Paper D19

Study on influence of leather fatliquor on the biodegradation of dye

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Abstract: The leather fatliquor and dyes are the two main types of chemical reagent in the leather production process. Most fatliquor are easily biodegradable organic compounds, but almost all dyes are refractory organic matter. In the leather production process, fat-liquoring and dyeing are in one bath, fatliquor and dyes are difficult to separate by the physical and mechanical methods in the sewage treatment process. In this paper, aiming to discuss influence of various commonly used fatliquor on the biodegradation, the decolorization and biological oxygen demand of dyes in the co-substrate with fatliquor were measured after static culture. And influence of fatliquor on the anaerobic decolorization of dyes was also examined. Promotion of oxidation-sulfited fatliquor to the anaerobic degradation of acid fuchsin and acid scarlet G. was certificated by the experiment results These works will provide a theoretical basis for more environmentally friendly material choice in the leather production process.

Keywords: fat-liquoring agent; dye; biodegradation; aerobic; anaerobic

1 Introduction

There are many kinds of dye used in the leather dyeing process, which have a complex structures and almost all the dyes are refractory organic pollutant. Remnants of the dye wastewater even in small concentrations can cause reduced water transmittance, thus leading to destruction of aquatic ecosystems [1]. Reason of biodegradation difficulty for the refractory organism is usually because that it is difficult for microbial growth in the substrate which refractory organic pollutants as sole carbon source, but easily biodegradable organic matters to be spiked in the medium can increase microbial activity and provide an effective carbon and energy for synthesis of enzymes to be involved with degradation of refractory organic pollutant, thus these easily biodegradable organic matters can improve the biological degradation of refractory organic pollutant. Previous studies in relation to the biodegradation of dyes have shown that sugar can effectively improve degradation of dye [2, 3]. But because of the particularity of leather manufacture, there are little sugars in tannery waste water, so it needs to add sugars in the process of waste treatment to promote the biodegradation of dyes if we used sugars as easily biodegradable organic matters, thus leading to increase of cost of wastewater treatment. Yet contents of protein and fat are higher in the tannery waste water, moreover, previous studies have shown that all anionic and nonionic fatliquor with neutral fat

or fatty acid ester as neutral oil are easily biodegradable substances, which there are the possibility using fatliquor to provide effective carbon and the energy to promote the biodegradation of the dye.

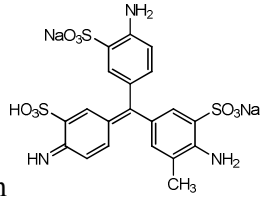
The leather fatliquor is mainly composed of two compositions of neutral oil and emulsion composition. The neutral oil is mainly with the structure of neutral fat and fatty acid esters or hydrocarbon, and the emulsion composition is composed mainly of amphiphilic molecular structure, that is surfactant. In the biodegradation process of fatty-based fatliquor, first fatliquor has been hydrolyzed into fatty acids and low molecular alcohol under the action of lipase, meanwhile the alcohol transmit directly into bacterial cells and then stimulate bacterial growth. Simultaneously fatty acids is broken down into CO_2 and H_2O in aerobic conditions, the aerobic biodegradation will release large amounts of energy and reduce the oxygen content in water. The degradation of fatty acid in the anaerobic conditions will produce large amounts of H_2 which can promote the broken of azo bond in azo dyes. Moreover, the emulsion composition can promote transmittal of gaseous matter in the bottom of water medium, and thus affecting degradation of dye. Therefore, the paper studied on influence of several fatliquor to be commonly used in the leather production on the biodegradation of three dyes, in order to discuss the possibility of utilizing fatliquor to promote the biodegradation of dye.

2 Materials and methods

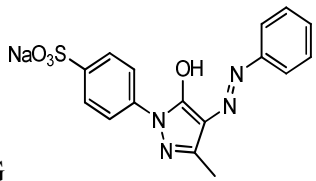
2.1 Reagents and instruments

2.1.1 Chemicals and reagents

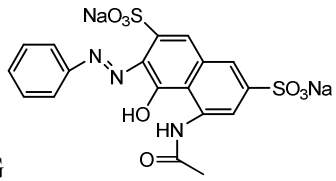
Dyes: Acid Fuchsin, Acid Light Yellow G and Acid Scarlet G are commercial pure from Tianjin Yadong Chemical Dye of China.



