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Nutrient Removal and Algal Community Variation from Urban River with the Isolated Microalgal Strains *Chlorella* sp. and *Scenedesmus* sp.

Weiju Zhu[1], Yajun Li[1], Xiaowen Fei[2], Xiaodong Deng[1]

[1]Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Science, Haikou, China
[2]School of Science, Hainan Medical College, Haikou, China
Email: gdzxzwj@163.com

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Abstract

The objectives of this study were to determine nutrient removal rates and algal community variation using the isolated microalgal strains *Chlorella* sp. and *Scenedesmus* sp. from an urban river water. The concentration of total nitrogen (TN) and total phosphorus (TP) in river water declined after pouring into *Chlorella* sp. and *Scenedesmus* sp., it was indicated that the *Scenedesmus* sp. had respective advantage in removing nitrogen (86% removal rate) and *Chlorella* sp. in removing phosphorous (95% removal rate). The algae community composition showed extreme sensitivity to change in the joint of the *Scenedesmus* or *Chlorella*, respectively, the lower diversity and higher dominance of algae can be observed in *Scenedesmus* group, there existed an opposite tendency in *Chlorella* group. The results demonstrated that the high potential of using *Chlorella* sp. and *Scenedesmus* sp. for nutrient removal from riverwater.

Keywords

Nutrient Removal, Algae Community, Chlorella, Scenedesmus, Eutrophication

1. Introduction

Eutrophication caused by nutrient enrichment in most freshwater, coastal marine and transitional waters has become the tricky issues all over the world since the mid-20th century [1]. The adverse ecological impacts caused by eutrophication, such as reduction of biodiversity, increment of algae bloom, increased turbidity of the water, decreased crop of aquatic products, economic loss, have made tremendous efforts to control eutrophication [2] [3]). Nitrogen and phosphorus have been considered to be the key of eutrophication [4] [5] [6]. So the reducing the impacts of eutrophication especially nitrogen and phosphorus in water bodies is urgently need [7] [8].

Compared to the chemical and physical methods for the treatment of wastewater, biological treatment method is economical, especially bio-treatment with microalgae. There are extensive studies of nutrient removal based on algae growth in municipal [9] [10], agricultural [11] [12], and industrial wastewaters [13] [14]. Microalgae have been proved to be as a potential biological treatment material for wastewater [15] [16]. Tremendous efforts have been put into research of *Chlorella* or *Scenedesmus* removing nutrient from different wastewater. It was confirmed that, based on the nitrogen and phosphorous removal efficiencies, there was a range from 8% to 100% and from 30% to 100% in *Chlorella* and *Scenedesmus*, respectively. Lau *et al.* (1996) [17] found that *Chlorella vulgaris* can remove 86% inorganic N and 70% inorganic P in wastewater.

However, there is little attention for nitrogen and phosphorous removal in river. Because of industrialization and rapid economic development, rivers are been imposed severe risks. In this study, we applied the isolated microalgal strains *Chlorella* sp. and *Scenedesmus* sp. by experiments to river water from Meishe River in Haikou City, Hainan Province. The primary objectives of this study were to test the ability of microalgae removing nutrients in river, to identify the change of algae community. To our opinion, the microalgae can be as a candidate for application of river-water treatment.

2. Methods

2.1. Sample Collection and Experiments Design

The Meishe River with a drainage area of 50.16 km^2, originates from the southern of Haikou City, Hainan Province, which flows into the Qiongzhou Strait after 23.86 km. The water quality of river has deteriorated because of discharge of untreated sewage. Although the program for comprehensive management of Meishe River were launched, there are some challenges for improving water quality of river. So we collected the river water for nutrient removal experiments, the experiment used 250ml triangle vase filled with 200ml river water.

The experiments were divided two groups: one was planted with *Chlorella* sp., the other one was planted with *Scenedesmus* sp. The cell density of *Chlorella* sp. was set three level: 2×10^5 cells/ml (C1), 6×10^5 cells/ml (C2), 12×10^5 cells/ml (C3). The cell density of *Scenedesmus* sp. was set three level: 2×10^5 cells/ml (S1), 4×10^5 cells/ml (S2), 6×10^5 cells/ml (S3). Each experiment had 3 replications. Before each replication poured into algae, *Chlorella* sp. and *Scenedesmu*s sp. were rinsinged with sterile water for eliminating the effects of nutrient from medium. The experiment lasted for 15 days, TN and TP measurement were conducted every five days. One the fifteenth day water samples were collected for quantitative analysis of algae community.

