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Feasibility study and process optimization of citric acid wastewater treatment and biomass production by photosynthetic bacteria

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ABSTRACT

Using photosynthetic bacteria (PSB) in wastewater treatment could not only eliminate environmental pollutants, but also recycle bacteria biomass. This paper studied the feasibility of using PSB in citric acid wastewater treatment. Some important factors which influence the pollutants removal efficiency and biomass were also examined to determine the optimal conditions. The results showed that PSB could effectively remove pollutants, and biomass production was also achieved. The optimal conditions were initial pH at 7.0, ratio of carbon/nitrogen at 10, initial chemical oxygen demand (COD) of 2,400 mg/L, natural light-micro aerobic condition, and hydraulic retention time of 40 h. Under these conditions, the removal rate of COD and the biomass could reach 89.4% and 2,250 mg/L, respectively, and the biomass yield was 0.76 $mg_{\text{-biomass}}/mg_{\text{-COD-removal}}$. By process evaluation, compared with anaerobic wastewater treatments, this process had better effluent quality and simpler process, compared with aerobic wastewater treatment, this process had the advantage of biomass recovery.

Keywords: Photosynthetic bacteria; Citric acid wastewater; Biomass growth; Optimization; Biomass yield

1. Introduction

As the world's largest producer and exporter of citric acid production, China produced approximately one million tons of citric acid as an output of product fermentation and around 50–60 million tons of citric acid wastewater in 2010 [1]. Direct discharging citric acid wastewater can cause serious pollution to the receiving water bodies. Therefore, large amounts of citric acid wastewater need to be properly treated before discharged. Commonly, citric acid wastewater was treated by up-flow anaerobic sludge bed, sequencing batch reactor, high-temperature anaerobic digester, Fenton oxidation, and so on [2–5]. Although these methods are effective in reducing organic pollutions, their treatment process are often long and complicated, and they could generate large quantities of excess sludge and lack of renewable resource cycle. Excess sludge is a serious environmental problem and the treatment of excess sludge increases the treatment cost. Therefore, using a new microbial wastewater treatment method with less or no excess sludge

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production is a direction in citric acid wastewater treatment. The method using photosynthetic bacteria (PSB) in wastewater treatment is qualified with this feature, and has attracted increasing attentions.

Since PSB were first reported in industrial wastewater treatment by Kobayashi and Tchan [6], they have been applied to treat heavy metal wastewater [7], dye wastewater [8], olive milk wastewater [9], pharmaceutical wastewater [10], dairy wastewater [11], latex wastewater [12], swine wastewater [13], sewage wastewater [14], slaughterhouse wastewater [15], food processing wastewater [16], oil-containing sewage [17], etc. Studies show that PSB wastewater treatment can remove pollutants effectively. More importantly, the method is characterized by a simple treating process and no excess sludge pollution. Some researchers have proposed to combine wastewater treatment with biomass recovery [18–20]. The PSB biomass is rich in single-cell protein, biopolymers, antimicrobial agents, carotene, pantothenic acid, and therapeutic compounds. It can be recycled as useful raw materials in food, medical, and agriculture industries. The reducing of excess sludge together with the potential of biomass recovery has made PSB promising in wastewater treatment [21].

Above researches suggest that PSB may have the potential to treat citric acid wastewater as well as recover biomass. However, related research is rare. The purpose of this work is to examine the feasibility of PSB for citric acid wastewater treatment and to optimize the treatment conditions for its application.

2. Materials and methods

2.1. Micro-organism and wastewater

Rhodopseudomonas sphaeroides is a strain of PSB isolated from soil [22]. It was cultured in a thermostat shaker (120 rpm, 26–30℃) with HCH medium [23]. The inoculated PSB was in the logarithmic growth phase, which began at 48 h after the cultivation and was the best time to treat wastewater. The bacterial solution was stored at 4℃ after reaching that period. The citric acid wastewater had a chemical oxygen demand (COD) of 4,800–6,000 mg/L, total nitrogen of 30–100 mg/L, and pH of 5.0–6.0.

