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Applied Research of Microbial Degradation of High-Concentration Organic Wastewater

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As high-concentration organic wastewater is high in proteins, polysaccharides and its decomposition products of organic acids and other ingredients, direct emissions will lead to environmental pollution. In this paper, under light and anaerobic conditions, as the photosynthetic bacterium Rhodopesudomonas HY21 has removal characteristics of three high-concentration organic wastewater—soybean wastewater, citric-acid wastewater and distillery wastewater, studies have shown that after HY21 strains were treated for 9d, the COD removal rates of soybean wastewater, citric-acid wastewater and distillery wastewater were 58.17%, 84.38% and 86.84%, respectively. In addition, inoculation of HY21 strains had only a slight impact on performance of degrading organic wastewater, while organic wastewater pH could seriously affect the HY21 biodegradation ability of high-concentration organic wastewater, and the optimal pH for HY21 strains was between 6 and 8.

1. Introduction

In recent years, with the development of the chemical industry, there are a growing number of various types of chemical wastewater, sludge and waste residues emitted in the production process. In particular, high concentrations of toxic and non-biodegradable organic wastewater have posed serious threat to the environment (Zhao and Zhang, 2014; Yi, 2010). Therefore, how to effectively deal with high-concentration organic wastewater caused by chemical engineering, metallurgical, coking, light manufacturing and food industries, timely and effectively remove harmful substances in water, lower the chemical oxygen demand (COD) in the water and restore ecological balance of water as soon as possible are the keys to environmental protection (Jin et al, 2012). Studies have shown that microorganisms and other bioremediation technologies have unique advantages to treat high-concentration organic wastewater and have been widely applied for removing high-concentration organic wastewater and played an important role (Wanget al, 2015). Currently, microbial species used include photosynthetic bacteria, white rot fungi, oxidation ponds, activated sludge and nitrobacteria (Suwanet al, 2014; Cuiling, 2008). Since photosynthetic bacteria (PSB) are a kind of photoheterotrophic bacteria that are widely distributed in lakes, oceans, soil and other natural environments and carry out photosynthesis under anaerobic conditions using light as the energy source, small organic molecules in nature, H2S and ammonia as nutrients. At present, research has shown that photosynthetic bacteria have strong abilities of decomposing and transforming a variety of organic compounds, excellent resistance to toxicants such as chlorine, salts, cyanides and phenol, are capable of synthesizing cell materials by transforming organic matter (acid, alcohol), H2S and ammonia produced by heterotrophic microbial metabolism in the water under light or dark, aerobic or anaerobic conditions, and reduce the COD in water (Zhouet al, 2015). There are researchers (Amezagaet al, 2014) using rotating bioreactor using immobilized photosynthetic bacteria for treating monosodium glutamate industrial wastewater, with the COD removal rate of 92%. A study by Yu Ji'an et al. (Takenoet al, 2005) has also exhibited that photosynthetic bacteria has a strong ability to degrade citric acid wastewater. Further, some experimental studies have shown that the removal rate fetched 92.7% after soybean wastewater which had a COD of 52840 mg/L was treated by photosynthetic bacteria for 12 h, and 99.5% after soybean wastewater which had a COD of 3860 mg/L was treated by photosynthetic bacteria for 72 h, indicating that photosynthetic bacteria have good application value in treatment of high-concentration organic wastewater.

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This study group got a HY21 strain, which was a purple non-sulfur bacterium isolated from polluted water and identified as Rhodopesudomonas HY21. The strain featured fast growth, strong adaptability and good ability to remove organic wastewater. In view of this, this paper treated high-concentration organic wastewater such as soybean wastewater, citric acid wastewater and distillery wastewater by using Rhodopesudomonas HY21 in the light anaerobic conditions, respectively and studied the performance of photosynthetic bacteria to treat organic wastewater, in the hope of providing references to remove high-concentration organic wastewater by using microorganisms.

2. Materials and methods

2.1 Experimental materials

2.1.1 Tested strains and culture media

The purple non-sulfur bacteria derived from isolation and purification of wastewater after several times of enrichment cultures in September 2010. The modified Ormerod medium were placed in a light and anaerobic condition in which the temperature was 28±2℃ for 8 d, and were illuminated at 3000 lux with incandescent lamps. The bacteria were collected by centrifugation at 8,000 g for 5 min and washed 5 times in saline to prepare a bacterial suspension with an OD660 of 2.4.

