

# Purification Efficiency of Eight Aquatic Plant Species in an Artificial Floating Island System in Relation to Extracellular Enzyme Activity and Microbial Community

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## Research Article

**Keywords:** Floating island, Extracellular enzyme, Aquatic plant, Microorganisms, Wastewater, purification efficiency

**Posted Date:** April 12th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-381642/v1>

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# Abstract

An artificial floating island is an ecological restoration technology that aims to create sustainable ecosystems and improve biodiversity. Aquatic plants play an important role in wastewater purification. The floating island system exploits the combination of aquatic plants, microorganisms, and extracellular enzymes to purify wastewater. We investigated the purification efficiency of eight aquatic plant species (*Ceratophyllum demersum*, *Elodea nuttallii*, *Eichhornia crassipes*, *Iris pseudacorus*, *Iris sibirica*, *Myriophyllum verticillatum*, *Thalia dealbata* and *Oenanthe javanica*) cultured in wastewater. The relationships of plant purification capacity with extracellular enzyme activity and microbial community were analyzed to explore the crucial factors that affect the plant purification capacity and the mechanism of pollutants removal in different plant systems. Three plant species, namely *Oenanthe javanica*, *Thalia dealbata*, and *Iris pseudacorus*, were the most effective for purification of ammonium-nitrogen ( $\text{NH}_4^+\text{-N}$ ), total phosphate (TP), and chemical oxygen demand (COD) with maximum efficiencies of 76.09%, 85.87%, and 89.10%, respectively. Urease, alkaline phosphatase (AP), and  $\beta$ -glucosidase activities were significantly and positively correlated with root system development ( $P < 0.05$ ). Activities of urease and AP were positively correlated with  $\text{NH}_4^+\text{-N}$  and TP removal, respectively. The magnitude of urease and AP activity was generally consistent with the plant's capacity to remove  $\text{NH}_4^+\text{-N}$  and TP.  $\beta$ -Glucosidase activity and COD removal were not significantly correlated. The dominant microbial phylum in each species treatment was Proteobacteria. Alphaproteobacteria and Bacteroidia showed > 1% relative abundance and greater involvement in degradation of pollutants in the experimental system. The results provide a scientific and theoretical basis for improvement of the plant purification efficiency of artificial floating island systems.

## 1. Introduction

An artificial floating island is an ecological restoration technology aimed at creating sustainable ecosystems conducive to enhancement or restoration of biodiversity (Ware, Callaway 2019). The approach has proved to be remarkably effective in the treatment of polluted rivers, lakes, and reservoirs (Kong et al. 2019). The strategy is advantageous in being space efficient compared with the spatial requirements of an artificial wetland (Guo et al. 2019). Artificial floating island systems remove pollutants through absorption by aquatic plant roots, and mitigates nitrogen and phosphorus enrichment in the water by means of microbial degradation (Hubbard et al. 2011; Kong et al. 2019; Sun et al. 2009). Many biopolymers, such as proteins, nucleic acids, and polysaccharides, cannot be transported across cell membranes and used directly by microorganisms in wastewater (Gonnelli et al. 2013). However, such biopolymers can be used by microorganisms if degraded into simpler compounds or inorganic substances as a result of hydrolysis by extracellular enzymes (Kong et al. 2009; Steen, Arnosti 2011).

The floating island system uses a combination of aquatic plants, microorganisms, and extracellular enzymes for wastewater purification. Extracellular enzymes are synthesized within the cell and subsequently are translocated across the cell membrane; these enzymes are either localized around the cell membrane or diffuse into the environment and function outside the cell (Münster et al. 1992). Extracellular enzymes are complex in origin and are synthesized by diverse sources in different ecosystems, including plant roots, phytoplankton, and microorganisms. Such enzymes play important roles in the biogeochemical cycling of carbon, nitrogen, and phosphorus in ecosystems. Research on artificial floating island systems has typically focused on improvement of the selected materials and technological innovation, and less frequently on extracellular enzymes, microorganisms, and aquatic plants. In the present study, analyses of microbial diversity and the relationships between extracellular enzyme activities and pollutant removal efficiency, and between plant root growth and extracellular enzyme activities, were conducted to investigate the influence of extracellular enzyme activity and microorganisms on the purification effectiveness of artificial floating islands. The results provide a scientific and theoretical basis for improvement of the purification efficiency of aquatic plants in artificial floating island systems.

## 2. Materials And Methods

### 2.1 Experimental materials

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The experiment was conducted in a greenhouse in the laboratory of Shandong Analysis and Test Center. Eight aquatic plant species were selected, comprising *Ceratophyllum demersum*, *Elodea nuttallii*, *Eichhornia crassipes*, *Iris pseudacorus*, *Iris sibirica*, *Myriophyllum verticillatum*, *Thalia dealbata* and *Oenanthe javanica*. The plants were incubated under a photoperiod of 14 h/10 h (light/dark), light intensity 300–330  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , temperature  $\sim 25^\circ\text{C}$ , and relative humidity 50%–70%. Plant seedlings were first cultured for 2 weeks in Hoagland's nutrient solution for acclimation to an aquatic environment and then transferred to the experimental device. A total of 27 miniature floating island devices of size 40 cm  $\times$  22 cm  $\times$  15 cm were established (Fig. 1).

**Figure 1** Schematic diagram of the artificial floating island device used in the study

## 2.2 Experimental method

### 2.2.1 Experimental design

Table 1  
Physicochemical indicators of the wastewater used in the study

indicators	COD	TN	$\text{NH}_4^+\text{-N}$	TP	$\text{PO}_4^{3-}\text{-P}$	pH	TS
concentration	232	251.24	50.56	18.06	17.57	6.9	0.078

Experimental water samples were collected from the oxidation pond of the wastewater treatment system of a large livestock farm in Linyi, Shandong Province, China (Table 1). Well-grown plants with intact leaves of relatively similar size and biomass were selected for the experiment. The experiment used a total of 27 groups of plants, with 10 plants per group. The total mass of each group was similar. The roots of each plant were rinsed three times with distilled water and then blotted dry with absorbent paper. Ten liters of the prepared wastewater was placed in the miniature floating island devices and the water level was marked. A plastic plate perforated with 10 holes was placed on top of the container, and 10 plants of the same species were secured in place with planting baskets. Three replicate groups for each plant species were established for each treatment. A simulated floating island lacking plants with the same volume of wastewater was used as the control (CK). Samples of the water were collected at 7-day intervals during the experimental period, that is, at the time points 0, 7, 14, 21, and 28 d, respectively. Reduction in water volume as a result of evaporation, plant transpiration and absorption, and sampling necessitated that the water was replenished with distilled water to the original level before and after sampling. At the end of the experiment, the root weight and length were measured for each plant.

On day 28, a water 500-ml sample was collected from the water in the vicinity of the root system at a depth of 10 cm below the water surface. The water samples from the three replicates of each group were mixed thoroughly. Microbial membrane filters were used for extraction to enrich microorganisms on the membranes. The filter membranes were immediately stored at  $-80^\circ\text{C}$  and transported to a gene sequencing company for 16S rDNA sequencing.

