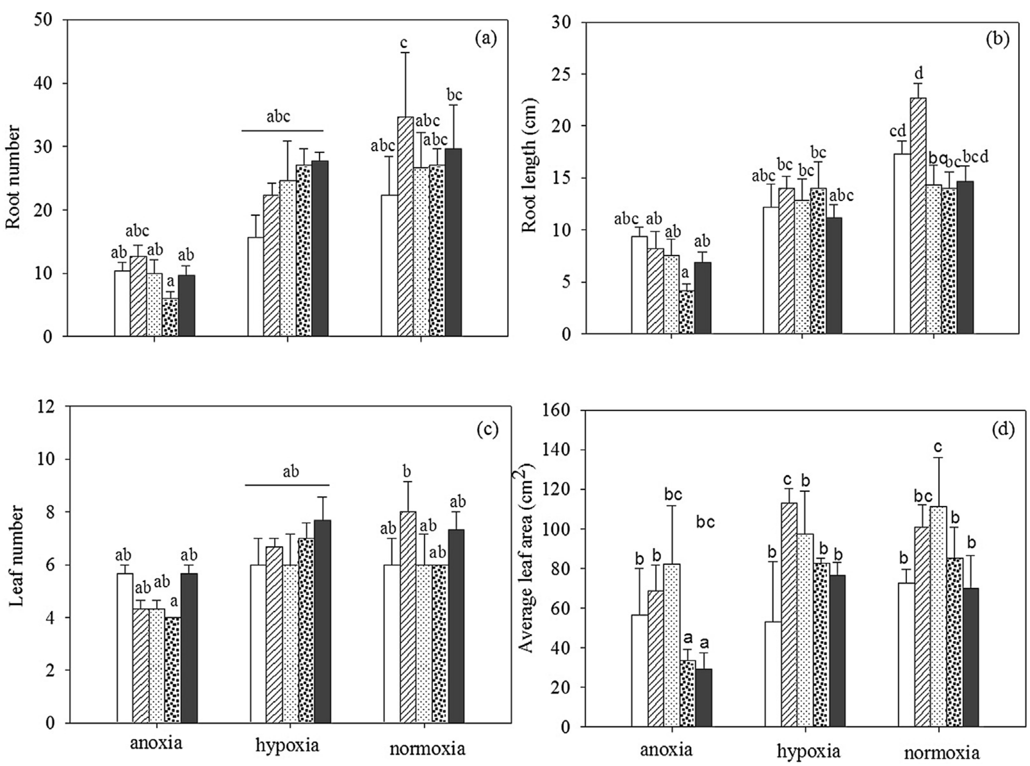

Fig. 2. Root number (a), root length (b), leaf number (c) and average leaf area (d) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) (mean ± SD) grown on different NH_4^+ concentrations (□ 0.5, ▨ 1, ⬚ 5, ▩ 10, ■ 15 mM) and O_2 levels (anoxia, hypoxia, normoxia). Different letters above columns indicate significant differences between treatments.

for example, switchgrass in the US, *Miscanthus* in Europe and Napier grass in South-East Asia (Dzantor et al., 2000; Somerville et al., 2010). The potential of these plants to grow and take up nutrients, especially ammonium under low O_2 available found in the effluent from the anaerobic digesters, had not been intensively studied, but it was an important criterion used in plant selection for tropical wastewater treatment systems.

Hybrid Napier grass (*Pennisetum purpureum* Schumach × *Pennisetum americanum* (L.) Leeke cv. Pakchong1) was introduced to Thailand to be used as animal feed because of its fast growth, high yield (100 tonnes FW rai^{-1} 60 days^{-1} with 18–19% DM), high protein content (12–16%), lack of itchy hairs and high palatability (Kiyothong et al., 2013). However, using this plant in constructed wetland (CW) systems for wastewater treatments had rarely been recorded compared with other commonly used species such as *Phragmites*, *Cyperus* and *Typha* (Leto et al., 2013; Vymazal, 2013). At present, the hybrid Napier grass has also been introduced for treating wastewater. This hybrid Napier grass had a potential for high yield and a very high crude protein content for livestock, but there was no evidence concerning its ability to grow with high NH_4^+ concentrations and low available O_2. The hybrid Napier grass was positively affected by inorganic nitrogen, especially NH_4^+ (Jampeetong et al., 2014). Moreover, high NH_4^+ uptake rate and high N in the plant tissues have been found. However, studies