2.1.2 Wastewater types for test

Soybean wastewater was derived from a soybean plant in the city; the wastewater was a light yellow emulsion and filtered by three layers of gauze to remove granular insoluble materials. The initial pH and COD values of the wastewater were measured. It was then naturally placed in a 5.0L glass vessel with an open mouth for 48h, during which the original microorganisms grew and reproduced, thus degrading macromolecular substances in the wastewater to be lower molecular substances. Several 500ml flasks were placed with 300ml acidified soybean wastewater each, and the photosynthetic bacterium tests were performed.

Citric acid wastewater was derived from a chemical raw material manufacturing plant in the city and filtered by three layers of gauze to remove granular insoluble materials. The initial pH and COD values of the wastewater were measured and subsequent operations are the same as with the soybean wastewater in 1.1.3.1.

Distillery wastewater was derived from a winery plant in the city and filtered by three layers of gauze to remove granular insoluble materials. The initial pH and COD values of the wastewater were measured and subsequent operations are the same as with the soybean wastewater in 1.1.3.1.

2.2 Experimental Methods

2.2.1 Determination of biomass and absorption spectra of living cells

In the bacterial culture process, absorption spectrometry nephelometry was employed for measurement, and OD660 represented biomass of bacteria. After the end of incubation, 200 ml bacterial suspension was taken for by centrifugation at 8,000 g for 5 min. Bacteria were collected and absorbent paper was used to absorb the residual liquid. The wet weight of the bacteria was measured and indicated by ρ(g/L). Absorption spectrum measurement of living cells: the cells washed 5 times in saline were suspended in the sucrose solution of 60%. The sucrose solution of 60% was taken as the control group to calculate the absorption spectra.

2.2.2 Determination of pH and COD

Mettler Toledo 320 pH Meter was used to determine the pH value.

The potassium dichromate method was adopted to determine the COD value

3. Experimental results

3.1 Identification of Rhodopesudomonas HY21—purple non-sulfur bacterial strains

3.1.1 Morphology

A HY21 strain was gram-negative on a pure culture, and its view under a microscope is shown in Figure 1: cells were rod-shaped, slightly curved and had a changeable shape; they had a cell size of 0.8×1.1μm and were suitable for growing under light and anaerobic conditions; the bacterial liquid was red or purple red. And by ultrastructure and 16sRNA, it was identified as a purple non-sulfur bacterium Rhodopesudomonas HY21.

3.1.2 Growth curves and absorption spectrum of living cells

The growth curve and absorption spectrum of living cells of Rhodopesudomonas HY21 are shown in Figure 2. It can be seen from the figure that HY21 strains grew well under light and anaerobic conditions; the characteristic absorption peaks of its living cells were 371nm, 596nm, 821nm and 871nm.

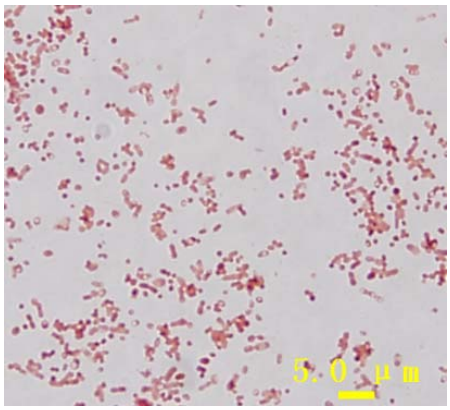

Figure 1: Strain microscopy of Rhodopesudomonas HY21

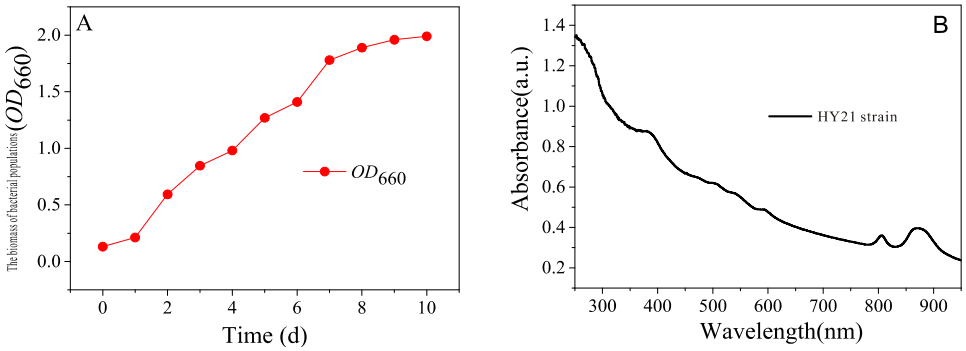

Figure 2: The growth curve of Rhodopesudomonas HY21 strain (A) and the absorption spectrum of living cells (B)

