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KINETICS OF NUTRIENT UPTAKE BY ECONOMICAL VEGETABLE SPECIES GROWN IN CONSTRUCTED WETLANDS

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ABSTRACT

Constructed wetlands are used to treat wastewater, however, their performance and usage can be improved by selecting a suitable substrate. Various plants may differ in their capacity to assimilate nutrients in a constructed wetland system. In this research, Michaelis-Menten kinetic (Michaelis-Menten constant (K_m) and maximum uptake rate (I_{max})) provides a useful tool for the identification of plant nutrient uptake efficiency and selection of plants. Six economical plant species (*Phyllostachysheteroclada, Medicago hispidaGaertn, Ipomoea aquatica, Brassica junceavar, Colocasiaesculenta L. Schoot, and A. tubersomrottle ex spreng*) were selected to investigate the uptake kinetics. *I. aquatica* showed a higher maximum uptake capacity for all three nutrients (PO_4-P, NO_3-N and NH_4-N). The second highest uptake capacity for PO_4-P, NO_3-N and NH_4-N was found for *A. tuberosum, B. juncea* and *M. hispida*. *I. aquatica* has the highest I_{max} and lowest K_m values for all three nutrients and it has good capabilities to adjust in high PO_4-P, NO_3-N and NH_4-N nutrient condition. I_{max} and K_m of *P.heteroclada* were relatively small and suitable for the low nutrients concentration environment, whereas the I_{max} and K_m of *A. tuberosum, M. hispida*and *B. juncea* were relatively large, which is suitable for treating wastewater with high nutrient concentration.

Keywords: Uptake kinetics; Michaelis-Menten kinetic; Michaelis-Menten constant (K_m); Maximum uptake rate (I_{max}); Constructed wetland.

INTRODUCTION

Adequate sanitation and access to clean water become one of the biggest problems that affect human health, especially for developing nations (Bisung and Elliott, 2014). On the other hand, over-exploitation of freshwater resources to meet water demand is a common practice (Hoekstra, 2014; Schyns and Hoekstra, 2014). These approaches cause negative environmental effects and deterioration of the ecosystems (Lemaire *et al.*, 2014; Peng *et al.*, 2016; Wang *et al.*, 2016a). The gap between the supply and demand for water and food is widening with the increase of world population, reaching alarming levels (Bogardi *et al.*, 2012). Experts around the globe are seeking new means to increase food productivity as well as to conserve water (Cai *et al.*, 2016). In this concern, recycling of wastewater and nutrients through constructed wetland systems involved the growth of economical vegetation is desired (Turcios and Papenbrock, 2014). Constructed wetland systems are used around the world for treating different type of wastewater (Zhang *et al.*, 2014; Wang *et al.*, 2016b). Plants may affect the efficiency of wastewater treatment through nutrients uptake and by the influence on microbial activity (Li *et al.*, 2013). The nutrients assimilating capacity of plants may differ according to plant species (Wu *et al.*, 2015). There is an important need to understand the efficiency of economical plants roots to acquire nutrients in constructed wetland system. It has been shown that the plant biomass is an important parameter affecting root zone processes and nutrient dynamics (Vymazal and Kröpfelová, 2011).

Michaelis-Menten kinetic provides a useful tool for the identification of plant nutrient uptake efficiency, the elucidation of the mechanism of absorption, the identification, and screening of plants (Bucher, 2007). The kinetics of nutrients uptake have been reported in many plant species such as wheat rice (Youngdahl *et al.*, 1982; Goyal and Huffaker, 1986), barley (Kronzucker*et al.*, 1999), eucalypts (Garnett *et al.*, 2003), citrus (Cerezo *et al.*, 2007) and dotted duck meat (*Landoltiapunctata*) (Fang *et al.*, 2007a). However, there is insufficient literature on the pollutants absorption kinetics of constructed wetland plants, and especially economical vegetable plants. The study on the kinetics of nutrient uptake in constructed wetland system can provide scientific basis and support to understand the mechanism of nitrogen and phosphorus removal in water bodies and selection of vegetable type macrophytes with high economic value in the local market regarding daily consumption.

