



# Analysis of Water Treatment Effectiveness of algae, Bacteria and Their Co-Culture Systems as Affected by Carbon Sources

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**Abstract.** The development of industry and agriculture has led to increasingly serious eutrophication of water bodies, and the study of methods for the effective treatment of wastewater is of great significance. This study was conducted to investigate wastewater treatment using *Chlorella pyrenoidosa*, *Bacillus licheniformis*, and microalgal-bacterial consortia under various carbon sources (glucose, sodium acetate, and citrate). Subsequently, the nutrient removal rate of each treatment was evaluated. The results showed that citrate was the optimal carbon source for *Bacillus licheniformis* in single culture, and the optimal carbon source for *Chlorella pyrenoidosa* was glucose. Microalgal-bacterial consortia could be effectively applied in wastewater treatment, since co-cultivation of these microorganisms could promote mutual growth and nutrients removal from wastewaters. The microalgal-bacterial consortia with glucose as carbon source had the highest treatment efficiency, the removal rates of TN, TP and TOC reached 86.05%, 93.71%, and 82.19%, respectively. The wastewater treatment effects reached the wastewater discharge standard one day earlier than the microalgae culture system alone. Due to the effectiveness demonstrated by the studied consortia, it could be concluded that carbon source would be important factors on effects of microalgal-bacterial consortia.

**Keywords:** Carbon source; Microalgal-bacterial consortium; Wastewater treatment

## 1 Introduction

China is a country with shortage of water resources, and with the development of industry and agriculture, a large amount of ineffectively treated sewage enters water bodies, resulting in increasingly serious eutrophication of water bodies, the structure and function of water ecosystems are affected, and human health is threatened<sup>[1]</sup>. Therefore, effective control of water pollution and deep treatment of wastewater is an urgent problem faced today. It has been shown that the use of biological methods for wastewater treatment has the advantages of being green and economically efficient compared to

traditional physical and chemical methods (e.g., coagulation and sedimentation, advanced oxidation technology, and activated carbon adsorption, etc.)<sup>[2]</sup>. Physicochemical methods consume large amounts of energy and materials, resulting in unnecessary waste. The use of microalgae for wastewater treatment not only reduces greenhouse gas emissions, but also accumulates biomass while removing nutrients<sup>[3]</sup>. However, in the process of single microalgae treatment of wastewater, the industrialization of microalgae treatment of wastewater is limited due to the long cultivation time of microalgae and the instability of the system; while co-cultivation of bacteria and microalgae can effectively make up for the shortcomings of the single microalgae treatment system and improve the wastewater treatment efficiency of the system<sup>[4]</sup>.

In the algal-bacterial co-culture system, microalgae provide oxygen as well as organic matter to heterotrophic bacteria through photosynthesis, while bacteria can also provide CO<sub>2</sub> and growth-promoting factors to microalgae. For example, both bacteria *E. asburiae* and *R. ornithinolytica* can promote the growth of *Chlorella vulgaris*, and the biomass of *Chlorella vulgaris* was increased by 58% and 42%, respectively, after 7 d of incubation compared to the culture system alone<sup>[5]</sup>. The algal-bacterial co-culture system has a good potential for application in the deep disposal and resource utilization of wastewater, which is worthy of in-depth study. Carbon is an essential nutrient element in microbial metabolism, so the carbon source has an important influence on the growth and metabolism of microorganisms<sup>[6]</sup>. In algal-bacterial co-culture systems, bacteria are mostly heterotrophic, whereas microalgae are nourished in a variety of ways, and some microalgae can not only grow autotrophically but also undergo mixotrophy, using both CO<sub>2</sub> as well as organic carbon sources (e.g., acetate, glucose, glycerol, and ethanol, etc.)<sup>[7]</sup>. Although it has been shown that wastewater treatment can be carried out using a *Chlorella* (*C. vulgaris*)-*Bacillus licheniformis* (*B. licheniformis*) co-culture system<sup>[8]</sup>, there are no in-depth studies reported on the utilization of different carbon sources by algal-bacterial co-culture systems, and due to the different nutrient modes of microalgae and bacteria, when constructing an algal-bacterial co-culture system, it is especially important to select appropriate culture conditions. Therefore, it is necessary to carry out research on co-cultured bacteria that are beneficial to the growth of *Chlorella vulgaris* and their suitable carbon sources.