3.2 Removal characteristics of strains on three types of organic wastewater in suitable conditions

HY21 strains were adopted to treat soybean wastewater, citric acid wastewater and distillery wastewater, respectively. The COD reached more than 2000 mg/L, as shown in Table 1, from which we can know that after a 9-d treatment, the removal rates of soybean wastewater, citric acid wastewater and distillery wastewater by HY21 strains were 58.17%, 84.38% and 86.84%, separately. Meanwhile, during the treatment, wastewater was regularly collected and processed; cells were removed by centrifugation and the COD value of wastewater was determined. The results are shown in Figure 3. We can see that in wastewater treatment by HY21 strains, COD jumped within 24 h, followed by a sharp fall. The results have shown that HY21 strains had good abilities to treat organic wastewater and grew well in the three types of wastewater; in which HY21 strains presented the best abilities to dispose distillery wastewater and also grew relatively well in citric acid wastewater and distillery wastewater.

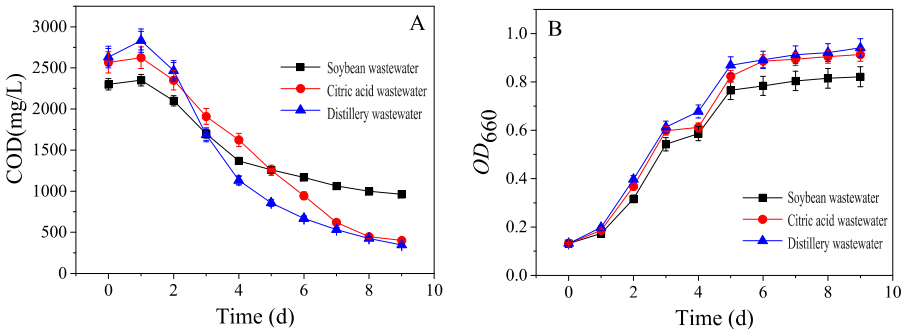

Figure 3: Change characteristics of removal of three types of organic wastewater by HY21 strains

3.3 Impacts of different inoculation sizes on removal of three types of organic wastewater by strains

To explore the impacts of different inoculation sizes on removal of organic wastewater by HY21 strains, this paper recorded the changes when organic wastewater was removed by HY21 strains at inoculation sizes (calculated by strain biomass OD660) of 0.13, 0.32, 0.51, 0.73 and 1.12, respectively. The results are shown in Figure 4, from which we can see that with an increase in the inoculation sizes, there were increased removal rates of organic wastewater by the strains, but after 9 days of treatment, there was only a slight increase and the overall disposal effect was not obvious.

Table 1: A comparison of removal rates of three types of organic wastewater by HY21 strains

No.	Type of wastewater	Strains under treatment	Pre-treated COD concentration mg/L	Treatment time (d)	Post-treated COD concentration mg/L	Removal rate
1	Soybean wastewater	HY21 strains	2300	9	962	58.17%
2	Citric acid wastewater	HY21 strains	2568	9	401	84.38%
3	Distillery wastewater	HY21 strains	2630	9	346	86.84%

