Toxicity Effect of Organic Solvents in Pharmaceutical Wastewater on Activated Sludge

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***Abstract* — Dehydrogenase can activate and transfer the hydrogen atom of the oxidized organic matter to a specific hydrogen body, therefore, the activity of dehydrogenase can reflect the degradation activity of microorganisms in the sewage treatment system. In this paper, the toxicity of organic compounds in pharmaceutical wastewater was determined by measuring the dehydrogenase activity of sludge. The toxicity of organic compounds was determined by measuring the absorbance of the reaction using TTC as artificial hydrogen acceptor. The more using solvents in the pharmaceutical process is triethylamine, DMF, acetone. Through calculating the experimental data to get the EC50 of each of the three substances respectively: trimethylamine 453.2mg/L, DMF 337mg/L, acetone 470927.1mg/L. The toxicity order of the three substances to the sludge is DMF> triethylamine >> acetone.**

Keywords - pharmaceutical wastewater; organic solvents; activated sludge; Toxicity effect

I. INTRODUCTION

Chemical pharmaceutical is a method of synthesizing Pharmaceutical by using chemical method to make organic substance or inorganic substance to produce other substance by chemical reaction. The main pollutants of pharmaceutical wastewater are suspended soild, COD, BOD, ammonia, cyanide, volatile phenols and other toxic substances [1, 2]. Pharmaceutical industry wastewater is generally belong to one of the more difficult to deal with the high concentration of toxic organic sewage. Pharmaceutical industry waste has the following characteristics: complex component and organic species, high concentration, the high value and high volatility of COD and BOD, the difference of the ratio of BOD/COD of wastewater, high concentration of ammonia nitrogen, deep color, high toxicity, and high concentration of SS [3, 4].

With the rapid development of the pharmaceutical industry, Pharmaceutical wastewater pollution is increasingly intensified to the environment, is also a serious threat to human health. Accurate determination of the toxicity of pharmaceutical wastewater is essential for the control of water pollution. The activity of sludge in pharmaceutical wastewater basically reflects the toxicity of toxic substances in wastewater. Microbial dehydrogenase is an essential enzyme for the degradation of organic pollutants and energy, which can reflect the activity of living organisms. In the field of academic research and production practice, dehydrogenase activity detection method is a sensitive, rapid and simple method to detect the activity of activated sludge. Study on the toxicity of three kinds of organic solvents in the pharmaceutical process widely used, it can draw the toxicity of triethylamine, DMF, acetone on sludge dehydrogenase activity. The experimental results are helpful to improve the selection of pharmaceutical technology or materials, and also have a great effect on the later treatment of pharmaceutical wastewater.

II. EXPERIMENT METHODS

A. TTC- reduction assay

Dehydrogenase activity was determined by TTC-reduction assay [5, 6]. This method adopts 2, 3, 5 - three phenyl tetrazole chloride (TTC) as a hydrogen acceptor. TTC in the oxidation state can accept hydrogen activated by dehydrogenase, and be reduced to the red triphenyl formazan (TF). TF was extracted with acetone, the absorbance was measured at 485nm wavelength, and then dehydrogenase activity was calculated. The toxicity of organic compounds to sludge dehydrogenase was quantitatively characterized by EC_{50} in the usual cases. The inhibition rate was regressed with Origin8.1 to obtain EC50.

Preparation a series of different concentrations of TTC using liquid: 1, 2, 3, 4, 5, 6mL from 1mg/mL TTC solution were pipetted into a set of 50ml volumetric flask, respectively. With distilled water content to 50mL, TTC concentrations in each bottle were 20, 40, 60, 80, 100, 120ug/mL.

Seven 25mL Colorimetric tubes were taken, 2mL Tris - HCl buffer, 1ml 0.36% sodium sulfite solution, 1ml distilled water, 1ml TTC solution were added into them. No TTC was added into the control. And then 5mg natrium hydrosulfurosum were added into each colorimetric tube respectively. The colorimetric tubes were putted into 30^0C bath oscillator, and taken out after 10min. 5mg acetone were added into each colorimetric tube respectively, then the tubes were putted into 30^0C bath oscillator for 10min. The absorbance was determinate at 485nm, the reagent blank was as the control.

B. Determination of dehydrogenase activity of activated sludge

To ensure that the external matrix is depleted, aeration was operated in activated sludge mixture liquid. A certain

amount of sludge mixture liquid was taken, diluted after washing with distilled water 2 times, the MLSS was controlled about 2000mg/L. In order to eliminate the effect of pH on experimental results, the pH of the sample was adjusted to 7.2 with phosphate buffer solution.

