Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Environmental Sciences 18 (2013) 540 – 546

Procedia Environmental Sciences

2013 International Symposium on Environmental Science and Technology (2013 ISEST)

Effect of different plants on azo-dye wastewater bio-decolorization

Xiaobai Zhou[a,b], Xuemin Xiang[a,b,]*

[a]*Key laboratory of Industrial Ecology and Environmental Engineering, Ministry of Education, Dalian University of Technology, Dalian 116024,China*

[b]*School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China*

Abstract

Two salt-tolerant plants (*Medicago sativa* L. and *Sesbania cannabina Pers*) as well as a kind of salt-tolerant azo-dye decolorization bacteria GTY (*Gracilibacillus sp.*GTY) were selected to treat acid red B or acid scarlet GR contaminated water. Results showed that *Medicago sativa* L. was more tolerant to the azo dyes and more helpful in promoting the azo-dye wastewater bio-decoloration than *Sesbania cannabina Pers*, but GTY density was higher in the root exudates of *Sesbania cannabina Pers* than that of *Medicago sativa* L. This indicated that the increase of GTY density only partially presented the azo-dye decolorization promoted by plants.

Keywords: plant; azo dyes; decolorization; root exudates; salt

1. Introduction

The textile industry produces a huge volume of azo-dye wastewater every year [1]. The inappropriate disposal of azo dyes to water bodies currently causes great concern since it can disturb the ecosystem and constitutes a potential environmental and health problem due to their toxicity and carcinogenicity. Chemical, physical and biological methods have been used to treat the azo-dye wastewater, and biological technique has been recognized as a promising technique owing to its inexpensive, environmental friendly and sustainable properties [2].

Decolorization, the process which the azo bond being broken down, is the first step of azo dye degradation [3]. Various bacteria and fungi have decolorization abilities, few, however, have been accepted by the textile industries [4]. Their lack of implement is mainly attributed to the low efficiency of

* Corresponding author: Xuemin Xiang. Tel.: +0-086-411-84706240; fax: +0-086-411-84706252.
E-mail address: hjyjs@dlut.edu.cn.

doi:10.1016/j.proenv.2013.04.073

the technique, for the growth and activity of microorganisms are inhibited owing to the toxicity and salinity of azo-dye wastewater.

Recently, the ability of plants to stimulate bio-decolorization of azo-dye has received much attention [5]. Plants are supposed to promote the bio-decolorization through the following ways: firstly, around 10% of the photosynthetic carbon is released by rhizo-deposition, which increases the growth of microorganisms in the wastewater. Secondly, the roots' exudes contained enzymes such as lignin peroxidase, manganese dependent peroxidase and laccase, which have been reported to stimulate dyes decolorization [6]. Thirdly, the roots could pump oxygen into the rhizoshpere, and form the anaerobic and aerobic microenvironment, which is essential for the biodegradation of the azo dyes [7]. Fourthly, the plants could adsorb the dye from wastewater, which could decolorize the water, and increase the contact of the microorganisms to the azo dyes. Some researches stated that algae, such as *Oscillatoria*, *Chlorella pyrenoidosa* and *Chlorella vulgaris* had the capability of decolorizing dye wastewater, and suggested to use these algae to treat the lake and river contaminated by azo dyes [8]. Constructed wetlands, which were previously used for sewage treatment, could successfully decolorize the azo-dye wastewater. The research of Mbuligwe et al. showed that the decolorization rate of constructed wetland with plants was more than twice as high as that of the unplanted bed [9]. The phytoremediation effect varied greatly with the character of the plants, however, the investigation on plants selection for azo-dye wastewater treatment was limited, and the mechanisms were still uncovered.

The present study was carried out to investigate the effect of two salt-tolerant plants, *Medicago sativa* L. and *Sesbania cannabina Pers*, on the bio-decolorization of two widespread azo dyes acid red B and acid scarlet GR. A species of salt-tolerant bacteria GTY (*Gracilibacillus sp.* GTY) with high azo-dye decolorization capability is utilized for azo-dye decolorization, and three treatments, wastewater inoculated with plants, GTY and plant-GTY were conducted to evaluate their effects on the azo-dye wastewater decolorization.

2. Materials and Methods

2.1. Materials

Two kinds of azo-dye, acid red B and acid scarlet GR, were purchased from Tianjin Shengda Chemical Factory (China, Tianjin) and the purity was above 97%. The molecular weight of acid red B and acid scarlet GR are 511 and 508, and the structures of the two azo dyes are shown in Fig. 1.