2.2. Analysis of TN and TP

TN and TP concentration was measured according to Chinese state standard testing methods [18].

Removal rates (%) were calculated using relation shown in (1).

$$R(\%) = \{(Rt - R0)/Rt\} \times 100\% \quad (1)$$

where, R is nutrient removal efficiency and Rt and $R0$ are the nutrient concentration at day t and day 0, respectively.

2.3. Analysis of Algae Community

The water samples were preserved with Lugol's iodine and sedimented for more than 48 h. The alga density was counted from 0.1 mL of the sediment through a 0.1 mL counting chamber using a microscope at 40 × 10 magnification. Alga taxa was identified to species or varieties according to Hu and Wei (2006) [19], and alga biomass was estimated according to the closest geometric shape of each taxa.

Species diversity index was calculated following Shannon-Wiener (H), Simpson indices (P) and Pielou evenness (J) formula.

$$H = -\sum (n_i/N)\log_2 (n_i/N)$$

$$P = 1 - \sum (n_i/N)^2$$

$$J = H/\ln S$$

Ni = number of individuals of a species i, N = total number of individuals.

3. Results

3.1. The Change of TN and TP Concentration during the Experiment

The TN concentration in river water declined after pouring into *Chlorella* sp. and *Scenedesmus* sp., S1 group obtained the higher ability for nitrogen removal on the tenth day, the TN concentration was only 0.81 ± 0.20 mg/l, TN removal rates reached 86%. The TN concentration varied between 11.28 mg/l and 11.83 mg/l in river water at the beginning of experiment.

Because of the different initial cell density, the variation of TN concentration in *Scenedesmus* groups and *Chlorella* groups demonstrated different tendency along with the experiment time. TN concentration declined on the fifth day and the tenth day, then increased on the fifteenth day in the group of S1, S2 and C2. TN concentration had a peak on the tenth day in the group of C1 and C3. However, TN showed a trend of increasing in the group of S3 (**Figure 1**).

The TP concentration in river water declined after pouring into *Chlorella* sp. and *Scenedesmus* sp., C1 group obtained the higher ability for phosphorous removal on the fifth day, the TP concentration was only 0.35 ± 0.08 mg/l, TP

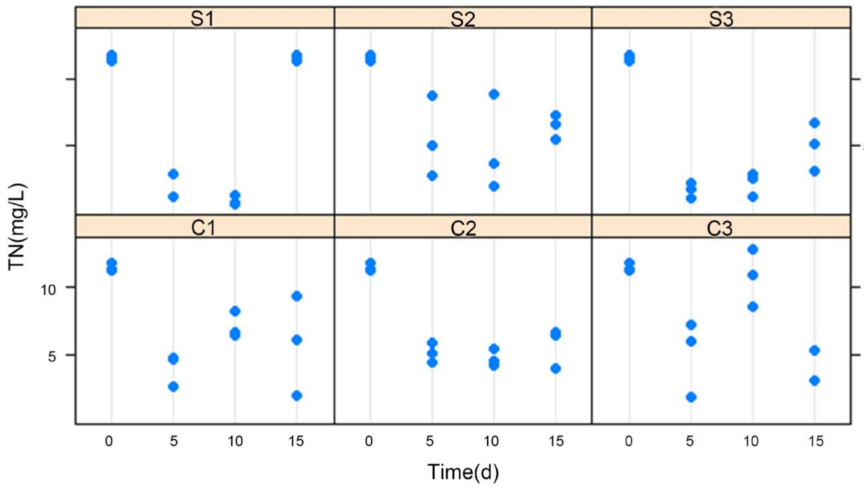

Figure 1. The variation of TN concentration with different initial cell density of *Chlorella* sp. and *Scenedesmus* sp.

removal rates reached 95%. The TP concentration varied between 6.61 mg/l and 6.68 mg/l in river water at the beginning of experiment.