Acid Fuchsin



Acid Light Yellow G



Acid Scarlet G

Inorganic salt medium: K_2HPO_4 21.75mg/L, KH_2PO_4 8.5mg/L, Na_2HPO_4 16.58mg/L, NH_4Cl 1.7mg/L, $MgSO_4$ 11.14mg/L, $CaCl_3$ 27.5mg/L, $FeCl_3$ 0.15mg/L.

Co-substrate: phosphorylated fatliquor (Limited Dermino SF Liquid of Clariant Chemicals (China)); oxidation-sulfited fatliquor (DT-F210 of Tingjiang new material INC.); non-ionic fatliquor (Limited Dermafimish LB Liquid of Clariant Chemicals (China)).

2.1.2 Instrument

DZF-6021 vacuum oven; 303-1 type electric incubator; QYC-200-type shock incubator;

TGL-16G-type centrifuge; UV-2000 type and UV-2550 UV/VIS spectrophotometer; self-made COD-measured instrument.

2.2 Analysis

2.2.1 Estimating biomass concentration X (mg/L) [4]

The supernatant of a sample which was centrifuged 10 min at 10,000 rpm is as the blank. Absorbance at 600 nm wavelength (OD600) of the sample was measured using a UV/VIS spectrophotometer, and then the optical density was converted to biomass concentration using a dry weight calibration curve.

2.2.2 Determination of dye concentration

The concentration of dye was detected spectrophotometrically by reading the supernatant culture at its specific maximum wavelength after centrifuging at 10,000 rpm for 10 min. The dye concentrations were determined from the attenuance (OD) of the culture at maximum absorption wavelength of the dye. Determination of absorption wavelength of dye acid fuchsin λ = 525nm, Acid Yellow G λ = 390nm, acid red G λ = 510nm.

2.2.3 COD_{Cr} measurement

Using the method of GB 11914-89 (Water quality-Determination of the chemical oxygen demand-Dichromate method).

2.2.4 Dissolved oxygen (DO) measurement

Using the method of GB 7489-1987(Water quality-Determination of dissolved oxygen-Iodometric method).

2.3 Methods

2.3.1 Decolorization of dyes under different substrates

The dewatering activated sludge used in the study was attained from a tannery wastewater treatment plant in China's Shandong Province. The sludge can provide aerobic and anaerobic microbial activity. The diluent of the activated sludge is as inoculum. Samples of the test are Acid Fuchsin (AF), Acid Light Yellow G (ALY), Acid Scarlet G (AS), phosphorylated fatliquor (SF), oxidation-sulfited fatliquor (DT) and non-ionic fatliquor (LB).

Test Control 1: AF + inorganic substrate + inoculum;
Test Control 2: ALY + inorganic substrate + inoculum;
Test Control 3: AS + inorganic substrate + inoculum;
Test 1.1: AF + SF + inorganic substrate + inoculum;
Test 1.2: AF + DT + inorganic substrate + inoculum;
Test 1.3: AF + LB + inorganic substrate + inoculum;
Test 2.1: ALY + SF + inorganic substrate + inoculum;
Test 2.2: ALY + DT + inorganic substrate + inoculum;
Test 2.3: ALY + LB + inorganic salt substrate + inoculum;
Test 3.1: AS + SF + inorganic substrate + inoculum;

Test 3.2: AS + DT + inorganic substrate + inoculum;
Test 3.3: AS + LB + inorganic substrate + inoculum.

In the substrates, the concentrations of dyes all were 50mg/L, the concentrations of fatliquor were 200mg/L, the dry weight concentrations of biological cells to be inoculated into substrate were 42mg/L. All the samples were divided in duplicate, one was at 30 ℃ after 15 days shaking culture in 110 rpm, another was at 30 ℃ after 15 days static culture. The absorbance of the dye in the medium before culture (A_1) is as the culture, the absorbance of the supernatant culture (A_2) after shaking and static culture is respectively measured, then decolorization activity was calculated as follows:

$$\text{Decolorization}(\%) = \frac{\text{Initial absorbance } (A_1) - \text{Observed absorbance } (A_2)}{\text{Initial absorbance } (A_1)} \times 100\%$$