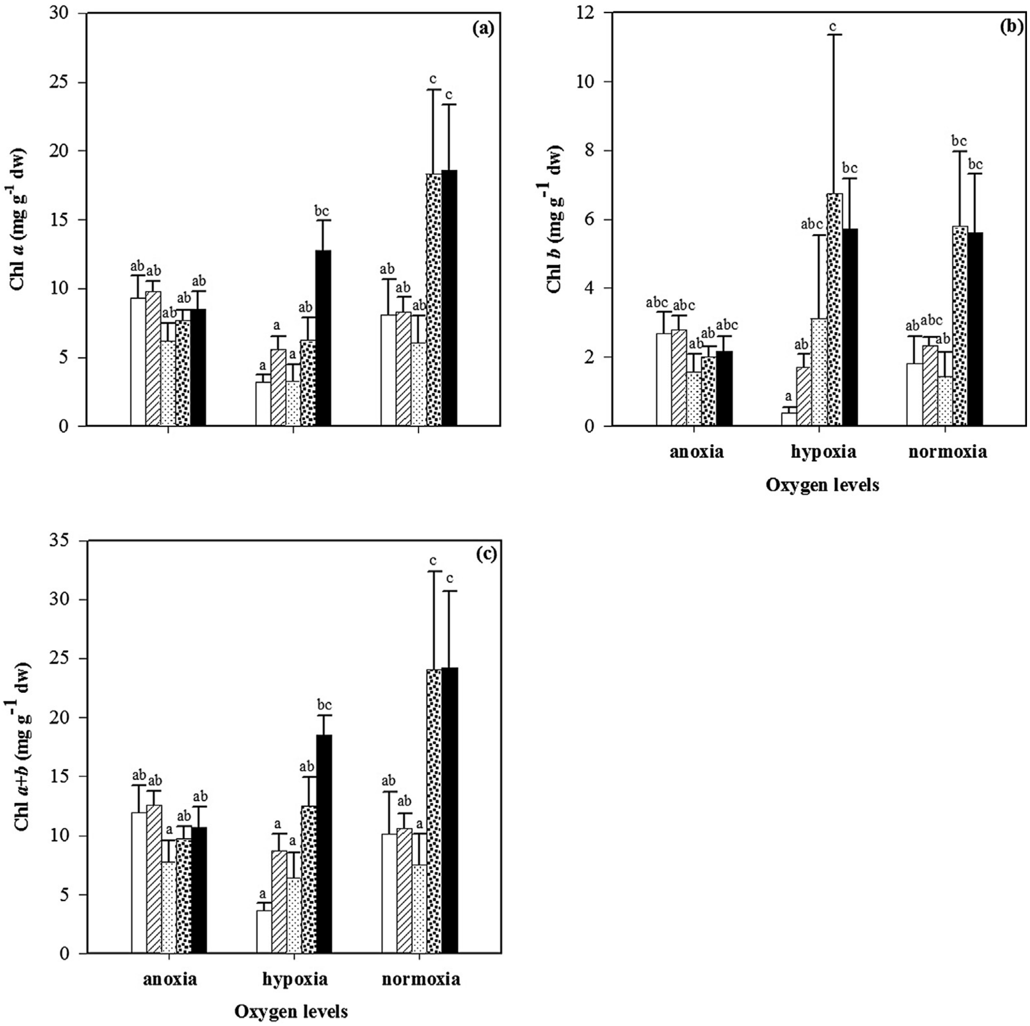

Fig. 3. Chl *a* (a), Chl *b* (b) and Total chl (*a* + *b*) (c) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) (mean ± SD) grown on different NH_4^+ concentrations (□ 0.5, ▨ 1, ▤ 5, ▩ 10, ■ 15 mM) and O_2 levels (anoxia, hypoxia, mormoxia). Different letters above columns indicate significant differences between treatments.

on interactive effects of NH_4^+ concentration and O_2 levels were lacking. Therefore, responses of the hybrid Napier grass to NH_4^+ concentration and O_2 availability focused on growth, morphology and mineral accumulation in the plant tissue were conducted to assess ability of this plant for using in CWs to treat wastewater run off from the biogas production tank and for its use as fertilizer after harvesting the biogas.

2. Materials and methods

2.1. Plant material and experimental set-up

New plants of the hybrid Napier grass (*P. purpureum* × *P. americanum* cv. Pakchong1) were prepared from 200 to 300 mm stem sections of mother plants. Approximately 1 month after soaking these sections in tap water, the new plants were separated from the stem nodes. The new plants were then placed in plastic buckets containing a full strength standard N and P free growth medium prepared according to Smart and Barko (1985) to which 100 μM KH_2PO_4 and a commercial plant micronutrient solution (Tropica, Egaa, Denmark) were added. Nitrogen was added as $(NH_4)_2SO_4$ at 500 μmol N L^{-1}, and pH was adjusted to 6.5 ± 0.2 using HCl and NaOH. All the plants were incubated in the greenhouse at the Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The temperature and light regimes in the greenhouse were 26 -32°C: 18-21°C day:night temperature and approximately 10 h light/14 h dark. The growth medium was renewed every 3 days to minimize the depletion of nutrients from the growth medium.

After 14 days of plant acclimation, similar-sized plants (approximately 20–30 g fresh mass and 200–250 mm tall) were selected (n = 45). Fifteen treatments were created based on combinations of different NH_4^+ concentrations (0.5, 1, 5, 10, 15 mM) and O_2 levels (anoxia, hypoxia, normoxia). In each of the treatments (n = 3), all the plants were placed in separated 5-L plastic buckets (diameter 0.25 m; height 0.22 m) containing a full strength standard N and P free growth medium prepared according to Smart and Barko (1985) to which 100 μM KH_2PO_4 and a commercial plant micronutrient solution (Tropica, Egaa, Denmark) were added, and pH was adjusted to 6.5 ± 0.2. The NH_4^+ solutions were prepared from $(NH_4)_2SO_4$. The three O_2 levels were set-up: (i) the normoxic treatment was created by continuous bubbling with atmospheric air (ii) the hypoxic treatment was created by continuous bubbling with N_2 gas, and (iii) the anoxic treatment was established by bubbling with N_2 gas and addition of a reducing reagent, $Na_2S_2O_4$ (0.1 g L^{-1} growth medium). In the anoxic treatment, the water surface was covered with foam and gaps between the plants and the plate form were sealed using plasticines to prevent O_2 diffusion from the air into the solutions. All the treatments were placed in the greenhouse at the Department of

