

*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, whereas almost all dyes are refractory organic matter. Fatliquoring and dyeing are in one bath in the leather production, and fatliquor and dyes are difficult to be separated by the physical and mechanical methods in the sewage treatment process. The study, aiming to determine the influence of various commonly used fatliquor on the biological decolorization of dyes, measured the decolorization rates of three dyes in the various co-substrates after shaking and static culture. To verify the influence mechanism of the fatliquor on the decolorization, the paper had measured the biological oxygen demand of the various cultures and UV-Vis spectra of the various co-substrates after static culture. The results certificated promotion of oxidation-sulfited fatliquor on the anaerobic degradation of acid fuchsin dye and acid scarlet GR. These works will provide a theoretical basis for more environmentally friendly material choice in the leather production.

Keywords: fatliquor; dye; biodegradation; aerobic; anaerobic.

1 Introduction

There are many kinds of dyes used in the leather dyeing process, which have a complex structures and almost all the dyes are refractory organic pollutant. Remnants of the dye in wastewater, even in small concentrations, can decrease the transmittance of the effluent and thus result in the destruction of aquatic ecosystems [1]. Reason of biodegradation difficulty for the refractory organism is usually the difficult growth of microbe using refractory organic pollutants as sole carbon source. Adding of some easily biodegradable organic matters can increase microbial activity and provide an effective

carbon source and energy for synthesis of enzymes to be involved with degradation of refractory organic pollutant, result in an improvement of refractory organic pollutant in the biodegradation. Previous studies on the biodegradation of dyes have shown that sugar can effectively improve degradation of dye 2, 3. However, because of the particularity of leather manufacture, there are little sugars but considerable organic pollutant in tannery wastewater. Adding sugars in the process of waste treatment would lead to increase of the treatment cost and the load of reactors certainly. Yet contents of protein and fat are higher in the tannery wastewater. Moreover, previous studies have shown that all anionic and nonionic fatliquor, which are prepared via the hydrophilic modifications of natural oil, are easily biodegradable substances. So it is possible that the biological decolorization of the dyes could be promoted by the fatliquor in the effluent.

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 are transported directly into bacterial cells and then stimulate bacterial growth. Simultaneously the reduced nicotinamide adenine dinucleotide (NADH) and the reduced flavin (FADH₂) have been produced in the degradation of the fatty acids. Under aerobic condition these reduced matters react with oxygen to decrease the dissolved oxygen concentration rapidly and then cause the culture transferred to anaerobiosis. Under anaerobic conditions the reduced matters produced from the degradation of the fatty acid could promote the broken of azo bond in azo

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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 the influence of several fatliquor to be commonly used in the leather production on the biological decolorization of three dyes, in order to discuss the possibility of utilizing fatliquor to promote the biodegradation of dye.

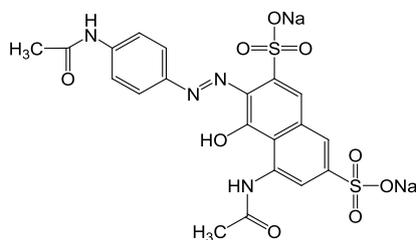
2 Materials and methods

2.1 Reagents and instruments

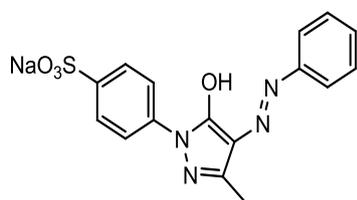
2.1.1 Chemicals and reagents

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

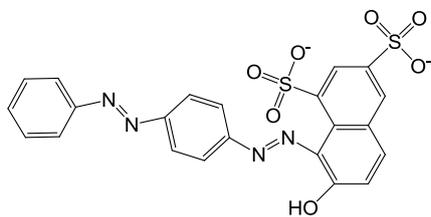
Acid Fuchsin Dye



Acid Light Yellow G



Acid Scarlet GR



Inorganic salt medium: K₂HPO₄ 21.75mg/L, KH₂PO₄ 8.5mg/L, Na₂HPO₄ 16.58mg/L, NH₄Cl 1.7mg/L, MgSO₄ 11.14mg/L, CaCl₂ 27.5mg/L, FeCl₃ 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 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, which is X (mg/L) = 135.267A_{600nm} (R²=0.9994).