2.3.2 To estimate biological oxygen demand of dyes in different substrates

50 mg/L dye, 50 mg/L dye + 200 mg/L SF, 50 mg/L dye + 200 mg/L DT, 50 mg/L dye + 200 mg/L LB solutions were prepared and as the test group, respectively corresponding distilled water, 200 mg/L SF, 200 mg/L DT, 200 mg/L LB solution as a control test, and the inorganic salt medium inoculated after aeration as the inoculated dilution water, test and control water samples were diluted according to the dilution ratio of 1 ∶ 50. And then the two culture flasks were filled with dilution to overflow by siphon and then covered with lid. The culture flasks were divided into two groups, and each group contains the test group and control group, one group is put in the incubator and in the dark for 5 days. On the starting point in time, dissolved oxygen concentrations of another group was measured. After cultured 5 days, dissolved oxygen concentrations of the group in the incubator were measured, then calculating biological oxygen demand of dye after 5 days according to the following formula.

$$\text{Biochemical oxygen demand of dye} = \left[(c_1 - c_2) - 0.98 \times (c_3 - c_4)\right] \times 50$$

Where: C_1 is dissolved oxygen concentration in water sample of a dye test in the initial time, its unit is mg/L; C_2 is dissolved oxygen concentration in water sample of the dye test after 5 days culture, its unit is mg/L; C_3 is dissolved oxygen concentration in water sample of respective control test in the initial time, its unit is mg/L; C_4 is dissolved oxygen concentration in water sample of the control test after 5 days culture, its unit is mg/L.

2.3.3 Analysis of UV/Visible Spectrophotometer

Samples contain 50 mg/L of dye and then were in static culture 15 days. UV/Visible spectra of culture supernatants of the initial time and 15 days were compared and possible degradation products were speculated.

2.3.4 Experimental design of biodegradation kinetics of

In the paper, the highest and lowest concentration point of fatliquor was set in 80mg/L

and 8mg/L respectively, and 10 points were set averagely. Accessing to 200mg/L of oxidation-sulfited fatliquor (DT) were as the test group, no-accessing to DT were as the control test group, and then medium had been inoculated with bacteria and inorganic nutrient solution. The dry weight concentration of bacterial cells is 78 mg/L. The samples were cultured 10 days at a static state in constant temperature of 30 ℃. Then biomass change and the residual concentration of dye in the medium were measured. The specific degradation rate was calculated according to following formula.

$$\text{The specific degradation rate } q_S = \frac{(C_1 - C_2)}{t \cdot X_0}$$

Where: qS is specific degradation rate, it means COD concentration (mg/L) of organic matter biodegraded by unit concentration (mg/L) of activated sludge per unit time (day), its unit is d-1, it reflects the biodegradability of fatliquor in different concentrations; C_1 and C_2 is the concentration of tester in the medium before and after static culture respectively, their unit are mg/L; X_0 is the initial biomass concentration in the solution, its unit is mg/L; t is the degradation time (day).

3 Results and discussion

3.1 The influence of fatliquor on decolorization of dyes

Experimental results of decolorizations of different dyes in shaking culture are shown in Table 1:

It can be seen from Table 1, after 15d shaking culture, all medium to contain acid fuchsin and acid scarlet G have decolorizations, while substrates adding a fatliquor as carbon source, decolorizations of acid fuchsin and acid scarlet G were more than the respective comparison test, but increasing decolorization is not large, this may be because acid fuchsin and acid scarlet G were more difficult to be biodegraded under aerobic conditions, and the decolorization of dyes are achieved mainly by adsorption of the bacterial gel in medium. So the substrates to be added fatliquor, under stimulation of fatliquor, increase microbial growth, thus lead to enhance biological adsorption of dyes. The previous studies have shown that phosphorylated fatliquor can promote a large number of bacteria growth [5], which can explain decolorization of mediums containing phosphorylated fatliquor are greatest under aerobic conditions.

Table 1 Decolorizations (%) of different dyes in shaking culture under different substrate

Co-substrate \ Dye	AF	ALY	AS
Blank	14.99	- ①	21.55
SF	19.07	0.47	33.57
DT	16.88	-	29.39
LB	17.82	-	24.91

① it means that substrates don't have decolorization after shaking culture.

Experimental results of decolorizations of different dyes in static culture are shown in Table 2:

Table 2 Decolorizations (%) of different dyes in static culture under different substrate

Co-substrate \ Dye	AF	ALY	AS
Blank	35.29	- ①	14.16
SF	39.88	-	33.57
DT	77.39	-	87.53
LB	35.59	-	14.48

① it means substrates don't have decolorization after static culture.