Nitrogen is one of the most important nutrients for plants and microbes exists in the inorganic forms of ammonium (NH_4^+), ammonia (NH_3), nitrate (NO_3^-), and nitrite (NO_2^-) (Lambers *et al.*, 2008). Ammonium and nitrate are the two forms, which are available for plant uptake and some plant species showing a strong preference for one ionic form over the other (Kronzucker*et al.*, 1997; Forde and Clarkson, 1999). Variation in nutrient removal between different plant species is likely to reflect differences in growth phases, the efficiency of nutrient uptake and use (Tanner, 1996; Güsewell and Bollens, 2003). Nitrogen removal mechanism is complicated and redox potential is one of the important parameter that play important role in the oxidation and reduction of nitrogen in constructed wetland (Saeed and Sun, 2012). The objective of this research to identify the performance of economical vegetation for nutrient uptake in a constructed wetland.*P.heteroclada, M. hispida, I. aquatica, B. juncea, C. esculenta, A. tuberosum*were selected on the basis of their economic value in the local market. This approach can make the application of constructed wetland broader as well as efficiently reuse of wastewater and nutrients in it.

MATERIALS AND METHODS

Plant materials: Experiment was conducted at Southeast University, Wuxi campus, China in a controlled environment (75-85% relative humidity, temperature 25 ± 1°C, 12-h photoperiod). Six plant species (Table 1) were selected from hybrid constructed wetland of Southeast University. After collection, the plants were rinsed with deionized water and transplanted into vessel contained solution culture system. N and P concentrations in the solution were similar to wastewater that is treated in the constructed wetland in the Wuxi campus of the Southeast University. The solution contained following macronutrients (mmol L^{-1}): 1.25 (1:1= NH_4–N:NO_3–N) and P = 0.32 (KH_2PO_4), K^+ = 0.64, Ca^{2+} = 0.25, Mg^{2+} =0.21 and SO_4^{2-} = 0.22, and micronutrients (mmol L^{-1}): Zn^{2+} = 0.6, Cu^{2+} = 0.15, Fe^{2+} = 6.1, Mn^{2+} = 0.9, BO_3^{3-} = 24 and MoO_4^{2-} = 5. The pH value was adjusted and maintained between 5.0 and 6.0 (most of the plants prefer a slightly acidic growing environment) and the nutrient solutions were replaced every 3 days to avoid substantial changes in pH as well as nutrients. After 2 weeks of growth, the uptake kinetics of PO_4-P, NO_3-N and NH_4-N by plants were measured separately.

Uptake kinetics: A depletion method (Barber, 1995) and serial concentration method were used to measure nutrient uptake kinetics. Plants with uniform size were selected, rinsed with deionized water and placed into nutrient solution for 2 days to achieved starvation induces maximal uptake response. The concentration in nutrient solution was similar as described earlier except absence of N or P concentration. Same controlled environmental conditions were used.

After a starvation period, plants were shifted to 2 L beaker contained 0.8 L nutrient solution with concentration of total phosphorus 2.2 mg / L, nitrate nitrogen 20 mg / L and the ammonium nitrogen 15 mg / L. Each treatment had three replicates. Samples were taken at 0, 0.5, 1, 2, 3, 4, 6, 8 and 10 hours. Immediately after the end of the absorption test, the roots were separated from the rhizomes and rinsed with deionized water and dried to a constant weight at 70˚C for 5 days in the oven and weighed.

Analytical method: PO_4-P (phosphate), NO_3-N (nitrate)NH_4-N (ammonium), were analyzed according to standard methods (Federation and Association, 2005).

Calculations and statistical analyses: Ion consumption dynamic equation is a quadratic polynomial equation (Mao, 1995):

$$Y = ax^2 + bx + c \quad (1)$$

Where

Y = is the treated ion concentration; a = initial ion concentration; x = the absorption time; b = maximum rate in concentration change; c = quadratic equation constant

For the derivative of Eq. 1, we can get the rate equation of concentration change:

By taking derivative from eq. (1)

$$Y' = 2ax + b \quad (2)$$

For equation 2, let x = 0, then Y′ = b is the maximum change in concentration, which can be the maximum absorption rate:

$$I_{max} = b \text{ x } V / \text{root dry weight} \quad (3)$$

where

I_{max} is the maximum absorption rate for ions.

V = the volume of solution added to the absorption test

Y′ = 1 / 2b into equation 2 to find x, and the value of Y obtained from equation 1 is K_m (the Michaelis-Menten constant, which is the concentration of ions in the solution at half of the maximum rate of the absorbed ions).

Initially, the data was recorded in MS-excel (Office package- 16) and then SPSS version-18.0 (SPSS Incorporation Chicago, Illinois, USA) program was used to perform one-way analysis of variance (ANOVA), whereas Duncan Multiple Range test was used to find the mean difference among various treated groups.