2.2. Operation and evaluation

For all experiments, the bioreactors were 250 mL flasks. These flasks were sterilized at 121℃ for 30 min before use. 150 mL synthetic citric acid wastewater was added to the bioreactor each time. PSB in logarithmic growth were inoculated and the initial bacteria concentration in wastewater was 580.0 mg/L (dry weight). The citric acid wastewater and PSB were rotated at 120 r/min with treatment temperature around 25–30℃ under sterile conditions.

For the feasibility study, pH was set at 7.0 and natural light–microaerobic condition was used (set as in Table 1) according to previous literature reports [23].

For the optimization studies, four sets of experiments were done. Five levels of initial pH tested were 5.0, 6.0, 7.0, 8.0, and 9.0; NaOH was used to adjust the initial pH. Six levels of carbon/nitrogen (C/N) ratio tested were 0.5, 5, 10, 15, 20, and 40; $(NH_4)_2SO_4$ was used as nitrogen source. Four levels of initial COD tested were 600, 1,200, 2,400, and 4,800 mg/L. Three different light–oxygen conditions, namely light–anaerobic, natural light–microaerobic, and dark–aerobic conditions, were set as in Table 1. Light intensity was controlled using a 40 watt incandescent. For anaerobic condition, the flask was saturated with nitrogen (99% purity), and then sealed with sealing membrane to keep it absolute anaerobic.

2.3. Analysis methods

Samples were collected from bioreactors and were centrifuged at 9,000 r/min for 10 min. The supernatant was used to test the COD according to the national standard methods; the collected PSB were used to measure the biomass (dry weight). The pH was measured by pH tester and the dissolved oxygen was measured by a dissolved oxygen meter (YSI-DO200, YSI Corporation Company, USA).

2.4. Statistical analysis

Parallel experiment, parallel samples, and parallel detections were conducted in order to ensure the accuracy of data. All the reported values were obtained from three or more experimental data. Tukey's test was adopted to analyze the significance of the values.

Table 1
Light–oxygen conditions for PSB citric acid wastewater treatment

Light–oxygen condition	Light–anaerobic	Natural light–microaerobic	Dark–aerobic
Light intensity (lux)	2,000	500–1,000	<0.1
Dissolved oxygen (mg/L)	<0.5	0.5–1.0	2.0

3. Results and discussion

3.1. Feasibility study

The results of this feasibility study could be seen in Fig. 1. Clearly COD decreased with time continuously. The final COD was 209.7 mg/L ($t = 90$ h), and reached the required discharge standard of citric acid wastewater. Meanwhile, the biomass increased from the original 580 to 2,375 mg/L. The result indicated that citric acid wastewater was feasible for PSB growth. Therefore, it was feasible to use PSB to treat citric acid wastewater as well as to recover biomass.

3.2. Effects of initial pH on PSB citric acid wastewater treatment

The solution pH is considered to have a huge influence on micro-organism. Generally, the best pH for biological treatment is 6.5–7.5. Since the citric acid wastewater was acidic (5.0–6.0), pH adjustment was necessary. The effects of different initial pH (5.0–9.0) on COD removal rate and biomass were studied. The results showed that COD removal rate was higher than 80% when the initial pH was 6.0–9.0, but pH of 7 was optimal for PSB growth. So pH of 7.0 was used for following experiments.

3.3. Effects of C/N on PSB citric acid wastewater treatment

The bacterial growth needs suitable C/N proportion [24] and the optimal ratio for traditional activated sludge treatment is 20. However, the citric acid wastewater was high in organic content and low in nitrogen content. Therefore, extra nitrogen was needed. The effects of PSB treatment with different C/N ratios were examined, and the results were shown in Fig. 2.

PSB could treat citric acid wastewater with C/N increased from 0.5 to 40.0 (COD removal rate > 75%) (Tukey's test $P < 0.05$). The optimal C/N was 10.0, and the corresponding COD removal rate was 95.6%. At the same time, under C/N of 10.0, the biomass production was the highest. When the C/N was lower than 10.0, the nutrients were not suitable for the growth of PSB, and the biomass was low.

Biomass yield was calculated according to Fig. 2. Biomass yield was defined as biomass-increase/COD-removal. The biomass yields were 0.17, 0.24, 0.39, 0.37, 0.32, and 0.23 $mg_{-biomass}/mg_{-COD-removal}$ with C/N increased from 0.5 to 40. The largest biomass yield was at C/N of 10. Hence, the optimal C/N for PSB citric acid wastewater treatment was 10, which was used in the following experiments.