### 2.2.2 DNA extraction and sequencing processing

DNA extraction kits were applied to extract total DNA. Primers were designed according to the conservative region. PCR amplification was performed, and the product was purified, quantified, and homogenized to create a sequence library. After the library was qualified, it was sequenced by Illumina HiSeq. The initial sequences were splice filtered and divided into operational taxonomic units for species annotation and abundance analysis. Diversity indexes (Simpson, Chao1, abundance-based coverage estimator (ACE) and Shannon) were calculated using Mothur. QIIME was used for representative sequence species annotation. In addition, significant species difference analysis was conducted to explore the differences among samples. Biological function of the microbiota was predicted using PICRUST (<http://picrust.github.com/picrust/>).

### 2.2.3 Chemical analysis

The wastewater quality indicators ammonium-nitrogen ( $\text{NH}_4^+\text{-N}$ ) concentration, total phosphorus (TP) concentration, and chemical oxygen demand (COD) in the experimental water samples were measured in accordance with the protocols of the State Environmental Protection Administration (SEPA) (Administration 1989). Urease (URE) activity was determined using the sodium phenate and sodium hypochlorite colorimetric method. The content of  $\text{NH}_4^+\text{-N}$  in 1 mL of the water sample after 24 h ( $\mu\text{g}$ ) was determined to express URE activity. Alkaline phosphatase (AP) activity was measured using the disodium phosphate–benzene colorimetric method. The amount of phenol released from 1 mL of the water sample after 12 h ( $\mu\text{g}$ ) was measured to express AP activity. The  $\beta$ -glucosidase ( $\beta$ -GLU) activity was determined using the *p*-nitrophenyl colorimetric procedure. The  $\beta$ -GLU activity was measured as the amount of *p*-nitrophenyl released from 1 mL of the water sample after 1 min (nmol) at pH 5.0 at 37°C.

## 2.2.4 Data analysis

Using the experimental data, a regression analysis of wastewater concentration and treatment duration was conducted. The regression equation for the pollutant concentration versus time for each group was obtained. The general equation for the regression of  $\text{NH}_4^+\text{-N}$ , TP, or COD concentration versus time ( $t$ ) was  $y = ae^{-bx}$  ( $a > 0$ ,  $b > 0$ ), where  $y$  is the indicator concentration (mg/L) and  $x$  is the time (d). In this equation, the value of  $b$  determines the magnitude of  $y$  when the value of  $a$  is certain, hence the value of  $b$  characterizes the removal capacity of each group of pollutants.

Expression of the growth rate of the pollutant metric was calculated with the following formula:

$$y = (C_{28} - C_0) / C_0 \times 100\% \quad (1)$$

where  $C_0$  is the concentration of  $\text{NH}_4^+\text{-N}$ , TP, or COD in the wastewater at the beginning of the experiment, and  $C_{28}$  is the concentration of  $\text{NH}_4^+\text{-N}$ , TP, or COD in the water at the end of the experiment.

The root growth status of plants was expressed as the root quality index (RQ), which represented the degree of root development. The RQ was calculated using the formula:

$$\text{RQ} = \text{RM} / \text{RL} \quad (2)$$

where RW is the root biomass (g) and RL is the root length (cm). The roots of submerged plants were degraded and contact with water was all biomass; therefore, the RL of the three submerged plant species (*Myriophyllum verticillatum*, *Elodea nuttallii*, and *Ceratophyllum demersum*) was recorded as 1.

The experimental data were processed using SPSS 17.0 statistical software and graphs were generated using Origin 2017 software. The Pearson product-moment correlation test was used for calculation of correlation coefficients.

## 3. Results

### 3.1 Relationship between extracellular enzymes and pollutant removal effectiveness

The enzymes URE, AP, and  $\beta$ -GLU are obligate hydrolases (Zhao 2012). Activity of URE plays an important role in nitrogen mineralization by hydrolyzing urea to ammonia and carbon dioxide. The AP activity is associated with the conversion of organic phosphorus and the release of phosphate from hydrolyzed phospholipids (Yang, Metcalf 2004; Zhao 2012). Activity of  $\beta$ -GLU catalyzes the hydrolysis of  $\beta$ -glucosidic bonds in dimeric and oligomeric glucosides and polysaccharides to yield glucose. The activity of each enzyme was compared separately with the corresponding pollutant concentration in the water samples.

#### 3.1.1 Relationship between URE activity and $\text{NH}_4^+\text{-N}$ removal rate

**Table 2** Ammonium-nitrogen (NH<sub>4</sub><sup>+</sup>-N), Total phosphate (TP) and Chemical oxygen demand (COD)–time (*t*)

regression equation and associated parameters for each plant species

pollutants	Plants	The formula	<i>r</i>	<i>b</i>
NH <sub>4</sub> <sup>+</sup> -N	<i>Oenanthe javanica</i>	$y=42.893e^{-0.0514x}$	0.984**	0.0514
	<i>Iris pseudacorus</i>	$y=45.407e^{-0.0540x}$	0.989**	0.0540
	<i>Thalia dealbata</i>	$y=37.683e^{-0.0496x}$	0.842*	0.0496
	<i>Eichhornia crassipes</i>	$y=44.560e^{-0.0487x}$	0.987**	0.0487
	<i>Iris sibirica</i>	$y=40.356e^{-0.0474x}$	0.963**	0.0474
	<i>Myriophyllum verticillatum</i>	$y=41.155e^{-0.0460x}$	0.966**	0.0460
	<i>Elodea nuttallii</i>	$y=46.004e^{-0.0453x}$	0.970**	0.0453
	<i>Ceratophyllum demersum</i>	$y=47.038e^{-0.0475x}$	0.870*	0.0475
TP	<i>Oenanthe javanica</i>	$y=19.056e^{-0.0771x}$	0.979**	0.0771
	<i>Iris pseudacorus</i>	$y=19.630e^{-0.0621x}$	0.969**	0.0621
	<i>Thalia dealbata</i>	$y=22.958e^{-0.0879x}$	0.929**	0.0879
	<i>Eichhornia crassipes</i>	$y=18.881e^{-0.0769x}$	0.980**	0.0769
	<i>Iris sibirica</i>	$y=20.161e^{-0.0607x}$	0.972**	0.0607
	<i>Myriophyllum verticillatum</i>	$y=20.636e^{-0.0591x}$	0.965**	0.0591
	<i>Elodea nuttallii</i>	$y=18.523e^{-0.0472x}$	0.975**	0.0472
	<i>Ceratophyllum demersum</i>	$y=19.519e^{-0.0479x}$	0.967**	0.0479
COD	<i>Oenanthe javanica</i>	$y=208.637e^{-0.0948x}$	0.930**	0.0948
	<i>Iris pseudacorus</i>	$y=240.870e^{-0.0825x}$	0.963**	0.0825
	<i>Thalia dealbata</i>	$y=230.551e^{-0.0864x}$	0.943**	0.0864
	<i>Eichhornia crassipes</i>	$y=244.810e^{-0.0774x}$	0.971**	0.0774
	<i>Iris sibirica</i>	$y=219.349e^{-0.0912x}$	0.928**	0.0912
	<i>Myriophyllum verticillatum</i>	$y=221.676e^{-0.0895x}$	0.929**	0.0895
	<i>Elodea nuttallii</i>	$y=229.508e^{-0.0814x}$	0.956**	0.0814
	<i>Ceratophyllum demersum</i>	$y=236.034e^{-0.0770x}$	0.969**	0.0770

\*, significant at the  $P < 0.05$  level; \*\*, significant at the  $P < 0.01$  level

During the experimental period, the concentrations of NH<sub>4</sub><sup>+</sup>-N, TP, and COD in the wastewater of each group were reduced. The ability of the eight plant species to remove the three pollutants from the wastewater differed significantly ( $P < 0.05$ ).

