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Nutrient removal efficiency and biomass production of different bioenergy plants in hypereutrophic water

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ABSTRACT

The purpose of this study was to investigate the feasibility and efficiency of nutrient removal from eutrophic water along with biomass production by standing plants grown on floating beds. Results indicated that the average removal efficiency for total nitrogen, ammonium nitrogen, nitrate and nitrite nitrogen and total phosphorus by six standing plant species were respectively 50.3%, 59.4%, 82.4% and 86.5%, during a 16-day experiment on floating beds. Among six tested plant species grown on floating beds in the field experiment, *Miscanthus sinensis Anderss (sp.)* and *Vetiveria zizanioides* were dominant in growth, annual biomass production, nitrogen phyto-uptake, phosphorus phyto-uptake, sulfur phyto-uptake and carbon sequestration. Neutral-detergent fiber, acid-detergent fiber, acid-detergent lignin, cellulose and hemicellulose contents of these species were similar to swithgrass. *M. sinensis Anderss (sp.)* and *V. zizanioides* were most promising plant species for biomass production and nutrient removal grown on eutrophic water with floating beds.

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1. Introduction

The eutrophication of inland water is caused by human activities including rapid urbanization, industrialization and intensive agricultural production [1]. The inclusion of wastewater enriched with phosphorus and nitrogen from point- and nonpoint-sources into natural waters have become a severe water pollution problem in many countries [2]. Over the last two decades, rapid population growth and economic development in China, nutrient-laden effluents have increasingly discharged into the inland water [3]. A recent survey indicates that up to 66% and 22% of lakes in China have become eutrophic or hypereutrophic [4]. To prevent the impacted inland water from further

deterioration, cost-effective remediation methods for eutrophication control are urgently needed in China as well as in other developing countries.

The conventional constructed wetlands (CWs) are considered as low cost systems for treating municipal, industrial and agricultural wastewater [5,6]. CWs with emergent plants are efficient for trapping particulate P and assimilating dissolved nutrients from the influent. However, CWs occupy a large area of lands, which are limiting resources in the countries where human population density is high. This is particularly true in the developed areas such as Yangtze River Delta and the Pearl River Delta of China.

The floating plant treatment system is perhaps more efficient to remove dissolved forms of nutrients from the inland

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water and is capable of adapting at variable water depths [7]. Use of floating plant system to assimilate and remove nutrients from water is an attractive phytoremediation approach [8]. However, few macrophytes are available for water treatment [9]. Some aquatic plants such as water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) are notorious in freshwater ecosystems despite of their rapid growth, high reproductive ability and high nutrient removal capacity [10]. The potential of rapid spread of these species on water surface limits their applications.

Floating beds of emergent plants (wetland or terrestrial plants) have been widely used in wastewater treatment in recent years. Floating beds differ from the conventional CWs in which the microbes and macrophytes grow on and within floating platforms, which are installed in the waterways needed for treatment. The macrophytes extend their roots into the water column where nutrients are assimilated hydroponically [11]. Several studies have investigated the efficiency of the floating beds [11,12]. On the other hand, the naturalized treatment systems have significant potentials for both wastewater treatment and resource recovery. There are many potential economic opportunities for biomass production associated with terrestrial or aquatic macrophytes, including bioenergy and traditional feeding materials for livestock [13].

The development of renewable energy and reduction of greenhouse gas (GHG) emissions are now established priorities within EU policy, USA, China, and many other countries. The main sources of anthropogenic CO₂ emissions accounting for approximately two thirds of the total in the United States are based on fossil-based power generation and transport means [14]. Biomass can be converted into energy by thermochemical processes, including combustion, pyrolysis, and gasification, or by fermentation of carbohydrates to produce methane and ethanol [15]. Sources of lignocellulosic biomass include wood, paper waste, crop residues, and herbaceous energy crops. Perennial herbaceous energy crops have been used as a feedstock because once established they do not require annual reseeding. They also have environmental benefits including reduced soil erosion, enhanced carbon sequestration, and providing wildlife habitat [16].

To explore an approach with multiple benefits including the treatment of polluted water, environmental protection and the development of renewable energy, the present work aimed to (1) analyze the efficiency of nitrogen and phosphorus removal by floating beds (2) assess the responses of aquatic plants in eutrophic water (3) evaluate the potential environmental benefits for alleviation of CO₂ and (4) assess the value of the plant biomass as bioenergy feedstock.