RESULTS

Kinetics of PO_4-P uptake: The kinetic of phosphorus uptake by the experimental plants are shown in Fig. 1. In all cases the concentration of PO_4–P decreases gradually with increasing the time of exposure. The absorption of PO_4–P by *I. aquatica* was the lowest while absorption by *B. juncea* was the highest. Table 2 shows significant differences in the kinetic characteristic of PO_4-P absorption among plants. I_{max} range was between 3.08 – 79.73 µg PO_4-P/g root DW·h and K_m was between 0.1529 – 1.5948 mg/l for all plant species. I_{max} and K_m patterns for PO_4-P uptake were: *I. aquatica>A. tuberosum>M. hispida> P.heteroclada> B. juncea>C. esculenta;* and *C. esculenta>M. hispida>A. tuberosum>B. juncea> P.heteroclada>I. aquatica,* respectively. There was no significant difference in K_m value between *C. esculenta, M. hispida*and*A. tuberosum* (P> 0.05).

Kinetics of NO_3-N uptake: Uptake of NO_3 - N gradually decreased as contact time increased (Fig. 2) with *B. juncea*showing high absorption of nitrate compared to other plants. The values for I_{max} varied between 27.08 and 713.41 mg/ L while values for K_m varied between 15.0501 and 16.8377 µg NO_3 - N /(g root DW · h) (Table 2). $I_{max}$for NO_3-N uptake decreased in the order of *I. aquatica>A. tuberosum>B. juncea>P.heteroclada>M. hispida>C. esculenta, while* K_m decreased in the order of *A. tuberosum>C. esculenta>P.heteroclada>M. hispida>B. juncea> I. aquatica*, respectively (Table 2).

NH_4-N kinetics: Fig 3 shows the absorption kinetics of NH_4 -N for various plants species. There is a great variation in NH_4-N uptake among all studied plants. Uptake kinetics for NH_4-N uptake, The K_m varied between 9.33 and 13.14 mg / L and decreased in the order of *I. aquatica>M. hispida>A. tuberosum>B. juncea>P.heteroclada>C. esculenta*. I_{max} ranged between 59.03 and 853.57µg NH_4 -N / (g root DW · h) and decreased in the order of *B. juncea>C. esculenta>M. hispida>A. tuberosum>P.heteroclada> I. aquatica*.

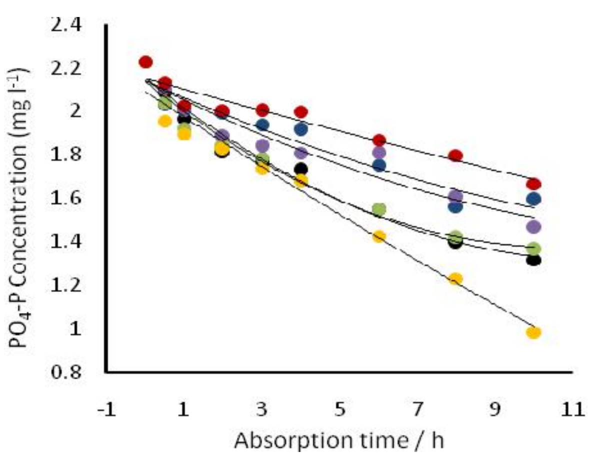

Figure 1.Decrease of PO_4-P concentration during the experiments with various plant species.
A. tuberosum ● *C. esculenta* ● *P.heteroclada* ●
M. hispida ● *B. juncea* ● *I. aquatica* ●

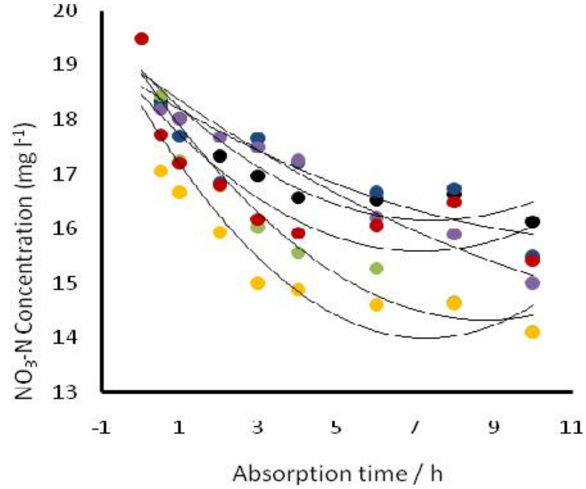

Figure 2. Decrease of NO_3-N concentration in the water in the presence of surveyed plant species.
A. tuberosum ● *C. esculenta* ● *P.heteroclada* ●
M. hispida ● *B. juncea* ● *I. aquatica* ●