Although the different initial cell density, the variation of TP concentration in *Scenedesmus* groups and *Chlorella* groups demonstrated same tendency along with the experiment time. TP concentration had a peak on the tenth day in all groups (Figure 2).

3.2. Algae Community Composition

Species of algae in *Chlorella* group were significantly more than river-water, but those in *Scenedesmus* group were less than river samples (Table 1). A total of 27 taxa were detected in river samples, including 4 divisions 21 genera. In S group, only 9 taxa were detected, including 3 divisions 6 genera. In C group, a total of 62 taxa were detected, including 4 divisions 36 genera. Comparing to river samples, most algae (e.g. *Melosira*, *Cyclotella*, *Navicula*) disappeared in S group. However, many genera (e.g. *Merismopedia*, *Anabaena*, *Fragilaria*, *Carteria*, *Tetraëdron*, *Oocystis*, *Pediastrum*, *Scenedesmus*) came out in C group.

The total of cell density was higher and Chlorophyta contributed more in *Scenedesmus* group (Figure 3). Bacillariophyta and Chlorophyta were the dominant groups in *Chlorella* group, Bacillariophyta contributed more in CK group (Figure 4). *Nitzschia* was the dominant species in CK, which contributed 54% - 72%, *Nitzschia* and *Chlorella* were the dominant species in C group, which contributed 37%, 21%, respectively. *Scenedesmus* was the dominant species in S group, which contributed 97%.

3.3. Algae Community Diversity Index Change

Compared to CK group, algae community diversity indexes increased in C group and declined in S group. The average Shannon-Wiener, Simpson indices and Pielou evenness increased 37%, 28%, and 18%, respectively in C group. The

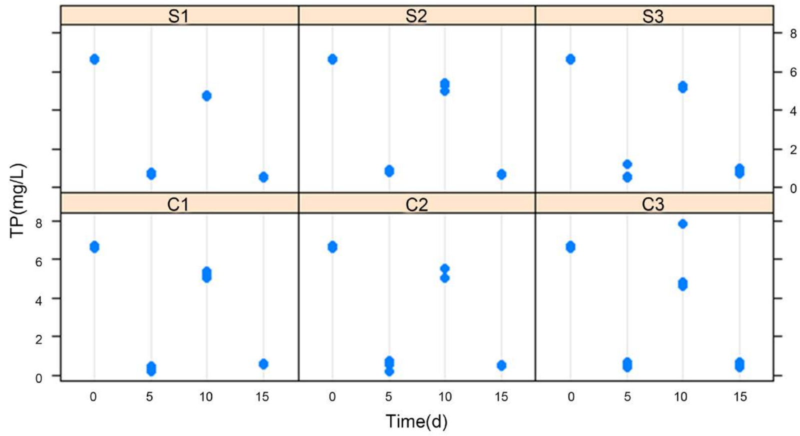

Figure 2. The variation of TP concentration with different initial cell density of *Chlorella* sp. and *Scenedesmus* sp.

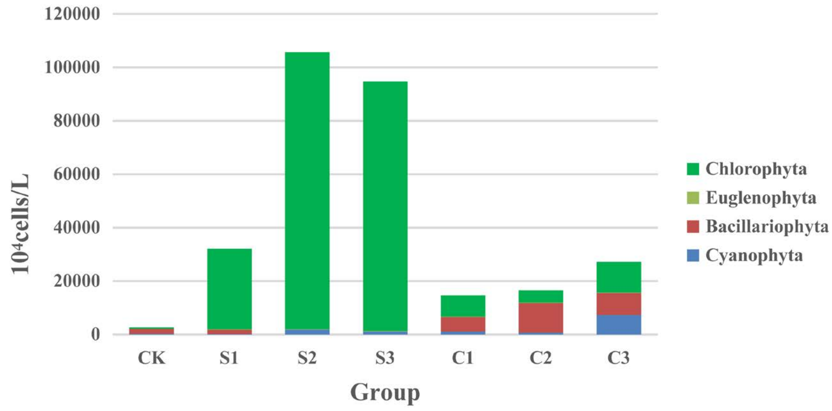

Figure 3. The variation of algae community with different initial cell density of *Chlorella* sp. and *Scenedesmus* sp.