2. Materials and methods

2.1. Experiment I

The tested water in this experiment was taken from Huajiachi Pond (30°16' N, 120°11' E) in Zhejiang University, Hangzhou, China. The pond water was high in total nitrogen ($8.12 \pm 0.37 \text{ mg L}^{-1}$), ammonium nitrogen ($2.0 \pm 0.18 \text{ mg L}^{-1}$), nitrite and nitrate nitrogen ($4.07 \pm 0.41 \text{ mg L}^{-1}$), and total

phosphorus ($2.28 \pm 0.19 \text{ mg L}^{-1}$). Experiment I was carried out under greenhouse conditions with a natural temperature (12–25 °C) and photoperiod from April 15 to April 30, 2009. A total of six species of promising plants were chosen and the seedlings of vegetative propagation were supplied by the Institution of Green Life Modern Agriculture and Ecological Engineering (Hangzhou, China). *Triarrhena lutarioriparia* and *Miscanthus sinensis* Anderss (sp.) are perennial plants native to Zhejiang Province, and have excellent fiber properties for paper making. Other four test plants have proved to be effective in removing nutrients, i.e. *Zizania caduciflora* [17,18], *Thalia dealbata* [19], *Vetiveria zizanioides* [20], and *Acorus calamus* [21]. Preliminary tests demonstrated that all the six plants can grow well on the floating beds.

The experimental design included six treatments and a control with floating bed but no plants. Seedlings of each plant species were selected from the nursery of the research center of Zhejiang University. The seedlings with an average height of $10 \pm 5 \text{ cm}$ were grown in one-third strength Barco solution for seven days [22], then rinsed with deionized water and transplanted into each floating bed. Each floating bed was made of rigid polyurethane foams with a thickness of 2 cm and a diameter of 50 cm. There were 12 holes with a diameter of 5 cm in rigid foams. One seedling was fixed within every hole made by rolling the flexible sponge made from foamed plastic polymers. The experiment was performed in a plastic tank with a diameter of 50 cm and a height of 60 cm. Water losses due to evaporation and evapotranspiration were controlled by adding deionized water to the original level at every alternate day.

Water samples were taken from the upper, middle and lower portion of the water column in the tanks between 08:00 and 10:00 on days 1, 6, 11 and 16. These were then mixed to produce a composite sample that was stored at 4 °C until analyzed generally within 48 h. All water samples were analyzed for ammonia nitrogen, nitrate and nitrite nitrogen, total nitrogen and total phosphorus according to the methods of Zhao et al. [23].

2.2. Experiment II

An in situ experiment was conducted in Huajiachi Pond with the above mentioned six plant species grown on artificial floating beds from April 3 to November 3, 2009. The total area of the pond was about 6000 m² and the average water depth was about 1.8 m. Each plant species was planted on three floating beds constructed with PVC pipes and bamboo tablets with a dimension of 0.6 m × 1.0 m. Floating beds were fastened into bamboo pillars with a 10 cm diameter to prevent them from free-floating in the pond. Plant seedlings were arranged into four rows with 8 seedlings (or clusters) per row. Plant biomass was harvested and fresh weight of each species was recorded. Aboveground tissues of each plant species were collected randomly and the flowers and seeds were picked out. Approximately 200 g of samples were oven dried to constant weight at 80 °C to determine the moisture content. Subsamples of the oven dried plants were ground to pass a 0.25 mm mesh sieve and then were analyzed.

Nitrogen, carbon and sulfur content were determined with a CNS analyzer (Vario MAX CNS Macro Elemental

Analyzer, Elementar Analysensystem GmbH, Hanae, Germany). After plant tissue samples were acid-digested ($\text{HClO}_4:\text{HNO}_3 = 5:1$), major mineral nutrients in the biomass samples were determined by an Agilent 7500a ICP–MS system (Agilent Technologies). Crude protein content was estimated by $\text{N} \times 6.25$. Crude lipid content was determined by exhaustive extraction with diethyl ether [24]. Total ash content was measured by the loss of weight after combustion at 450°C for 16 h in a muffle furnace. Acid-detergent fiber (ADF), acid-detergent lignin (ADL) and neutral-detergent fiber (NDF) were determined by a Raw Fiber Extractor (Fire3, Velp Scientifica, Milan, Italy). Hemicellulose (HE) and cellulose (CL) were calculated as: $\text{HE} = \text{NDF} - \text{ADF}$, $\text{CL} = \text{ADF} - \text{ADL}$.