It can be seen from Table 2, after 15d static culture, substrates adding a fatliquor as carbon source, decolorizations of acid fuchsin and acid scarlet G were more than the respective comparison test, but the promotion of phosphorylated fatliquor on biological decolorization of the two dyes obviously is less than that of the oxidation-sulfited fatliquor. This may be because under anaerobic conditions, acid-producing bacteria ferment low molecular weight dissolved organic matters into acetate, and phosphorus-accumulating bacteria hydrolyze phosphorus accumulation to exist in self-cell, the hydrolysis release the partial energy to be utilized for absorption of acetic acid, H^+ and e^-, these matter form PHB to be stored in the bacteria, while the release of phosphate, but the biodegradation of phosphorylated fatliquor produce large amounts of phosphate to be released into solution, which affects the physiological activity of phosphorus-accumulating bacteria under anaerobic conditions. Moreover, in the medium of adding the oxidation-sulfited fatliquor, decolorizations of acid fuchsin and acid scarlet G is 77.39% and 87.53% respectively, indicating that efficacious promotion of oxidation-sulfited fatliquor on biodegradation of these two dyes. Furthermore the medium with acid fuchsin after the static culture appear color transformation and trend purple color. Color of acid fuchsin substrate with inoculum and non-inoculum after 15d static culture was shown in Figure 1.

This should be because chromophore and auxochrome of acid fuchsine have been broken and some colored intermediate appeared under anaerobic condition, so that the medium only containing the acid fuchsin and also that being added a non-ionic fatliquor appear a purple color. But degradation of phosphorylated and oxidation-sulfited fatliquor consumes a lot of oxygen in the initial time, the medium become quickly in anaerobic conditions, thus promoting the biodegradation of these intermediate, leading to the medium containing these two fatliquor showed light red. It indicated that fatliquor had promoted biodegradation of dyes under anaerobic conditions consequently promoted the biological decolorization of dye.

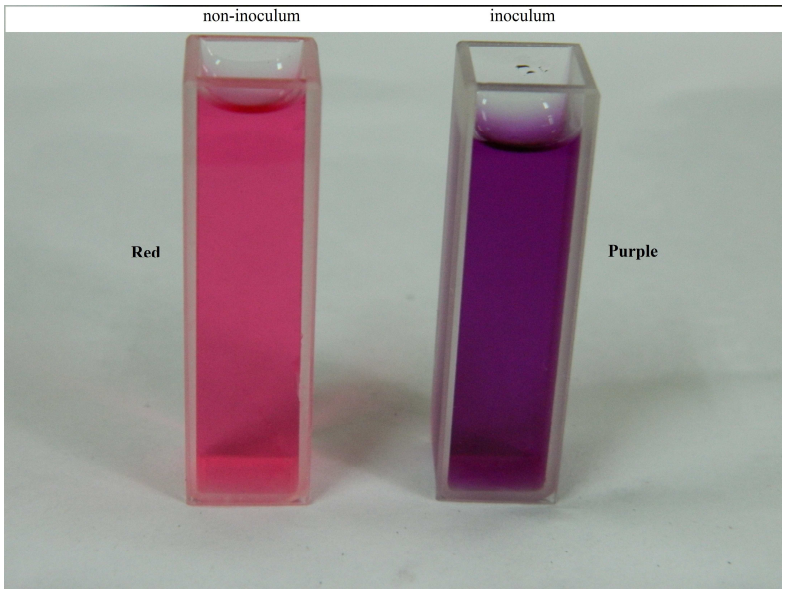


Figure 1 The color of acid fuchsine with inoculum and non-inoculum after 15d static culture

From the Table 1 and Table 2, it can be found that fatliquor had no significant effect on promoting biological decolorization of acid light yellow G in shaking and static culture conditions on, which may be because that acid light yellow G is not biodegradable in aerobic or anaerobic conditions, while adsorption capacity of micro-organisms for the dye is too small to promote adsorptive decolorization of acid light yellow G to utilize fatliquor. From the Table 1 and Table 2 can also be found that the non-ionic fatliquor don't have obvious effect on promoting the decolorization of dyes in shaking or static culture conditions , it is because the non-ionic fatliquor in this research is composed of a mineral oil and an emulsifier, and mineral oil is mostly straight-chain alkanes structure, it is difficult to be biodegradable, its biodegradability is substantially lower than anionic fatty-based fatliquor [6], and it does not stimulate a lot of microbial growth in substrate, while providing adequate energy and carbon source is difficult for synthesis of enzymes in relation to degradation of dyes. Therefore, promotion of fatty-based fatliquor to biological decolorization of dye is greater than mineral-oil-based fatliquor.

3.2 Biological oxygen demand of dyes in different substrates

In order to determine whether the biodegradation of dye exists under aerobic conditions, the paper has estimated biological oxygen demand of dyes in different substrates; the results are shown in Table 3.