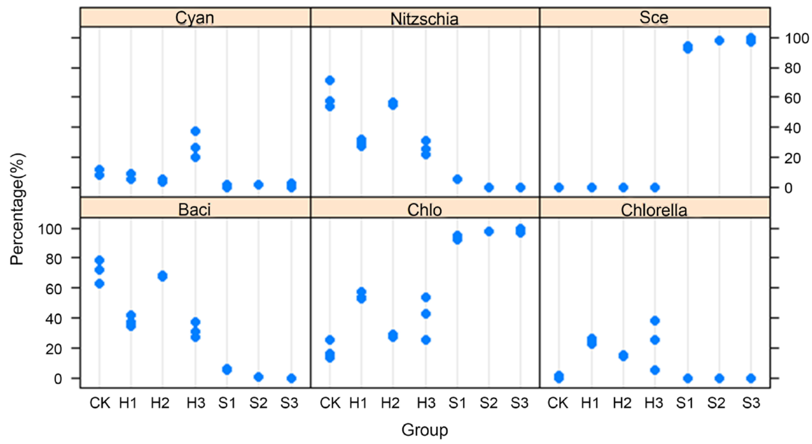

Figure 4. The percentage of algae community with different initial cell density of *Chlorella* sp. and *Scenedesmus* sp.

average Shannon-Wiener, Simpson indices and Pielou evenness decreased 90%, 90%, and 77%, respectively (**Figure 5**).

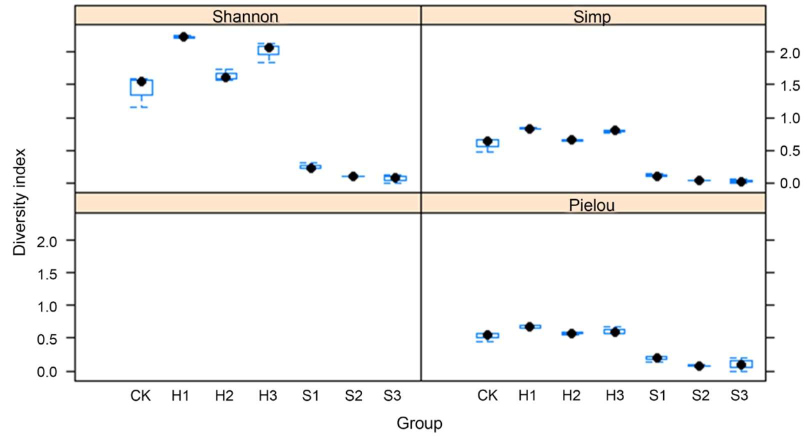

Figure 5. The change of algae community diversity with different initial cell density of *Chlorella* sp. and *Scenedesmus* sp.

Table 1. The list of algae in CK, S group and C group.

Species list	CK	S group			C group		
		1	2	3	1	2	3
Cyanophyta							
Chroococcus limneticus Lemmermann						+	
Merismopedia minima G. Beck					+	+	+
Merismopedia punctata Meyen							+
Lyngbya hieronymusii Lemmermann		+	+				
Oscillatoria princeps Vaucher							+
Oscillatoria sp.	+			+	+	+	
Anabaena sphaerica Bornet et Flahault						+	+
Bacillariophyta							
Melosira granulata (Ehr.) Ralfs					+		
Melosira granulata var.*angustissima* O. Müller	+				+	+	
Melosira varians Agardh					+		
Cyclotella meneghiniana Kützing	+				+	+	+
Synedra acus Kützing	+				+		
Synedra ulna (Nitzsch.) Ehrenberg	+						+
Synedra sp.			+				
Fragilaria capucina Deamaziéres					+		
Diploneis ovalis (Hilse) Cleve	+						
Navicula cryptocephala Kützing	+	+			+	+	+
Navicula sp.	+					+	
Pinnularia gibba Ehrenberg						+	+
Pinnularia sp.							+
Amphora ovalis (Kütz.) Kützing	+						
Encyonema laten (Krasske) Mann							+
Cocconeis placentula Ehrenberg							+

