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DECOLORIZATION OF ORANGE 16 BY BACTERIA

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ABSTRACT

A bacterial strain Aer2-1, identified as *Aeromonas* sp. Aer2-1, was isolated from sediment from a textile wastewater treatment plant in Indonesia. The strain could decolorize an azo dye, Orange 16 under the static condition after the shaking culture. In order to increase cell concentration by the shaking culture, the optimum culture condition was investigated for following the decolorization incubation. The dye was decolorized completely up to concentration of 750 mg/*l* for 50 hr under static condition by using 12 hr-cultured cells at optimal shaking culture condition.

KEYWORDS

Aeromonas sp.; Azo dye; biodegradation; decolorization; Orange 16.

INTRODUCTION

Azo dyes are the most commonly used synthetic dyes in dyeing and textile industries worldwide. About 10-15% of the dyes used in dyeing processing are discharged in effluent (Vaidya & Datye 1982). Even at very low concentrations, azo dyes can cause waste stream to become highly colored. The industrial effluents often contain residual dyes which may become harmful to human health because some azo dyes and their metabolic intermediates are carcinogenic and mutagenic (Chung and Cerniglia 1992). Therefore, removal of these pollutants should be avoided.

Biodegradation of azo dyes by environmental microorganisms has been described (Chung & Stevens 1993). In the microbial degradation of azo dyes, the initial process is their decolorization. The azo bond should be cleaved for decolorization to take place. Various azo dyes were decolorized by microbial consortium (Brown & Laboureur 1983, Brown & Hamburger 1987, Weber & Wolfe 1987) and by the culture of purified bacteria (Mayer 1981, Haug et al., 1991) under unaerobic process. However these dyes are generally resistant to bacterial attack under aerobic condition.

A bacterial strain, Aer2-1 isolated from sediment of a textile industrial wastewater treatment plant, showed strong ability to remove the color of Orange16 under static condition. Preliminary experiments of this bacterial cultivation suggested that the initial cell concentration was a very important factor to decolorize Orange 16 following static incubation.
So we employed a serial 2-step culture condition. After growing the bacterial cells in the medium aerobically with the low concentration of the dye, the cultured medium was shifted to the static condition with addition of the dye for the decolorization.

A set of experiments has been carried out in batch culture to optimize the cell growth under aerobic condition. The effect of several culture conditions such as temperature, initial pH, and nutrient concentration of the medium were investigated.

MATERIALS AND METHODS

Measurement of dye and cell growth

Orange 16 (Figure 1) was obtained from Nissei Co. Ltd, Japan. Concentration of Orange 16 in the medium was determined by measuring the absorbance at 492 nm corresponding λmax of this dye. Bacterial growth of the cultures was measured as OD at 600 nm.

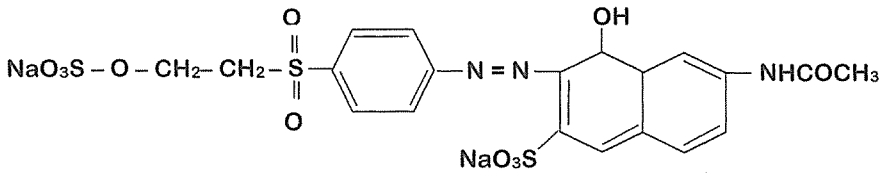

Figure 1. Structure of Orange 16 used in this study

Microorganism and culture condition

Bacterial strain Aer2-1 used in this work, was isolated from sediment of the wastewater treatment facilities of a textile industry in Bandung, Indonesia. The medium used for screening of bacteria with 0.02 g/*l* Orange 16 decolorizing potential contained 5 g/*l* glycerol, 4 g/*l* yeast extract, 0.5 g/*l* ammonium sulfate, 0.2 g/*l* $MgSO_4 \cdot 7H_2O$, 0.5 g/*l* KH_2PO_4, and 1.5 g/*l* K_2HPO_4. The initial pH was adjusted to 8.0. The strain was cultivated in a reciprocal shaker at 30°C. The culture conditions including the medium composition were varied to determine the optimum condition for the bacterial growth.

Batch culture experiments under the aerobic condition were carried out in 500 m*l* Sakaguchi flask, containing 100 m*l* medium inoculated with a loopful of 1-day grown-cells from the slant. Flasks were shaken at 125 rpm in a reciprocal shaker. Aliquots collected at intervals were used for measurement of the cell growth.

For decolorization experiments, the bacterial cells under optimal growth condition were transferred to 100 m*l* of Erlen-Meyer flask. Orange 16 was added to give 0.15 – 2 mg/*l* and further incubated at 30°C under the static condition.

