



Optimized Design of Acid Red B for Degradation by *Corynebacterium*

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Abstract

The optimized experimental parameters of degradation obtained with a four-factor at three-level orthogonal array experimental design L18(3⁶) were temperature, rotation speed, inoculum size and liquid level by *Corynebacterium variabile* in the shaking bottle as 33°C, 22h after shaking at the same temperature and 120 rpm for 14h, 4%, 80mL in 150mL triangle bottle, respectively. Among four factors, liquid level is paramount.

Keywords: *Corynebacterium variabile*, Acid Red B, Orthogonal design

1. Introduction

Azo dyes, as the mainly dyeing material in the world (Yuzhu, 2001, PP.251-262), are often used in the colouring process of several textiles, dyestuff and paper-making products. Relatively recently it has been recognised that some azo dyes agents may bring a large amount of waste water, which flow abroad in aqueous solutions. The researches in the field have led to laboratory demonstration of the applicability of technique, and to industrial pilot plant and full-scale established technologies for treating effluents of dyeing factories. Dye wastewater is usually treated by physical- or chemical-treatment processes such as flocculation, absorption and electrolysis etc, by most factories in China, nevertheless, both physical and chemical methods have their shortcomings which incurs less decolorization, more electric power and thus high operating expenses. In recent years, there have been an intensive research focused on the degradation of dyes by microorganism in the world (Ping, 2002, P.59, Wenwen, 2008, PP.120-123, Xinjiao, 1999, PP.220-224, Chunlong, 1998, PP.41-58, Long, 2007). In aerobic or anaerobic condition, azo dyes were likely degraded to low molecular aromatic structures which could be further used. The reaction rate of degradation was usually quite slow, and thus promotion of the reaction could be helpful to the application of these techniques. In the present paper, *Corynebacterium variabile*, as a microbial agent, was undertaken to investigate the degradation effect in Acid Red B and the experimental parameters of degradation were also optimized. Hence data obtained from these experiments would give some novelty in the application of dye degradation by this fungus.

2 Materials and methods

2.1 Fungus

Microorganism used in this study was separated and purified from sullage sample of printworks effluent contaminated sites in Wuqing District, Tianjin with a good effect of dye degradation, and was identified and named as *Corynebacterium variabile*, briefed as fungus B.

2.2 Medium

Domesticated medium: Beef grease 3g, Peptone 10g, Acid Red B 0.04g, pH 7.2, sterilized for 30 min under 0.10Mpa.

Inorganic medium: $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 0.5g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2g, K_2HPO_4 0.5g, $(\text{NH}_4)_2\text{SO}_4$ 2g, CaCl_2 0.1g, Glucose 10g, Acid Red B 0.04g, Distilled water 1000ml.

Acid Red B powder was commercially available dye used without further purification. The maximum absorption wavelength was 515 nm in UV-vis measurements.

2.3 Culture condition confirmation

14 150ml taper flask was arranged for seven groups, each group 2 in order to compare. Every flask was filled with 50ml inorganic medium, sterilized for 20min under 0.05Mpa and then was added with fungus B. After that, the first group was incubated at 33°C and the other six were incubated in the shaking bottle at the speed of 120r/min at 33°C for 12 h, then every 2h, one group was transferred to incubator until decolorization.

2.4 Culture method

150ml taper flasks were filled with different dose inorganic medium respectively. Based on orthogonal array experimental design, flasks added with fungus B was incubated at the optimal condition for 36h.

2.5 Orthogonal design

To obtain the optimised experimental parameters, a four-factor at three-level orthogonal array experimental design $L_{18}(3^4)$ was adopted by Orthogonal Design Assistant and the absorbance of degradation in each test were measured. The four factors were temperature, rotation speed, inoculum size and liquid level. Based on the experimental results of the previous orthogonal design and ANOVA analysis, the optimal ranges for each factor and their degradation effect could be obtained, and speculated the optimal conditions for fungus B in the degradation of Acid Red B. The factors studied and the assignments of the corresponding levels are listed in Table 1.

2.6 Measurement

The fungus solution incubated till decolorization was centrifuged to remove cells, and supernate was undertaken to determine the absorbance of Acid Red B before or after degradation by UV-vis measurements (spectrophotometer 731, China). Decolorization rate was defined as follows:

$$q = \frac{A_0 - A_t}{A_0} \times 100\%$$

Where A_0 is the initial absorbance combined with fungus B and A_t is the absorbance after incubated for 36h.

3. Results and discussions

3.1 Incubating condition

As seen in Table 2, efficiencyes of decolorization was not good when fungus was incubated always in incubator or shaking apparatus, however, when incubated first in shaking apparatus for some time, then transferred to incubator was all good. At a time ratio of 14:22 (shaking /quiescence), efficiency of decolorization was the highest and was not increased by adding the time in shaking apparatus, but decreased inversely. Therefore, time ratio of 14:22 (shaking /quiescence) was the optimal condition for growth of fungus and degradation of Acid Red B.