3.4. Effects of initial COD on PSB citric acid wastewater treatment

Previous study showed that the initial COD affected organic matter degradation and microbial growth [23]. High initial COD might increase biomass production, but decreased the effluent quality. In this study, the raw citric acid wastewater was diluted to 600, 1,200, and 2,400 mg/L, respectively. And the effects of initial COD and the growth of biomass were shown in Fig. 3.

By diluting the raw citric acid wastewater, the hydraulic retention time (HRT) was shortened. At the 40th hour, the COD of the diluted wastewater were all below 300.0 mg/L, which already met the required discharge standard of citric acid wastewater.

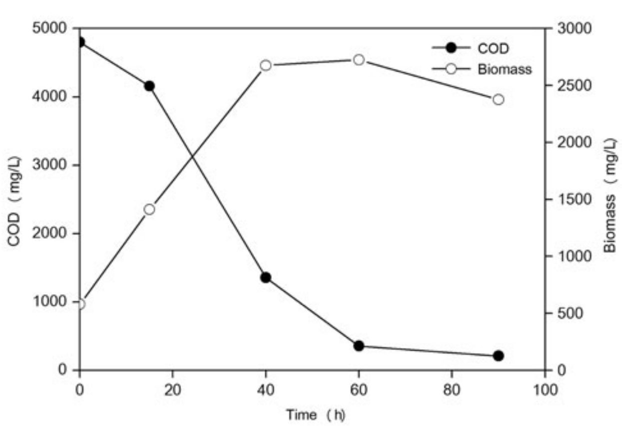

Fig. 1. COD removal rate and biomass increase in citric acid wastewater treatment, natural light–microaerobic condition, initial pH 7.0.

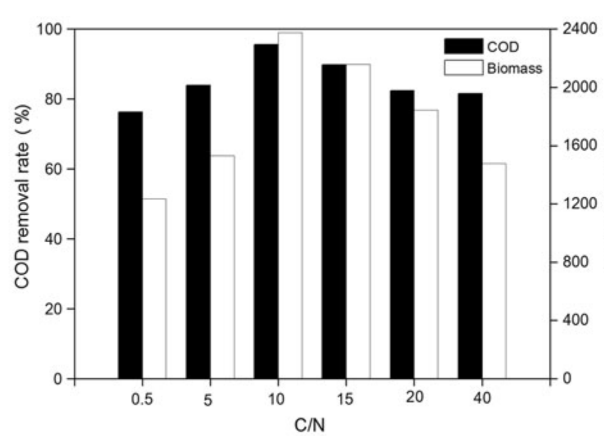

Fig. 2. Effects of different ratios of C/N on COD removal rate and biomass growth, natural light–microaerobic condition, $t = 90$ h.