Identification of bacterial strain Aer2-1

The taxonomical studies were carried out according to Bargey's manual (Sneath *et al.*, 1994). The 16*s* rRNA gene in this strain was amplified by 30 cycle of PCR using genomic DNA from strain Aer2-1 as the templete and two specific primers, 20F (5'-GATTTTGATCCTGGCTCA-3') and 1500R (5'-GTTACCTTGTTACGACTT-3'). Each cycle was carried out at 95°C for 30 sec, at 55°C for 20 sec, and at 72°C for 90 sec. An amplified DNA fragment was sequenced by the dideoxynucleotide chain termination method using BigDye terminator Cycle Sequencing Kit (Perkin-Elmer, Foster City, California) and a DNA sequencer (model 310, Perkin-Elmer). Homology of the DNA sequence was searched by using the BLASTn program at DDBJ.

RESULTS AND DISCUSSION

Isolation of bacteria with high dye-decolorizing potential

A small portion of the sediment samples was inoculated to 100 ml of the basal medium and cultivated at 30°C for 24 hr with shaking and then flasks were subsequently incubated at 30°C for 24 hr under the static condition. The samples showing decolorization of Orange 16 were spread on the agar plate containing Orange 16 to observe clear zone formation. Isolated bacteria were cultivated again as described above to check the decolorization potential. Isolated strain Aer2-1 showed the highest decolorization rate.

Identification of a bacterial strain Aer2-1

Isolated bacterial strain Aer2-1 was rod-shaped and negative in Grum-staining. The nucleotide sequence of 16*s* rDNA gene showed high homology to *Aeromonas caviae*, *A. trota*, and *A. hydrophila*, respectively. From these results, the bacterial strain Aer2-1 was identified as *Aeromonas* sp.

Effect of initial pH and temperature on the growth of *Aeromonas* sp. Aer2-1

With various initial pHs from 6 to 10 of the medium, the cell growth was tested at 30°C for 12 hr as shown in Figure 2. The bacterium showed slow growth at pH 10 with OD_{600} 8.5. Since, there was not much difference on the growth between pH 7 and 9 with OD_{600} 12.1 to 12.7 for 12 hr cultivation period. We employed pH 7 as initial pH in further experiments.

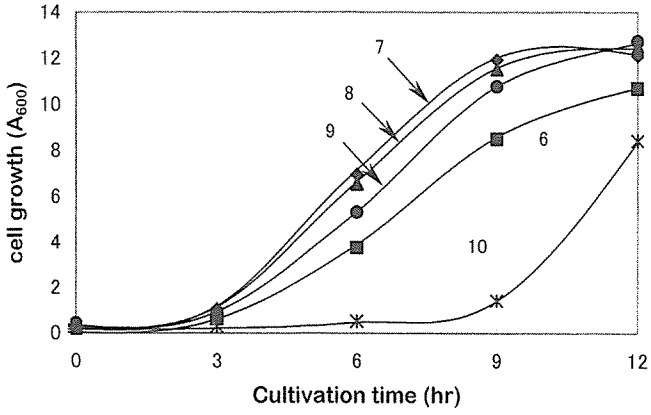

Figure 2. Effect of initial pH on the growth in the shaking culture.
Initial pH of the medium are indicated in the figure.

Aeromonas sp. Aer2-1 was grown in the medium at 25°C, 30°C, and 35°C. The initial pH of the medium was adjusted to 7.0. The highest growth was observed when the cultivation temperature was 30°C for 12 hr of cultivation as shown in Figure 3.

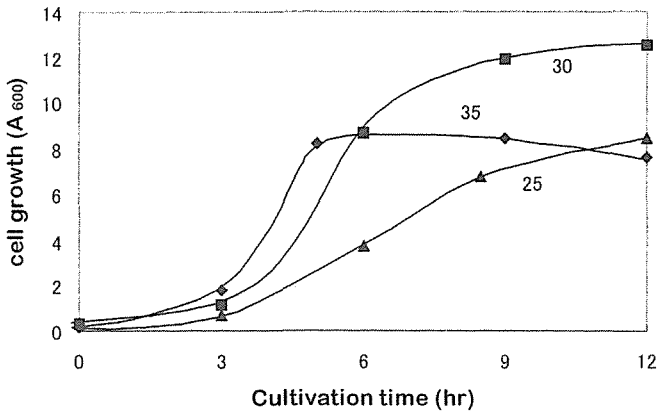

Figure 3. Effect of temperature on the growth in the shaking culture.
Cultivation temperature (°C) is indicated in the figure.

Effect of nutrients on the growth of *Aeromonas* sp. Aer2-1

The effects of yeast extract, glycerol, and ammonium sulfate concentration in the medium on the growth were investigated. The culture was carried out at 30°C and initial pH of 7 with shaking. *Aeromonas* sp Aer2-1 was cultivated in the medium containing yeast extract from 0 – 4 g/*l*. As shown in Figure 4, cells did not grew in the absence of yeast extract but growths were stimulated, and the highest growth was obtained with 4 g/*l* yeast extract for 12 hr cultivation.