In order to investigate the effects of different carbon sources on algae, bacteria and their co-culture system, a strain of *Bacillus licheniformis* selected in the preliminary test and *Chlorella vulgaris* were chosen as the objects, *Bacillus licheniformis* is a probiotic and has certain absorption capacity for nutrients; *Chlorella vulgaris*, as a kind of economic microalgae with fast reproduction and rich nutrients, has been widely used in various industries. Examining the effects of glucose, sodium acetate and trisodium citrate on the effluent treatment effect of *Chlorella*, *Bacillus licheniformis* and their co-culture system can provide technical support for the treatment of wastewater by algal-bacterial co-culture system.

## 2 Materials and Methods

### 2.1 Algae and Bacteria Species

*Chlorella* is one of the microbial foods approved by the European Union, and it is more effective in research and application in the field of water treatment. *Bacillus licheniformis* is a pro-biotic bacterium of *Chlorella*. *Chlorella pyrenoidosa* FACHB-5 was obtained from the Freshwater Algae Species Bank of the Institute of Aquatic Biology, Chinese Academy of Sciences, and *Bacillus licheniformis* was obtained from the State Key Laboratory of Microbiology, Huazhong Agricultural University.

### 2.2 Experimental Methods

#### 2.2.1 Pre-Culture of Microalgae and Bacteria.

After isolation and purification, *Chlorella vulgaris* was inoculated in 1L conical flasks containing 750 mL of BG11 medium sterilized at 121°C for 20 min<sup>[9]</sup>, and incubated in an incubator with continuous aeration for 5 d at 25°C, a light intensity of 50  $\mu\text{mol}/(\text{m}^2\text{-s})$ , and a light-to-dark ratio of 12h:12h, and the aeration volume (via a 0.22  $\mu\text{m}$  filter membrane air aeration from the bottom of the conical flask) was 400 mL/min.

*Bacillus licheniformis* was activated and inoculated in LB medium sterilized at 121°C for 20 min<sup>[10]</sup>, and incubated for 12 h in a 250 mL conical flask (100 mL filling volume) in a thermostatic shaker at 30°C and 100 r/min.

#### 2.2.2 Artificial Effluent Configuration.

The BG11 medium without nitrogen and phosphorus was used as the substrate, and 151.76 mg/L  $\text{NaNO}_3$  (25 mg/L  $\text{NO}_3\text{-N}$ ), 13.17 mg/L  $\text{KH}_2\text{PO}_4$  (3 mg/L  $\text{PO}_4\text{-P}$ ) were added to configure a simulated effluent, and the initial pH was adjusted to 7.0–7.5. The simulated effluent was sterilized by 121°C for 20 min. The simulated effluent was sterilized at 121°C for 20 min, then cooled and prepared for use. Glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ), sodium acetate ( $\text{C}_2\text{H}_3\text{NaO}_2$ ), and trisodium citrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\text{-}2\text{H}_2\text{O}$ ) filtered through 0.22  $\mu\text{m}$  membrane were added to the simulated effluent on the aseptic bench to serve as the source of organic carbon, and the TOC was adjusted to 125 mg/L, respectively.

#### 2.2.3 Inoculation Treatment and Culture Conditions.

*Bacillus licheniformis* and *Chlorella* were cultured to the logarithmic stage by centrifugation at 3000 r/min for 10 min, the supernatant was removed, and the centrifugation was washed and centrifuged twice repeatedly with distilled water, and then inoculated into culture bottles, so that the initial inoculum volume of bacteria was  $(1.0 \pm 0.5) \times 10^7$  CFU/mL, and the initial inoculum volume of *Chlorella* was  $(2.0 \pm 0.3) \times 10^6$  /mL. Bacteria alone, *Chlorella* alone, and algae-bacteria co-culture tests were conducted under different carbon source cultures, and three parallels were set up for each group of tests.

Bacteria alone, Chlorella alone and algal co-culture tests were conducted with artificial sewage as the culture medium, and were incubated in a light incubator with continuous aeration for 7 d. The incubation conditions were the same as those of the Chlorella pre-culture, and samples were taken at 24-h intervals for testing.

#### 2.2.4 Biomass Determination.

Chlorella biomass was determined by microscopy using the hemocyte counting plate counting method, and Bacillus licheniformis biomass was determined by plate colony counting method.

Specific growth rate:

$$\mu = \frac{\ln B_t - \ln B_0}{t} \quad (1)$$

Where:  $B_t$  is the biomass concentration after incubation for  $t$  time;  $B_0$  is the initial biomass concentration;  $t$  is the incubation time (d).

