Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(1):327-331

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The mechanism of evans blue removal by sunflower

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ABSTRACT

Dyeing wastewater has caused serious synthetic dyes pollution on both water bodies and soil in China. This study investigated the mechanism of azo dye removal by different vegetative organs of hydroponically grown sunflowers. In all treatments, the percent of dye removal by living organs was higher than corresponding dead organs. The results show that decolorization of Evans blue by tissues of sunflowers was partly attribute to degradation and partly due to sorption. Moreover, the biodegradtion played a greater role than sorption in the dye removal. The biodegradation rate of the dye was estimated. Realistic values of Km and Vmax were estimated by a computer program using non-linear regression treatment and Lineweaver–Burk model. In both methods, the rate of azo dye removal from the aqueous solution by roots was the highest, followed by leaves and stems.

Key words: Azo dye; Biodegradation; Biosorption; Phytoremediation

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INTRODUCTION

Total colorant production in China is estimated to be above 420,000 tons per annum, which account for 42% of the total World colorant production [1]. According to incomplete statistics, daily discharge of printing and dyeing wastewater reached 4 million m^3 in China [1]. In China, a large number of organic dyes have escaped and seeped into the environment due to backward dye-manufacturing technology and dye-using by sporadic and small-scale rural textile and synthetic dye industries. This situation made serious synthetic dyes pollution on both water bodies and soil [2]. The development of efficient and environmentally friendly technologies to decrease dye content in wastewater to acceptable levels at affordable cost is of utmost importance [3]. Phytoremediation is a cost-effective technology, and it takes advantage of the fact that a living plant can be compared to a solar driven pump, which can extract and concentrate particular elements from the environment. It entails the use of plants for uptake, sequestration, detoxification, or volatilization of inorganic and organic pollutants from soils, water, sediments, and possibly air [4].

Studies have discovered that peroxidases and polyphenol oxidases from plants could effectively degrade many kinds of synthetic dyes [5-8]. Some researches have confirmed that constructed wetlands can effectively degrade azo dye wastewater [9, 10]. Aquicultural plant system experiments showed that plants can decolorize wastewater containing dyes [11-14]. Some aquicultural plant system experiments further showed that plants can degrade dyes [13-15]. In an early research, the authors founded that aquicultural sunflowers seedlings can decolorized 20 mg L^{-1} Evans blue wastewater completely. By visual inspection, it was found that Evan blue appeared a very little in root surface and didn't accumulate in the vascular. The authors inferred that Evans blue was mostly degraded [16]. Although plants have been demonstrated to remediate dyes contamination [9-14], little information has been obtained concerning the enzyme kinetics parameters of different species used in dye metabolism. In order to explore the role of biodegradation and biosorption in the process of dye removal, dye removal tests with detached living organs and

dead organs was conducted. The percent of biosortion and biodegradtion were estimated. Michaelis–Menten kinetics of Evans blue removal by vegetative organs of sunflowers was also estimated.

EXPERIMENTAL SECTION

Materials

The Evans Blue (CI, 23860) was purchased from Shanghai Huanlan Chemical Company, China. Commercial oilseed type sunflower (*Helianthus annuus* L.) seeds, Xinza number 5 were purchased from market of Changji City, Xinjiang Uygur Autonomous Region, China. Sunflower seeds were sowed in vermiculite moistened with distilled water. After 7 days, the healthy sunflower seedlings, whose roots were washed with running tap water to remove adherent vermiculite, were placed in conical beakers containing half strength Hoagland's hydroponic nutrient solution. Those conical beakers covered by black plastic film to deter algal growth. The seedlings were suspended in the conical beakers using rubber plugs with a hole so only the roots were in contact with the hydroponic growth solution. The seedlings were cultured under 40-watt cool white fluorescent tubes with continuous light at room temperature (24-25℃) for 10 days.

Removal tests with detached living vegetative organs

Sealed glass flasks containing Evans blue solution and plant organs were used as a reactor. Four different treatment levels of Evans blue were tested, namely 20 mg L-1, 40 mg L-1, 80 mg L-1, 100 mg L-1. The leaves, stems and roots of sunflowers were cut into small pieces, precisely weighed (1.0 g fresh weight for leaves and stems; 0.5 g fresh weight for roots) and placed in each flask containing 100mL Evans blue solution. For each organ, three separate flasks containing plant tissue and aqueous spiked solution were used for each treatment concentration. The flask reactors were closed with glass stoppers and were shaking with hands for a while, and then placed in an incubator kept at a constant temperature of 25±0.5℃ for 31 hours. During incubation period, aliquots (1.0 mL) solutions were withdrawn from each flask at 4, 8, 12, 23, 27 and 31 hours after incubation. The aliquots were centrifuged at rotation speed 5000 r/min for 10 min and supernatant was separated. Decolorization of the dye was monitored by measuring the absorbance of the supernatant of dye solutions at its absorption maxima 610 nm by a Hitachi U-1100 Spectrophotometer. The standard curve of the dye was made. The remaining concentration of each dye was calculated through the standard curve regression equation. The decolorization percentage was calculated as:

$$\text{Percent decolorization}=\frac{\text{Concentration of untreated dye}-\text{Concentration of treated dye}}{\text{Concentration of untreated dye}}\times 100$$