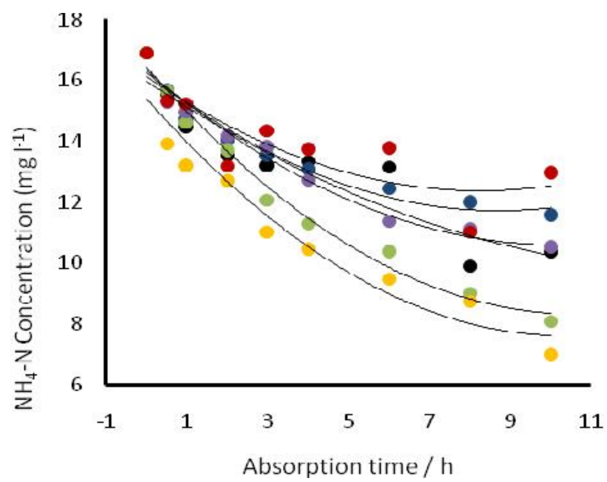

Figure 3. Decrease of NH_4-N concentration in the water in the presence of surveyed plant species.
A. tuberosum ● *C. esculenta* ● *P.heteroclada* ●
M. hispida ● *B. juncea* ● *I. aquatica* ●

Table 1. Selected plants used in the experiments.

S. no	Common name	Scientific name	Used name
1	Water bomboo	*Phyllostachysheteroclada Oliver*	*P.heteroclada*
2	Chinese cabbage	*Medicago hispidaGaertn*	*M. hispida*
3	Water spinach	*Ipomoea aquatica*	*I. aquatica*
4	Chinese mustard	*Brassica junceavar*	*B. juncea*
5	Taro	*Colocasiaesculenta L. Schoot*	*C. esculenta*
6	Chinese Leek	*A. tubersomrottle ex spreng*	*A. tuberosum*

Table 2. Estimated values of K_m and I_{max} for PO_4–P, NO_3–N and NH_4–N uptake kinetics.

No.	Plant Species	Nutrients	Ion consumption curve equation	Nutrients Kinetics	
				K_m (µg/g root DW·h;)	I_{max} ($mg·L^{-1}$)
				Mean ± sd**	
1	*P.heteroclada*	PO_4–P	$0.0066x^2 - 0.1421x + 2.0327$	1.45 ± 0.100^{a,b}	32.48 ± 1.03^{b}
		NO_3 - N	$0.0587x^2 - 1.0299x + 18.842$	15.48 ± 2.00^{a,b}	262.47 ± 1.98^{b}
		NH_4 + -N	$0.0719x^2 - 1.525x + 16.415$	10.31 ± 1.00^{a,b}	331.54 ± 1.95^{b}
2	*M. hispida*	PO_4–P	$0.0029x^2 - 0.0916x + 2.1367$	1.53 ± 0.30^{a,b}	39.57 ± 0.95^{c}
		NO_3 - N	$0.0141x^2 - 0.5103x + 18.835$	15.32 ± 2.00^{a,b}	216.00 ± 2.00^{c}
		NH_4 + -N	$0.055 x^2 - 1.1193x + 15.006$	11.37 ± 1.00^{a,b}	600.32 ± 5.03^{c}
3	*I. aquatica*	PO_4–P	$0.001x^2 - 0.1186x + 2.7903$	0.15 ± 0.05a ^{a}	79.73 ± 1.00^{f}
		NO_3 - N	$0.0926x^2 - 1.2663x + 18.297$	15.05 ± 3.00^{a}	712.80 ± 2.55^{f}
		NH_4 + -N	$0.0597 x^2 - 1.3337x + 14.913$	9.32 ± 0.32^{a}	853.52 ± 3.00^{f}
4	*B. juncea*	PO_4–P	$0.0004x^2 - 0.0504x + 2.6505$	1.41 ± 0.20^{a,b}	23.28 ± 3.00^{d}
		NO_3 - N	$0.0466x^2 - 0.637x + 16.771$	15.13 ± 1.00^{a,b}	366.20 ± 4.01^{d}
		NH_4 + -N	$0.0552 x^2 - 0.9131x + 16.139$	13.14 ± 2.00^{a,b}	496.31 ± 4.03^{d}
5	*C. esculenta*	PO_4–P	$0.0022x^2 - 0.08x + 2.1403$	1.56 ± 0.15^{b}	3.08 ± 0.02^{a}
		NO_3 - N	$0.0177x^2 - 0.4479x + 18.602$	16.42 ± 2.00^{b}	27.02 ± 3.00^{a}
		NH_4 + -N	$0.044 x^2 - 1.0729x + 17.741$	12.81 ± 4.00^{b}	59.01 ± 2.00^{a}
6	*A. tuberosum*	PO_4–P	$0.006x^2 - 0.1423x + 2.1512$	1.50 ± 0.20^{a,b}	73.67 ± 3.4^{e}
		NO_3 - N	$0.0503x^2 - 0.7438x + 18.9$	16.81 ± 1.00^{a,b}	394.02 ± 6.00^{e}
		NH_4 + -N	$0.0311 x^2 - 0.8867x + 15.983$	11.21 ± 4.00^{a,b}	533.11 ± 3.00^{e}