#### 2.2.5 Methods of Water Quality Determination and Removal Rate.

TN was determined by alkaline potassium persulfate-dissolved ultraviolet spectrophotometry; TP was determined by ammonium molybdate spectrophotometry; TOC was determined directly by total organic carbon analyzer.

Nutrient removal rate:

$$R = \frac{S_0 - S_t}{S_0} \times 100\% \quad (2)$$

Where:  $S_t$  is the nutrient concentration (mg/L) after incubation for  $t$  time;  $S_0$  is the initial concentration (mg/L);  $t$  is the incubation time (d).

### 2.3 Data Processing

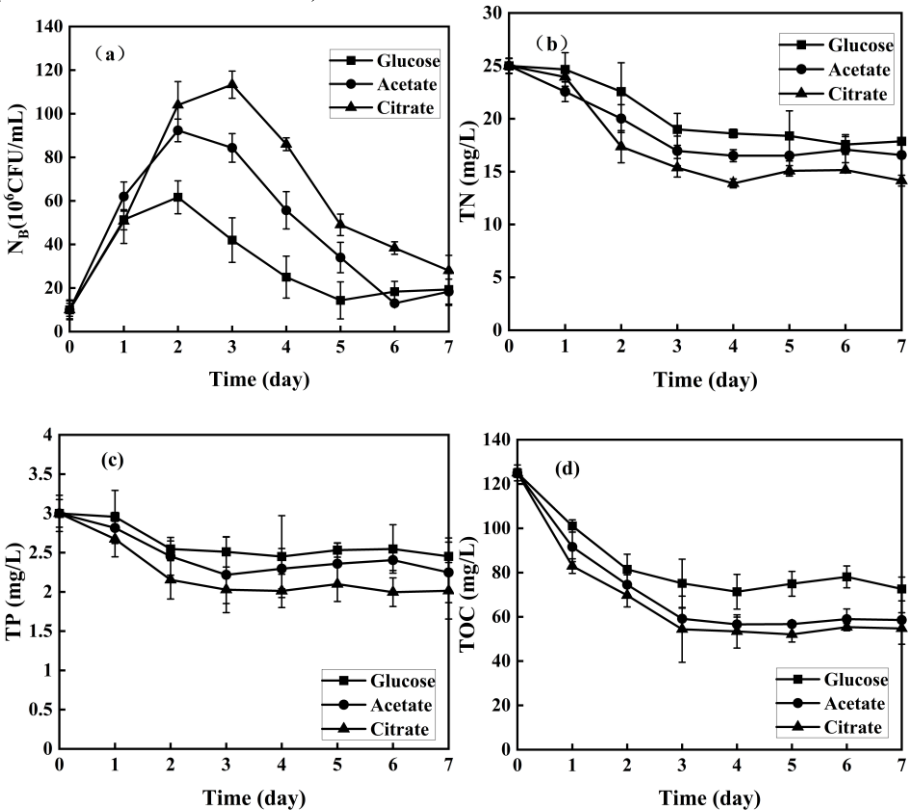
The data were analyzed by one-way ANOVA using the statistical software SPSS, and the statistical test was performed using the LSD method ( $P < 0.05$ ), and Origin 8.5 was used to graph the data.

## 3 Results and Analysis

### 3.1 Effect of Carbon Source on the Culture of Bacillus Licheniformis Alone

Figure 1-a shows that *B. licheniformis* can utilize organic carbon for aerobic growth, but the growth characteristics under the three carbon sources were significantly differ-

ent, in which the growth period of *B. licheniformis* was maintained for 3 d when trisodium citrate was used as the carbon source, and bacterial decline was observed on the 4th day. By maximum biomass analysis, the maximum bacterial biomass was significantly lower ( $P < 0.05$ ) when glucose was used as the carbon source than when sodium acetate and trisodium citrate were used as the carbon sources, and the highest bacterial biomass was found when trisodium citrate was used as the carbon source (NB.max=1.13×10<sup>7</sup> CFU/mL).



**Fig. 1.** Dynamic changes of biomass(a), TN(b), TP(c) and TOC(d) in bacterial culture system under different carbon source culture conditions.