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 absorbance of the culture at maximum absorption wavelength of the dye. The formulas for the dyes concentrations are as follow: Acid fuchsin dye (mg/L) = 36.941A_{525nm} (R²=0.99904); Acid Yellow G (mg/L) = 24.888*A_{390nm} (R²=0.99965) and Acid Scarlet GR (mg/L) = 24.6244A_{510nm} (R²=0.99841).

2.2.3 CODCr measurement

Chemical oxygen demand was measured by the standard Potassium dichromate method.

2.2.4 Dissolved oxygen (DO) measurement

The values of Dissolved oxygen in various synthetic wastewaters were measured by the Winkler method.

2.3 Methods

2.3.1 Decolorization of dyes under different substrates

The dewatered 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 the inoculum. Samples of the test are Acid Fuchsin Dye (AF), Acid Light Yellow G (ALY), Acid Scarlet GR (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 50 mg/L and the concentrations of fatliquor were 200 mg/L. the dry weight concentrations of biological cells to be inoculated were 42 mg/L. All the samples were divided in duplicate, one was at 30°C after 15 days shaking culture in 110 rpm, another was at 30°C after 15 days static culture. To compare the absorbance of the dye in the medium before culture (A1), the absorbance (A2) of the supernatant of the cultured medium was measured, and then decolorization activity was calculated as follows:

$$\text{Decolorization(\%)} = \frac{\text{Initial absorbance (A}_1\text{)} - \text{Observed absorbance (A}_2\text{)}}{\text{Initial absorbance (A}_1\text{)}} \times 100\%$$

2.3.2 Estimation of biological oxygen demand of dyes in different substrates

On the experiment, the dye concentrations were 50 mg/L and the fatliquor concentrations were 200 mg/L in various medium. 5 ml of initial medium containing dye solution, no-containing the dye solution and distilled water was added to airtight BOD bottles Sample 1, Sample 2 and Blank respectively. Place desired volume of water in a suitable bottle and 1 ml of each of Phosphate buffer, MgSO₄, FeCl₃ and seeding/L of water. Before using, dilution water temperature was

brought to 30°C. Dilution water was aerated with organic free filtered air. All the bottles are kept in the shaking incubator at 30°C for 5 days. The DO concentrations in the diluted mediums before and after shaking culture were measured, and then biological oxygen demand of dye after 5 days was calculated according to the following formula.

$$\text{Biochemical oxygen demand of dye} = [(OD_1 - OD_2) - (OD_3 - OD_4)] \times 50$$

OD1 and OD2 – the DO concentration of Sample 1 before and after culture respectively;

OD3 and OD4 – the DO concentration of Sample 2 before and after culture respectively;

50 – the dilution factor of Sample and Blank.

The BOD values were compared between the initial medium containing various fatliquor.

2.3.3 Analysis of UV/Visible Spectrophotometer

Under static conditions, the mediums containing 50 mg/L of dye were cultured for 15 days. UV/Visible spectra of supernatants of the mediums before and after the culture were compared and possible degradation products were speculated.

2.3.4 Experimental design of biodegradation kinetics of the dyes in co-substrate

On the experiment, the highest and lowest concentration point of fatliquor was set in 80 mg/L and 8 mg/L respectively, and 10 points were set averagely. The mediums containing 200 mg/L of oxidation-sulfited fatliquor (DT) were as the test group, the mediums no containing DT were as the control test group, and then all mediums had been inoculated with bacteria and inorganic nutrient solution. The dry weight concentration of bacterial cells in the cultures is 78 mg/L. Under static conditions, the samples were cultured at 30°C for 10 days. The changes in biomass and the degraded concentration of the dye were measured. The specific degradation rate was calculated according to following formula.

$$\text{The specific degradation rate } q_c (d^{-1}) = \frac{\Delta C_{dye}}{X_0 \times \text{the cultured time}}$$

C_{dye} – the degraded concentration of the dye, the unit is mg/L;

X₀ – the initial biomass concentration (the dry weight of cells), the unit is mg/L.