The Table 3 shows that biological oxygen demands of dyes after 5d culture are all negative, i.e. the biological oxygen demand of test group added a dye are less than the control group. It indicates that the promotion of fatliquor on biological decolorization of dyes under aerobic conditions mainly is because it promotes bacterial adsorption of dye by increasing microbial growth and dye decrease biological activity in the medium leading to decrease the biodegradation of fatliquor.

Table 3 Biological oxygen demands of dyes in different substrates after 15d culture

Co-substrate \ Dye	AF	ALY	AS
Blank	-1.79824	-5.84427	-7.64251
SF	-29.43	-23.8266	-2.69736
DT	-58.2341	-26.4251	-56.1949
LB	-26.3154	-26.9736	-68.333

3.3 Result of UV/VIS-spectra analysis

The UV-VIS absorption spectra corresponding to initial and final samples of decolorization experiments for acid fuchsin and acid scarlet G respectively from 350 to 630 nm are shown in Figure 2 and Figure 3. The initial acid fuchsin and acid scarlet G solution respectively showed high peak at the wavelength of 524nm and 509nm. The decolorized dye trended to disappearance of peak after static culture, and the absorbance peak of the dyes in the medium containing DT almost disappeared, which indicate that the decolorization is due to dye degradation [7].

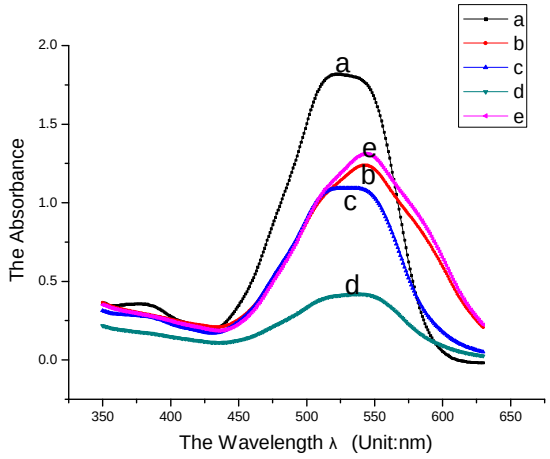


Figure 2 The UV-visible absorption spectra of acid fuchsin

a - UV/VIS-spectra of the initial acid fuchsin solution;
b - UV/VIS-spectra of the acid fuchsin in the medium which is not added fatliquor;
c - UV/VIS-spectra of the acid fuchsin in the medium which is added SF;
d - UV/VIS-spectra of the acid fuchsin in the medium which is added DT;
e - UV/VIS-spectra of the acid fuchsin in the medium which is added LB.

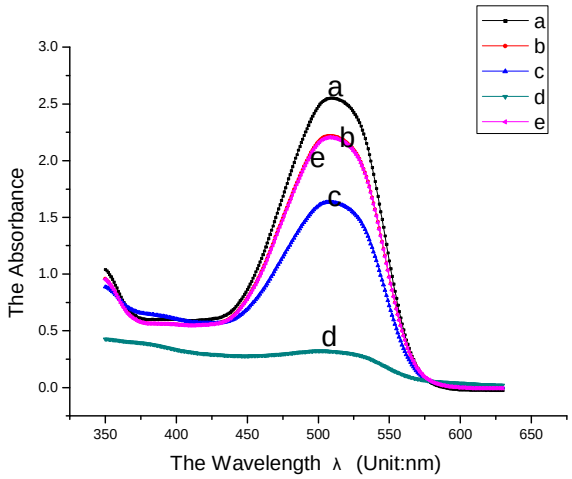


Figure 2 The UV-visible absorption spectra of acid scarlet G

a - UV/VIS-spectra of the initial acid scarlet G solution;

b - UV/VIS-spectra of the acid scarlet G in the medium which is not added fatliquor;

c - UV/VIS-spectra of the acid scarlet G in the medium which is added SF;

d - UV/VIS-spectra of the acid scarlet G in the medium which is added DT;

e - UV/VIS-spectra of the acid scarlet G in the medium which is added LB.