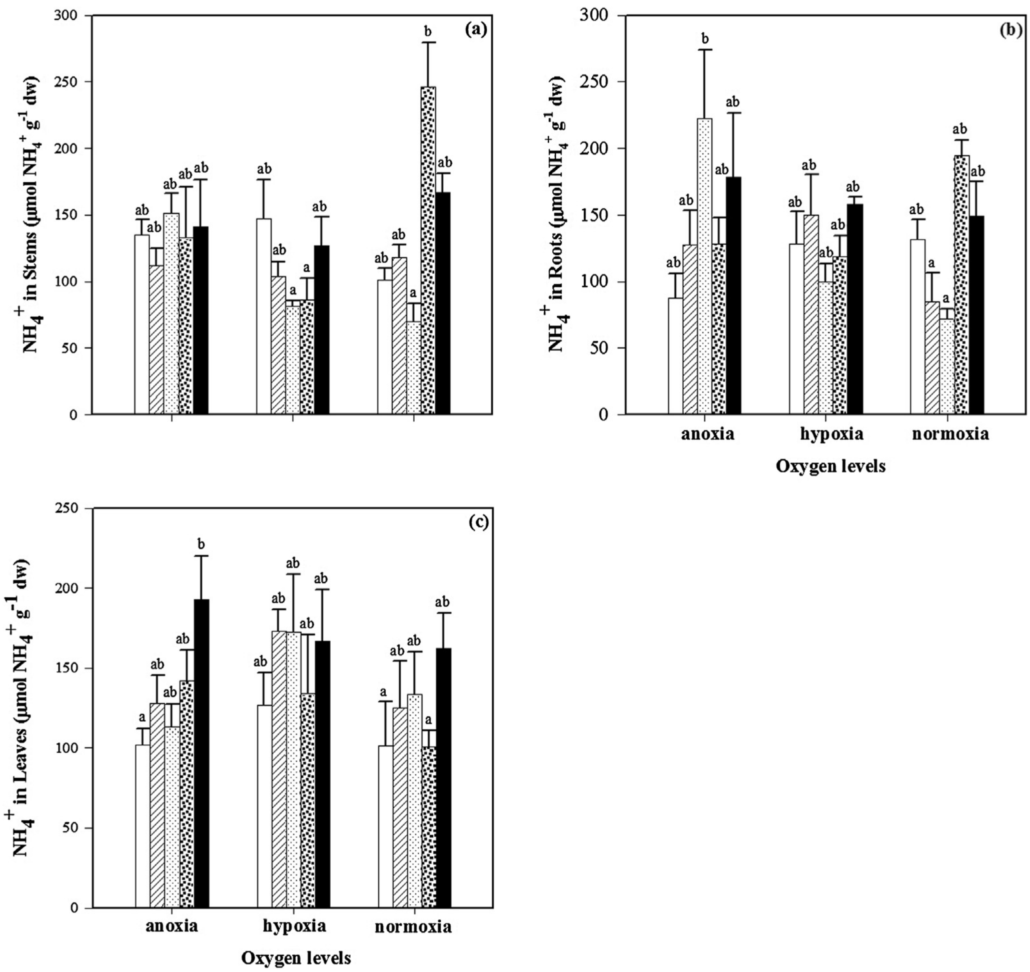

Fig. 4. NH_4^+ in stems (a), roots (b) and leaves (c) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) (mean ± SD) grown on different NH_4^+ concentrations (□ 0.5, ▨ 1, ⬚ 5, ▩ 10, ■ 15 mM) and O_2 levels (anoxia, hypoxia, normoxia). Different letters above columns indicate significant differences between treatments.

Biology, Faculty of Science, Chiang Mai University, Thailand. During the experimental period, the temperature and light regimes in the greenhouse were 26-32 °C: 18-21 °C day:night temperature and approximately 10 h light/14 h dark. The growth media were changed daily.

2.2. Growth and morphological study

After 16 days, all the plants were harvested and cleaned. Total biomass, plant height and some morphological characteristics (number of new shoots, number of leaves, average leaf area, root number and the maximum root length) were recorded. Then, the plants were separated into roots, stems and leaves and freeze dried. The relative growth rate (RGR; g g^{-1} d^{-1}) in all the treatments were calculated using the formula: RGR = $(\ln W_2 - \ln W_1)/(t_2 - t_1)$, where W_1 and W_2 were the initial and final dry masses (DM), and t_1 and t_2 are initial and final times (days). The shoot elongation rate (SER; mm d^{-1}) was calculated as the total increase in shoot length throughout the experiment divided by the number of days.