*Bacillus licheniformis* showed some nitrogen removal efficiency under all three sets of carbon source culture conditions. In Figure 1-b, TN removal reached 43.4% when trisodium citrate was used as carbon source, which was 52.17% and 28.78% higher than that of glucose and sodium acetate, respectively. From the analysis of Figure 1-c, it was found that the phosphorus removal efficiency of *Bacillus licheniformis* was significantly lower than the nitrogen removal efficiency ( $P < 0.05$ ), and the maximum removal rate of TP was 33.70%. The organic carbon was effectively taken up by *Bacillus licheniformis* in all the three carbon sources, and up to the 3rd day of incubation, the content of organic carbon in the three groups of systems reached a stable level, among which,

the TOC removal rate of the incubation system with sodium acetate and trisodium citrate as the carbon source was larger, respectively, at 53.12%, and 56.21%. At the end of incubation, the C/N in the three carbon source systems were 4.06, 3.54 and 3.86, respectively, which were not significantly different ( $P>0.05$ ).

### 3.2 Effect of Carbon Source on Chlorella Alone Culture

As shown in Figure 2-a, Chlorella was able to utilize glucose for parthenogenetic growth in the presence of light in all three groups of carbon sources, in which the maximum biomass of Chlorella reached  $13.37 \times 10^6$  organisms/mL when glucose was used as a carbon source, and the maximum biomass was significantly lower than that of the glucose group in both the sodium acetate and trisodium citrate groups when sodium acetate and trisodium citrate were used as a carbon source ( $P<0.05$ ). At the early stage of incubation (0-2 d), the specific growth rates of Chlorella with glucose and sodium acetate as carbon sources were 0.58 and 0.53, respectively, which were both significantly higher than those of the incubation system with sodium acetate ( $\mu = 0.24$ ) as carbon source ( $P<0.05$ ).

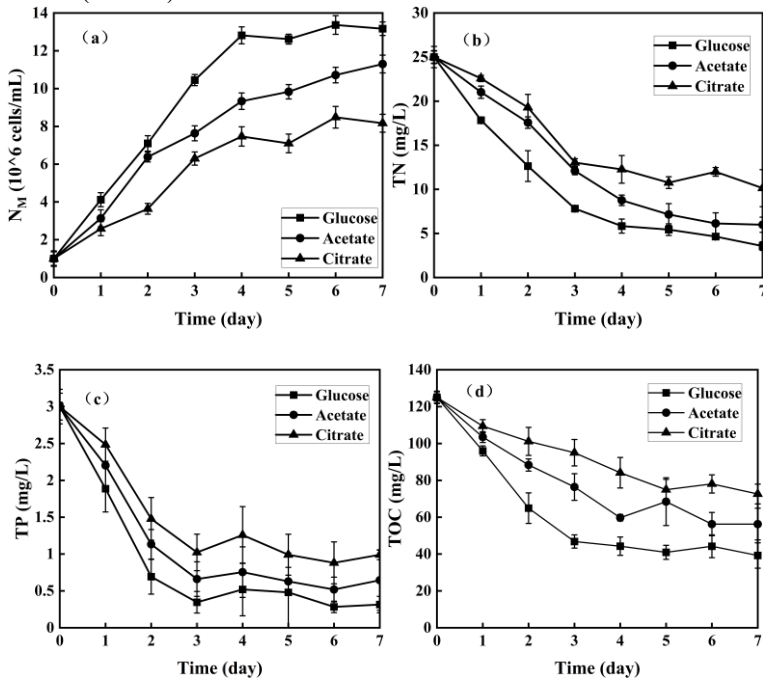


Fig. 2. Dynamic changes of biomass(a), TN(b), TP(c) and TOC(d) in microalgae culture system under different carbon source culture conditions.

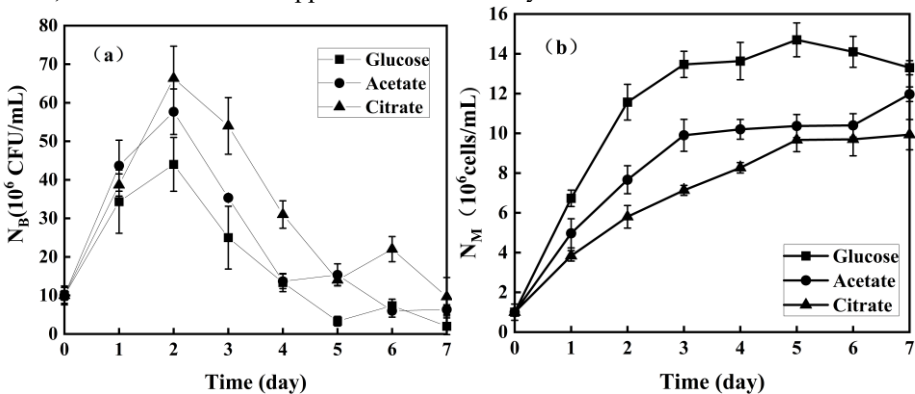
In Fig. 2-b, Chlorella alone was cultured with maximum TN removal of 85.74% when glucose was used as carbon source, which was significantly higher than that when sodium acetate and trisodium citrate were used as carbon sources (total nitrogen removal of 76.05% and 59.44%). In the three groups of carbon source culture systems,