Removal tests with detached dead vegetative organs

To aid in distinguishing biodegradation from sorption by plant organs, a sorption experiment with cell wall skeletons of organs of sunflowers was performed. Organs of sunflowers as those used in the uptake experiment described above were transferred to a 2:1 (v/v) solution of methanol: chloroform (MC) for 3 d. This treatment strips the biotic material from the organs, leaving a cell wall skeleton that can be used to examine sorption processes at the organs wall level in isolation from biological processes [17]. The 1.0 g fresh weight of leaf, stem and 0.5 g fresh weight of root after this treatment was flushed with distilled water then used in dye removal experiment. The other procedure and method are same as experiment with living vegetative organs.

RESULTS AND DISCUSSION

Removal efficiency of Evans blue by detached living and dead vegetative organs

The concentration of Evans Blue in the aqueous solution was analyzed over time of incubation. Fig.1 presents an illustrative example of the measured concentrations of 20 mg L^{-1} dye solution treated with living or dead organs. In the 20 mg L^{-1} dye solution treatment, the decolorization percentage treated with living leaves, stems and roots was 48.7%, 47.7% and 40.6% respectively after 31 hours, while that of the dead leaves, stems and roots was 14.2%, 20.2% and 14.1% respectively. In the 40, 80 and 100 mg L^{-1} dye solution treatment, results were similar to that of 20 mg L^{-1} dye solution treatment groups. The removal efficiency of living organs was significantly higher than that of corresponding dead organs in all treatments. It can be inferred that the decolorization of dye was partly attribute to degradation and partly due to sorption.

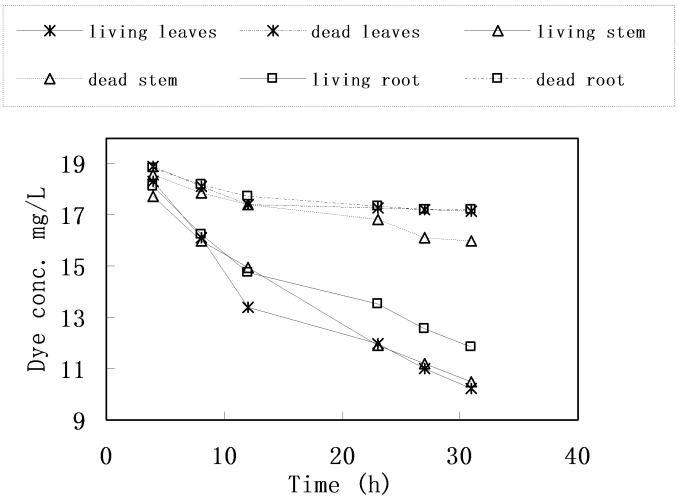

Fig. 1 Measured concentrations (mg L^{-1}) of dye in closed vessel with living or dead organs of sunflowers at 20 mg L^{-1}. The values are the mean of three replicates. Standard deviations are not shown in figures.

Estimation the percent of dye biodegradation and biosorption in the solution

The removal of dye by dead organ is just a physical biosorption process, while the removal of dye by living organ involves not only physical biosorption process but also biodegradation process. The fraction of representing presumed Evans blue biodegradation was estimated as the difference between the dye concentrations removal by living organs of sunflowers and that by corresponding

Table 1 The percent of dye biodegradation and biosorption in the solution at the end of the experiment

Dye concentration	Biodegradtion [%]			Biosorption [%]		
[mg/L]	leave	stem	root	leave	stem	root
20	70.9	70.3	65.1	29.1	29.7	34.9
40	61.2	55.5	66.7	38.8	44.5	33.3
80	53.6	39.3	67.4	46.4	60.7	32.6
100	48.2	35.3	67.8	51.8	64.7	32.2

dead organs of sunflowers. The percent of dye biodegradation and dye biosorption in the solution at the end of the experiment was calculated (Table 1). In average, biosorption of leaves, stems and roots accounted for 41.5%, 49.9% and 33.3% respectively of the total dye removal during 31 hours, while biodegradation of leaves, stems and roots accounted for 58.5%, 50.1% and 66.8% respectively. It was found that up to 19% of the decolorization was caused by biosorption of the acid anthraquinone dye tectilon blue onto the biomass, with the majority of the decolorization caused by utilization of the dye by bacteria [18]. In our experiment, the percent of dye removal of the biosorption by organs of sunflowers were much higher than that of bacteria. One of the reasons may be that enzyme activity of detached plant organs was reduced.