Continued

Gomphonema gracile Ehrenberg	+				+		
Gomphonema gracile Ehrenberg					+		
Achnanthes exigua Grunow	+				+	+	
Nitzschia palea (Kütz.) W. Smith		+	+		+	+	+
Nitzschia scalpelliformis (Grunow) Grunow	+						
Nitzschia sp.	+		+		+	+	
Nitzschia sp.	+	+			+	+	+
Nitzschia sp.	+				+	+	+
Nitzschia sp.					+		
Nitzschia sp.		+			+	+	+
Euglenophyta							
Trachelomonas sp.	+						
Chlorophyta							
Chlamydomonas globosa Snow					+	+	+
Carteria multifilis Dill					+		
Micractinium crassisetum Hortobagyi	+				+	+	+
Golenkinia radiata Chodat	+						+
Schroderia setigera (Schroed.) Lemmermann					+		+
Chlorella vulgaris Beijrinck	+				+	+	+
Chodatella citriformis J. W. Snow					+		
Tetraëdron trilobulatum (Reinsch) Hansgirg					+		
Ankistrodesmus acicularis (A. Braun) Korschikoff	+				+		+
Ankistrodesmus angustus Bernard	+				+		+
Kirchneriella lunaris (Kirch.) Moebius	+				+	+	+
Kirchneriella obesa (W. West) Schmidle					+		
Quadrigula lacustris (Chodat) G. M. Smith							+
Oocystis lacustris Chodat						+	+
Oocystis sp.					+		
Dictyosphaerium ehrenbergianum Nägeli					+		+
Dictyosphaerium pulchellum Wood	+				+	+	
Pediastrum duplex Meyen						+	
Pediastrum tetras var. *tetraodon* (Corda) Rabenhorst					+		
Scenedesmus sp.		+	+	+			
Scenedesmus acuminatus (Lag.) Chodat							+
Scenedesmus bicaudatus (Hansgirg)Chodat					+	+	+
Scenedesmus biguga (Turp.) Lagerheim					+	+	+
Scenedesmus denticulatus Lagerheim					+		

Continued

Scenedesmus dimophus (Turp.) Kützing				+	+
Scenedesmus javaensis Chodat			+		
Scenedesmus protuberans Fritch			+		
Scenedesmus platydiscus (G. M. Smith) Chodat			+		
Scenedesmus quadricauda (Turp.) Brébisson	+		+	+	+
Scenedesmus serratus (Corda) Bohlin			+		
Tetrastrum elegans Playfair	+		+	+	+
Crucigenia tetrapedi (Kirchn.) West & West			+		+
Crucigenia apiculata (Lemm.) Schmidle	+				
Actinastrum hantzschii Lagerheim			+		
Coelastrum sphaericum Nägeli	+		+		+
Closterium gracile Brébisson			+		
Staurastrum sp.				+	
	27	9		62	

4. Discussion

The success of applying microalgae to remove nitrogen or phosphorus from different wastewater has been demonstrated extensively [1] [20]). In our study, it was indicated that the nutrient removal rate from river water was different between *Scenedesmus* group and *Chlorella* group, the *Scenedesmus* sp. had respective advantage in removing nitrogen and *Chlorella* sp. in removing phosphorous (**Figure 1**). S1 group had the highest removal rate for TN on the tenth day, this suggests that *Scenedesmus* sp. with the initial cell density of 2×10^5 cells/ml is likely to have a better TN removal effect. Álvarez-Díaz *et al.* (2017) [21] reported that *Scenedesmus obliquus* achieved higher daily nitrogen removal from wastewater than *Chlorella kessleri*, *Chlorella vulgaris*. Compared to free-living cells of *Scenedesmus*, the chitosan immobilized cells can accomplished a 70% nitrate and 94% phosphate removal within 12 h of incubation [22]. In the nitrogen/phosphorus ratio of 5:1 - 12:1, 83% - 99% nitrogen and 99% phosphorus could be removed. The cells of *Scenedesmus* have the benefit of being equipped with spines and bristles, which make them more buoyant, increased nutrient uptake and avoid predation in the water [23] [24].