3 Results and discussion

3.1 The influence of fatliquor on decolorization of dyes

Experimental results of decolorization activities of different dyes in various substrates after shaking culture are shown in Table 1.

The acid fuchsin dye and acid scarlet GR had been decolorized after shaking culture for 15 days, and adding of various fatliquor increased the decolorization activities of the two dyes in various degrees. But all increased degrees were not great. It is because the decolorization of the dyes is achieved mainly by adsorption of the bacteria under aerobic condition. Adding of the fatliquor in the substrates stimulated microbial growth and thus led to enhance biological adsorption of the bacteria for the dyes. The previous article had shown that phosphorylated fatliquor can promote considerable growth of the bacteria in the mediums⁵, so the decolorization activities of the mediums containing phosphorylated fatliquor are greatest under aerobic conditions.

Table 1 Decolorization activities (%) of different dyes in various substrates after shaking culture

| Dye Co-substrate | 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 the substrates don't have decolorization after shaking culture.

Experimental results of decolorization of different dyes in various substrates after static culture are shown in Table2:

Table 2 Decolorization activities (%) of different dyes in various substrates after static culture

| Dye Co-substrate | 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 the substrates don't have decolorization after static culture.

After static culture, three fatliquor all enhanced the decolorization activities of the acid fuchsin dye and acid scarlet GR in various degrees. Comparing to the oxidation-sulfited fatliquor, the promotion of the phosphorylated fatliquor on the decolorization activities of the two dyes were not obvious. Respectively on the acid fuchsin dye and acid scarlet GR, the different value of the decolorization activities between the medium no-containing the phosphorylated fatliquor and containing the phosphorylated fatliquor was 4.59% and 19.41%, which is general accords with the experimental result under aerobic condition. It is because the biodegradation of phosphorylated fatliquor produce large amounts of phosphate to be released into solution, which promote the physiological activity of phosphorus-accumulating bacteria. The acid fuchsin dye and acid scarlet GR are azo dyes, and under anaerobic condition, the kind of dye are reduced azo linkage to produce two aromatic amines resulted in the decolorization of the dyes. Nicotinamide adenine dinucleotide (NADH) functions as cofactors in the azo reduction. But under anaerobic condition phosphorus-accumulating bacteria consume NADH to transform short-chain fatty acids into poly- β -hydroxybutyrate (PHB) ⁶. There is a competitive inhibition between the form of PHB and the decolorization of azo dyes. Moreover, in the medium of adding the oxidation-sulfited fatliquor, decolorization activities of acid fuchsin dye and acid scarlet GR is 77.39% and 87.53% respectively. The oxidation-sulfited fatliquor has an efficacious promotion on biodegradation of these two dyes. Furthermore

the medium with acid fuchsin dye after the static culture appear color transformation and trend purple color. Color of acid fuchsin dye substrate with inoculum and non-inoculum after 15d static culture was shown in Figure 1.

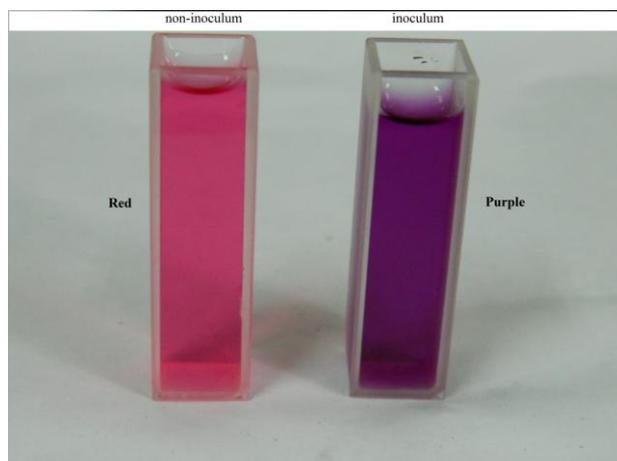


Figure 1 The color of acid fuchsin dye with inoculum and non-inoculum after 15d static culture

This should be because chromophore and auxochrome of acid fuchsin dye have been broken and some colored intermediate appeared under anaerobic condition, so that the medium only containing the acid fuchsin dye 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 mediums change-over anaerobic conditions and thus promote the biodegradation of these intermediate, resulted in that 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.