According to the curvilinear regression model for NH<sub>4</sub><sup>+</sup>-N–*t*, the Pearson correlation coefficients (*r*) for each group ranged from 0.842 to 0.989 and showed a good fit. Based on the value of *b*, the ranking of the species by their ability to degrade NH<sub>4</sub><sup>+</sup>-N was (in descending order) *Iris pseudacorus* > *Oenanthe javanica* > *Thalia dealbata* > *Eichhornia crassipes* > *Ceratophyllum demersum* > *Iris sibirica* > *Myriophyllum verticillatum* > *Elodea nuttallii*. *Iris pseudacorus* and *Oenanthe javanica* showed the maximum removal rates of 76.09% and 75.08%, respectively; *Myriophyllum verticillatum* and *Elodea nuttallii* showed the lowest NH<sub>4</sub><sup>+</sup>-N removal rates of 69.50% and 69.31%, respectively. The difference between *Elodea nuttallii* (with the lowest removal rate) and *Iris pseudacorus* (with the highest removal rate) was 6.78%, which accounted for 9.76% of the NH<sub>4</sub><sup>+</sup>-N removal by *Iris pseudacorus*.

During the experimental period, URE activity increased in all groups and increased significantly in all treatments with plants compared with the control (Fig. 2). The enzyme activity in each treatment increased at a more rapid rate in the early stages of incubation and at a lower rate in the later stages of the experimental period. The URE activity in the control (CK) in the absence of plants increased by 13.16% during the experimental period, indicating that the enzyme at least partially originated from a source other than the plant root system. The relationship between increase in URE activity in the wastewater and the  $\text{NH}_4^+$ -N removal rate by the different plant species is shown in Fig. 3. Among the eight aquatic plant species selected for this experiment, seven showed a positive correlation between  $\text{NH}_4^+$ -N removal rate and URE activity in wastewater. The  $\text{NH}_4^+$ -N removal rate was enhanced with increase in URE activity for each group. In the median scatter plot (Fig. 3), three species were located in the second quadrant, namely (from highest to lowest URE activities) *Iris pseudacorus*, *Oenanthe javanica*, and *Thalia dealbata*, which showed URE activities of 2.45, 2.32, and 2.31  $\mu\text{g}/\text{mL}$ , respectively. The fourth quadrant contained *Eichhornia crassipes*, *Iris sibirica*, *Myriophyllum verticillatum*, and *Elodea nuttallii*; the latter showed the lowest URE activity of 2.19  $\mu\text{g}/\text{mL}$ . However, *Ceratophyllum demersum* did not conform with this general pattern and showed relatively high URE activity (2.52  $\mu\text{g}/\text{mL}$ ) but a low  $\text{NH}_4^+$ -N removal rate.

### 3.1.2 Relationship between AP activity and TP removal rate

According to the curvilinear regression model  $\text{TP}-t$  (Table 2), the Pearson correlation coefficients ( $r$ ) for each group ranged from 0.929 to 0.980 and showed a good fit. From the value of  $b$ , the ranking of the species' ability to degrade TP was (in descending order) *Thalia dealbata* > *Oenanthe javanica* > *Eichhornia crassipes* > *Iris pseudacorus* > *Iris sibirica* > *Myriophyllum verticillatum* > *Ceratophyllum demersum* > *Elodea nuttallii*. *Thalia dealbata* and *Oenanthe javanica* showed the maximum TP removal rates of 85.87% and 80.22%, respectively. *Ceratophyllum demersum* and *Elodea nuttallii* showed the lowest TP removal rates of 70.84% and 69.04%, respectively. The difference between *Elodea nuttallii* (with the lowest removal rate) and *Thalia dealbata* (with the highest removal rate) was 16.83%, which accounted for 19.60% of the TP removal by *Thalia dealbata*.

During the experimental period, AP activity increased in all groups and increased significantly in all treatments with plants compared with the control (Fig. 4). The AP activity was highest in the *Thalia dealbata* treatment, which was 12-fold higher than that of the control (CK). The relationship between the increase in AP activity and TP removal rate during the experimental period for each plant species is shown in Fig. 5. Four species were located in the second quadrant, namely (from highest to lowest AP activities) *Thalia dealbata*, *Iris pseudacorus*, *Eichhornia crassipes*, and *Oenanthe javanica*, with AP activities of 1.00, 0.98, 0.94, and 0.90  $\mu\text{g}/\text{mL}$ , respectively. *Elodea nuttallii* and *Ceratophyllum demersum* showed the weakest AP activities of 0.79 and 0.78  $\mu\text{g}/\text{mL}$ , respectively.

### 3.1.3 Relationship between $\beta$ -GLU activity and COD removal rate

According to the curvilinear regression model  $\text{COD}-t$  (Table 2), the Pearson correlation coefficients ( $r$ ) for each group ranged from 0.928 to 0.969 and showed a good fit. From the value of  $b$ , the ranking of the species' ability to degrade COD was (in descending order) *Oenanthe javanica* > *Iris sibirica* > *Myriophyllum verticillatum* > *Thalia dealbata* > *Iris pseudacorus* > *Elodea nuttallii* > *Eichhornia crassipes* > *Ceratophyllum demersum*. *Oenanthe javanica* and *Iris sibirica* showed the maximum COD removal rates of 89.10% and 87.88%, respectively. *Ceratophyllum demersum* and *Oenanthe javanica* showed the lowest COD removal rates of 79.65% and 80.05%, respectively. The difference between *Ceratophyllum demersum* (with the lowest removal rate) and *Oenanthe javanica* (with the highest removal rate) was 9.45%, which accounted for 10.60% of the COD removal of *Oenanthe javanica*.

During the experimental period,  $\beta$ -GLU activity increased in all groups and increased significantly in all plant treatments compared with that of the control (Fig. 6). The highest  $\beta$ -GLU activity of *Iris pseudacorus* was as high as 1.12 nmol/mL. The  $\beta$ -GLU activity in the control (CK) increased by 91.67%. The relationship between  $\beta$ -GLU activity and COD removal rate for each plant species is shown in Fig. 7. The relationship between COD removal rate and  $\beta$ -GLU activity was not significant among the eight plant species and no significant correlation between the removal efficiency of COD and  $\beta$ -GLU activity in each plant species was observed.

