Synergic Effects of Anabaena and Light on Biological Degradation of Basic Oranges

Received for publication, June 25, 2011
Accepted, April 20, 2012

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Abstract

Anabaena is a dominant kind of cyanobacteria bloom, which has high propagation speed, adaptability and exists abundantly in the natural water bodies. The process of visible light-synergized degradation of basic orange by anabaena was investigated in this paper. The results show that anabaena and visible light have effective synergy to the degradation of basic orange. The pH value is a key factor of the synergistic degradation. Algae concentration and visible light intensity has a direct effect on the speed and degree of the synergistic degradation. This paper shows the feasibility of using cyanobacteria to dispose dyeing wastewater with sunlight and make them become useful resource.

Keywords: dye, Anabaena, visible light, synergistic degradation

Introduction

Dye is one of the major pollutants in dyeing wastewater, and also one of the important water pollution sources [1-2]. Effective degradation and treatment technology of them is a prerequisite for dyeing wastewater disposal. Current treatment methods include physicochemical method, biological method and physicochemical-biological joint method [3-5]. Although single physicochemical and biological methods have their advantages, they are limited by running costs, post treatment process, etc. Therefore, the developing direction of treating dyeing wastewater is transferring to the combination of treatment technology, materials modification, and operating technical, especially the joint of physicochemical and microbial methods [6-7]. Otherwise, algal blooms in lakes outbreak frequently, and the algae pollution have become a global problem. In the 13th World Lake Conference held in November, 2009, cyanobacteria treatment and resource management, as one of the conference topics, has become the hottest issue for the global expert’s discussion. In this paper, the visible light-synergized degradation of dye by Anabaena, one kind of the cyanobacteria, and the influence factors were investigated by combining photodegradation and microbial technology, and the corresponding kinetic analysis was also studied.

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Experimental Section

Materials
The Anabaena species was purchased from Institute of Hydrobiology (IHB), Chinese Academy of Sciences (CAS). Selenite Enrichment Medium, Ascorbic acid, NaOH, Na₂CO₃ and HCl of grade AR were obtained from Sinopharm Chemical Reagent Co, Ltd. (SCRC) and used without further purification. A set of pH buffer agents and basic orange were obtained from Changqing Chemical Market.

Instruments
GF-9070 drying cabinet, LD5-2A centrifuge, SPD-20A High Performance Liquid Chromatography (HPLC), HP400G thermostatic light incubator, pH-4 digital pH meter, BS124S analytical balance, PR22-50 quartz photochemical reactor, XB-K-25 blood counting chamber, high pressure mercury lamp, SMZ-PM130/200 electron microscopy, and UV-1800 UV-visible spectrophotometer were used in this study.

Methods

The pure culture of Anabaena. The standard stock solution of SE medium was adjusted to pH value of 7.0-7.2, and sterilized by high temperature at 121 ºC for 20 minutes. Anabaena was placed in a thermostatic light incubator after its sterile inoculation. The concentration of algae was controlled by blood counting chamber.

Pretreatment of Anabaena suspension. The Anabaena suspension which was in the exponential growth was adjusted to pH value of 3.0 by adding ascorbic acid solution. After stirring 30 minutes, they were separated by centrifugation. The algae were washed three times with double distilled water. Finally, the liquid culture of Anabaena was prepared for study after cleaning and counting.

Visible light-synergized degradation of basic orange by Anabaena at different pH values. Eight sets of mixed solutions with 4×10⁸ cells/L Anabaena and 20 mg/L basic orange were prepared, and the pH values were 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0, respectively. They reacted under the 250w high-pressure mercury lamp illumination with light intensity of 38,000 lux for ten hours, while the dark reaction was also processing. The samples were centrifuged before and after light, respectively. Then, the UV-visible absorption spectra of the supernatant and its absorbance under λ_max were measured. Therefore, the optimum pH value of the synergistic degradation could be determined.

Photosensitization test of basic orange without Anabaena at the optimum pH value. The 20 mg/L basic orange solution was prepared, and its pH was adjusted to the optimal value according to the above experimental results. Then, the samples reacted under the 250w high-pressure mercury lamp illumination for ten hours. Meanwhile, the UV-visible absorption spectra and the absorbance under λ_max of the sample supernatant after centrifugation were measured in every two-hour sampling time.