TP removal was 89.52%, 78.51% and 66.98%, respectively, and TOC removal were 68.64%, 62.29% and 41.92%. When glucose was used as the carbon source, the content of TN and TP in the effluent on the 3rd day of treatment reached the Class A standard (TN<15 mg/L, TP<0.5 mg/L) in the "Pollutant Emission Standards for Wastewater Treatment Plants (GB 18918-2002)", which can be used for water reuse; while *Chlorella vulgaris* TP removal could not reach the standard when trisodium citrate was used as the carbon source during the whole experimental cycle. As can be seen from Figure 2-b, d, the TN, TP and TOC removal rates in *Chlorella* alone culture system with different carbon sources were significantly higher than that of *Bacillus licheniformis* alone culture system ( $P<0.05$ ), which indicated that the nutrient removal efficiency of *Chlorella* was better under the same culture conditions.

### 3.3 Effect of Carbon Sources on *Chlorella Vulgaris*-*Bacillus Licheniformis* Co-Culture System

#### 3.3.1 Biomass.

Analysis of bacterial biomass in separate culture (Fig. 1-a) and co-culture (Fig. 3-a) revealed that the maximum bacterial biomass in algal co-culture was significantly smaller than that in bacterial separate culture when glucose, sodium acetate and trisodium citrate were used as the carbon sources ( $P<0.05$ ), which were  $4.40 \times 10^7$  CFU/mL,  $5.77 \times 10^7$  CFU/mL and  $6.63 \times 10^7$  CFU/mL. during co-culture with trisodium citrate as carbon source, the bacterial decline time was 1 d earlier than that of the bacteria alone, and cellular decline appeared on the 3rd day.



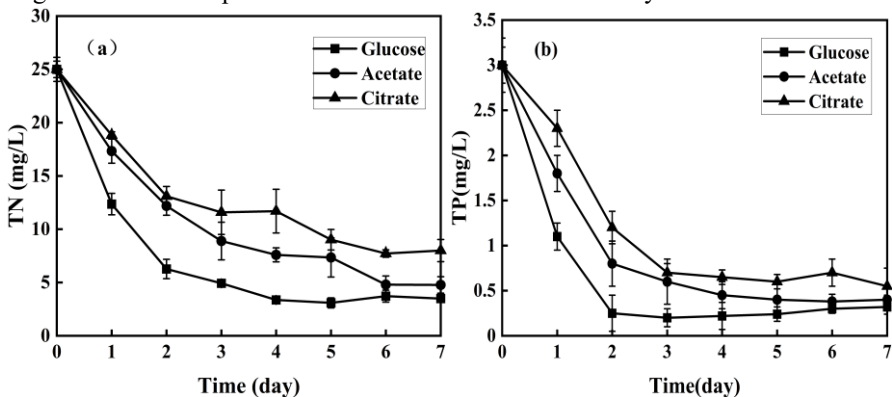
**Fig. 3.** Dynamic changes of bacteria (a) and microalgae (b) biomass in symbiotic systems under different carbon source culture conditions

The analysis of microalgae biomass in separate culture (Fig. 2-b) and co-culture (Fig. 3-b) revealed that in the co-culture system, the growth of microalgae was promoted by the presence of bacteria under all three carbon source culture conditions. The maximum biomass of microalgae in algal-bacterial co-culture under the three carbon source culture conditions were  $14.70 \times 10^6$  cells/mL,  $11.97 \times 10^6$  cells/mL and  $9.70 \times 10^6$  cells/mL, respectively. The specific growth rates of microalgae were 0.27, 0.24 and 0.21, respectively, with no

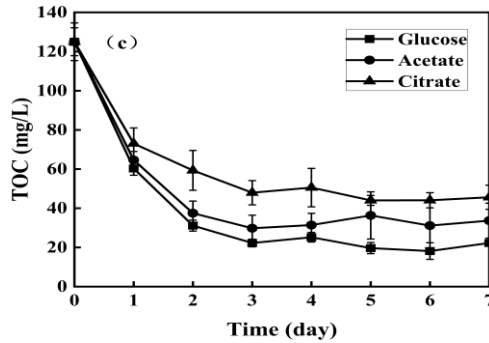
significant difference ( $P>0.05$ ) compared with the microalgae alone culture system; whereas, by analyzing the growth curves, the specific growth rates of *Chlorella* in the co-culture were significantly higher than that of the microalgae alone culture system ( $P<0.05$ ) in the early stage of the incubation (0-2 d) when the bacteria were in the logarithmic growth phase, which were increased by 29.65%, respectively, 14.83% and 48.93%, respectively.