### 3.2 Correlation of RQ and extracellular enzyme activities

Correlation analysis revealed that extracellular enzyme activities in each treatment were positively correlated with the RQ of each plant species. The URE activity was significantly and positively correlated with the RQ of *Oenanthe javanica*, *Eichhornia crassipes*, *Elodea nuttallii*, and *Ceratophyllum demersum*, and was highly significantly correlated with the RQ of *Thalia dealbata*, *Eichhornia crassipes*, and *Myriophyllum verticillatum*. The AP activity was highly significantly and positively correlated with the RQ of *Oenanthe javanica*, *Iris pseudacorus*, *Eichhornia crassipes*, *Elodea nuttallii*, and significantly and positively correlated with RQ of *Thalia dealbata*, *Eichhornia crassipes*, *Myriophyllum verticillatum*, and *Ceratophyllum demersum*. The  $\beta$ -GLU activity was highly significantly and positively correlated with RQ for all plant species. Therefore, the development of the root system of each plant species had a significant effect on the relationship with URE, AP, and  $\beta$ -GLU activities.

Table 3  
Relationship of extracellular enzyme activity and root quality index (RQ) for each group of samples

	<i>Oenanthe javanica</i>	<i>Iris pseudacorus</i>	<i>Thalia dealbata</i>	<i>Eichhornia crassipes</i>	<i>Iris sibirica</i>	<i>Myriophyllum verticillatum</i>	<i>Elodea nuttallii</i>	<i>Ceratophyllum demersum</i>
URE	0.887*	0.798	0.961**	0.950**	0.889*	0.984**	0.870*	0.846*
AP	0.987**	0.977**	0.889*	0.876*	0.952**	0.896*	0.923**	0.865*
$\beta$ -GLU	0.984**	0.966**	0.954**	0.965**	0.976**	0.865*	0.891**	0.914**

### 3.3 Microbial alpha-diversity index and species composition structure

Table 4  
Microbial alpha-diversity index statistics for the water samples

Sample Name	Shannon	Simpson	Chao	ACE
OJA	4.081645	0.085279	1522.25	1624.791
IPS	4.038492	0.065041	1434.468	1507.270
TDA	3.398401	0.206564	2569.329	2693.314
ECS	3.188804	0.118222	2008.564	2180.314
ISA	3.866869	0.060667	2370.075	2522.965
MVM	4.881961	0.27461	6256.653	6643.259
ENI	4.285435	0.065629	2041.67	2190.688
CDM	3.823341	0.160164	4501.815	4788.791

Notes: CDM: *Ceratophyllum demersum*; ENI: *Elodea nuttallii*; ECS: *Eichhornia crassipes*; IPS: *Iris pseudacorus* ISA: *Iris sibirica*; MVM, *Myriophyllum verticillatum*; TDA, *Thalia dealbata* and OJA, *Oenanthe javanica*

In this study, data for microorganisms detected in water samples on day 28 of incubation were used to calculate the alpha-diversity index at the 97% similarity level (Table 4). The Shannon and Simpson indices are influenced by the abundance and evenness of species in the sample community. Thus, for the same species abundance, the greater the evenness of the

species in the community, the greater the diversity of the community. The Chao1 and Ace indices reflect the number of species in the community. The *Myriophyllum verticillatum* samples showed the highest alpha-diversity and number of species, followed by *Thalia dealbata* and *Ceratophyllum demersum* (Table 4). The species diversity and number of species were low in the *Oenanthe javanica*, *Iris pseudacorus*, and *Elodea nuttallii* samples. The *Iris sibirica* samples showed the lowest species diversity but not the lowest number of species, which indicated that one or more microorganisms constituted a large proportion of the microbiota in this treatment.

Species annotation was performed for each sample, and the specific colony structure of each sample was classified and organized. Certain differences in species composition structure at the phylum and class levels were observed among the samples (Fig. 8). Only the first six and the first 10 taxa are shown for the phylum and class levels, respectively. At the phylum level, most microorganisms in the samples belonged to the Proteobacteria. Bacteroidetes showed relative abundance greater than 1% in all samples. In addition, the relative abundance of Actinobacteria was greater than 1% in samples for all plant species except *Eichhornia crassipes* (Fig. 8A). At the class level, the samples for *Eichhornia crassipes*, *Iris sibirica*, *Myriophyllum verticillatum*, and *Ceratophyllum demersum* contained a high abundance of Gammaproteobacteria, the samples for *Iris pseudacorus* and *Thalia dealbata* contained a high abundance of Alphaproteobacteria, and the samples for *Oenanthe javanica* and *Elodea nuttallii* contained a high abundance of Bacteroidia. In all cases, these classes showed greater than 10% relative abundance. The relative abundance of Campylobacteria was also high (4.8%) in *Elodea nuttallii* samples (Fig. 8B).

Species composition information was obtained by comparing 16S sequence data and PICRUSt software was used to infer the functional gene composition in the samples. KEGG analysis revealed no significant differences ( $P < 0.05$ ) in the metabolic pathways predicted to show higher abundance among the 12 samples. The metabolic pathways were associated with basal transcription factors. The KEGG pathway analysis predicted the up-regulation of five pathways (basal transcription factors, proteasome, betalain biosynthesis, mRNA surveillance pathway, and vasopressin-regulated water reabsorption) and down-regulation of 10 pathways (e.g., phosphonate and phosphinate metabolism, *Staphylococcus aureus* infection, and chlorocyclohexane and chlorobenzene degradation). In addition, metabolism of xenobiotics by the cytochrome P450 metabolic pathway was predicted to be significantly up-regulated in samples for *Eichhornia crassipes*, *Iris sibirica*, *Thalia dealbata*, and *Oenanthe javanica* relative to the abundance in the other samples ( $P < 0.05$ ) (Fig. 9). No significant differences in the predicted genes functions associated with nitrogen and phosphorus metabolism were observed, probably because transformation of nitrogen and phosphorus in an aqueous environment is a complex process in which multiple microorganisms interact to produce different responses.

The dominant phylum in each species treatment was Proteobacteria. The dominant class in samples for *Eichhornia crassipes*, *Iris sibirica*, *Myriophyllum verticillatum*, and *Ceratophyllum demersum* was Gammaproteobacteria. The dominant class in *Iris pseudacorus* and *Thalia dealbata* samples was Alphaproteobacteria, and that in *Oenanthe javanica* and *Elodea nuttallii* samples was Bacteroidia. Considering the aforementioned differences in the purification capacity of each species treatment, the Alphaproteobacteria and Bacteroidia phyla may contain microorganisms important for degradation of pollutants. In addition, the phylum Bacteroidetes was abundant in all groups; taxa of this phylum play an important role in the degradation of organic matter (Zhang et al. 2018). Plant allelopathic effects and secreted polyphenols are among the factors responsible for differences in the microbial composition rhizosphere (Pang et al. 2016).