2.3. Data Analysis

All compositional analyses were performed in triplicate, and data were corrected to a 100% dry matter (DM) basis. The data are expressed as mean \pm standard errors. One-way ANOVA and Tukey's comparison of means were performed with SPSS (version 16.0). The significance level was set at $P < 0.05$.

3. Results

3.1. Effects of plant species on nitrogen removal efficiency

As for experiment I, total nitrogen (TN) concentrations of all treatment systems were lower than that of the control (CK) except for the initial measurement (Fig. 1A). After 16-day

treatment, TN concentrations with floating bed treatments decreased from 8.1 mg L^{-1} to 4.0 mg L^{-1} , which was significantly lower than that measured in the CK (6.8 mg L^{-1}). The removal rates of TN by *Z. caduciflora*, *T. lutarioriparia*, *T. dealbata*, *V. zizanioides*, *M. sinensis anderss* and *A. calamus* ranged from 43.8 to 59.9%, which was significantly higher than those of CK treatment. The highest removal was found in the treatment with *M. sinensis anderss* (59.9%).

The reduction in ammonium nitrogen ($\text{NH}_4^+ - \text{N}$) was less than nitrite and nitrate nitrogen ($\text{NO}_x - \text{N}$), as measured in the 16 days (Fig. 1B and C). The $\text{NH}_4^+ - \text{N}$ concentration in the control decreased from 2.0 mg L^{-1} to 1.4 mg L^{-1} , whereas $\text{NO}_x - \text{N}$ concentration decreased from 4.1 mg L^{-1} to 1.9 mg L^{-1} . The average removal of $\text{NH}_4^+ - \text{N}$ and $\text{NO}_x - \text{N}$ by the plant treatments were 59.4% and 82.4%, respectively, which were significantly higher than CK. However, there were no significant differences in removal between plant treatments ($P > 0.05$).

3.2. Effects of plant species on phosphorus removal efficiency

Total phosphorus (TP) concentration did not change significantly between CK and plant treatments in the initial six days of experiment I (Fig. 1D). After 16-day treatment, except for *M. sinensis anderss* which had a significantly higher removal (91.5%) than *A. calamus* (79.1%), there was no significant difference in percent removal between the other five plant species. The average removal rate of TP for all the plant treatments was equivalent to $\text{NO}_x - \text{N}$, but significantly higher than the removal of TN and $\text{NH}_4^+ - \text{N}$.