From the Table 1 and Table 2, it can be found that fatliquor had not significant effect on promoting biological decolorization of acid light yellow G under shaking and static conditions, which may be because that acid light yellow G is not biodegradable under aerobic or anaerobic conditions, while adsorption capacity of micro-organisms for the dye is too small to utilize fatliquor promoting adsorptive decolorization of acid light yellow G. From the Table 1 and Table 2 it 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 reason that the non-ionic fatliquor is the mineral-oil-based fatliquor with poor biodegradability 7, which does not stimulate a

lot of microbial growth in substrate and provide efficacious energy and carbon source for synthesis of enzymes in relation to degradation of the dyes. Therefore, promotion of neutral-oil-based fatliquor on biological decolorization of dye is greater than that of mineral-oil-based fatliquor.

3.2 Biological oxygen demand of dyes in different substrates

In order to determine whether the biodegradation of the dyes existed under aerobic conditions, the paper had estimated biological oxygen demand of dyes in different substrates, and the results are shown in Table 3.

The Table 3 shows that biological oxygen demands of the dyes after 5d culture are all negative, i.e. the biological oxygen demand of Sample 1 added a dye are less than that of the Sample 2 which no-containing the dye. It indicates that the promotion of fatliquor on biological decolorization of dyes under aerobic conditions was achieved mainly by the increased adsorption of the bacteria on the dyes. Because the dyes inhibited biological activity in the cultures and decreased the biodegradation of the fatliquor, the BOD all appeared the negative values.

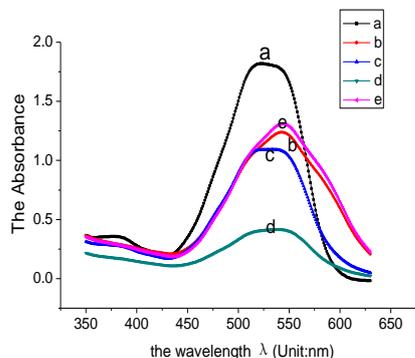
Table 3 Biological oxygen demands of dyes in different substrates after 5d culture

| Dye Co-substrate | AF | ALY | AS |
|---------------------|---------|---------|---------|
| None | - | - | - |
| SF | 1.79824 | 5.84427 | 7.64251 |
| | -29.43 | - | - |
| DT | - | 23.8266 | 2.69736 |
| | - | - | - |
| LB | 58.2341 | 26.4251 | 56.1949 |
| | - | - | -68.333 |
| | 26.3154 | 26.9736 | |

3.3 Result of UV/VIS-spectra analysis

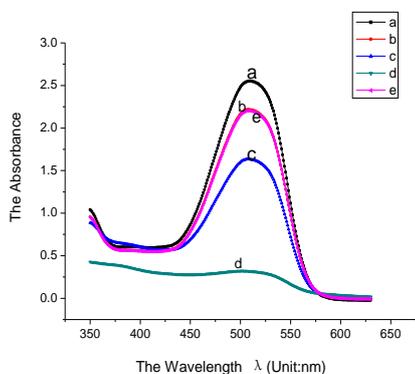
the UV-VIS absorption spectra corresponding to initial and final samples of decolorization experiments on acid fuchsin dye

and acid scarlet GR is shown in Figure 2 and Figure 3 respectively. The absorbance analyzed from 350 to 630nm. The initial acid fuchsin dye and acid scarlet GR solution showed the maximum absorption peak is at the wavelength of 524nm and 509nm respectively. The decolorized dye showed disappearance of the peaks after static culture, which indicate that the decolorization is due to dye degradation 8.



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

Figure 2 The UV-visible absorption spectra of acid fuchsin dye



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

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

2.3.4 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 dye and acid scarlet GR in static culture have been established. For microbial growth in the co-substrate, 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 9. Assuming that the degradation of dye and fatliquor is a case of concurrent substrate utilization, the dyes do not support the growth of microbial cells, 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 10.