## 4. Discussion

In water  $\text{NH}_4^+\text{-N}$  is an essential nutrient that is absorbed by aquatic plants to synthesize proteins and organic nitrogen, which is ultimately converted into biomass. The stems and roots of aquatic plants also provide a suitable substrate and habitat for nitrification and denitrification by microorganisms (Miyoko et al. 2018; Zuo et al. 2020). The removal of phosphorus is predominantly reliant on absorption by aquatic plants and physicochemical adsorption, whereby microorganisms degrade insoluble phosphorus into soluble phosphate (Brown et al. 2018), which is directly absorbed by plants. The purification

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on wastewater in the present study showed that root growth differed among the

species and the purification of  $\text{NH}_4^+\text{-N}$ , TP, and COD among the treatments differed. *Thalia dealbata*, *Oenanthe javanica*, and *Iris pseudacorus* produced better-developed root systems and showed superior purification performance, whereas the root systems of *Elodea nuttallii* and *Ceratophyllum demersum* developed slowly and were less effective for wastewater purification. Organic matter is hydrolyzed by URE, AP, and  $\beta\text{-GLU}$  to produce inorganic nitrogen, inorganic phosphorus, and glucose, respectively (Zhao 2012). Plant root metabolites may also promote microbial growth, and enhance enzyme synthesis and activity (Kong et al. 2009). In the present study, the activities of URE, AP, and  $\beta\text{-GLU}$  varied significantly with time in the different treatments and increased significantly compared with activities in the control ( $P < 0.05$ ). The patterns of change were generally similar, which is consistent with the results of a previous study of enzyme activities in artificial wetlands (Kumar et al. 2020). It is evident that the efficacy of extracellular enzymes is consistent in different environments or in different species treatments in the same environment. The above analyses indicated that the magnitude of URE and AP activities was generally consistent with the strength of the plant's ability to remove  $\text{NH}_4^+\text{-N}$  and TP from the water, and was positively correlated with  $\text{NH}_4^+\text{-N}$  and TP removal. However, no significant correlation between  $\beta\text{-GLU}$  activity and COD removal was observed, indicating the factors that influence the capacity for COD purification are complex and require further investigation. A feedback mechanism may exist between enzymes and associated physicochemical factors. In the current study, the rate of increase in URE activity was larger in each treatment at an early stage of the incubation period, probably because  $\text{NH}_4^+\text{-N}$  uptake by plant roots was more rapid in the early stages. Thereafter, the  $\text{NH}_4^+\text{-N}$  concentration decreased rapidly, which would trigger a regulatory signal that promoted the increase in URE activity to accelerate the hydrolysis of organic nitrogen. The slow growth in the later stages of the incubation period may be due to decline in the nitrogen source, which slows down its conversion to inorganic nitrogen and the mechanism would thus induce a weakening signal to regulate the balance between organic and inorganic nitrogen. Alkaline phosphatase is a compensatory mechanism for microbially absorbable phosphorus sources in water (Rose, Axler 1997). The AP activity was positively correlated with TP removal in the present experiment, and thus microorganisms would be induced to produce phosphatase when the inorganic phosphorus concentration was reduced. For this reason, AP is useful as a diagnostic marker for marine phosphorus deficiency (D.Cembella et al. 1982; Lin et al. 2012). The higher the  $\beta\text{-GLU}$  activity, the more readily biopolymers are hydrolyzed to small organic molecules. The predominant source of glucosidase production in the water column is plankton, which is also the primary source of energy for microorganisms (Ryszard, Chróst 2003; Turner et al. 2002). The optimum pH for  $\beta\text{-GLU}$  activity in water is in the acidic range, but the enzyme is stable at a pH exceeding 7.0 (Chen et al. 2000; Li, Gao 2007). The pH of the water environment in the present experiment ranged between 7 and 8, thus the effect of pH on  $\beta\text{-GLU}$  activity was minimal. The present results revealed a significant negative correlation between COD removal and  $\beta\text{-GLU}$  activity in the same treatment ( $P < 0.05$ ), although the relationship differed among the species treatments. The URE, AP, and  $\beta\text{-GLU}$  activities were strongly and positively correlated with the degree of root development in each group of plants. In addition, root secretion and dissolved oxygen enhance enzyme activities (Rosa, Isabel 2008).

Bacteria constitute the dominant group of microorganisms in water and play an important role in material transformation and energy cycling in a floating island system. The highest microbial diversity and number of species among the present species treatments was observed for *Myriophyllum verticillatum*, followed by *Thalia dealbata* and *Ceratophyllum demersum*. Microbial diversity and species number were not significantly correlated with the activities of URE, AP, and  $\beta\text{-GLU}$  ( $P < 0.05$ ), which indicated to some extent that not all three enzymes originated from bacteria, but also from plants, which is consistent with the results of previous studies (Monica, K 2009; Ou et al. 2010; Rose, Axler 1997). Leaf abscission occurred in submerged plants during the present experiment; leaf decomposition leads to an increase in microorganism abundance, which impacts on the degradation of pollutants (Mille-Lindblom, Tranvik 2003). Thus, although microbial diversity was higher for *Myriophyllum verticillatum* and *Ceratophyllum demersum*, the effectiveness of the two species for pollutants purification was not outstanding.

An additional consideration is that the present experiment was conducted at an ambient temperature of 25°C. The activity of hydrolytic enzymes varies with temperature (Kim et al. 2012; Qi et al. 2010). Whether the temperature was optimal for the three enzymes monitored in this experiment and thus whether activities were maximal requires further investigation. The

temperature of the water environment is crucial to improve the purification capacity with regard to plant growth, microbe survival, and extracellular enzyme activities.

## 5. Conclusion

This study provides an important theoretical basis for improvement of the effectiveness of aquatic plants, cultured using an artificial floating island system, for purification of pollutants in wastewater. The results demonstrate the relationships among plant species, microorganism diversity and abundance, and extracellular enzyme activity with regard to purification capacity.

During the purification of wastewater by artificial floating islands, an inhibitory relationship is observed between extracellular enzyme activity and  $\text{NH}_4^+\text{-N}$ , TP, and COD concentrations. The URE and AP activities are positively correlated with the purification capacity of plants for removal of  $\text{NH}_4^+\text{-N}$  and TP, respectively, whereas  $\beta\text{-GLU}$  activity is not significantly correlated with the purification efficiency of plants for COD removal. The activities of URE, AP, and  $\beta\text{-GLU}$  are significantly and positively correlated with root system development of the plant ( $P < 0.05$ ). Plant roots and microorganisms use water as a medium for secretion of extracellular enzymes, and the roots release substances that enhance enzyme activity.

Microbial diversity in water bodies is strongly influenced by the environment and the associated plant species. The dominant bacterial phylum in each species treatment was Proteobacteria. The relative abundance of Alphaproteobacteria and Bacteroidia was greater than 1%. At the class level, the treatment with *Eichhornia crassipes*, *Iris sibirica*, *Myriophyllum verticillatum*, and *Ceratophyllum demersum* showed a high abundance of Gammaproteobacteria, Alphaproteobacteria were more abundant in the *Iris pseudacorus* and *Thalia dealbata* treatments, whereas Bacteroidia were more abundant in the *Oenanthe javanica* and *Elodea nuttallii* treatments. Each of these classes showed greater than 10% relative abundance. This variation in microbial species abundance is one possible reason for the influence of enzyme activity and purification efficiency. In addition, microbial metabolism of xenobiotics by cytochrome P450 was predicted to be up-regulated in the *Eichhornia crassipes*, *Iris sibirica*, *Thalia dealbata*, and *Oenanthe javanica* treatments.

The present artificial floating island system achieved maximum purification efficiencies of 76.09%, 85.87%, and 89.10% for  $\text{NH}_4^+\text{-N}$ , TP, and COD by *Iris pseudacorus*, *Thalia dealbata*, and *Oenanthe javanica*, respectively. In general, the degree of root development and plant adaptability are important factors affecting the purification capacity of artificial floating island systems. Plant survival and root growth contribute to enhanced extracellular enzyme activity and to the survival and reproduction of systemic microorganisms.