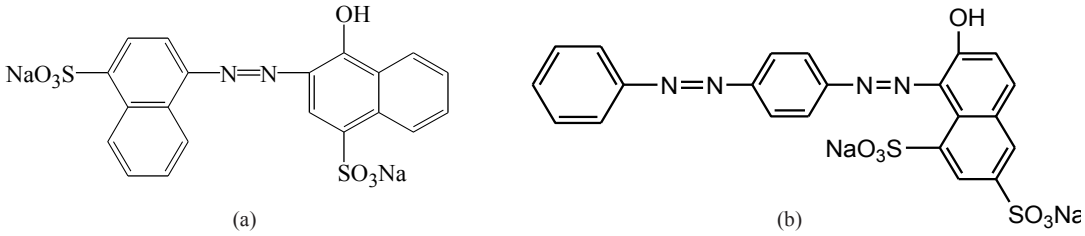


Fig. 1. Chemical structure of (a) acid red B and (b) acid scarlet GR.

Two species of plants, *Medicago sativa* L. and *Sesbania cannabina Pers* are used as experimental plants. *Medicago sativa* L. seeds are purchased from Shenyang Agricultural University, and the *Sesbania*

cannabina Pers seeds are purchased from Dongying Halophytes Garden. Plants are cultivated in the Hoagland's solution.

The salt-tolerant bacteria GTY are isolated from the activated sludge of the seafood processing plants in Dalian. According to the investigation of the Institute of Microbiology Chinese Academy of Science, the optimal growth conditions of this bacteria are as follow: PH = 7.2, temperature = 30ºC, rotational speed = 150 r•min^{-1}. The bacteria were cultivated in the Luria-Bertani (LB) medium.

2.2. Methods

2.2.1. Plants tolerance

The tolerance of the two plants was evaluated by seed germination test and root elongation test in the Hoagland's solution with different NaCl / azo-dye concentration.

Seed germination test. NaCl solution (5.0, 8.0, 10.0, 12.0 g•L^{-1}) and azo-dye solution (0.1, 0.5, 1.0, 5.0 g•L^{-1}) were prepared for the salt tolerance test and azo-dye tolerance test separately, and distilled water was used as control. Three replicates of 10 seeds were used for each condition. Each set of seeds was placed in 10-cm diameter tightly sealed petri plants with 3-layer filter water soaked in 15 ml solution. The seeds were incubated under a thermoperiod of 23/18ºC, and the germination of the seeds was calculated after two weeks incubation.

Root elongation test. The same salt and azo-dye stresses as seed germination test were used in the root elongation test. Three replicates of 10 germinated seeds were used for each condition. The seedlings were cultivated in vermiculite under a thermoperiod of 23/18 ºC (light/dark) and an 18/6 h photoperiod (light/dark, 50 μmol•sec^{-1}•m^{-2}). The root elongations of the seedlings were measured after 1 month incubation.

2.2.2 Plants and GTY for azo-dye decolorization

Azo dyes and NaCl were added into the Hoagland's solution to prepare the artificial wastewater with the final azo-dye concentration of 0.1, 0.5 and 1.0 g•L^{-1} respectively, and NaCl concentration of 5.0 g•L^{-1}. Three treatments, plant, GTY and plant-GTY treatments were conducted for azo-dye decolorization. For the planted treatments, three plant-seedlings of 10 cm were cultured in each of the Erlenmeyer flasks containing 100 ml of wastewater. The experiment lasted for 30 days. The decolorization of the dyes was monitored using UV-Vis spectroscopic (JASCO V-560) analysis, and the measure wavelength of acid red B and acid scarlet GR were 516 and 510 nm respectively.

2.2.3 The effect of root exudates on GTY density

The root was washed by de-ion water before root exudates collection. The roots was soaked in the de-ion water for 2 days, and the root exudates were collected by rotary evaporation. 5 ml GTY bacteria liquid was added into 100 ml de-ion water, 100 ml Medicago sativa L. root exudates and Sesbania cannabina Pers root exudates, and GTY was cultured for 1 day at 30ºC, 150 r•min^{-1}. The OD660 of solution was evaluated (UV-Vis spectroscopic (JASCO V-560)).

2.3. Data analysis

Statistical analysis of the data was performed using one way (azo-dye decolorization and bacteria quantity) ANOVA followed by a Newman-Keuls (SNK) test on SPSS 17 to determine significant differences between treatments ($P < 0.05$).