Visible light-synergized degradation of basic orange by Anabaena at different Anabaena concentrations. Two sets of mixed solutions with 4×10⁸ cells/L, 8×10⁸ cells/L Anabaena concentration and 20 mg/L basic orange concentration were prepared at the optimal pH value, respectively. They reacted under the 250w high-pressure mercury lamp illumination for ten hours, meantime, the dark reaction was also carried out for comparing. In the process, the UV-visible absorption spectra, the absorbance under λ_max and high performance liquid
chromatography (HPLC) of the sample supernatant after centrifugation were measured in every two-hour sampling time.

Visible light-synergized degradation of basic orange by Anabaena with different light intensities at the optimal pH value. The mixed solution with $4 \times 10^8$ cells/L Anabaena and 20 mg/L basic orange were prepared at the optimal pH value. They reacted under the 250w and 125w high-pressure mercury lamp illumination, respectively, while their dark reaction was also carried out for comparing. Meantime, the UV-visible absorption spectra, the absorbance under $\lambda_{max}$ and high performance liquid chromatography (HPLC) of the sample supernatant after centrifugation were measured in every two-hour sampling time.

Results and discussion

Visible light-synergized degradation of basic orange by Anabaena at different pH values

Figures 1 shows the absorption spectra of the solution including basic orange and Anabaena before and after irradiation at different pH values. Before irradiation, firstly, the intensity of the main absorption decreases markedly as the pH value increases, especially the absorbance under $\lambda_{max}$, then the curve increases when pH > 5.5. After irradiating for ten hours, all the basic orange solutions were degraded to some degree in the presence of Anabaena at different pH values. The decoloration of the solutions was also observed in different levels. Seen from the results, the neutral samples degrade and decolor faster and more effectively, indicating that pH values play an important role on the visible light-synergized degradation of basic orange in the presence of Anabaena. Therefore, the optimum pH value of pH = 5 was determined. Meanwhile, the shape of the UV-VIS spectra during the process is almost changeless. It could be concluded that there weren’t novel complex functional groups formed...
in the process of synergized degradation and sample post-treatment.

Zepp\textsuperscript{[8]} pointed out that the colloidal Fe (OH)\textsubscript{3} particles possessed photosensitization, and it could be a catalyst for the degradation of organic compounds under sunlight. In order to eliminate this disturbance, the saturated solution of ascorbic acid was employed to clean the Anabaena suspension and remove the possible colloidal Fe (OH)\textsubscript{3} particles adsorbed on the surface of algal cells.

\textbf{Photosensitization of basic orange solution without Anabaena}

From \textbf{Figure 2}, it can be seen that there isn’t any change for the curve of UV-Vis absorption spectrum during illuminating the basic orange solution without Anabaena at the optimum pH value for ten hours. \textbf{Figure 3} shows that the absorbance of the basic orange solution without Anabaena at pH = 5 under $\lambda_{\text{max}}$ changes very little, and decoloration behavior doesn’t happen, indicating that basic orange has strong resistance to light and its degradation by itself does not affect the experimental results. As for the decoloration process of dye by photo-oxidation, it is complex, and it could be affected by various factors including molecular structure of dye, matrix, light wavelength and intensity, temperature, humidity, etc.
Biological degradation of basic orange solution by Anabaena (dark reaction)

The comparison curves of the UV-Vis absorption spectra before and after degradation without light were presented in Figure 4. It was found that the curve of basic orange solutions in the presence of Anabaena at different pH values almost didn’t decrease and its shape remained unchanged during the process. The results showed that the basic orange was not degraded, and no novel complex intermediate product was formed in the process of dark reaction. However, this does not mean that the dye solution can’t be biodegraded by Anabaena. Algae are simple photosynthetic organisms, which can not only absorb inorganic nutrient salts from the environment and synthesize their own cell material through photosynthesis, but also gain carbon source through degrading organic compounds if the culture time is long enough. In the experiments, the illumination time was controlled to less than ten hours in order to eliminate the effect of biodegradation of basic orange by Anabaena.