## Declarations

### Acknowledgements

Special thanks to Boao Biotechnology company for technical assistance with molecular work. The authors greatly acknowledge the laboratory facility provided by Shandong Analysis and Test Center. The work was funded by the National Key Research and Development Program of China (2017YFD0801403) and the National Natural Science Foundation of China (41877041;42077051). We thank Robert McKenzie, PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

**Ethics approval and consent to participate** Not applicable

**Consent for publication** Not applicable

**Availability of data and materials** Not applicable

**Competing interests** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Funding** The work was funded by the National Key Research and Development Program of China (2017YFD0801403) and the National Natural Science Foundation of China (41877041;42077051). We thank Robert McKenzie, PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

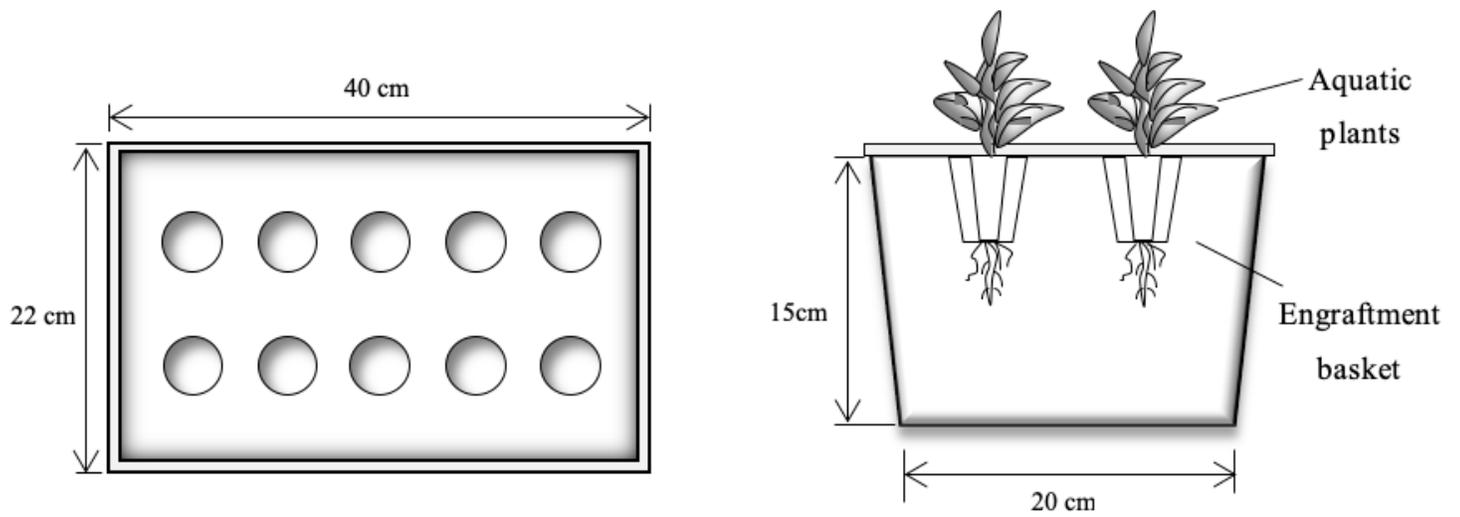
**Authors' contributions** QC: Methodology, Resources, Supervision, Writing–review and editing, Funding acquisition. YF: Conceptualization, Formal analysis, Investigation, Methodology, Data curation, Writing–original draft, Writing-review & editing. JL: Investigation, Supervision, Writing - review & editing. QL: Data curation, Investigation. TL: Project administration, Supervision.

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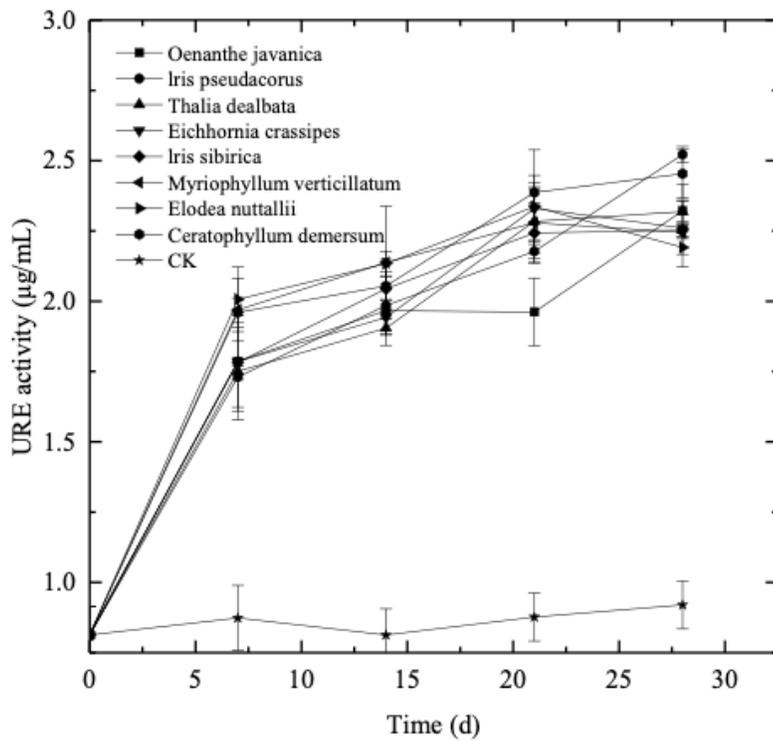
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# Figures