### 3.3.2 Efficiency of Wastewater Treatment.

As can be seen from Table 1, the removal efficiency of TN, TP and TOC in the co-culture system was significantly higher than that of bacteria alone when glucose, sodium acetate and trisodium citrate were used as carbon sources ( $P<0.05$ ), and the nutrient removal efficiency of the algal-bacterial co-culture system was the highest when glucose was used as a carbon source (TN, TP and TOC removal rates were 86.05%, respectively, 93.71% and 82.19%, respectively). In the co-culture system, the removal efficiencies of TN and TP were not significantly different from those of microalgae alone ( $P>0.05$ ), but were significantly higher than those of bacteria alone ( $P<0.05$ ), suggesting that the final removal efficiencies of TN and TP in the co-culture system were mainly limited by microalgae. In the co-culture system, the TOC removal rates under all three carbon source culture conditions were significantly higher than that in the microalgae alone culture system ( $P<0.05$ ), indicating that the addition of bacteria to the microalgae culture species could help to improve the TOC removal efficiency of the system; whereas, in the co-culture system, the TOC removal efficiency was not only significantly higher than that of the algal and bacterial alone culture using glucose as the carbon source ( $P<0.05$ ), but also significantly higher than that of the algal and bacterial alone culture using glucose as the carbon source ( $P<0.05$ ), amounting to 82.19%, and was significantly higher than the algal-bacterial co-culture system with sodium acetate and trisodium citrate as carbon sources (73.07% and 63.52%) ( $P<0.05$ ), indicating that glucose was the optimal carbon source in the co-culture system.







**Fig. 4.** Dynamic changes of TN (a), TP(b) and TOC(c) in symbiotic systems under different carbon source conditions.

**Table 1.** Nutrients removal efficiencies under different carbon source culture conditions.

nutrient	culture-condition	nutrient removal under different carbon source incubation conditions/%		
		glucose C6H12O6	sodium acetate (chemistry)	trisodium citrate
TN	single microalgae	28.52±0.45 <sup>a</sup>	33.70±1.29 <sup>a</sup>	43.40±2.05 <sup>a</sup>
	single bacterium	85.74±1.98 <sup>b</sup>	76.05±3.43 <sup>b</sup>	59.44±8.42 <sup>b</sup>
	algal bloom	86.05±1.01 <sup>bc</sup>	80.93±3.09 <sup>bc</sup>	68.02±4.16 <sup>bc</sup>
TP	single microalgae	18.24±7.81 <sup>a</sup>	25.05±12.86 <sup>a</sup>	33.44±6.56 <sup>a</sup>
	single bacterium	89.52±3.71 <sup>b</sup>	78.51±13.61 <sup>b</sup>	66.98±2.22 <sup>b</sup>
	algal bloom	93.71±2.22 <sup>bc</sup>	89.52±3.71 <sup>bc</sup>	81.66±9.29 <sup>bc</sup>
TOC	single microalgae	41.92±4.28 <sup>a</sup>	53.12±1.18 <sup>a</sup>	56.21±5.70 <sup>a</sup>
	single bacterium	68.64±5.49 <sup>b</sup>	62.29±6.89 <sup>b</sup>	41.92±4.28 <sup>b</sup>
	algal bloom	82.19±1.24 <sup>c</sup>	73.07±7.12 <sup>ac</sup>	63.52±4.90 <sup>ac</sup>

Note: Different lowercase letters for the same nutrient in the same column indicate statistically significant differences (P<0.05).