A series of 10ml sludge mixture samples were taken to 4000r/min centrifugal 5min, supernatant was removed. Three kinds of organic matter were added in, and then diluted with distilled water to 10ml, oscillated in the 30^0C water bath for 2h. Each sample was measured 3 times in parallel. Sludge mixed liquid sample 1mL was placed in 10ml centrifuge tube. Tris-HCl buffer 2mL, 0.1mol/L glucose solution 0.5mL and 0.4% TTC solution 0.5mL were added in order, oscillated in the 30^0C water bath for 2h, then added formaldehyde 0.5mL to terminate the reaction. Samples were taken to 4000r/min centrifugal 5min to remove supernatant, added 5mL acetone, oscillated in the 30^0C water bath for 10min. After extraction, samples were taken to 4000r/min centrifugal 5min. The supernatant was taken to measure absorbance at 485nm. The settling sludge was taken to dry 2h at about 105^0C to measure dry weight. The sludge dehydrogenase activity was calculated by Equation (1).

$$ETS_t = \frac{D_{485}V}{k_t W t} \quad (1)$$

ETS_t-dehydrogenase activity; D_{485}-absorbance of solution at 485nm; V-volume of the extraction agent; k_t-slope of the standard curve; W-dry weight of sludge; t-sludge culture time.

III. Experiment Results

A. TTC standard curve

After the treatment of the above standard curve, the absorbance of the solution under different TTC concentration was determined, and then the standard curve was drawn.

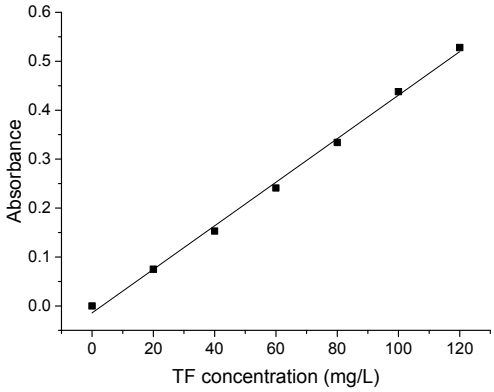

Fig.1 Dependence of absorbance on TTC concentration

From the experimental results, the linear relationship between the absorbance and the concentration of the solution is good. By linear regression, the standard curve equation was Y=0.0044X-0.0142, correlation coefficient was R=0.9971.

B. The inhibition of trimethylamine to dehydrogenase activity

A series of 10ml sludge mixture samples were taken to 4000r/min centrifugal 5min. 0, 0.5, 1, 0, 2.7, 3.3, 4, 5, 6, 6.7, 7.3, 8mL 1500mg/L trimethylamine were added to the samples in order, then diluted to 10mL by distilled water, oscillated in the 30^0C water bath for 2h. The dehydrogenase activity after inhibition was determined. Each sample was measured 3 times in parallel.

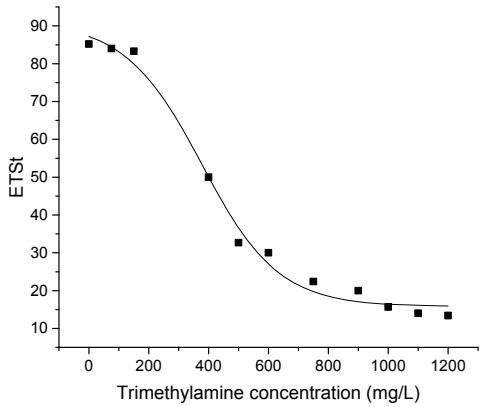

Fig.2 Dependence of ETSt on trimethylamine concentration

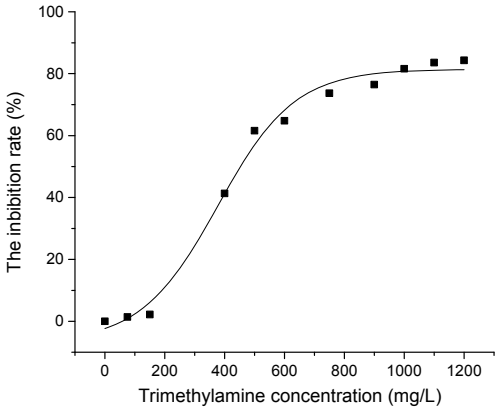

Fig.3 Dependence of the inhibition rate on trimethylamine concentration

As shown in Fig.3, when the concentration was between 150mg/L to 400mg/L, the inhibition of sludge dehydrogenase activity increased rapidly with the increase of the concentration of trimethylamine. When the concentration of trimethylamine was greater than 1000mg/L, the inhibition of the activity of the sludge dehydrogenase tended to be stable.

C. The inhibition of DMF to dehydrogenase activity

A series of 10ml sludge mixture samples were taken to 4000r/min centrifugal 5min. 0, 0.2, 1, 1.5、2、2.2、2.5、3、4、5mL 2000mg/L DMF were added to the samples in order, then diluted to 10mL by distilled water, oscillated in the 30^0C water bath for 2h. The dehydrogenase activity after

inhibition was determined. Each sample was measured 3 times in parallel.