2.3. Chlorophyll concentration

Chlorophyll concentration of the leaves in all the treatments were analyzed in samples (5 mg) of freeze-dried leaves. The leaves were cut and extracted in 96% ethanol in the dark at room temperature for 24 h. Then, the extracts were thoroughly mixed by vortexing and their absorbances were measured at 648.6 nm and 664.2 nm using a UV–vis spectrophotometer (Lambda 25 version 2.85.04, USA). Chlorophyll a (Chl *a*), chlorophyll b (Chl *b*) and total chlorophyll (total Chl $a+b$) were calculated according to Lichtenthaler (1987).

2.4. Mineral elements

Concentrations of total N, phosphorus (P), potassium (K), magnesium (Mg) and iron (Fe) in leaves, stems and roots were analyzed in subsamples (150–180 mg) of finely ground freeze-dried plant materials. The samples were digested in 7 ml acid solution (concentrated H_2SO_4 1 L, K_2SO_4 100 g and selenium 1 g) at a temperature range of 100–330 °C. Total N was analyzed by the Kjeldahl method (Hanlon et al., 1994) and the concentrations of P, K, Mg and Fe in the digests were analyzed using the method of Chapman and Pratt (1978).

2.5. Statistics

All the results were analyzed by one-way and two-way analysis of variance (ANOVA) using Statgraphics Plus version 4.1 (Manugistics, Inc., MD, USA). Differences between treatments were tested using the Tukey HSD post hoc procedure at the 5% significance level.

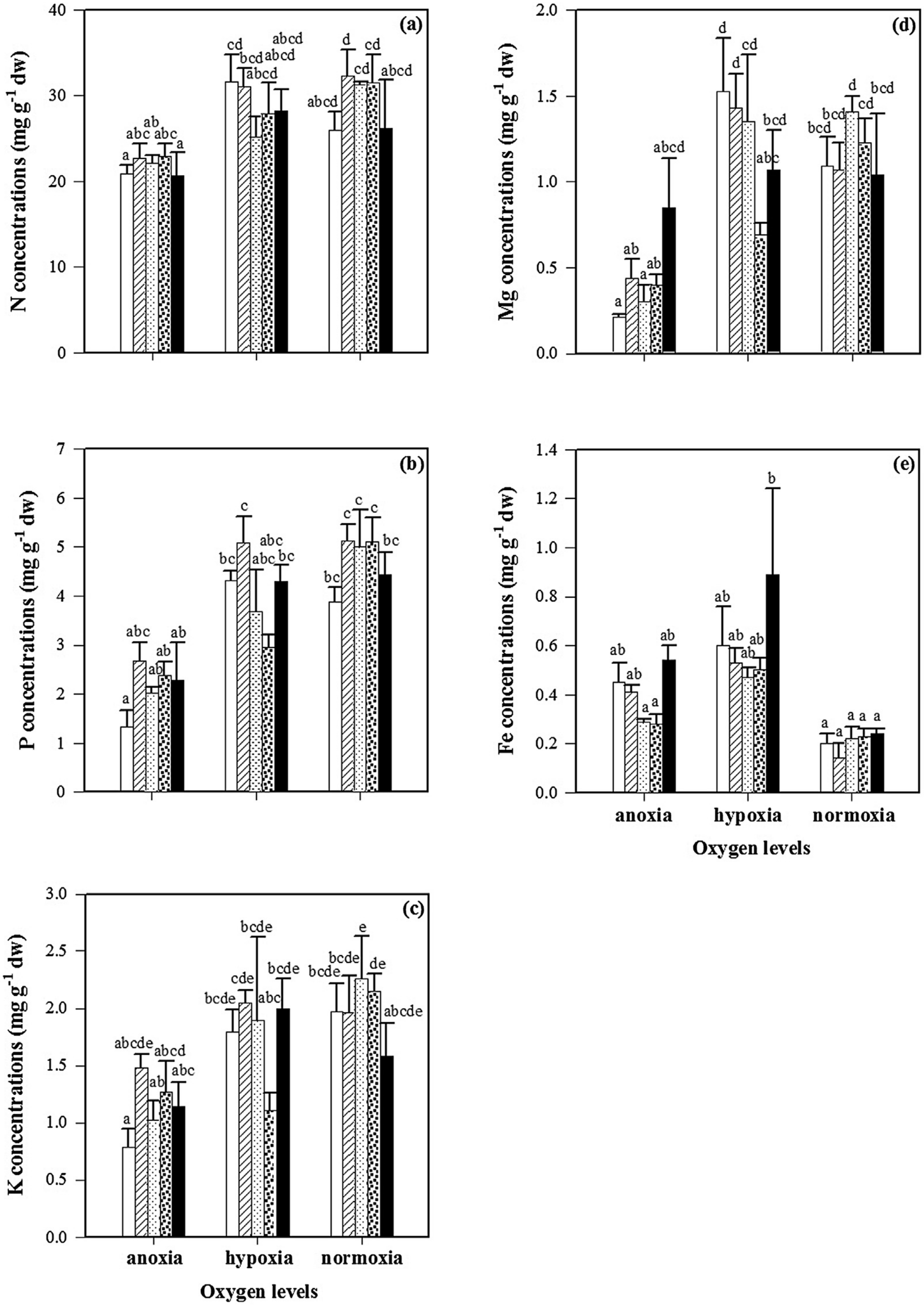

Fig. 5. Concentrations of N (a), P (b), K (c), Mg (d) and Fe (e) in roots of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) (mean ± SD) grown on different NH_4^+ concentrations (□ 0.5, ▨ 1, ⊡ 5, ▩ 10, ■ 15 mM) and O_2 levels (anoxia, hypoxia, normoxia). Different letters above columns indicate significant differences between treatments.