3.2 Effect of Acid Red B degradation by fungus B in different conditions

According to Orthogonal Design Assistant, 18 tests were performed. The analytical results are listed in Table 3. The average of the decolorization rate in each test were calculated. The values of $|k_{\max} - k_{\min}|$ and F in Table 3 and 4 indicate the effect of temperature, rotation speed, inoculum size and liquid level. The efficiency of these four factors were classified in the order of liquid level (D)>inoculun size(C)>temperature(A)>rotation speed(B). Thus, liquid level was the major factor affecting the decolorization rate, whilst inoculun size, temperature and rotation speed had a less obvious influence. More attention should be paid to liquid level and inoculun size two factors in the experiment. As seen in Figure 1, in order to obtain the maximum decolorization rate, temperature, rotation speed, inoculum size and liquid level were chosen as 33°C, 120r/min, 6%and 80mL, respectively, namely $A_2B_2C_3D_3$.

3.3 Validated experiment

According to the orthogonal design results, the optimized parameters of decorlorization were undertaken to validate the effect of acid red B degradation by fungus B. With three replicates, results were listed in the Table 5.As seen from Table 5, results were conformed to Table 4 and Figure 1 and the average decolorization rate was 95.4% under the optimized condition where temperature, rotation speed, inoculum size and liquid level were chosen as 33°C, 120r/min, 6%and 80mL, respectively. Decolorization rate was high and repeatable, which showed that the optimized condition was reasonable.

4. Conclusions

Fungus B separated from sullage has strong degradation activity of dyes. Through orthogonal design, degradation condition of acid red B by fungus B was optimized. Results showed that at a time ratio of 14:22 (shaking /quiescence), degradation efficiency of acid red B was best, and the decolorization was 94.6%; In this way, the optimized incubating parameters of temperature, rotation speed, inoculum size and liquid level were 33°C, 120r/min, 6% and 80mL, respectively, whilst the decolorization rate was above 95%.

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Table 1. Assignments of the levels to factors in orthogonal design

Level	Factors			
	A Temperature (°C)	B Rotating speed (r/min)	C Inoculation size (%)	D Liquid level (mL)
1	28	90	2	20
2	33	120	4	50
3	38	150	6	80

Table 2. Effect of the time ratio of shaking to resting

Time ratio of shaking to quiescence	0:36	12:24	14:22	16:20	20:16	24:12	36:0
Decolorization rate(%)	32.6	92.6	94.6	93.4	91.7	70.7	46.9

Table 3. The matrix associated with the analytical results

Line	1	2	3	4	5	6
Factors Number	Temperature (°C)	Rotating speed(r/min)	Inoculation size (%)	Liquid level(mL)	Error	Results(%)
1	1	1	1	1	1	47.1
2	1	2	2	2	2	95.5
3	1	3	3	3	3	94.8
4	2	1	1	2	2	81.2
5	2	2	2	3	3	95.9
6	2	3	3	1	1	96.5
7	3	1	2	1	3	74.6
8	3	2	3	2	1	95.2
9	3	3	1	3	2	95.6
10	1	1	3	3	2	95.7
11	1	2	1	1	3	51.1
12	1	3	2	2	1	95.7
13	2	1	2	3	1	96.2
14	2	2	3	1	2	96.0
15	2	3	1	2	3	84.3
16	3	1	3	2	3	96.7
17	3	2	1	3	1	96.2
18	3	3	2	1	2	62.2
Average1	79.983	81.917	75.917	71.250	87.817	
Average2	91.683	88.317	86.683	91.433	87.700	
Average3	86.750	88.183	95.817	95.733	82.900	
$ k_{\max}-k_{\min} $	11.700	6.400	19.900	24.483	4.917	

Table 4. Variances of orthogonal design test of azo dye degradation

Factors	Sum of squares of deviation from mean	Degree of freedom	F ratio	F critical value	Significance
Temperature	414.031	2	4.383	19.000	
Rotating speed	160.498	2	1.699	19.000	
Inoculation size	1190.698	2	12.606	19.000	
Liquid level	2050.581	2	21.710	19.000	*
error	94.45	2			

 $F_{0.05}(2, 2)=19.000$

Table 5. Results of comparison test

Technology conditions	Number	Decolorization rate(%)
A ₂ B ₂ C ₃ D ₃	1	94.9
	2	95
	3	96.2

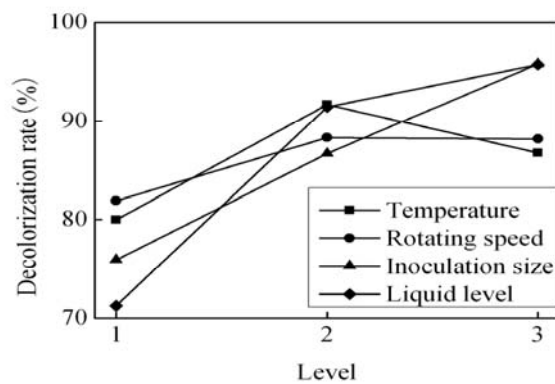


Figure 1. Effect Trend of the Four Factors