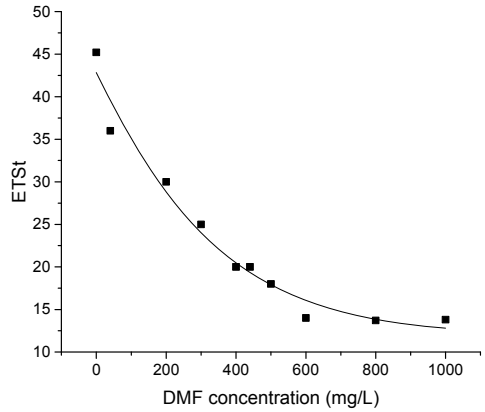

Fig.4 Dependence of ETSt on DMF concentration

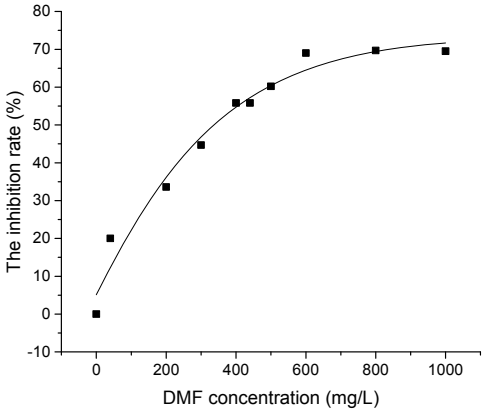

Fig.5 Dependence of the inhibition rate on DMF concentration

As shown in Fig.5, when the concentration was between 0mg/L to 600mg/L, the inhibition of sludge dehydrogenase activity increased rapidly with the increase of the concentration of DMF. When the concentration of DMF was greater than 600mg/L, the inhibition of the activity of the sludge dehydrogenase tended to be stable.

D. The inhibition of acetone to dehydrogenase activity

A series of 10ml sludge mixture samples were taken to 4000r/min centrifugal 5min. 0, 0.1, 0.25, 0.5, 0.75, 1, 1.25, 1.9, 2.5, 5, 8mL acetone were added to the samples in order, then diluted to 10mL by distilled water, oscillated in the 30^0C water bath for 2h. The dehydrogenase activity after inhibition was determined. Each sample was measured 3 times in parallel.

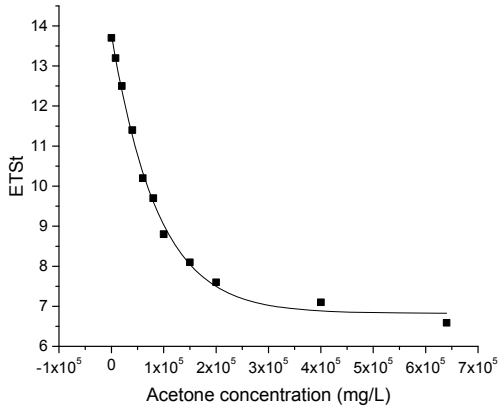

Fig.6 Dependence of ETSt on acetone concentration

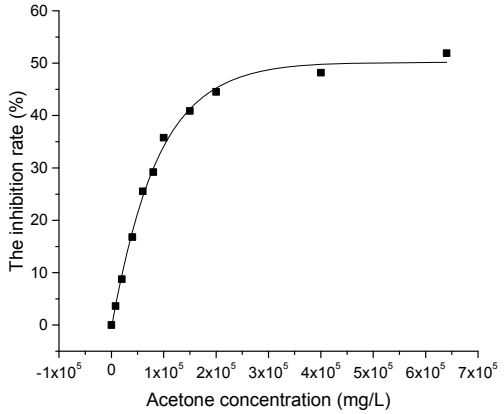

Fig.7 Dependence of the inhibition rate on acetone concentration

As shown in Fig.7, when the concentration was under 200000mg/L, the inhibition of sludge dehydrogenase activity increased rapidly with the increase of the concentration of acetone. When the concentration of acetone was greater than 200000mg/L, the inhibition of the activity of the sludge dehydrogenase tended to be stable.

E. Toxicity of organic solvents on activated sludge

Dose effect analysis of the three organic solvent to activated sludge dehydrogenase activity inhibition rate was performed through the above data. EC50 of the organic solvents could be calculate and compare each other.

TABLE I. DOSE EFFECT ANALYSIS OF THE THREE ORGANIC SOLVENTS

Solvent	**EC50（mg/L）**
trimethylamine	453.2
DMF	337.0
acetone	470927.1

Through calculating the experimental data to get the EC50 of each of the three substances respectively: trimethylamine 453.2mg/L, DMF 337mg/L, acetone 470927.1mg/L. The toxicity order of the three substances to the activated sludge is DMF> triethylamine >> acetone.

IV. CONCLUSION

From the above experiments, it can be seen that triethylamine, DMF and acetone had different degrees of inhibition on dehydrogenase activity of activated sludge. The toxicity of different solvents was increased with the increase of concentration, and the toxicity reached a steady state when the concentration reached a certain concentration. Due to the toxic effects of organic solvents, it is necessary to preprocess the pharmaceutical wastewater. Pretreatment can reduce the toxic substances to maintain the activity of microorganisms, and improve the treatment efficiency of the subsequent biochemical treatment.

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