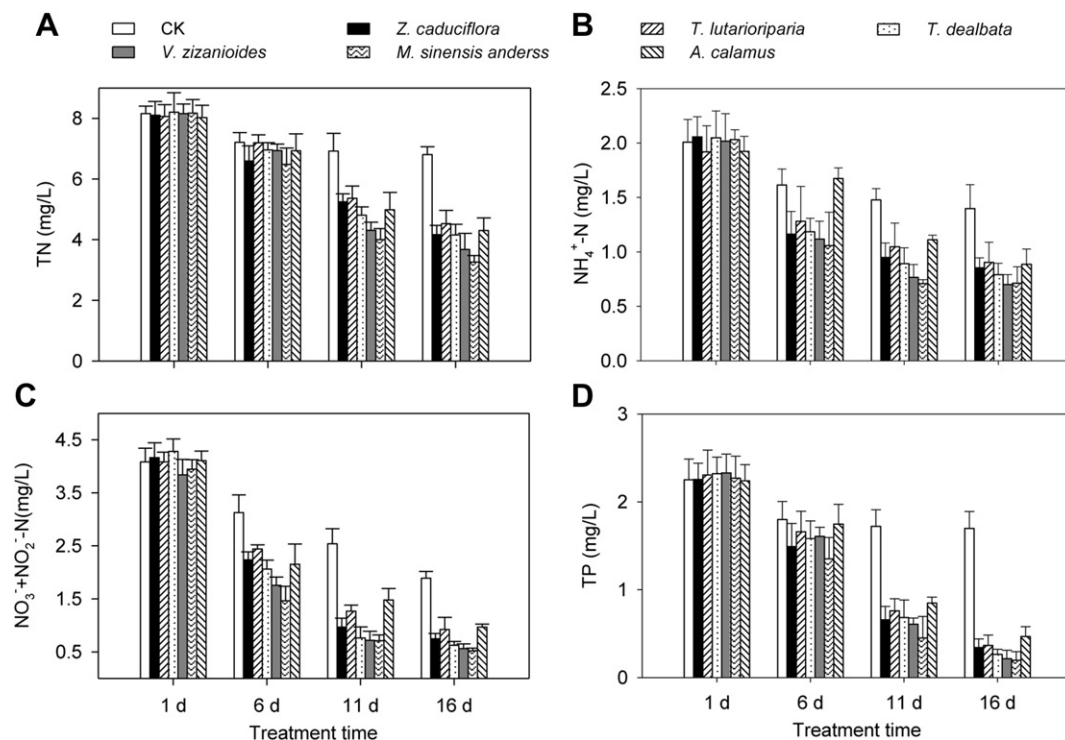


Fig. 1 – Changes of TN (A), $\text{NH}_4^+ - \text{N}$ (B), $\text{NO}_3^- + \text{NO}_2^- - \text{N}$ (C), and TP (D) concentrations of plant treatment system and control (CK) during the 16-day in experiment I. Each treatment was run in triplicates. Vertical bars represent standard error ($n = 3$).

3.3. Biomass response of different plants in eutrophic water

As the growing season of test plants in Zhejiang Province was from April to November, the biomass during the period of experiment II was treated as all-year biomass. Although each of the six plant species can grow in the eutrophic water, the biomass production was considerably different (Fig. 2). Annual aboveground fresh matter production was in the order of *M. sinensis anderss* > *V. zizanioides* > *T. dealbata* > *A. calamus* > *Z. caduciflora* > *T. lutarioriparia*. The water contents of the aboveground tissue were 71–73% (w/w). The plants with higher dry biomass were *M. sinensis anderss* (9.77 kg m⁻²), *V. zizanioides* (7.92 kg m⁻²) and *T. dealbata* (7.04 kg m⁻²), which were statistically different from each other in biomass production. Dohleman and Long also found that larger leaf area and longer duration were the main reasons for more biomass of *Miscanthus*, though maize had a higher maximum velocity of phosphoenolpyruvate carboxylation, velocity of phosphoenolpyruvate regeneration, light saturated rate of photosynthesis, and higher maximum quantum efficiency of CO₂ assimilation [25]. The difference in dry biomass among *T. dealbata*, *Z. caduciflora*, *T. lutarioriparia* and *A. calamus* were not significant ($P > 0.05$).

3.4. Chemical contents, phyto-uptake and sequestration

In the experiment II, nitrogen (N) content of aboveground tissue of *A. calamus* and *T. lutarioriparia* were 20.7 mg kg⁻¹ and 19.7 mg kg⁻¹, which were significantly higher than those of the other four plants (Fig. 3A). The lowest N content (9.1 mg kg⁻¹) was found in *V. zizanioides*. Because of higher biomass, the ability of *M. sinensis anderss* to extract N (46.9 mg m⁻²) was significantly higher than the other five species.

Phosphorus (P) content of aboveground tissue of *Z. caduciflora*, *T. lutarioriparia* and *A. calamus* were in range of 8.4–8.6 mg kg⁻¹ DM, significantly higher than that of *T. dealbata* (Fig. 3B). P phyto-uptake by *M. sinensis anderss* and

V. zizanioides was respectively 25.5 mg m⁻² and 20.0 mg m⁻², significantly greater than that by the other four plant species.

The amount of nutrient stored by standing biomass depends upon plant growth rate and nutrient content in tissues. The average N and P phyto-uptake of each plant species in experiment II were 29 mg m⁻² and 16 mg m⁻², respectively (Fig. 3A and B). The P phyto-uptake by *M. sinensis anderss* and *V. zizanioides* was higher than the other species. However, because of lower N content in aboveground tissue, N phyto-uptake by *V. zizanioides* was significantly less than *M. sinensis anderss*.