**Figure 1**

Schematic diagram of the artificial floating island device used in the study



**Figure 2**

Variation in urease (URE) activity for each aquatic plant species

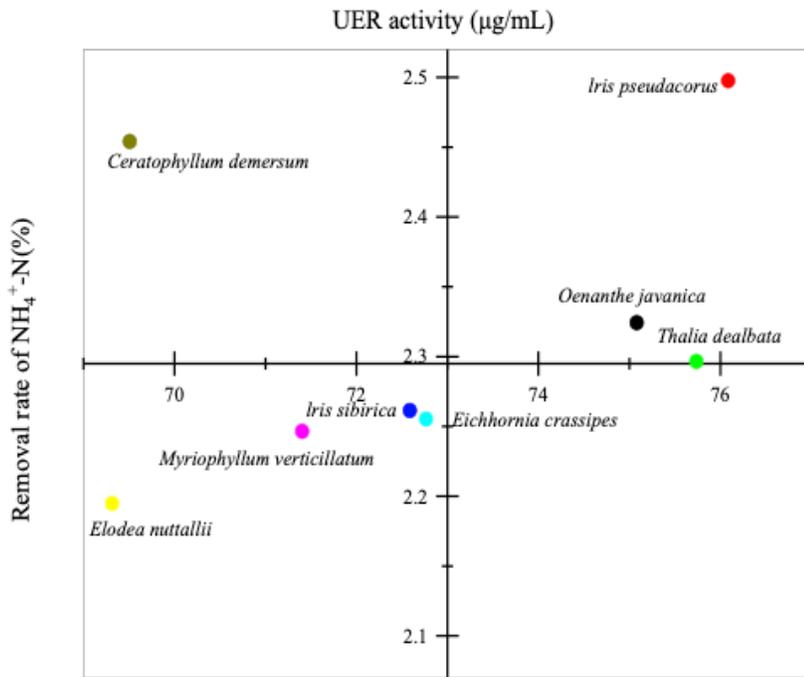


Figure 3

Relationship between URE activity and NH<sub>4</sub><sup>+</sup>-N removal rate

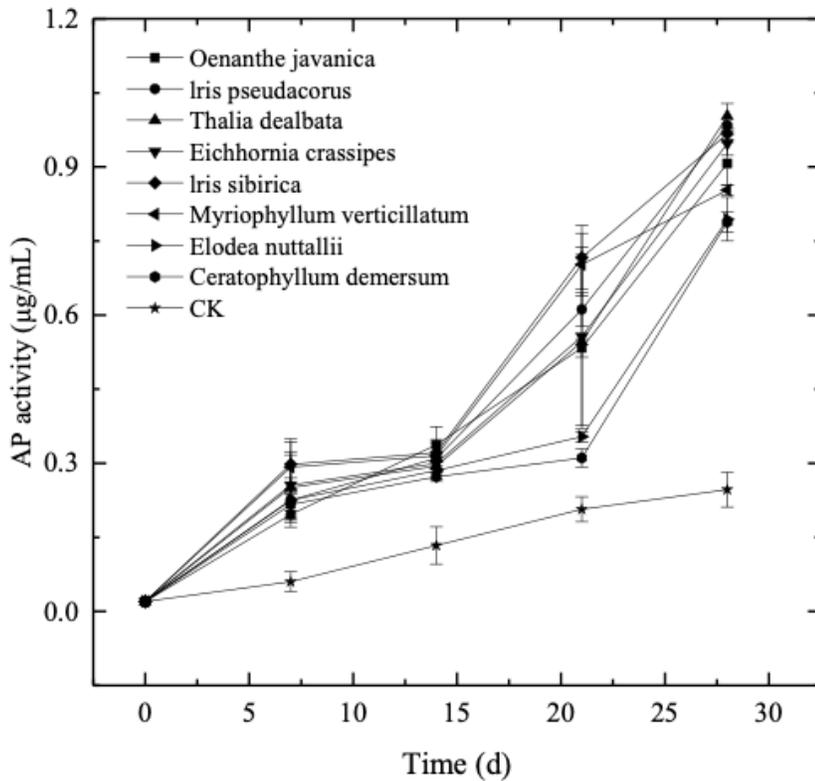


Figure 4

Variation in alkaline phosphatase (AP) activity for each aquatic plant species

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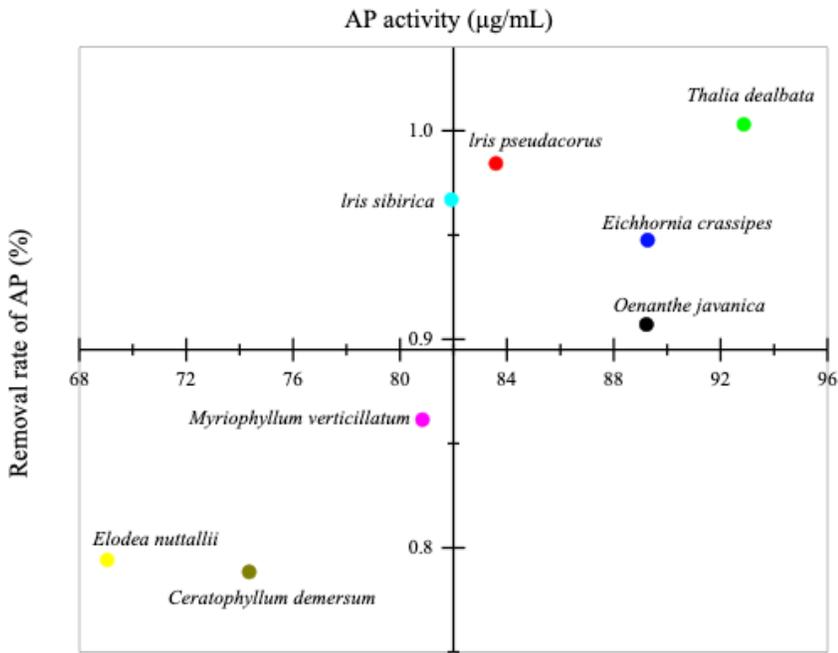


Figure 5

Relationship between alkaline phosphatase (AP) activity and total phosphorus (TP) removal rate

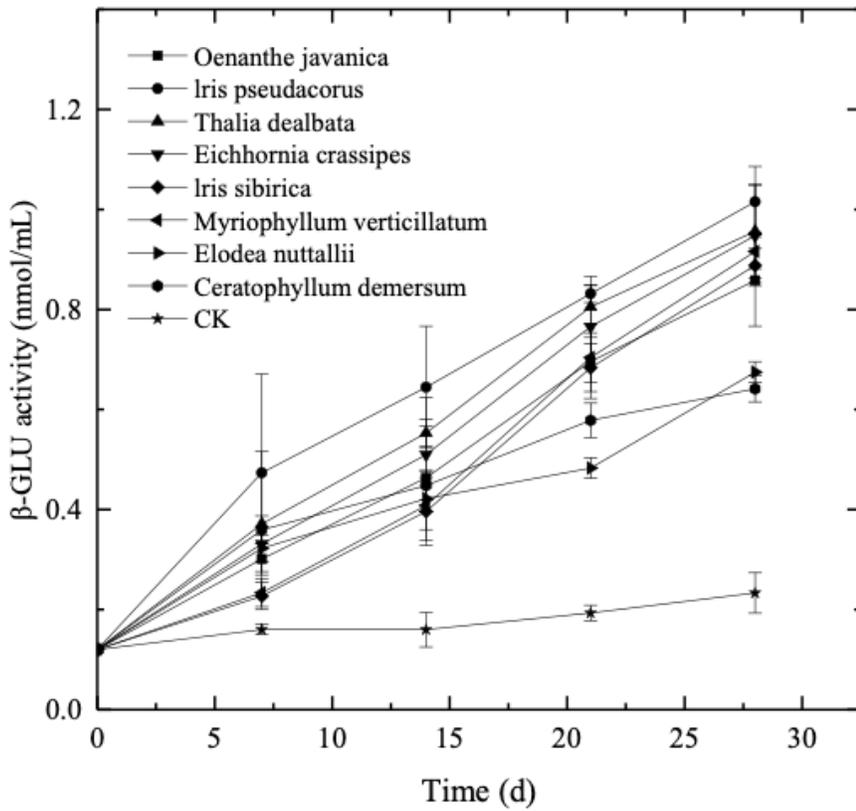


Figure 6

Variation in  $\beta$ -glucosidase ( $\beta$ -GLU) activity for each aquatic plant species