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

Where q_c and q_g is the specific degradation rate of the dye and the fatliquor respectively and the units are day⁻¹. Respectively S_c and S_g is the initial concentration of the dye and the fatliquor in the co-substrate and the units are mg/L. The K_c is the half-saturation coefficient of the dye in the medium which the dye as the single-limited substrate and the unit is mg/L. The T_{cg} is the transformation capacity coefficient of the growth substrate, which characterizes activity of the DT fatliquor to promote the degradation of the dye, and the unit is 1. Because all co-substrate on the experiment contained 200 mg/L of the DT fatliquor, the value of q_g is a constant. So the multiplication of T_{cg} by q_g could indicate the T_{cg} values. The K_g is the inhibition coefficient of the growth substrate to the degradation rate of non-growth substrate, which indicates the inhibition of the DT fatliquor on the dyes owing to the competitive inhibition. The unit of K_g is mg/L. The fitting curves of model for the experimental data of the acid fuchsin dye and acid scarlet GR is shown in the following Figure 4 and Figure 5 respectively.

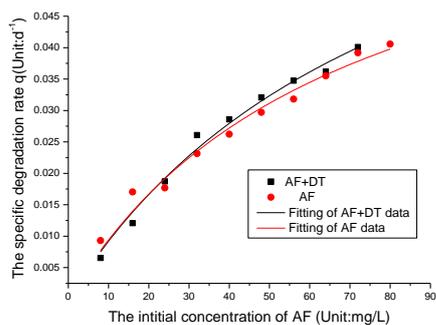


Figure 4 The fitting curve of model

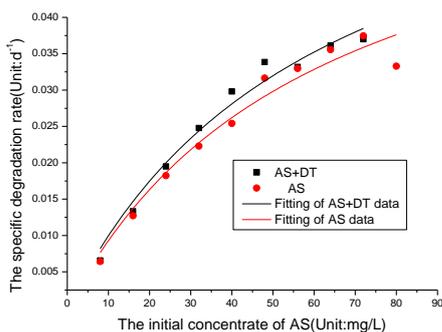


Figure 5 The fitting curve of model for acid fuchsin dye for acid scarlet GR

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 | qc | Kc | Tcgqg | Kg |
|-----------|--------|--------|---------|---------|
| Substrate | | | | |
| AF+DT | 0.0738 | 68.538 | 0.0133 | 847.481 |
| AS+DT | 0.0666 | 61.651 | 0.00498 | 55452.1 |

Table 4 shows that the transformation capacity coefficient of DT on the degradation

of acid fuchsin dye is greater than that on the degradation of acid scarlet GR, indicating that promotion of DT on biodegradation of acid fuchsin dye is more efficient. Moreover the inhibition coefficients of DT on the degradation rate of acid scarlet GR and acid fuchsin dye all are very great, which indicate there are little competitive inhibition between the biodegradation of the dyes and the DT fatliquor.

4 Conclusions

(1) Under aerobic and anaerobic conditions, the fatliquor all have stimulative effects on biological decolorization of acid fuchsin dye and acid scarlet GR, but to acid yellow G it is not as so. Moreover, the promotion of mineral-oil-based fatliquor on biological decolorization of dye is less than that of neutral-oil-based fatliquor.

(2) Under shaking condition, the decolorization of acid fuchsin dye and acid scarlet GR mainly depend on the biological adsorption, so the addition of fatliquor has improved the physical adsorption of dye via stimulating sludge microbial growth, and lead to promote aerobic biological decolorization of dyes.

(3) Under static condition, the decolorization of acid fuchsin dye and acid scarlet GR mainly depend on the biodegradation of dyes. The oxidation-sulfited fatliquor is efficient to promote biodegradation of the dyes and result in the promotion on the anaerobic biological decolorization of dyes.

(4) Promotion of DT on biodegradation of the acid fuchsin dye is more efficient than that of acid scarlet GR, while there are little the competitive inhibition between the biodegradation of the dyes and the DT fatliquor.

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