Sulfur (S) content in the aboveground tissue of *T. lutarioriparia* was 3.9 mg kg⁻¹ DM, significantly higher than that of *V. zizanioides* (2.6 mg kg⁻¹), followed by *M. sinensis anderss* (Fig. 3D). Due to higher biomass, the S phyto-uptake by *M. sinensis anderss* and *V. zizanioides* was 5.8 mg m⁻² and 5.3 mg m⁻², respectively. There were no significant difference in S uptake between the two species and *T. lutarioriparia* (5.7 mg m⁻²). However, the three plant species had a significant greater S phyto-uptake than the other three species.

As shown in Table 1, crude protein (CP) contents of plant dry tissues except *T. dealbata* and *V. zizanioides* were more than 100 mg kg⁻¹ and was of Grade One Standard Quality for Gramineous Hay [26]. *V. zizanioides* had the lowest content of CP and the highest contents of crude fiber (CF), neutral-detergent fiber (NDF) and acid-detergent fiber (ADF), and these differences were significant.

Z. caduciflora and *A. calamus* had significantly higher ash contents than *T. dealbata*. Crude ether extract (EE) of *V. zizanioides*, *M. sinensis anderss* and *A. calamus* was greater than 70 mg kg⁻¹ DM. *V. zizanioides* had significantly higher calcium (Ca) content, 2.5–4.4 times higher than the other species (Table 1)

Estimation of cellulose (CE), hemicellulose (HE), and acid-detergent lignin (ADL) contents in aboveground tissue of the six plant species are presented in Fig. 4. *V. zizanioides* and *M. sinensis anderss* had 284.4 mg kg⁻¹ and 263.3 mg kg⁻¹ of CE and 337.5 mg kg⁻¹ and 319.6 mg kg⁻¹ of HE, which were significantly higher than *A. calamus* and *T. dealbata*. However, ADL contents of *V. zizanioides* and *M. sinensis anderss* were lower than *A. calamus* and *T. dealbata*. The sum of CE and HE of *V. zizanioides* or *M. sinensis anderss* was approximately 600 mg kg⁻¹.

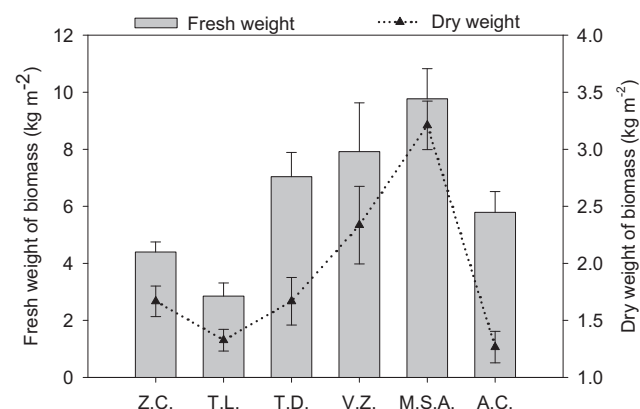


Fig. 2 – Aboveground biomass production of six plant species growing on floating beds in experiment II. Z.C., T.L., T.D., V.Z., M.S.A. and A.C. respectively stand for *Z. caduciflora*, *T. lutarioriparia*, *T. dealbata*, *V. zizanioides*, *M. sinensis anderss* and *A. calamus*. Vertical bars represent standard error ($n = 3$).

4. Discussion

4.1. Nutrient phyto-uptake from eutrophic water

Biomass yield varied with plant species and affected physiology and morphology of the plants [23]. This study also revealed that there were wide variations in biomass, nutrient concentration and accumulation among the six species. Although *M. sinensis anderss* did not display the highest N and P content in the plant tissue, N and P phyto-uptake were higher than any other plant species because of its greatest biomass among the tested plant species (Fig. 3). However, because of lower N content in aboveground tissue, nitrogen removal by *V. zizanioides* was significantly less than *M. sinensis anderss*. Unfavorable growth conditions seemed to be responsible for the less biomass of *T. lutarioriparia* and