C1 group had the highest removal rate for TP on the fifth day, this suggests that *Chlorella* sp. with the initial cell density of 2×10^5 cells/ml is likely to have a better TP removal effect (**Figure 2**) Chlorella is widely used in different type of wastewater treatment such as industrial wastewater, municipal wastewater, swine wastewater [25] [26], and it is shown to be effective in removing nitrogen and phosphorus. It is demonstrated that nitrogen and phosphorous removal efficiencies from the growth of Chlorella sp. range from 8% to 100% [1], and there exists some differences between different species of *Chlorella*. Some study confirm that *Chlorella vulgaris* has higher nutrient removal efficiencies than that of *Chlorella kessleri* when comparing their performances in artificial medium [27] [28]. In our study the TP removal efficiency of Chlorella reached 95%, it can be a candidate species for removing the TP in river water. Su *et al.* (2011) [29] found that *Chlorella pyrenoidosa* in soybean processing wastewater obtained the faster removal of nitrogen over phosphorus. The ratio of N/P should be considered in order to ensure the simultaneous utilization of both nitrogen and phosphorus [30], an optimal N/P ratio for *C. vulgaris* was reported to be 7 [31].

There existed some differences in S group, C group and river sample, algae community composition showed extreme sensitivity to change in the joint of the *Scenedesmus* or *Chlorella*. Comparing to river sample, most algae (e.g. *Melosira*, *Cyclotella*, *Navicula*) disappeared in S group. However, many genera (e.g. *Merismopedia*, *Anabaena*, *Fragilaria*, *Carteria*, *Tetraëdron*, *Oocystis*, *Pediastrum*, *Scenedesmus*) came out and the diversity of algae increased in C group.

5. Conclusions

The study tested nutrient removal rates and algal community variation using the isolated microalgal strains *Chlorella* sp. and *Scenedesmus* sp. from an urban river water. The results showed:

1) The TN and TP concentration in river water declined after pouring into *Chlorella* sp. and *Scenedesmus* sp., the *Scenedesmus* sp. had respective advantage in removing nitrogen and *Chlorella* sp. in removing phosphorous. *Scenedesmus* sp. with the initial cell density of 2×10^5 cells/ml is likely to have a better TN removal effect, TN removal rates reached 86%. *Chlorella* sp. with the initial cell density of 2×10^5 cells/ml is likely to have a better TP removal effect, TP removal rates reached 95%.

2) Species of algae in *Chlorella* group were significantly more than river-water, but those in *Scenedesmus* group were less than river samples (**Table 1**). A total of 27 taxa were detected in river samples, including 4 divisions 21 genera. In S group, only 9 taxa were detected, including 3 divisions 6 genera. In C group, a total of 62 taxa were detected, including 4 divisions 36 genera. Comparing to river samples, most algae (e.g. *Melosira*, *Cyclotella*, *Navicula*) disappeared in *Scenedesmus* group. However, many genera (e.g. *Merismopedia*, *Anabaena*, *Fragilaria*, *Carteria*, *Tetraëdron*, *Oocystis*, *Pediastrum*, *Scenedesmus*) came out in *Chlorella* group.

3) The total of cell density was higher and Chlorophyta contributed more in *Scenedesmus* group. Bacillariophyta and Chlorophyta were the dominant groups in *Chlorella* group, Bacillariophyta contributed more in CK group. *Nitzschia* was the dominant species in CK, which contributed 54% - 72%, *Nitzschia* and *Chlorella* were the dominant species in *Chlorella* group, which contributed 37%, 21%, respectively. *Scenedesmus* was the dominant species in *Scenedesmus* group, which contributed 97%.

4) Compared to CK group, algae community diversity indexes increased in

Chlorella group and declined in *Scenedesmus* group. The average Shannon-Wiener, Simpson indices and Pielou evenness increased 37%, 28%, and 18%, respectively in C group. The average Shannon-Wiener, Simpson indices and Pielou evenness decreased 90%, 90%, and 77%, respectively.

In conclusion, this study showed that the *Scenedesmus* sp. had respective advantage in removing nitrogen and *Chlorella* sp. in removing phosphorous, the lower diversity and higher dominance of algae can be observed in *Scenedesmus* group, there existed an opposite tendency in *Chlorella* group.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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