Analyzing the above results, it is concluded that basic orange solution can degrade markedly at pH = 5 under the synergized action of Anabaena and high-pressure mercury lamp illumination. Comparing to the separate illumination process of basic orange solution without Anabaena and the dark reaction process of basic orange solution in the presence of Anabaena, the degradation rate under the synergized action of Anabaena and illumination is significantly accelerated and faster, and the decoloration is more effective, suggesting that Anabaena and visible light illumination have an excellent synergy to the degradation of basic orange.
Effect of Anabaena concentration on visible light-synergized degradation of basic orange

Figure 5 shows the UV-Vis absorption spectra of the basic orange solutions in the presence of Anabaena at the optimum pH value. The concentration of Anabaena was $4.0\times10^8$ cells/L and $8.0\times10^8$ cells/L, respectively. The samples were illuminated for different time. It could be concluded easily from the results that the degradation rate of basic orange was markedly getting faster and the degradation action was more effective with the increase in Anabaena concentration.

There are two main absorption peaks, 260nm and 450nm, for unirradiated and irradiated basic orange solution in the presence of Anabaena (Figure 5). The wide and strong absorption peak at about 450nm belongs to the conjugated structure of basic orange molecule, which makes it characteristic orange [9]. To compare the UV-Vis absorption spectra of basic orange solution in the presence of Anabaena which have been irradiated for 0h, 2h, 4h, 6h, 8h, and 10h, it was found that the intensity of absorption peak of the conjugated structure was changed markedly, which significantly declined with increasing the illumination time. The absorption peak nearly disappeared after ten-hour illumination, indicating that the decoloration reaction of the basic orange solution in the presence of Anabaena was almost complete. However, the intensity of absorption peak at 260nm didn’t change much during illuminating process. It increased slightly as time increased, which might be due to red shift of $-\text{NH}_2$ auxochrome at benzene ring. Thus, it could be concluded that a small amount of amines were formed in the process of visible light-synergized degradation of basic orange by Anabaena.
In the process of visible light-synergized degradation of basic orange doped with different algae concentrations, its supernatant after centrifugation and removing Anabaena was also characterized by high performance liquid chromatogram (HPLC). From the change of height and area of characteristic peaks (Figure 6), it shows that the rate and efficiency of visible light-synergized degradation of basic orange would be improved by increasing Anabaena concentration, which is consistent with the analysis by UV-Vis absorption spectra. It is concluded that Anabaena concentration has a direct relationship to the degradation rate and efficiency of basic orange.

**Effect of light intensity on visible light-synergized degradation of basic orange in the presence of Anabaena**
The effect of light intensity on degradation of basic orange was directly reflected in Figure 7. The results indicate that the degradation rate and efficiency of basic orange solution in the presence of Anabaena at the optimal pH value under the high pressure mercury lamp of 250w were significantly improved when compared with that under the high pressure mercury lamp of 125w. Consequently, light intensity could directly affect synergized degradation of basic orange in the presence of Anabaena, and degradation rate and efficiency could be improved by increasing light intensity.

HPLC analysis of the supernatant of the basic orange degradation after centrifugation and removing Anabaena was made (Figure 8). The samples were at the optimal pH value and at the same Anabaena concentration, but the light intensity was different, which was 250w and 125w, respectively. From the change of height and area of characteristic peaks, it is concluded that the rate and efficiency of visible light-synergized degradation of basic orange in the presence of Anabaena are improved by increasing light intensity from 125w to 250w, which is in agreement with the analysis by UV-Vis absorption spectra.

Degradation kinetics

The obtained results confirm that Anabaena concentration plays an important role in the degradation of basic orange solution. Table 1 shows the degradation kinetics of basic orange solution without Anabaena and with different Anabaena concentration under the 250w high pressure mercury lamp. The analysis results were gained from the absorbance under $\lambda_{\text{max}}$ over time. From the data of Table 1, the correlation coefficient ($R^2$) of the regression equation of pseudo-zero order degradation kinetics for basic orange solution without Anabaena is larger than that of the pseudo-first order degradation kinetics, which can determine that the degradation kinetics of basic orange solution without Anabaena is pseudo-zero order reaction. After doped with Anabaena in a certain concentration, the correlation coefficient ($R^2$) of the regression equation of pseudo-zero order kinetics for the synergistic degradation of basic

![Figure 8](image-url)
orange solution is less than that of the pseudo-first order kinetics. Thus, it can be considered that the synergistic degradation kinetics of basic orange solution with Anabaena is pseudo-first order reaction. Meanwhile, the synergistic degradation rate increases with increasing Anabaena concentration.