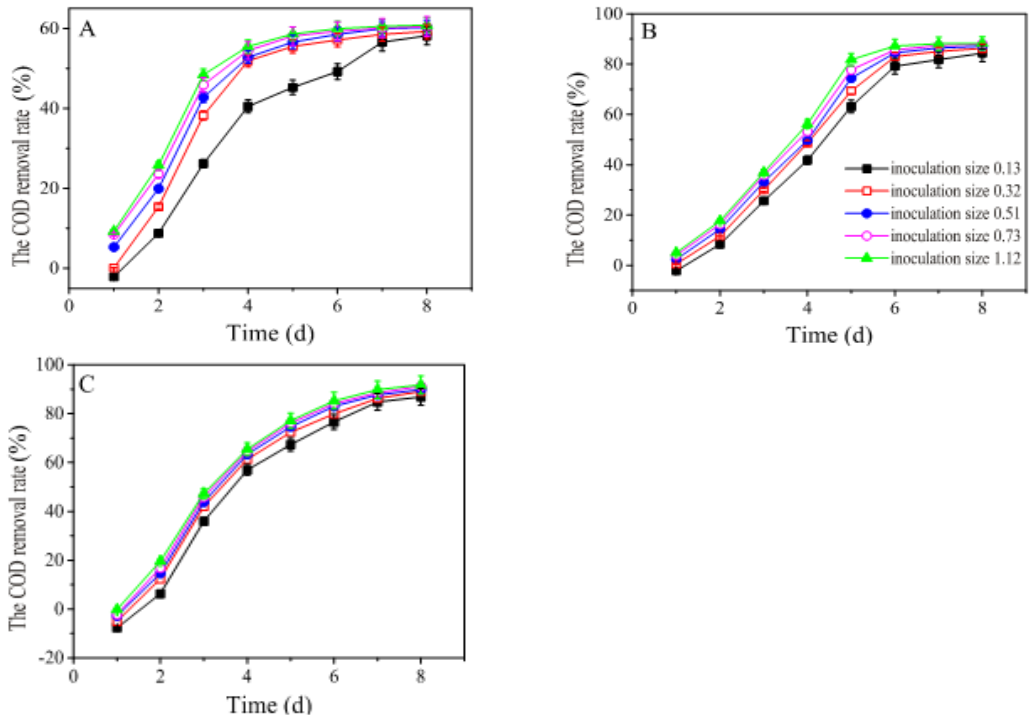

Figure 4: Impacts of different inoculation sizes on removal of soybean wastewater (A), citric acid wastewater (B) and distillery wastewater (C) by HY21 strains

3.4 Impacts of different pH on removal of three types of organic wastewater by strains

Subsequently, we explored the Impacts of different pH values on removal of organic wastewater by HY21 strains. When the pH was at 6, 7, 8, 9 and 10, respectively, the disposal effects of three types of organic wastewater by strains are shown in Figure 5. When the pH was 6, the strains demonstrated the best removal effects of soybean wastewater, citric acid wastewater and distillery wastewater, with the removal rates of 59.95%, 86.44% and 91.56%, separately. When the pH was 10, the removal rates of soybean wastewater, citric acid wastewater and distillery wastewater by HY21 strains were 6.4%, 8.6% and 10.8%, respectively, which were severely affected. This is because an excessively high pH value of the water would seriously affect the growth of strains, resulting in reduced abilities to dispose the polluted water.