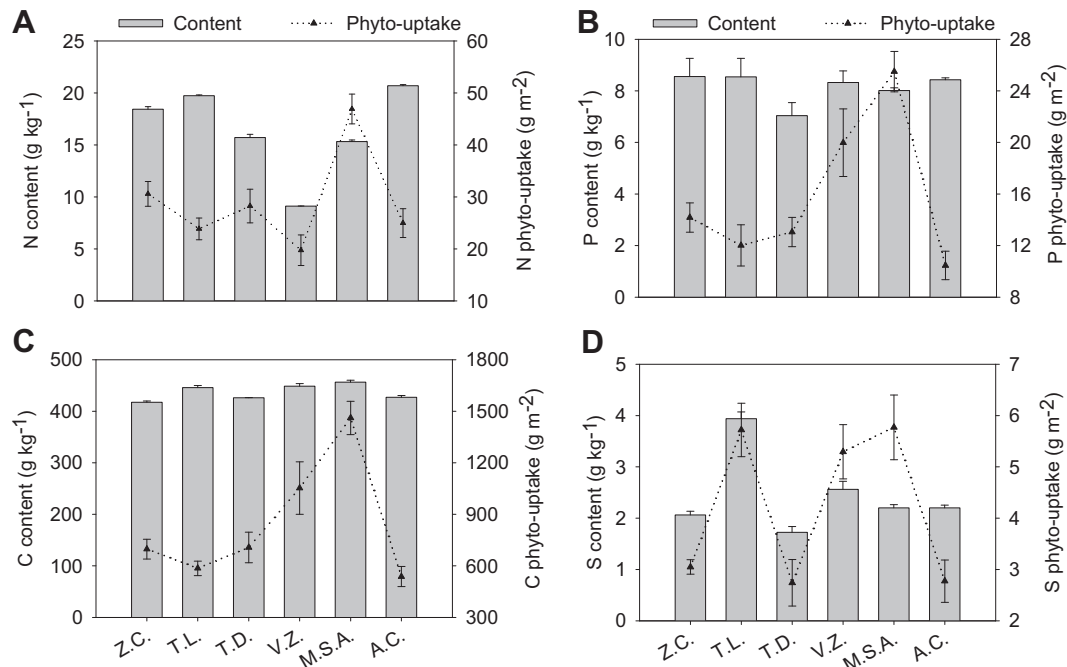


Fig. 3 – N content and phyto-uptake (A), P content and phyto-uptake (B), C content and sequestration (C) and S content and phyto-uptake (D) of six plant species growing on floating beds in experiment II. Z.C., T.L., T.D., V.Z., M.S.A. and A.C. respectively stand for *Z. caduciflora*, *T. lutarioriparia*, *T. dealbata*, *V. zizanioides*, *M. sinensis anderss* and *A. calamus*. Vertical bars represent standard error ($n = 3$).

A. calamus (Fig. 2) and the lower nutrient removal rates (Fig. 1). Both of *T. lutarioriparia* and *A. calamus* with rhizomes prefer to grow in wetlands rather than in water column directly.

4.2. Potential environmental benefits for alleviating greenhouse gas (GHG)

Emissions of GHG have contributed to global warming. Development of carbon-neutral biomass plants based has been promoted as one option to sequester CO_2 [27,28], where as water surface-based biomass plant cultivation can contribute to both the sequestration of CO_2 . Carbon (C) content of the aboveground tissues of the six plant species ranked from a lowest level of 417.2 g kg^{-1} for *Z. caduciflora* to a highest level of 456.3 g kg^{-1} for *M. sinensis anderss* (Fig. 3C). C sequestration of *M. sinensis anderss* was 1461.1 g m^{-2} , which

was significantly higher than *V. zizanioides* (1052.5 g m^{-2}), both of which were significantly higher than that of the other four plant species. Therefore, cultivating bioenergy plants on water surface showed a good alleviation to CO_2 .

4.3. Assessment of different plant species for bioenergy feedstock

It is reported that plant biomass currently provides 13–15% of the global energy demand and at least half of the world's population relies on plant biomass as their main source of energy [29]. Perennial biomass crops, such as grasses, have particular advantages as bioenergy feedstock. First of all, they are not food crops and show fast growth with the potential to produce large yields with low fertilizer and pesticide inputs. Secondly, there is no annual cultivation cycle. Finally,

Table 1 – Chemical contents (g kg^{-1} DM) in the aboveground tissue of different plant species in experiment II.