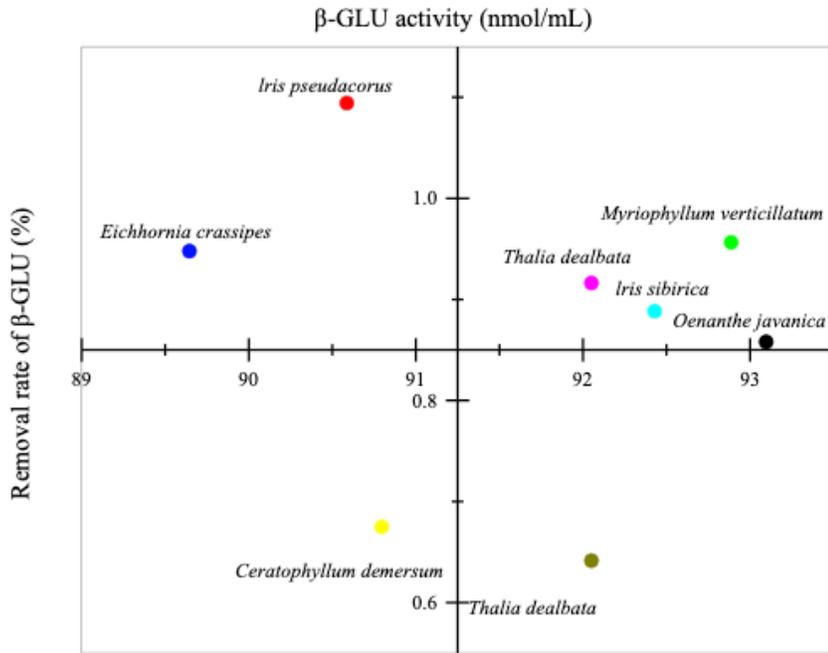


Figure 7

Relationship between  $\beta$ -glucosidase ( $\beta$ -GLU) activity and chemical oxygen demand (COD) removal rate

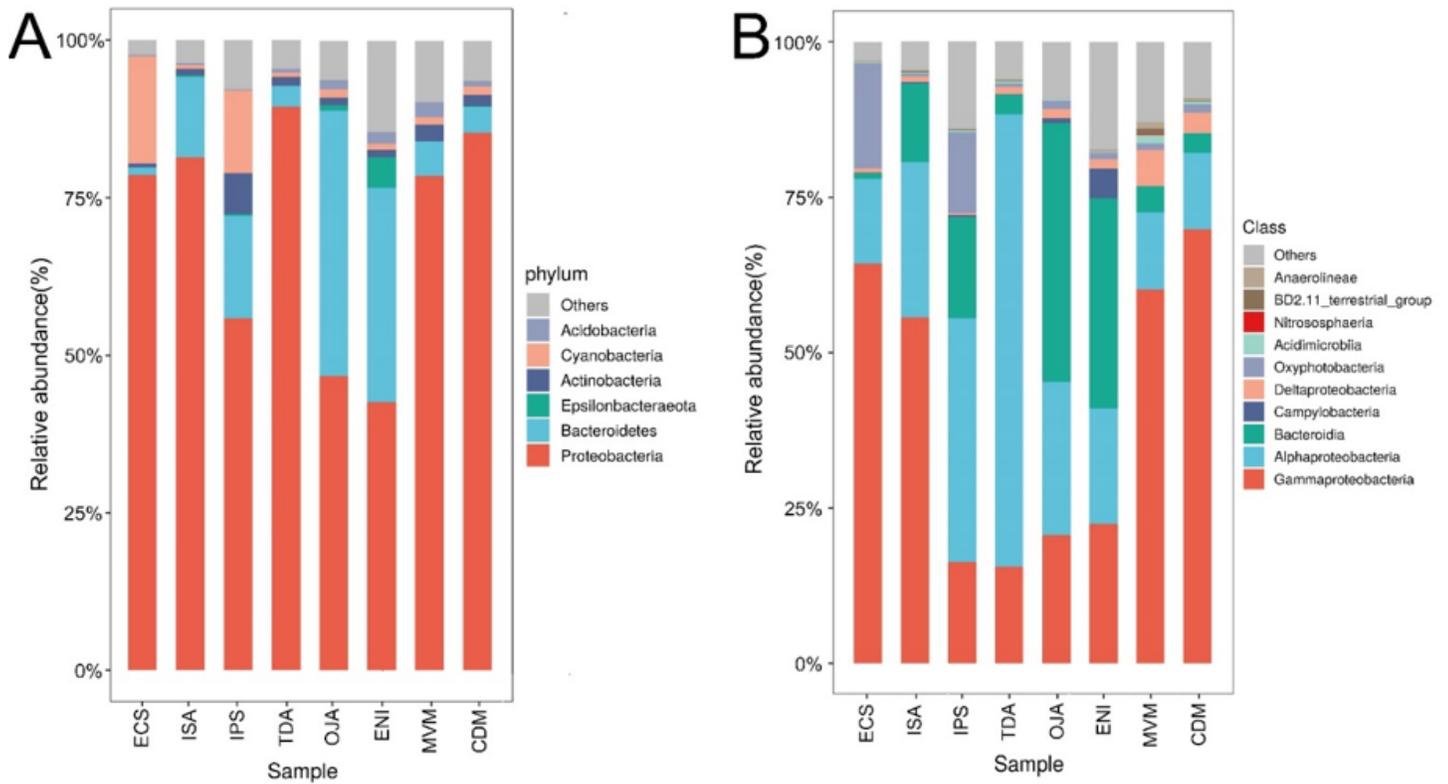


Figure 8

Relative abundance of microorganisms at the (A) phylum and (B) class levels among the water samples

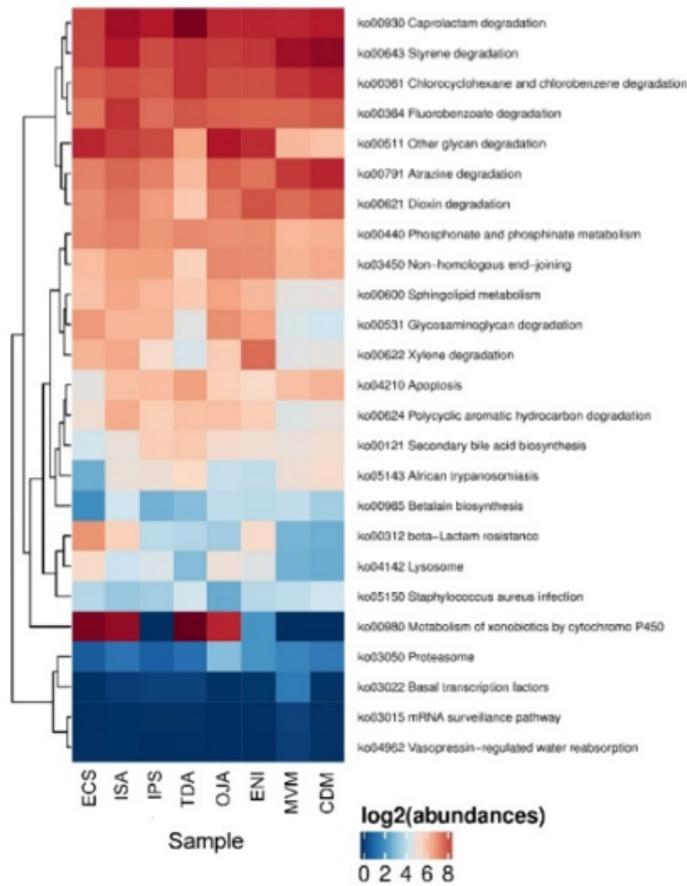


Figure 9

KEGG pathways associated with predicted gene functions

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