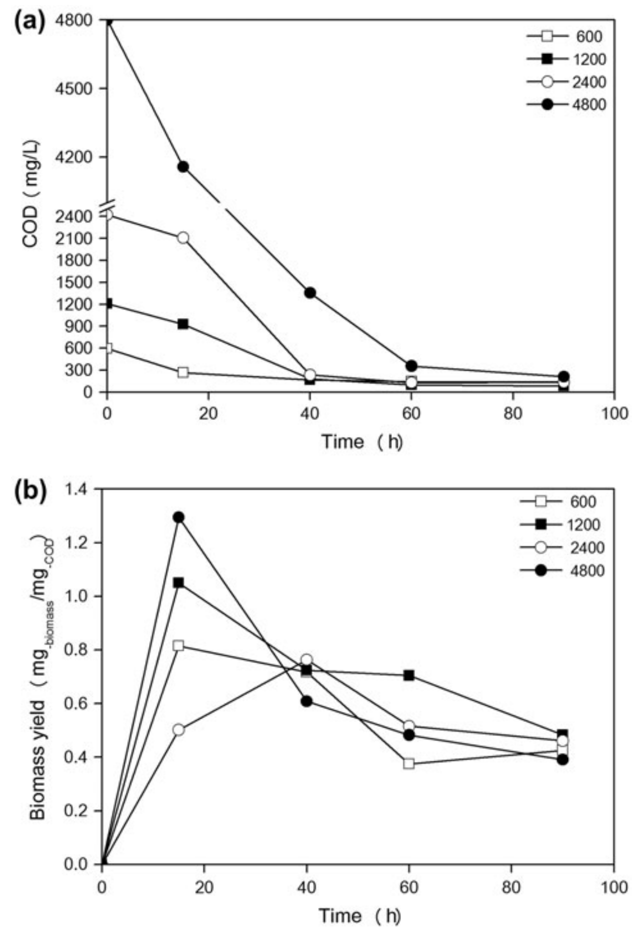

Fig. 3. Effects of initial COD on (a) COD removal rate and (b) biomass growth, natural light–microoxygen condition.

Meanwhile, at the 40th hour the highest biomass yield was 0.76 mg-biomass/mg-COD-removal, increased by 25.6% compared to the raw wastewater group (0.61). The corresponding wastewater with initial COD was 2,400 mg/L. The increase of biomass yield was very important since it meant that more biomass could be obtained with the same amount of wastewater COD. After 40th hour, the biomass yields all decreased. So, from the point of biomass yield, the 40 h was the optimal HRT, and 2,400 mg/L was the optimal concentration. The biomass yield increased by diluting.

It is important to note that diluting citric acid wastewater to 2,400 mg/L was beneficial. When reflux ratio was added to 100%, the raw citric acid wastewater was diluted to 2,400 mg/L.

3.5. Effects of light and oxygen conditions on PSB citric acid wastewater treatment

A unique characteristic of PSB is that they have two energy metabolic pathways and they can live in light, dark, anaerobic, and aerobic conditions. So light and oxygen are two important controlling factors in PSB wastewater treatment. In this study, three typical combinations of light–oxygen conditions were examined, namely light–anaerobic, natural light–microaerobic, and dark–aerobic condition, and the results were shown in Fig. 4.

COD removal rate increased with time under different light and oxygen conditions. Under natural light–micro aerobic condition, the COD removal rate had the fastest growth with a final highest removal of 93.7% at the 90th hour (Tukey's test $P < 0.05$). At the 40th hour, the COD removal rate was 89.4%, which was higher than the light–anaerobic (33.6%) and dark–aerobic condition (73.5%) group. At the same time, all three treatments at the 40th hour had a good biomass production. Thus, the optimal condition was natural light–micro aerobic condition.

Sigmaplot software was adopted to fit COD degradation curve. Results showed that the degradation of

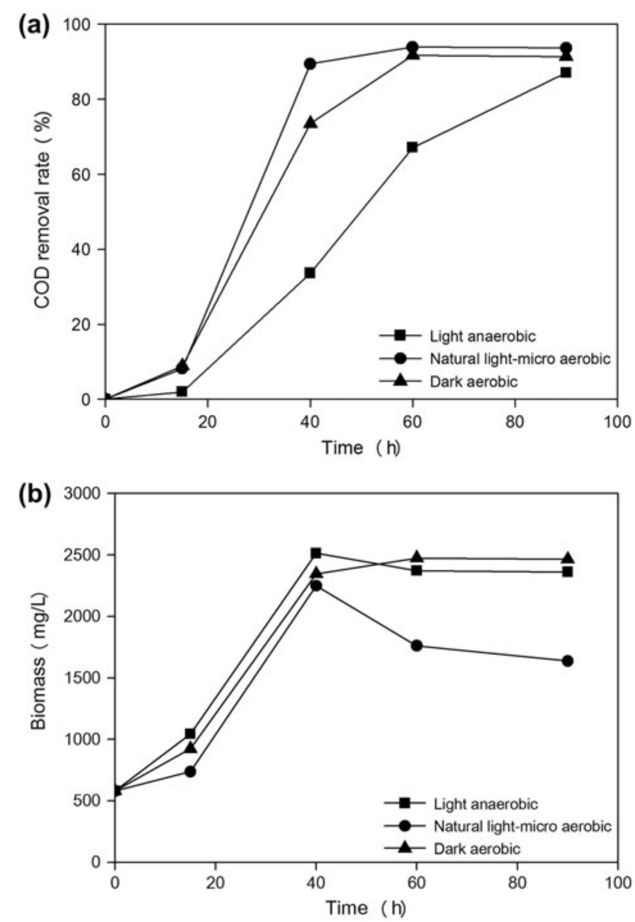

Fig. 4. Effects of different light and oxygen conditions on (a) COD removal rate and (b) biomass growth.