3. Results

3.1. Growth and morphological study

Both the NH_4^+ concentrations and O_2 levels affected growth and morphology of the hybrid Napier grass (*P. purpureum* × *P. americanum* cv. Pakchong1), but there was no interaction between these two factors (Table 1). Generally, growth of the plants decreased in anoxic conditions, especially at the high NH_4^+ concentrations whereas the relative growth rates of the plants in the hypoxic conditions did not significantly differ from the normoxic condition (Fig. 1). Decreasing O_2, particularly in anoxic conditions, significantly affected the root number, the root length, the number of leaves, the average leaf area and the new shoot number. Overall, the plants grown under the anoxic conditions were smaller than the plants grown under the hypoxic and normoxic conditions. Moreover, in the anoxic conditions, there was a trend of decreasing in

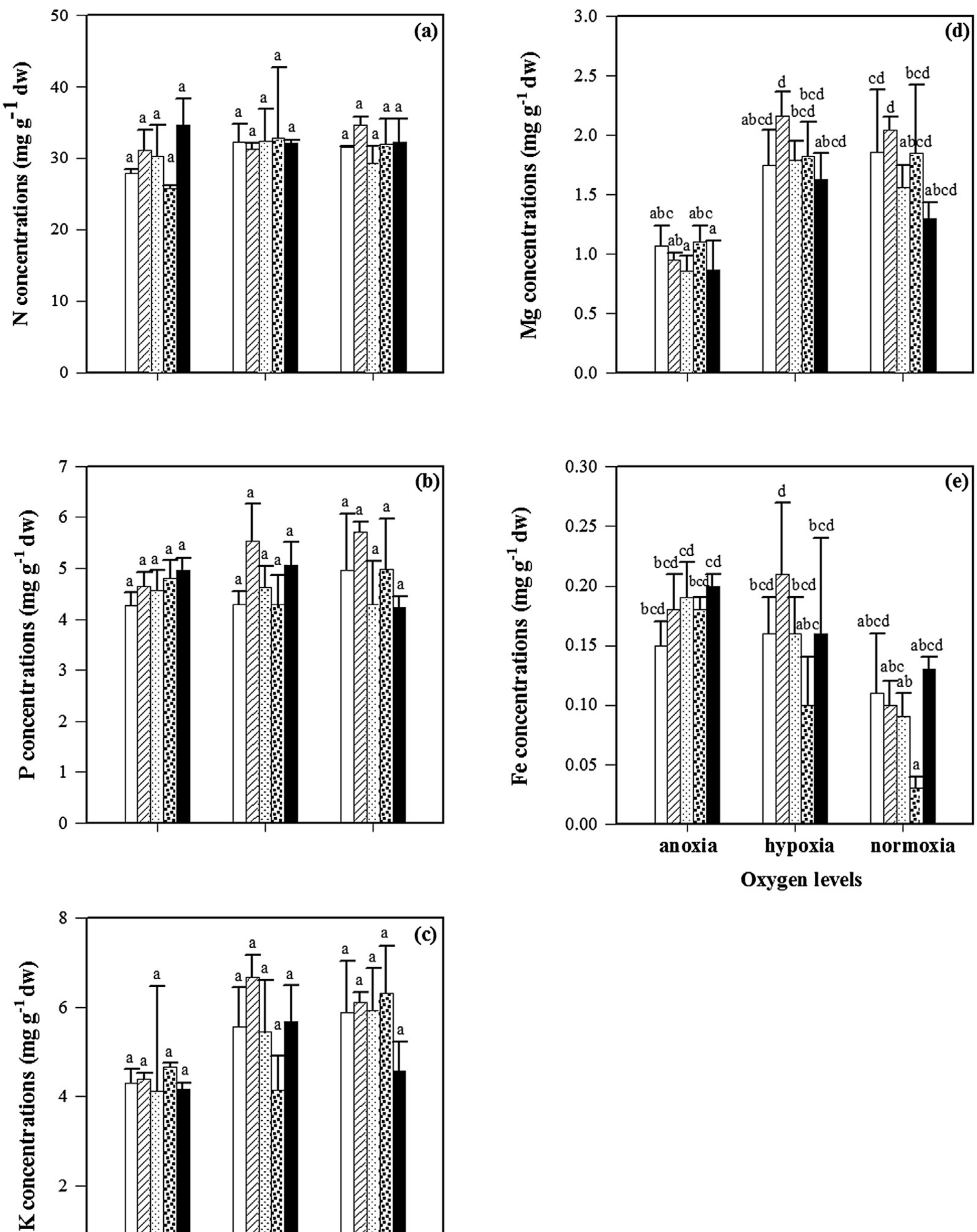

Fig. 6. Concentrations of N (a), P (b), K (c), Mg (d) and Fe (e) in leaves of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) (mean ± SD) grown on different NH_4^+ concentrations (□ 0.5, ▨ 1, ⬚ 5, ▩ 10, ■ 15 mM) and O_2 levels (anoxia, hypoxia, normoxia). Different letters above columns indicate significant differences between treatments.

root number and root length when growing the plants under the high NH_4^+ concentrations (10, 15 mM) (Fig. 2).