Table 1. Degradation kinetics of basic orange solution without Anabaena and with different Anabaena concentration under the 250w high pressure mercury lamp.

<table>
<thead>
<tr>
<th>Anabaena concentration</th>
<th>Pseudo-zero order reaction</th>
<th>Pseudo-first order reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>Equation</td>
</tr>
<tr>
<td>0</td>
<td>0.9870</td>
<td>c = -0.0022t + 0.6928</td>
</tr>
<tr>
<td>4×10⁸ cells/l</td>
<td>0.8234</td>
<td>c = -0.1054t + 0.5051</td>
</tr>
<tr>
<td>8×10⁸ cells/l</td>
<td>0.5712</td>
<td>c = -0.1025t + 0.4359</td>
</tr>
</tbody>
</table>

Table 2 shows the degradation kinetics of basic orange solution in the same Anabaena concentration under the 125w and 250w high pressure mercury lamp or in dark for ten hours. The analysis was based on the absorbance under λ_max over time. It was found that the correlation coefficient (R²) of the regression equation of pseudo-zero order degradation kinetics for basic orange solution in the presence of Anabaena in dark is larger than that of the pseudo-first order degradation kinetics, which can judge that the degradation kinetics of basic orange solution in the presence of Anabaena in dark is pseudo-zero order reaction. When the reaction is under light, the correlation coefficient (R²) of the regression equation of pseudo-zero order kinetics for the synergistic degradation of basic orange solution in the presence of Anabaena is less than that of the pseudo-first order kinetics. Thus, it can be considered that the synergistic degradation kinetics of basic orange solution doped with Anabaena in light is pseudo-first order reaction. Furthermore, the degradation rate increases with increasing light intensity.

Table 2. Degradation kinetics of basic orange solution in the same Anabaena concentration with different light intensity.

<table>
<thead>
<tr>
<th>Light intensity</th>
<th>Pseudo-zero order reaction</th>
<th>Pseudo-first order reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>Equation</td>
</tr>
<tr>
<td>Dark reaction</td>
<td>0.9613</td>
<td>c = -0.0004x + 0.6191</td>
</tr>
<tr>
<td>125w mercury lamp</td>
<td>0.9336</td>
<td>c = -0.0642t + 0.574</td>
</tr>
<tr>
<td>250w mercury lamp</td>
<td>0.8234</td>
<td>c = -0.1054t + 0.5051</td>
</tr>
</tbody>
</table>
Conclusions

1. Basic orange has a strong light resistance, and its self-degradation without Anabaena is very little.
2. Anabaena has little effect on the biological degradation of basic orange without light. In the process of dark reaction, the absorbance under $\lambda_{\text{max}}$ almost doesn’t change.
3. The pH value is one of the important factors on the visible light-synergized degradation of basic orange in the presence of Anabaena. On near neutral conditions (at about pH=5), the synergized degradation rate is the fastest, and the decoloration is the most effective, indicating that Anabaena and light have excellent synergized action for the degradation of basic orange at the optimum pH value.
4. Light intensity plays an important role on the degradation rate and efficiency. The stronger the light intensity, the faster the degradation rate, and the more effective the decoloration. Kinetics analysis shows that the synergistic degradation of basic orange solution doped with Anabaena in light is pseudo-first order reaction.
5. Anabaena concentration has a direct impact on the degradation of basic orange solution in light. The degradation rate and efficiency is improved with increasing Anabaena concentration. The synergistic degradation of basic orange solution doped with different Anabaena concentration in light belongs to pseudo-first order reaction.

Acknowledgements

This work is financially supported by the Young and Middle-aged Excellent Creative Team of Huangshi Institute of Technology, the Major Program of Education Bureau of Hubei Province, China (Z20104401), the Provincial Key Program of Natural Science Foundation of Hubei Province, China (2010CDA026), and the NSFC (21174047).

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