By analyzing the nutrient dynamics of algae, bacteria and their co-culture systems from Figure.4, it was found that the nutrient removal rates in the algal and bacterial co-culture systems at the beginning of the incubation period (day 2) were significantly higher than those in the separate culture systems at the same period (P<0.05). Moreover, in the co-culture system with glucose as the carbon source, the removal rates of TN, TP and TOC were the largest, reaching 74.99%, 92.66% and 75.09%, respectively, compared with the microalgae alone culture at day 2 (49.44%, 76.94% and 48.05% for TN, TP and TOC, respectively). The presence of bacteria significantly increased the early stage of culture in the co-culture system. nitrogen and phosphorus removal efficiency (P<0.05). The TN and TP removal efficiencies in the co-culture system stabilized from the 4th day to the end of the incubation period and were not significantly different from those of the microalgae incubation alone (P>0.05). It indicates that the activity of bacteria in algal-bacterial co-culture has an important influence on the efficiency of nitrogen and phosphorus removal in the co-culture system. Among the algal-bacterial co-culture systems, the highest efficiency of nitrogen and phosphorus removal

was found in the culture system using glucose and sodium acetate as carbon sources, both of which reached the primary A emission standard. Among them, with glucose as the carbon source, the effluent reached the discharge standard on the 2nd day, which was 1 d earlier than the microalgae alone system, indicating that the algal co-culture system helps to improve the efficiency of wastewater treatment and shorten the time for the effluent to meet the discharge standard.

## 4 Discussion

### 4.1 Effect of Carbon Source on Separate Culture of Algae and Bacteria

Under the same culture conditions, glucose, sodium acetate and trisodium citrate could be used as carbon sources for *Chlorella*, *Bacillus licheniformis* and algal-bacterial co-culture system, but there were differences in the effects of different carbon sources on the nutrient removal efficiency and maximum biomass of algae, bacteria and their co-culture system. In the bacteria-alone system, the highest bacterial biomass was observed when trisodium citrate was used as the carbon source, mainly due to the fact that the rate of bacterial nitrogen removal is not only affected by the concentration of the carbon source, but also by the nature of the electron donor itself <sup>[11]</sup>. Under aerobic conditions, bacteria preferentially use organic acids for growth and metabolism compared to sugars, and citrate as an intermediate product of the tricarboxylic acid cycle is more readily utilized by bacteria, thus contributing to the nitrogen removal efficiency. In this study, the bacteria had a higher nitrogen removal efficiency when sodium acetate was used as a carbon source, which may be due to the fact that a carbon source with a simple structure and smaller molecules is more conducive to nitrogen removal; in addition, according to the results of Krul et al. <sup>[12]</sup>, *Bacillus licheniformis* is an aerobic denitrifying bacterium, which can utilize nitrate nitrogen to carry out denitrogen removal under aerobic conditions. The present study showed that sodium acetate and trisodium citrate were more suitable for bacterial growth compared to glucose, which is consistent with the results of a study on the fine nitrogen removal performance of an aerobic denitrifying strain by Xinping Yang et al. Although glucose was at a disadvantage in terms of growth promotion for bacteria compared to the other two carbon sources, the data still showed that it had a certain growth-supporting ability for bacteria, suggesting that bacteria can utilize glucose for growth.

Under light conditions, *Chlorella* can utilize organic carbon for parthenogenetic growth. In this study, the fastest growth rate of *Chlorella* was observed when glucose was used as the carbon source, which was more favorable for nutrient uptake, and the removal rate of TN and TP could reach 85.74% and 89.52% by using glucose as the carbon source of microalgae, which was similar to the results of the study of Shen et al. <sup>[13]</sup> who used glucose as the carbon source for the treatment of municipal wastewater using microalgae. It has been shown that with glucose as a carbon source, microalgae respiration rate was higher than photosynthetic autotrophic and heterotrophic cultures under both parthenogenetic conditions and the rate of nutrient uptake was faster <sup>[14]</sup>. Glucose is more suitable for microalgae growth than sodium acetate and trisodium citrate, because sodium acetate as well as trisodium citrate reduces the level of chlorophyll

synthesis and the activity of ribulose 1,5-bisphosphate carboxylase, which leads to a decrease in the rate of photosynthesis, and the growth of microalgae is inhibited to a certain degree.