3. Results and discussion

3.1. The salt and azo-dye tolerance of the plants

The seeds germination rate and root elongation of plants at different NaCl concentration were shown in Fig. 2. The seeds germination rate and root elongation of plants at different concentration of acid red B and acid scarlet GR were shown in Fig. 3 and Fig. 4 respectively. Results showed that the germination rate and root elongation decreased with the NaCl concentration increased. This indicated that the increasing salt concentration hold back the growth of both plants. The *Medicago sativa* L. showed tolerance towards low concentration of azo dyes, since the germination rate and the root elongation showed no significant decrease when the concentration of azo dyes was below 1 g•L^{-1}, while they were significantly decreased when the concentration of azo dyes reached 5 g•L^{-1}. The root elongation of *Sesbania cannabina Pers* decreased with the addition of the azo dyes even the azo dyes were at a low concentration.

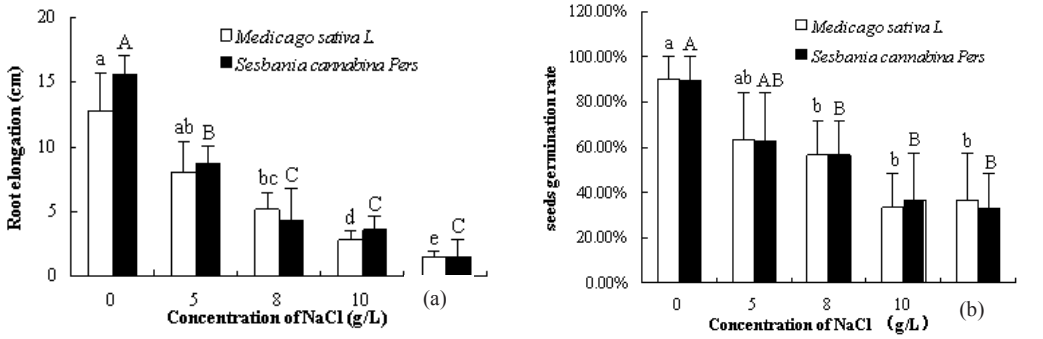


Fig. 2. Seeds germination rate (a) and root elongation (b) of plants at different NaCl concentration. Mean ± SD (n=3).

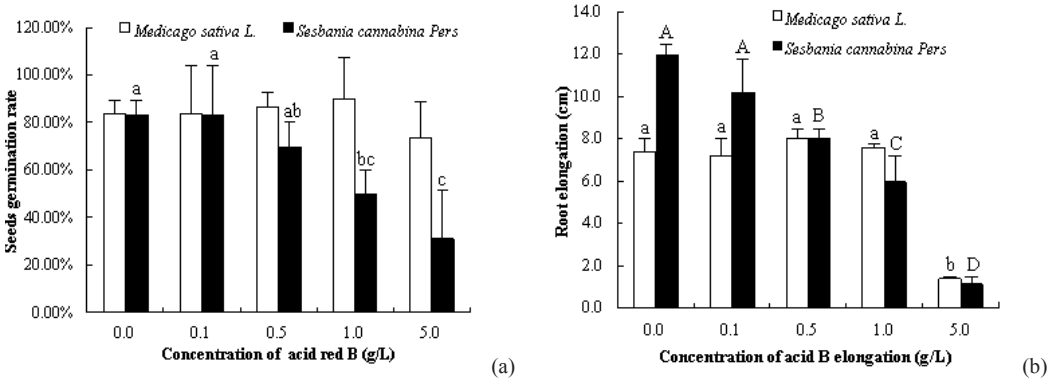
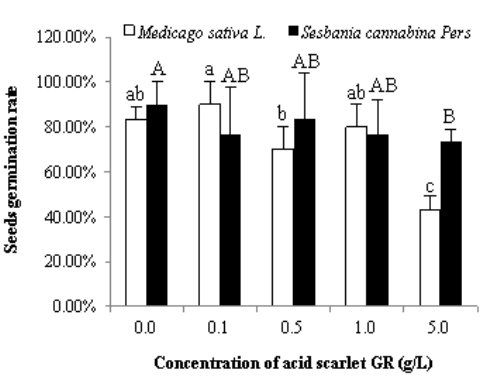


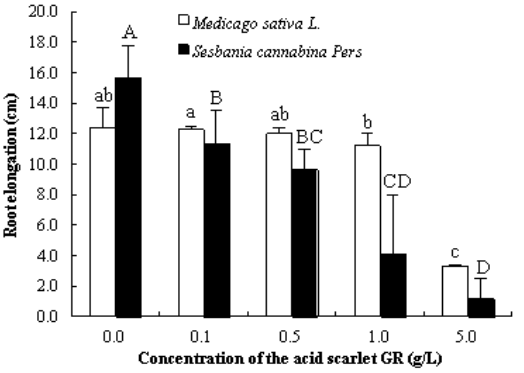
Fig. 3. Seeds germination rate (a) and root elongation (b) of plants at different acid red B concentration. Mean ± SD (n=3).