3.2. Chlorophyll concentration

Both the NH_4^+ concentrations and the O_2 level affected only Chl *a*, but there was no interaction between two these factors (Table 1). In the normoxic conditions, the Chl *a* increased with increasing external NH_4^+ concentration. However, the Chl *a* in the leaves of the plants grown in the growth medium under the hypoxic and anoxic conditions did not significantly differ between the treatments even though the external NH_4^+ concentration increased (Fig. 3).

3.3. NH_4^+concentration

The external NH_4^+ supply affected the NH_4^+ concentration in the stems, the roots and the leaves of the hybrid Napier grass and an interaction between the two factors was found in the stems and the roots (Table 1). In the stems, under the hypoxic and normoxic conditions, the NH_4^+ concentration of the plants grown with 0.5 and 1 mM NH_4^+ was no significant different and it decreased

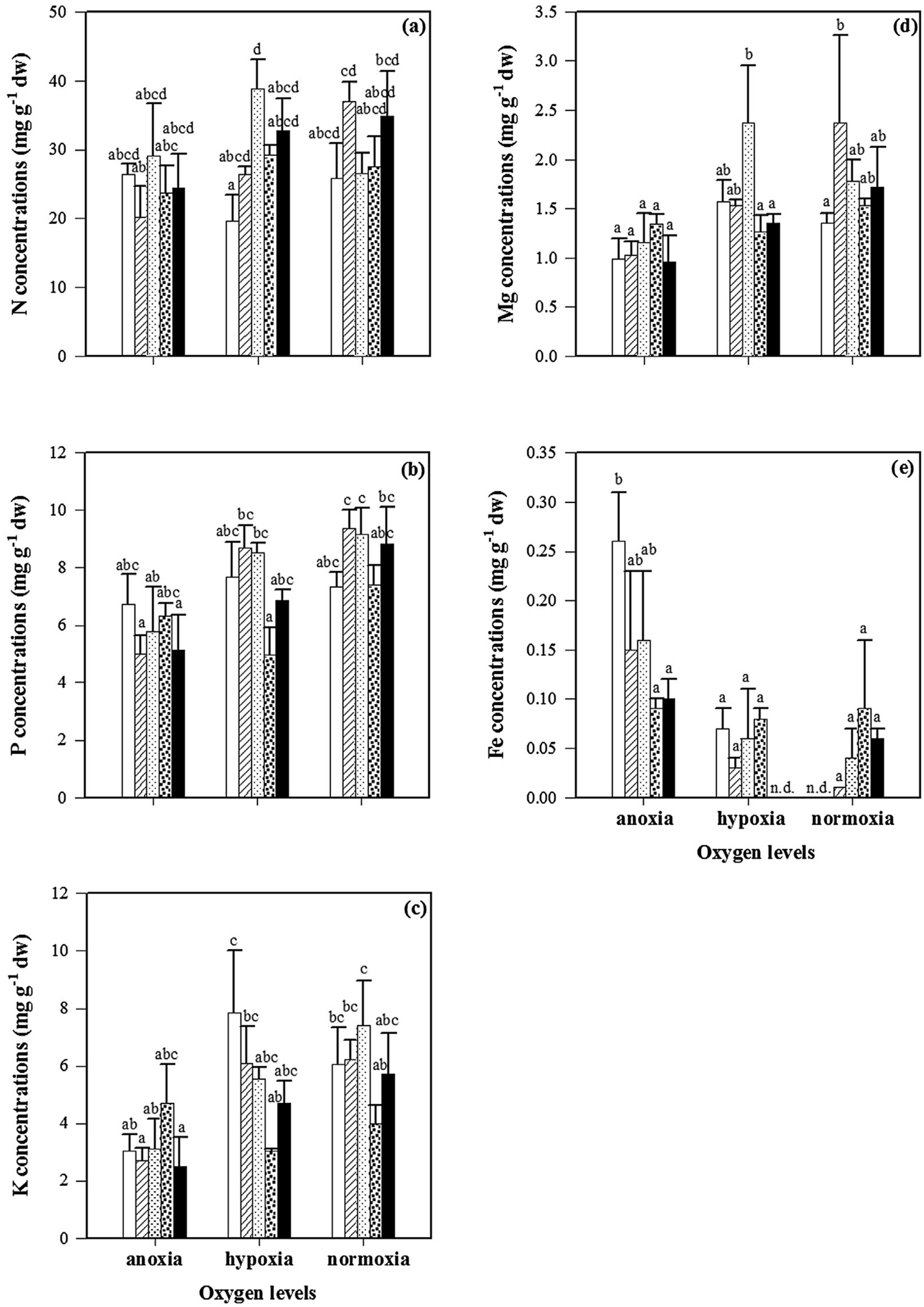

Fig. 7. Concentrations of N (a), P (b), K (c), Mg (d) and Fe (e) in stems of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) (mean ± SD) grown on different NH_4^+ concentrations (□ 0.5, ▨ 1, ▤ 5, ▩ 10, ■ 15 mM) and O_2 levels (anoxia, hypoxia, normoxia). Different letters above columns indicate significant differences between treatments. n.d. = not detected.