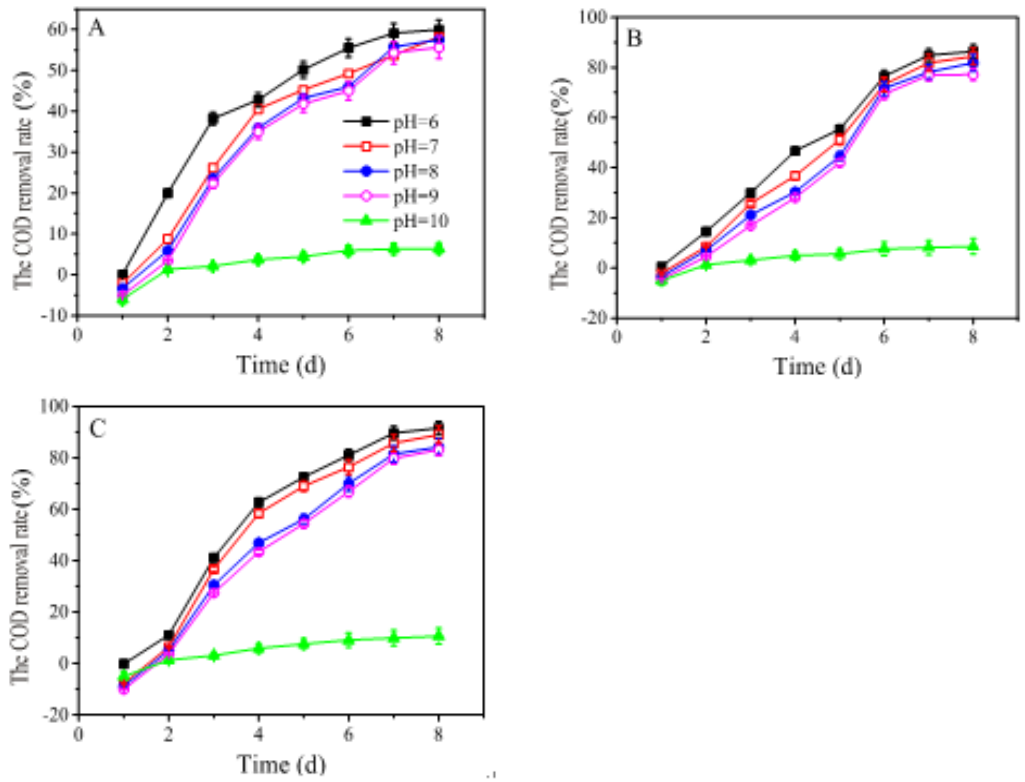

Figure 5: Impacts of different pH on removal of soybean wastewater (A), citric acid wastewater (B) and distillery wastewater (C) by HY21 strains

4. Result analysis and discussion

There is tremendous research on disposal of high-concentration organic wastewater by PSB, but due to the differences of strains used, environmental factors (lighting, pH and temperature) and culture media, the disposal performance varies to some extent. It has been reported that Liu Junyi et al. realized 58% total COD removal in treatment of citric acid wastewater with a density of 10 billion/mL of bacterial liquid mixing Rhodopseudomonaspalustris, Rhodopseudomonassphaeroides and Rhodopseudomonascapsulatus. Zeng Yu et al. disposed distillery wastewater by using photosynthetic bacteria mixture that they have isolated and cultured and found that the photosynthetic bacteria had high COD removal efficiency of disposing distillery wastewater, but after static natural culture and solubilization, photosynthetic bacteria could use monosaccharide, amino acids, volatile fatty acids and other small molecule compounds, but not wastewater containing macromolecular compounds or refractory dissolved organic matter. In micro-aerobic and anaerobic conditions, researchers explored the impacts of temperature, initial wastewater concentration, pH, inoculation size and other factors on PSB treatment of soybean wastewater, in which COD removal was the most efficient at the temperature of 30℃; COD removal absolute value was proportional to the initial concentration of wastewater; and an increase in the inoculum size could improve COD removal; PSB adapts to a wide pH range and the highest COD removal rate was recorded in a pH range of 6.5-8.0 of the wastewater.

Rhodopesudomonas HY21 strains used herein had significantly inferior abilities to dispose soybean than to dispose citric acid wastewater and distillery wastewater. This is consistent with related research reports. PSB can not only take advantage of organic matter in water (acid and alcohol), H_2S and ammonia to synthesize the cells substances, but also reduce the chemical oxygen demand in water, purify water and inhibit the growth of pathogenic bacteria. In addition, PSB also has abilities to remove toxicants in high-concentration organic wastewater. Meanwhile, in the process of disposing organic wastewater, it has been found that pH gradually increased in the water, which was because PSB could produce low-molecular-weight organic matter (such as sugars, organic acids, alcohols, peptides, amino acids, etc.) when utilizing and transforming organic matter, produce dissolved oxygen in water and pH changes and have a significant impact on the growth and metabolism of microbial populations in water.

5. Conclusion

This paper has expounded on the removal performance of Rhodopesudomonas HY21 strains on soybean wastewater, citric acid wastewater and distillery wastewater in the light and anaerobic conditions. HY21 strains could well reduce COD concentrations in citric acid wastewater and distillery wastewater but showed a decrease in the ability to remove soybean wastewater. Moreover, the inoculum size of photosynthetic bacteria had only a slight impact on the performance of degrading organic wastewater. A suitable pH value range would be 6-8; when the pH was too high, it would seriously affect the degradation of high-concentration organic wastewater by HY21 strains. As a result, the study shows that HY21 strains had good removal effects of high-concentration organic wastewater, which could provide some reference for further investigating the high-concentration organic wastewater by using microorganisms.

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