Calculation of dye biodegradation capacity

An enzyme-catalyzed reaction is most conveniently assessed by measuring the rate of appearance of the product or the rate of disappearance of a substrate. The rate of disappearance of dye introduced by biodegradation was used to calculate. In this investigation, the biodegradation rates of Evans blue were determined from the slope of the plot of the amount of aqueous dye degradation by plant tissues versus time (mgkg^{-1} h^{-1}) according to Yu's method [19]. The best fit was obtained with linear regression and judged by the regression coefficient R^2. Data of the exposure period from 0 to 12 h were used in the calculation. Table 2 shows the calculated degradation velocities at each treatment concentration of different organs. Of all organs, roots showed the highest degradation rate in all dye concentrations, followed by leaves, and stems. The dye degradation rate of leaves at the treatment concentration of 100 mg L^{-1} was 70.9 mg kg^{-1} h^{-1}， which was much higher than that of the lowest treatment concentration 20 mg L^{-1}.The degradation rate of stem and roots also had the similar phenomena. All these results showed that the rate of dye degradation from the aqueous solution by plant materials increased with applied dye concentration, indicating that the reaction was substrate controlled.

Table 2 Calculated dye removal capacity per sunflowers organ [mgkg^{-1}h^{-1}]at various treatment concentrations

Different organs	20 [mg/l]		40 [mg/l]		80 [mg/l]		100 [mg/l]	
	v	R^2	*v*	R^2	*v*	R^2	*v*	R^2
Leave	43.8	0.9923	52.9	0.9909	58.7	0.9885	70.9	0.9856
Stem	20.4	0.9704	24.6	0.9767	24.9	0.9974	28	0.9954
Root	57.2	0.9987	75.8	0.9578	79.8	0.9014	93	0.9749

Determination of enzyme kinetics (K_m and V_{max})

One of the most common mathematical function in use to relate enzyme activity (*v*) to substrate concentration (C) is the Michaelis-Menten equation [20]:

$$v = \frac{v_{\max} c}{k_m + c} \times M$$

where v (mg h^{-1}) is the degradation rate of the substrate concentration C (mg L^{-1}), V_{max} (mg kg^{-1} h^{-1}) is the maximal degradation velocity, K_m (mg L^{-1}) is the half-saturation constant (the substrate concentration where the disappearance velocity is half the maximum).

Since the relationship between the independent variable, C, and the dependent variable, v, is curvilinear, it has long been customary to facilitate estimation of the two parameters by plotting the experimental data according to the following linear transformations Equation [21]:

$$\bar{v} = \frac{k_m}{v_{\max}}\left(\frac{1}{c}\right) + \frac{1}{v_{\max}}$$

This is a linear equation in 1/v and 1/C. The slope of the line is K_m/V_{max}, the 1/v intercept is $1/V_{max}$ and the extrapolated 1/C intercept is $-1/K_m$. Unfortunately, linear equation introduces bias in the parameter estimates which are disproportionately influenced by the experimental error in data obtained at high and low substrate concentrations [22]. Therefore, kinetic data were also analyzed by computer software using non-linear regression plots, provided by Science Press [23]. Table 3 gives the details of the estimates of Evans blue metabolism kinetics for the investigated organs. Both linear regression and non-linear regression treatments, the highest V_{max} was observed with roots, followed by the leaves. The lowest V_{max} was observed with stem. Larsen et al. used a similar test system without consideration of sorption to determine Km and V_{max} of cyanide removal for willow leaves. The results showed that V_{max} with leaves is higher than V_{max} with roots [24].

Table 3 Calculated kinetics（vmax and Km）of dye for different organs

Different organs	Non-linear fit			Line weaver-Burk		
	V_{max}(mgkg^{-1}h^{-1})	$k_{m(mgh^{-1})}$	R^2	V_{max}(mgkg^{-1}h^{-1})	$k_{m(mgh^{-1})}$	R^2
Leave	76.72	16.41	0.9267	73.72	14.07	0.9559
Stem	29.21	8.46	0.9347	29.14	8.45	0.9554
Root	102.44	15.55	0.9541	102.24	15.55	0.9755

CONCLUSION

The results show that decolorization of Evans blue by tissues of sunflowers was partly attribute to degradation and partly due to sorption. Plant root has large root surface area that provides a good adsorption media. In average, biosorption of roots accounted for 33.3% of the total dye removal during 31 hours. Recent studies have shown that peroxidases and polyphenol oxidases from plants could effectively degrade many kinds of synthetic dyes [6-8].The calculated reaction constants indicated that roots and leaves degraded the dye at a faster velocity than stem. Because sunflowers are fast grown plants, it is efficient to use them as phytoremdiation tool.

Acknowledgements

The authors appreciate the financial support from projects of Special Forestry Public Welfare Industry Research Program “The Regional Characteristics and Construction Techniques of Beautiful Town Forest Landscape in Shandong Peninsula”（201404301）and Shandong province science and technology development plan “Research and Demonstration of ecological remediation for spring protection in southern mountain area of Jinan and NSFC Support（31170662）.

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