Plants selection is of great importance for a successful phytoremediation. One of the basic plant selection principle is that the plants could tolerant the pollutants. Pollutants containing in the wastewater, for example the azo-dye, nitrobenzene, and heavy metals, always adversely affect the growth of plants

[10,11]. The azo-dye wastewater in our experiment also inhibited the growth of both plants, since the germination and root elongation of both plants were all decreased in the wastewater with high concentration of the salt and azo dyes. The germination and root elongation of the *Medicago sativa* L. showed no significant decrease at the concentration of azo-dye below 1 g•L^{-1}, while the germination and root elongation of the *Sesbania cannabina Pers* decreased with the azo-dyes addition, even at a low pollutant concentration, indicating that the *Medicago sativa* L. had a higher pollutant tolerance than *Sesbania cannabina Pers*. Previous studies showed high salinity wastewater may bring with the water and nutrient stress, and finally decrease the plant growth. And the adverse result of azo-dyes on the plant growth may be attributed to their inhibition on the ATPase activity of plant, which could finally inhibit the photosynthetic oxygen evolution and plant growth.



(a)



(b)

Fig. 4. Seeds germination rate (a) and root elongation (b) of plants at different acid scarlet GR concentration. Mean ± SD (n=3).

3.2. The effect of plants on azo dyes bio-decoloration

The effects of the two species of plants and GTY on azo dye decolorization were shown in Fig. 4. According to the result, the percentage of azo dye decolorization in GTY, plants and GTY-plants treatments decreased with the increase of the azo dyes concentration. However, the quantity of the azo dye decolorization increased with the increase of azo dye concentration. After one month's incubation, combination of the GTY with the plants obtained higher azo-dye decolorization rate than GTY or plants alone at all the concentration of azo-dye. The acid red B (0.1, 0.5 and 1.0 g·L^{-1}) decolorization in *Medicago sativa* L.- GTY co-existent treatment was 2.0, 2.9 and 3.4 times higher than that of the GTY treatment, while in *Sesbania cannabina Pers*- GTY co-existent treatment was 2.0, 2.4 and 2.6 times higher than that of the GTY treatment. The acid scarlet GR (0.1, 0.5 and 1.0 g·L^{-1}) decolorization in *Medicago sativa* L.- GTY co-existent treatment was 3.3, 3.0 and 3.0 times higher than that of the GTY treatment, while in *Sesbania cannabina Pers*- GTY co-existent treatment was 2.5, 2.4 and 3.0 times higher than that of the GTY treatment. This indicates that both of the plants could promote the bio-decoloration of the azo-dye in the wastewater.

In the past decades, over thousand research papers supported the observation that the addition of plants to the soil and water bodies could enhance the removal of extra nutrients and the pollutants, and the mechanisms could be complex. One of the key reasons is that plant could stimulate the bacteria in the rhizosphere by secreting root exudates. The carbon resource and nutrients contained in the root exudates could support the growth of the bacteria contributing to the azo-dye decolorization. In our experiment, the

effect of root exudates on GTY growth was evaluated, and the GTY density in the solution follows the *Sesbania cannabina Pers* exudates > the *Medicago sativa* L. exudates > de-ion water. This indicated that both of the plants could promote the growth and multiplication of the GTY, which may partly explain the positive effect of plants on azo-dye wastewater decolorization.

The effects of plants on azo-dye decolorization were species-dependent. Results showed that the azo dye decolorization rate in *Medicago sativa* L. treatment was significantly higher than *Sesbania cannabina Pers* treatment, indicating that the *Medicago sativa* L. had higher decolorization capability. In consideration of the higher azo-dye tolerance of *Medicago sativa* L., it may be more suitable to be used in azo-dye wastewater decolorization than *Sesbania cannabina Pers*. It is worth noting that the GTY density was higher in root exudates of *Sesbania cannabina Pers* than *Medicago sativa* L., which indicating that the increase of the quantity of GTY wasn't the only reason for the increase of azo-dye decolorization of plants. Further investigation should focus on the other mechanisms, such as root adsorption, GTY activity stimulation, enzyme or other plant secondary metabolism secretion, which may all benefit the azo-dye decolorization [12].