Items	<i>Z. caduciflora</i>	<i>T. lutarioriparia</i>	<i>T. dealbata</i>	<i>V. zizanioides</i>	<i>M. sinensis anderss</i>	<i>A. calamus</i>
CP	120.3 ± 4.1	136.5 ± 1.1	88.1 ± 0.1	63.1 ± 0.3	109.3 ± 3.2	116.1 ± 0.2
CF	283.7 ± 0.4	267.3 ± 1.8	283.9 ± 1.8	342.8 ± 28.5	336.9 ± 12.7	316.6 ± 5.3
NDF	685.6 ± 19.3	691.7 ± 9.7	648.9 ± 11.1	749.8 ± 4.1	702.7 ± 8.2	550.2 ± 12.1
ADF	383.0 ± 9.6	404.7 ± 6.3	362.2 ± 24.4	412.3 ± 9.7	383.1 ± 4.2	329.5 ± 19.6
Ash	138.5 ± 3.9	63.4 ± 4.9	104.8 ± 1.0	63.3 ± 2.3	54.6 ± 1.1	140.6 ± 4.6
EE	43.6 ± 4.8	28.0 ± 1.4	38.6 ± 5.7	71.3 ± 10.8	79.2 ± 9.3	72.9 ± 13.8
Ca	5.1 ± 0.5	6.5 ± 0.3	3.7 ± 0.6	16.3 ± 0.7	4.8 ± 0.8	3.9 ± 0.6
P	8.6 ± 0.7	8.5 ± 0.7	7.0 ± 0.5	8.3 ± 0.5	8.0 ± 0.1	8.4 ± 0.1

Note: CP, crude protein; CF, crude fiber; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; EE, crude ether extract.

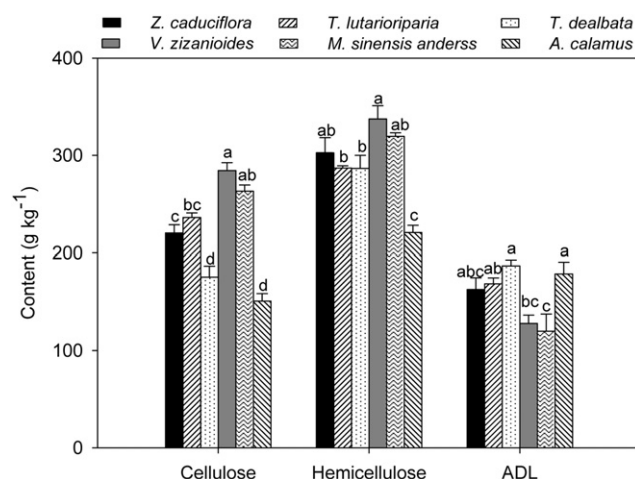


Fig. 4 – Cellulose (CE), hemicellulose (HE), and acid-detergent lignin (ADL) content of six plant species growing on floating beds in experiment II. Vertical bars represent one standard error ($n = 3$).

life-cycle analyses of heat, electricity and liquid biofuel production indicate both high energy savings and substantial reductions in GHG emissions [30].

However, the development of bioenergy feedstock production is slow in China. Many factors may have contributed to it, but the lack of incentives and available land is probably among the major ones [31]. Therefore, water surface-based biomass plant cultivation is an attractive alternative for the production of biomass energy in China. In the experiment II, except for *A. calamus*, the aboveground dry weight of all the experimental plants contained 649–750 g kg⁻¹ of NDF and 362–412 g kg⁻¹ of ADF (Table 1). Their NDF and ADF contents were similar to switchgrass [32], which was considered as a model energy crop. Moreover, *V. zizanioides* and *M. sinensis anderss* had equivalent CE, HE, and ADL contents [33,34].

5. Conclusion

Concerns about environmental pollution and energy for sustainable development have impelled us to look at a win-win approach. The low per capita cropland in China makes it almost impractical to purify eutrophic water far and wide using artificial constructed wetlands or convert croplands as energy crop cultivation. This study demonstrated that the six tested plant species grew well on floating beds in eutrophic water, and assimilated nutrients effectively by plants such as *M. sinensis anderss* and *V. zizanioides*. The large amounts of aboveground biomass produced from the water treatment processes can be valuable resources for bioenergy production.

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