Here, the superscripts ^{a,b,c,d,e,f} are the mean difference among various plants treated values according to Duncan Multiple Range test, whereas **is the probability value > 0.001

DISCUSSION

The results of nutrients uptake kinetics revealed differences between six economical plants in the uptake of PO_4-P, NO_3-N and NH_4–N (Table 2). The preference of different plant species for particular nutrient source can have important ecological and practical implications (Forde and Clarkson, 1999). *I. aquatica* showed higher maximum uptake capacity for all three nutrients (PO_4-P, NO_3-O and NH_4-N). The second highest uptake capacity for PO_4-O, NO_3-N and NH_4-N was *A. tuberosum, B. juncea* and *M. hispida*, respectively. Previously, it has been reported by many researchers that NH_4–N is the preference source of N for most wetland plants (Brix *et al.*, 2002; Tylova-Munzarova *et al.*, 2005; Fang *et al.*, 2007b; Jampeetong and Brix, 2009). Whereas *C. esculenta* showed minimum uptake capacity for all three nutrients. Fang *et al*. (Fang*et al.*, 2007b) studied four wetland plants and observed that two plant species (*B. monnieri* and *Azolla spp*.) had preference for NO_3–N, but *L. repens* required both nutrients for N. The absorption kinetics for nutrients by economical vegetables has not been reported, whereas many studies are reported on crop species (Rao *et al.*, 1993; Tylova-Munzarova *et al.*, 2005).

According to Zhenhua (Zhang *et al.*, 2009), the differential preferences for nutrient forms between plant species may because of their relative tolerance and habitat preference to inundation. Habitat preference can also relate to other factors such as pH, aeration, temperature, water and salt stress, composition of nutrients, plants growth stage and symbiosis (Bradley and Morris, 1990; Brix, 1994; DYHR‐JENSEN and Brix, 1996). These abiotic and biotic factors directly or indirectly may depend on design and operational conditions of wetland and wastewater characteristics, and nutrient uptake can vary from one wetland to another. (Ayaz and Akça, 2001; Coleman *et al.*, 2001; Luederitz *et al.*, 2001; Meuleman *et al.*, 2003).

According to (Cacco *et al.*, 1980), V_{max} and K_m are useful parameter to indexes the level of adaptation of the genotype in different nutrient conditions, and can be hypothesized; high V_{max} and low K_m, which represent the most favorable environment, fit in wide set of nutritional conditions; high V_{max} and high K_m, making the plants advantage at higher concentration of nutrients; low V_{max} and low K_m adapted to low nutrient condition; low V_{max} and high K_m, representing the most unsuitable situation of any nutrient conditions. On the proposed basis by using I_{max} and K_m value to evaluate the adoptability of the plant species in different nutrients conditions. I_{max} and K_m values reflect the nutrients affinity of plants; smaller the value has a strong affinity for a particular nutrient. *I. aquatica* has the highest I_{max} and lowest K_m values for all three nutrients and it has good capabilities to adjust in high PO_4-P, NO_3-N and NH_4-N nutrient condition. I_{max}

and K_m of *P.heteroclada* was relatively small and suitable for low nutrients concentration environment, whereas the I_{max} and K_m of *A. tuberosum, M. hispida* and *B. juncea* were relatively large, which is suitable for treating wastewater with high nutrient concentration.

Conclusion: The absorption kinetics of PO_4-P, NO_3-N and NH_4-N in selected economical plants species were unlike. *I. aquatica* showed maximum uptake capacity for all three nutrients (PO_4-P, NO_3-O and NH_4-N), whereas *A. tuberosum, B. juncea*and*M. hispida* were suitable for PO_4-O, NO_3-N and NH_4-N, respectively. The absorption kinetics parameters, $I_{max}$and K_m are importantindicator for selection of economical plants for constructed wetland system. By using constructed wetland for economical plants can reduce the gap between supply and demand for water and food as well as for economic, environmental and social benefits. This approach can make the application of constructed wetland broader.

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