when the external NH_4^+ concentration increased up to 5 mM. At the high NH_4^+ supply (10, 15 mM), the greater increased in the NH_4^+ concentration was found in the normoxic condition (Fig. 4a). The NH_4^+ concentration in the roots of the plants grown under the anoxia condition increased with the increasing of the external NH_4^+ concentrations up to 5 mM, but at the high NH_4^+ concentrations (10, 15 mM) slightly reduced in the NH_4^+ concentrations was found. However, under the hypoxic and normoxic conditions, the NH_4^+ concentration tended to decrease when the external NH_4^+ supply increased to 5 mM, and then it increased at high NH_4^+ concentrations (10, 15 mM) (Fig. 4b). Compared with the leaves, the NH_4^+ concentration of the plants grown in the anoxic condition increased with the increasing external NH_4^+ concentrations. Under the hypoxic and normoxic conditions, the plants also had high NH_4^+ concentration in the tissues except at 10 mM NH_4^+, where the reduction in the NH_4^+ concentration was found (Fig. 4c).

Table 1
F-ratio and results of two-way ANOVA of growth, morphological characteristics, chlorophyll concentrations in leaves, NH_4^+ in the plant tissue, and minerals in the tissue of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) grown on different NH_4^+ concentrations (0.5, 1, 5, 10, 15 mM) and O_2 levels (anoxia, hypoxia, normoxia).

		Main effects		Interaction
		NH_4^+ concentration	O_2 level	NH_4^+ concentration × O_2 level
RGR ($g\,g^{-1}\,d^{-1}$)		1.2	23.3***	1.9
SER ($mm\,d^{-1}$)		1.4	4.3*	0.3
Root number		1.1	22.6***	0.7
Root length (cm)		3.8*	45.1***	1.8
Internode number		1.8	2.2	0.3
New shoot		1.1	9.6**	1.1
Total root dw (g)		0.7	6.9*	1.4
Total leaf dw (g)		1.1	8.8**	0.9
Shoot:root ratio		0.8	1.4	1.3
Average leaf area (cm^2)		2.8*	3.7*	0.5
Leaf number		1.8	10.7***	0.9
Chl *a* ($mg\,g^{-1}$ dw)		5.2**	6.9**	1.9
Chl *b* ($mg\,g^{-1}$ dw)		2.6	0.9	1.0
Total Chl *a* + *b* ($mg\,g^{-1}$ dw)		5.7**	4.0*	1.9
NH_4^+ in tissue (μmol NH_4^+ g^{-1} dw)				
	Stems	3.5*	3.1*	4.8***
	Roots	1.7*	1.1	3.6**
	Leaves	2.8*	1.9	0.6
N ($mg\,g^{-1}$ dw)				
	Stems	1.5	2.4	1.6
	Roots	0.8	12.0***	0.8
	Leaves	0.3	0.6	0.4
P ($mg\,g^{-1}$ dw)				
	Stems	1.4	10.3***	1.6
	Roots	2.2	39.1***	1.5
	Leaves	1.0	0.1	0.6
K ($mg\,g^{-1}$ dw)				
	Stems	1.3	8.2**	1.5
	Roots	0.7	11.4***	1.3
	Leaves	0.4	3.3	0.6
Mg ($mg\,g^{-1}$ dw)				
	Stems	1.2	5.6**	1.1
	Roots	0.6	20.8***	1.9
	Leaves	1.3	15.4***	0.3
Fe ($mg\,g^{-1}$ dw)				
	Stems	1.3	6.5*	1.1
	Roots	2.2	16.6***	0.7
	Leaves	1.3	7.5**	0.5

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

3.4. Mineral contents

Generally, mineral accumulation in the plant tissue was affected by the O_2 level (Table 1). The concentrations of N, P, K and Mg in the roots of the plants grown in the anoxic conditions decreased approximately 26%, 50%, 43% and 63% respectively, compared to the control normoxic conditions. However, the concentrations of Fe in the roots increased 2–3 times (Fig. 5). In the stems, the concentrations of P and Mg decreased approximately 31% and 45%, respectively, compared to the control plants (Fig. 7). Whereas, the concentrations of Mg decreased approximately 43% in the leaves of the plants grown in the anoxic conditions (Fig. 6).