3.3. The effect of the azo-band number on decolorization

The structure and character of the pollutants always influence the efficiency of the phytoremediation. The two azo dyes investigated, acid red B and acid scarlet GR are of the similar molecular weight, but the acid red B has one azo band, well the acid scarlet GR has two azo bands. Results showed that the decolorization rate of the 0.1, 0.5 and 1.0 $g \bullet L^{-1}$ acid red B solution were 44.23%, 22.58% and 12.23% respectively, which were significantly higher than that of acid scarlet GR (24.32%, 15.91% and 8.66%). The same results were also found in planted treatment. This result was consistent with the research of Noonpui and Thiravetyan, which also found that the one azo-band dye decolorized faster than the two azo-band dyes [13]. This indicated that the structure and number of azo-band strongly affects the efficiency of dye bio-decolorization. And the high remaining concentration of acid scarlet GR probably because that the structure of the two azo-band dyes was steadier than the one azo-band dye, making the bio-decolorization more difficult.

4. Conclusions

The two species of plants, *Medicago sativa* L. and *Sesbania cannabina Pers*, demonstrated their ability to endure the salinity and the toxicity of the azo-dye wastewater and promote the wastewater bio-decolorization. However, there is little understanding of the mechanisms, although we found that root exudates, which could enrich the GTY, might contribute to the azo-dye removal. The *Medicago sativa* L. was more suitable for azo-dye wastewater treatment owing to its higher azo dyes tolerance and removal rate. Further investigation should examine possible phytochemicals of the *Medicago sativa* L. that increase the decolorization of azo-dye by GTY. And the decolorization products should also be investigated to reveal the decolorization pathway of the azo dyes, and to evaluate the possible environmental risk and feasibility of applying this technique in actual azo-dye wastewater treatment.

Acknowledgements

This research was financially supported by the ministry of science and technology of People 's Republic of China (No. 2012ZX07202-006).

References

[1] Selvam K, Swaminathan K, Chae KS. Decolourization of azo dyes and a dye industry effluent by a white rot fungus *Thelephora sp*.. *Bioresour Technol* 2003;**88**:115-119.
[2] Banat IM, Nigam P, Singh D, Marchant R. Microbial decolorization of textile-dye-containing effluents: a review. *Bioresour Technol* 1996;**58**:217-227.
[3] van der Zee FP, Villaverde S. Combined anaerobic-aerobic treatment of azo dyes - a short review of bioreactor studies. *Water Res* 2005;**39**:1425-1440.
[4] Yu L, Li WW, Lam MHW, Yu HQ, Wu C. Isolation and characterization of a *Klebsiella oxytoca* strain for simultaneous azo-dye anaerobic reduction and bio-hydrogen production. *Appl Microbiol Biotechnol* 2012;**95**:255-262.
[5] Ong SA, Uchiyama K, Inadama D, Ishida Y, Yamagiwa K. Treatment of azo dye Acid Orange 7 containing wastewater using up-flow constructed wetland with and without supplementary aeration. *Bioresour Technol* 2010;**101**:9049-9057.
[6] Vyas BRM, Molitoris HP. Involvement of an extracellular H_2O_2-dependent ligninolytic activity of the white rot fungus Pleurotus ostrus in the decolourization of remazol brilliant blue R. *Appl Environ Microbiol* 1995;**61**:3919-3927.
[7] Ong SA, Uchiyama K, Inadama D, Yamagiwa K. Simultaneous removal of color, organic compounds and nutrients in azo dye-containing wastewater using up-flow constructed wetland. *J Hazard Mater* 2009;**165**:696-703.
[8] EI-Sheekh MM, Gharieb MM, Abou-EI-Souod GW. Biodegradation of dyes by some green algae and cyanobacteria. *Biodegradation* 2009;**63**:699-704.
[9] Mbuligwe SE. Comparative treatment of dye-rich wastewater in engineered wetland systems (EWSs) vegetated with different plants. *Water Res* 2005;**39**:271-280.
[10] Novotny C, Dias N, Kapanen A, Malachova A, Vandrovcova M, Itävaara M, *et al*. Comparative use of algal and protozoan test to study toxicity of azo and anthraquinone dyes. *Chemosphere* 2006;**63**:1436-1442.
[11] Ko KS, Lee PK, Kong IC. Evaluation of the toxic effects of arsenite, chromate, cadmium, and copper using a battery of four bioassays. *Appl Microbiol Biotechnol* 2012;**95**:1343-1350.
[12] Singer AC, Crowley DE, Thompson IP. Secondary plant metabolites in phytoremediation and biotransformation. *Trends Biotechnol* 2003;**21**:123-130.
[13] Noonpui S, Thiravetyan P. Treatment of reactive azo dye from textile wastewater by burhead (*Echinodorus cordifolius* L.) in constructed wetland: Effect of molecular size. *J Environ Sci Heal A* 2011;**46**:709-714.