3.4 The degradation kinetics of dye in co-substrate

In order to discuss the specific effects of fatliquor on the biodegradation of dyes, the models in relation to effects of DT on the biodegradation of acid fuchsin and acid scarlet G in static culture have been established. For microbial growth in the mixture medium, the bacterial growths of double growth-limiting substrates can be divided into three cases with concurrent substrate utilization, sequential substrate utilization and sequential substrate elimination [8], and assuming that the degradation of dye and fatliquor is a case of concurrent substrate utilization, and dye does not support the growth of microbial cells including sequential use, i.e. the co-substrate degradation of dye and fatliquor is the co-metabolism, thus the following model have been built according to Criddle's previous studies [9].

$$q_c = \left(T_c^g q_g + q_{c,\max}\right)\left(\frac{S_c}{K_{S_c} + S_c + \frac{K_{S_c} S_g}{K_{ig}}}\right)$$

where q_c is the specific degradation rate of a non-growth substrate which is the dye (Unit: d^{-1}); q_g is the specific degradation rate of a growth substrate which is the DT (Unit: d^{-1}), and the concentration of DT is calculated by measuring solution COD_{Cr} in the medium where fatliquor is as single-limited substrate; $q_{c,\max}$ is the maximum specific degradation rate of dye in the medium where dye is as single-limited substrate (Unit: d^{-1}); K_{Sc} is the half-saturation coefficient of dye in the medium where dye is as single-limited substrate (Unit: mg/L); S_c is the concentration of the dye in the medium (Unit: mg/L); T_{cg} is the transformation capacity

coefficient of growth substrate, characterizing activity of DT in promoting the degradation of dye (Unit: 1); K_{ig} is the inhibition coefficient of the growth substrate (DT fatliquor) to the degradation rate of non-growth substrate (Unit: mg/L). The fitting curves of model for the experimental data of acid fuchsin and acid scarlet G are respectively shown in the following Figure 4 and Figure 5.

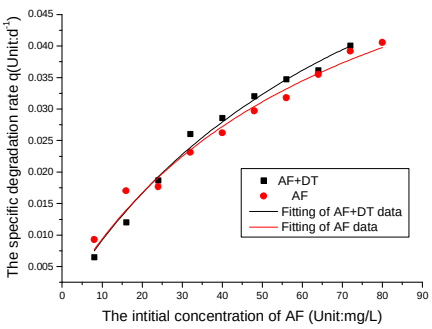


Figure 4 The fitting curve of model for acid fuchsin

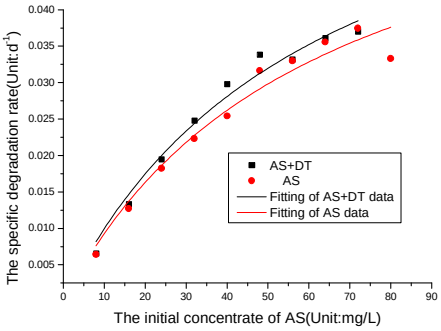


Figure 5 The fitting curve of model for acid scarlet G

The fitting parameters of the model were shown in the following Table 4:

Table 4 The fitting parameters of the model in relation to the co-metabolism of the dyes

Parameter / Substrate	$^{q}_{c}$	K_{Sc}	T_c^g	K_{ig}
AF+DT	0.07384	68.53795	0.0250844	847.481
AS+DT	0.06658	61.65168	0.0410785	55452.1

Table 4 shows that the transformation capacity coefficient of DT on the degradation of acid scarlet G is greater than on the degradation of acid scarlet G, indicating that promotion of DT on biodegradation of acid scarlet G is more efficient. Moreover the inhibition coefficient of DT on the degradation rate of acid scarlet G is greater than that of acid fuchsin; it indicates that the competitive inhibition of DT on the biodegradation of acid scarlet G is less than that of acid fuchsin.

4 Conclusions

(1) Both in aerobic or anaerobic conditions, fatliquor all have stimulative effects on biological decolorization of acid fuchsin and acid scarlet G, but to acid yellow G it isn't as so. Moreover, Stimulative effects of mineral-oil-based fatliquor on biological decolorization of dye are less than that of fatty-based fatliquor.

(2) In the shaking culture, the decolorization of acid fuchsin and acid scarlet G mainly depend on the biological adsorption, so added fatliquor has improved the physical adsorption of dye by stimulating sludge microbial growth, leading to promote aerobic

biological decolorization of dyes.

(3) In the static culture the decolorization of acid fuchsin and acid scarlet G mainly depend on the biodegradation of dyes. Oxidation-sulfited fatliquor is efficient to promote biodegradation of the dyes leading to promote anaerobic biological decolorization of dyes.

(4) Promotion of DT on biodegradation of acid scarlet G is more efficient than that of acid fuchsin, while the competitive inhibition of DT on biodegradation of acid scarlet G is less than that of acid fuchsin.

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