4. Discussion

This study showed that the hybrid Napier grass (*P. purpureum* × *P. americanum* cv. Pakchong1) was more sensitive to O_2 deprivation than to the NH_4^+ concentration. Overall, the RGRs of the plants grown under the anoxic conditions were reduced by 20–30% compared to the normoxic and hypoxic conditions, respectively. Under the anoxic conditions, the plants showed the greater decrease in the RGRs when the NH_4^+ concentration was more than 1 mM. Most plants grown under low O_2 or completely anoxia had reduced or stunted growth and other symptoms like root aging and leaf chlorosis (Drew and Sisworo, 1979; Jampeetong and Brix, 2009c). However, the hybrid Napier grass seemed to adapt well to grow under low O_2 that the plant growth rates were not significant difference from the plants grown on the normoxic conditions. This ability of the hybrid Napier grass to acclimate to low O_2 might be obtained from its parent, *P. purpureum* because the plant inhabits in water saturated soil for most of the year (Cheng et al., 2009). Likewise, several species of emergent plants inhabiting wetlands where O_2 is commonly low and NH_4^+ is the major N-source for plant growth could tolerate high NH_4^+ concentrations compared with submerged species which were sensitive to high NH_4^+ (Cao et al., 2007, 2009). Hence, trend to prefer NH_4^+ has been documented in the hybrid Napier grass, in which the plants had higher NH_4^+ uptake rates than NO_3^- (Jampeetong et al., 2014). In the present study, however, NH_4^+ toxicity symptoms including chlorosis, short and rotting roots, small leaves were evident at the NH_4^+ concentrations more than 5 mM that were commonly reported in other species (Britto and Kronzucker, 2002; Jampeetong and Brix, 2009b, Piwpuan et al., 2014) and this study found the serious symptoms in anoxic conditions.

Tolerance to high NH_4^+, the ability to detoxify NH_4^+ via assimilation in roots had been reported (Tobin and Yamaya, 2001; Omari et al., 2010). Sorghum–sudan grass hybrids (*Sorghum bicolor* L. × *S. bicolor* var. sudanense) increased capacity for nitrogen assimilation in the roots when growing the plants at high levels of NH_4^+, resulted in increasing of glutamine synthetase (GS) activity and protein accumulation in its roots. It was suggested that this mechanism might prevent NH_4^+ translocated to leaves and alleviate the plants from NH_4^+ toxicity (Omari et al., 2010). Moreover, it had been reported that most plants exhibited NH_4^+ efflux across membrane to maintain cytosolic NH_4^+concentrations in the root cells leading to high O_2 demanding for root respiration (Britto et al., 2001; Kronzucker et al., 2001; Piwpuan et al., 2014). In this study, the NH_4^+ accumulation in the leaves varied slightly across different O_2 levels and NH_4^+ concentrations, while the total nitrogen in the leaves did not significantly differ among the treatments. The findings suggested that most of the NH_4^+ taken up was probably assimilated mainly in the roots. However, inadequate O_2 might affect NH_4^+ maintenance and assimilation in the root cells. Here, the NH_4^+ accumulation in the roots tended to increase at the high NH_4^+ levels under the anoxic conditions while the total nitrogen was reduced. Compared to the normoxic and hypoxic treatments, the NH_4^+ concentration in the roots decreased with the increasing external NH_4^+ concentration up to 5 mM whereas the total nitrogen in the roots increased. This indicated that the hybrid Napier grass could regulate the excess NH_4^+ enhancing this species to tolerate the NH_4^+ concentration up to 5 mM even with low O_2 supply.

Oxygen in the rooting medium played an important role in root respiration as a terminal electron acceptor that can affect nutrient uptake. Reduction in nitrogen and phosphorus uptake under reduced conditions had been documented in many species such as *Typha domingensis* (Li et al., 2010), *Hordeum vulgare* L. (Leyshon and Sheard, 1974), *Zea mays* L. (Ferreira et al., 2008). In the present study, N and P accumulations in the roots were decreased by 26% and 50%, respectively, under the complete anoxic condition as compared with the normoxic conditions. Moreover, reductions in K and Mg were also been found in this study. Under anoxic conditions, the ferric form was reduced to the ferrous form that was soluble, resulting in tissue Fe concentration that was greater than found in the plants grown in normoxic conditions (Gries et al., 1990; Pezeshki, 2001; Li et al., 2010). In the hybrid Napier grass, the increasing of Fe was found in all parts of the plants particularly in the roots. Furthermore, the high accumulation of Fe in the leaves could cause chlorosis in the leaves (Tanaka and Yoshida, 1970) and might decrease photosynthesis efficiency that caused the decrease in growth rate.

The NH_4^+ tolerance and adaptation to low O_2 of the hybrid Napier grass suggested that this species may have a potential for being used to treat the effluent from the biogas production tank where NH_4^+ predominated associated with low O_2 availability. This indicated that the hybrid Napier grass (*P. purpureum* × *P. americanum* cv. Pakchong1) could be used in horizontal flow CWs where the substrate was water-saturated and the N mainly occurred in the reduced forms as organic N and NH_4^+.

In summary, under the anoxic conditions, the hybrid Napier grass showed decrease in the growth rate, the root numbers, the root length, the leaf numbers, the average leaf areas and the new shoot numbers. Moreover, the N and P concentration in plant tissues were reduced in the anoxic conditions. On the other hand, the hybrid Napier grass grew well in high NH_4^+concentrations under both the hypoxic and normoxic conditions. Therefore, the hybrid Napier grass (*P. purpureum* × *P. americanum* cv. Pakchong1) can be used for treating effluent with high NH_4^+ concentration and low O_2 from the biogas production tanks. In addition, using wastewater to grow the Napier grass is an integrating management to increase Napier crop, especially in Southeast Asia, where fresh water shortages usually occur.

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