#### 4.2 Interaction between Bacteria and *Chlorella Vulgaris*

In the algal-bacterial co-culture system, the maximum biomass of microalgae was increased to a certain extent under the conditions of three different carbon sources compared with the microalgae alone culture system, indicating that *Bacillus licheniformis* can promote the growth of microalgae under certain conditions. It is noteworthy that at the beginning of the experiment, bacteria in the logarithmic phase had a significant effect on the growth of *Chlorella*, which may be due to the fact that the bacteria in the logarithmic phase were in the active growth stage and had higher biomass, and the CO<sub>2</sub> emission caused by bacterial metabolism increased, which provided a good source of carbon for the photosynthesis of the microalgae [15], and facilitated the growth of the microalgae proliferation. However, in the algal-bacterial co-culture system, the maximum biomass of bacteria under the three carbon sources was lower than that in the bacteria-alone culture system. The possible reason for this may mainly come from the fact that the competition for nutrient salts in the algal-bacterial co-culture system is more intense, and microalgae may accelerate the heterotrophic proliferation through the rapid uptake of nutrients, which restricts the nutrient uptake by bacteria and inhibits the growth of the bacteria. It has been shown that *Chlorella* can perform both photosynthetic autotrophic as well as heterotrophic metabolism with high nutrient uptake efficiency in a culture system that provides both organic and inorganic carbon [16]; the results of the present study are similar.

It has been shown that bacteria can provide growth-promoting factors as well as vitamins to microalgae, stimulate their physiological metabolism, increase cellular biomass and delay cellular decay [17]. Liang et al. [18] showed that a co-culture system of *Bacillus licheniformis* and *Chlorella vulgaris* could achieve better nitrogen and phosphorus removal efficiency, and that *Bacillus licheniformis* could promote the growth of *Chlorella*. In the algal-bacterial symbiotic system, *Bacillus licheniformis* promotes the expression of c-di-GMP in microalgae [19]. c-di-GMP, as an important intracellular second messenger, regulates a variety of cellular physiological functions and directly affects cellular signaling [20], but the specific mechanism of interactions between algae and bacteria remains to be further research. When the bacteria entered the decline phase, there was no significant difference in the growth rate of microalgae relative to the microalgae-alone culture system, suggesting that the interrelationship between microalgae and bacteria changes with the growth cycle and culture time. The possible reasons for this phenomenon are that the rapid growth of algae and bacteria caused late nutrient salt limitation, and the microenvironment of algae and bacteria changed with the prolongation of the culture time, which resulted in the alteration of the interrelationships between algae and bacteria [21].

### 4.3 Selection of Carbon Source in Algal Co-Culture System

In the process of wastewater treatment, the carbon source has an important impact on the microbial colony structure and the efficiency of nitrogen and phosphorus removal, etc. [22]. Since this experiment is an artificial construction of bacterial colonies, its construction is mainly based on the exchange of substances and information between the colonies to improve the stability of the system to achieve specific functions [23]; and carbon source as a life-essential metabolism material, different organisms have specificity to the type of carbon source. Under algal-bacterial co-culture conditions, bacteria mainly promote the growth of microalgae to improve the efficiency of the system in removing nitrogen and phosphorus [7]. Huang et al. [2] compared the effluent treatment effect between the chlorella-bacteria co-culture system and the microalgae alone system, and found that in the algal-bacterial hybrid system chlorella plays a dominant role in the removal of nitrogen and phosphorus, and the bacteria mainly remove the organic matter from the wastewater. organic matter in the wastewater. The experimental results showed that glucose was the most suitable carbon source for microalgae alone culture and trisodium citrate was the most suitable carbon source for bacteria, but in the co-culture system, when glucose was used as the carbon source, not only the maximum microalgae biomass could be harvested, but also the effluent treatment effect was optimal, so it was appropriate to choose glucose as the carbon source for algal-bacterial co-culture system. Therefore, in order to improve the efficiency of wastewater treatment and obtain the maximum microalgal biomass, it is necessary to select a carbon source that is suitable for the growth of microalgae and satisfies the metabolism of bacteria.

## 5 Conclusions

This study shows that the type of carbon source has an important effect on the growth of *Bacillus licheniformis* and *Chlorella vulgaris* as well as the efficiency of wastewater treatment under three carbon source culture conditions. The results of the study showed that:

- (1) In the separate culture system, trisodium citrate was the most suitable carbon source for *Bacillus licheniformis*, while glucose was the most suitable carbon source for *Chlorella* growth;
- (2) In the algal co-culture system, *Bacillus licheniformis* can promote the growth of *Chlorella*, and the algal co-culture is conducive to improving the efficiency of nutrient uptake;
- (3) In the co-culture system of *Bacillus licheniformis* and *Chlorella*, glucose can be used as a suitable carbon source for wastewater treatment in the algal co-culture system.
- (4) Carbon source is considered to be an important factor influencing the effectiveness of microalgal-bacterial communities, and therefore further studies targeting the effect of carbon source are necessary for follow-up.

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