Table 2
Comparison of aerobic, anaerobic, and PSB wastewater treatment

Feature	Aerobic wastewater treatment	Anaerobic wastewater treatment	PSB wastewater treatment
Typical organic strength ($mg_{\text{-COD}}/L$)	<1,000	>4,000	600–5,000
Biomass (g/L)	1.5–10.0	3.0–20.0	2.0–3.0
Organic removal efficiency (%)	90–95%	>70%	>89%
Effluent quality	Meet the standard	After some post-process, the effluent meet the standard	Meet the standard
Organic load ($kg_{\text{-COD}}/m^3\,d$)	0.40–1.00	4.00–20.00	1.35
Biomass yield ($mg_{\text{-biomass}}/mg_{\text{-COD-removal}}$)	0.40–0.60	0.03–0.10	0.76
Bioenergy and nutrient recovery	No	Yes	Yes
Reference	[25–30]	This study	

COD followed the first-order degradation kinetics and the fitting reactor kinetics equations with three operational conditions were shown in the following:

Light–anaerobic condition: $\ln(C_t/C_o) = -0.019t$, $R^2 = 0.929$

Natural light–microaerobic condition: $\ln(C_t/C_o) = -0.037t$, $R^2 = 0.905$

Dark–aerobic condition: Condition C: $\ln(C_t/C_o) = -0.031t$, $R^2 = 0.937$

And the corresponding degradation kinetics constants (k) were as follows: k_1 was 0.019; k_2 was 0.037; and k_3 was 0.031.

The degradation kinetic constant of natural light–microaerobic the highest, and it meant the COD degradation rate was appropriate the fastest.

4. Process evaluation

A preliminary comparison of aerobic, anaerobic, and PSB wastewater treatment was carried out in Table 2. The organic removal efficiency by aerobic wastewater treatment was the highest, followed by PSB wastewater treatment, finally the anaerobic wastewater treatments. The effluent quality of aerobic and PSB wastewater treatment can directly meet the required discharge standards. But for the anaerobic wastewater treatments, some post-treatments, such as aerobic process or membrane separation, were needed to meet the discharge standards. The organic load of anaerobic wastewater treatments was the highest, followed by PSB wastewater treatment, and ended with the aerobic wastewater treatments. The biomass yield by PSB wastewater treatment was the highest, followed by the aerobic wastewater treatments, finally the anaerobic wastewater treatments. The PSB and anaerobic wastewater treatments both had bioenergy and nutrient recovery, while the aerobic wastewater treatment failed in resource recovery.

Compared with the anaerobic wastewater treatments, PSB wastewater treatment could reach effluent quality standard with simple process. Compared with the aerobic wastewater treatment, PSB wastewater treatment has the advantages of bioenergy and nutrient recovery. In a word, the PSB wastewater treatment is highly attractive.

5. Conclusions

This work analyzed the potential use of PSB for citric acid wastewater treatment as well as biomass growth, and evaluated the influences of some important factors. Generally, it was concluded that under certain conditions PSB were promising in treating citric acid wastewater.

(1) By the treatment of PSB, the effluent of citric acid wastewater could meet the required discharge standard of citric acid wastewater.
(2) The optimal conditions were initial pH at 7.0, ratio of C/N at 10 diluted to 2,400 mg/L, natural light-microaerobic condition, and HRT of 40 h, and the corresponding results were the final COD removal rate of 89.4%, biomass of 2,250 mg/L, and biomass yield of 0.76 $mg_{\text{-biomass}}/mg_{\text{-COD-removal}}$.
(3) Process evaluation showed that compared with anaerobic wastewater treatments, PSB wastewater treatment had the advantage of higher effluent quality and simpler process, and compared with aerobic wastewater treatment, PSB wastewater treatment had the advantage of biomass recovery.

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