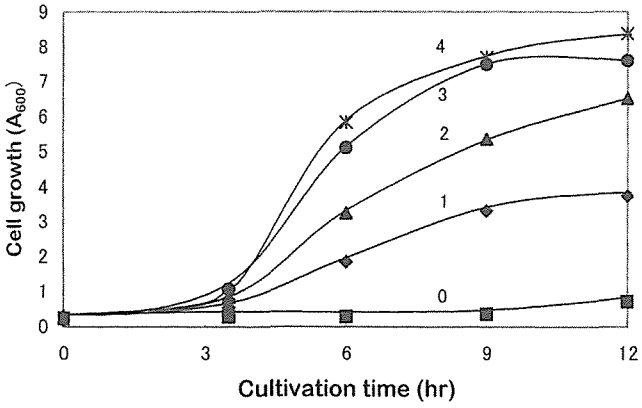

Figure 4. Effect of yeast extract concentration on the growth in the shaking culture
Yeast extract concentration (g/l) is indicated in the figure.

Glycerol concentration in the medium was also varied from 1 to 5 g/*l*. The result is shown in Figure 5. Glycerol concentration of 5 g/*l* gave the highest growth of the bacteria with OD_{600} 12 for 9 hr cultivation.

Variation of the ammonium sulfate from 0.1 to 0.5 g/*l* affected only slightly on the bacterial growth (data not shown). We employed 0.1 g/*l* of ammonium sulfate as the optimal concentration.

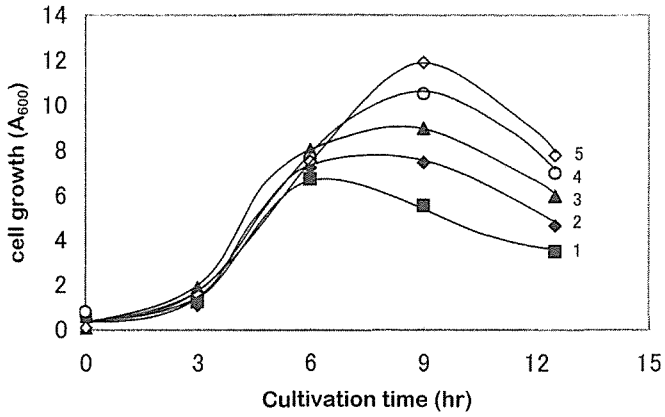

Figure 5. Effect of glycerol concentration on the growth in the shaking culture. Glycerol concentration (g/l) is shown in the figure..

Decolorization of Orange 16 under static condition

Aeromonas sp. Aer2-1 was cultivated in 100 m*l* of the medium in the 500-m*l* sakaguchi flask under the optimal condition as described above. After 12 hr cultivation, the culture was shifted to the static condition. Various final concentrations (150 mg/*l* to 2000 mg/*l*) of Orange 16 were added to each flasks and then incubated at 30°C for the decolorization of the dye. The results are shown in Figure 6. The efficient colour removal were shown with the initial dye concentration up to 1200 mg/*l*. Orange 16 was completely decolorized with initial concentration of dye up to 750mg/*l* around 50 hr. At the initial concentration of 2000 mg/*l*, the decolorization degree obtained was about 60%.

As shown in Figure 7, decolorization rate increased with the increasing Orange 16 concentration and reached constant (around 15.5 mg/*l*/hr) in the concentration of more than 750 mg/*l*. Through the decolorization experiments, the cell weight were not increased in the static condition and the cell surface still remained white, indicating that no Orange 16 was adsorbed to the cell surface.

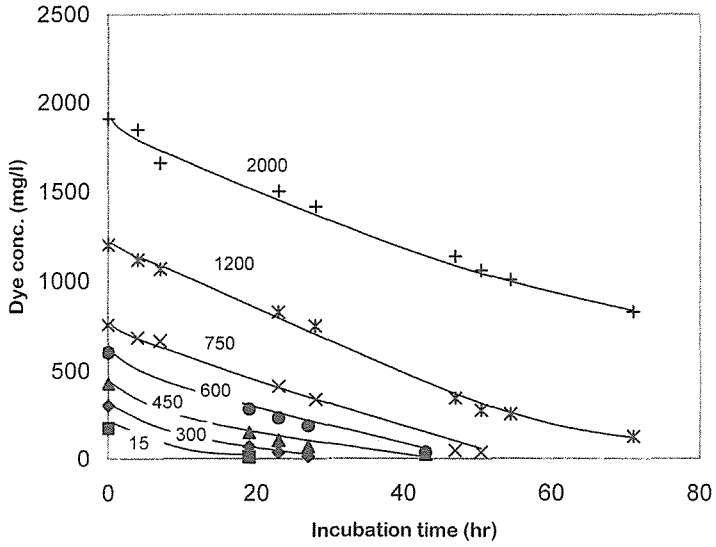

Figure 6. Effect of Orange 16 concentrations on the decolorization. Orange 16 concentration in the medium is indicated in the figure.

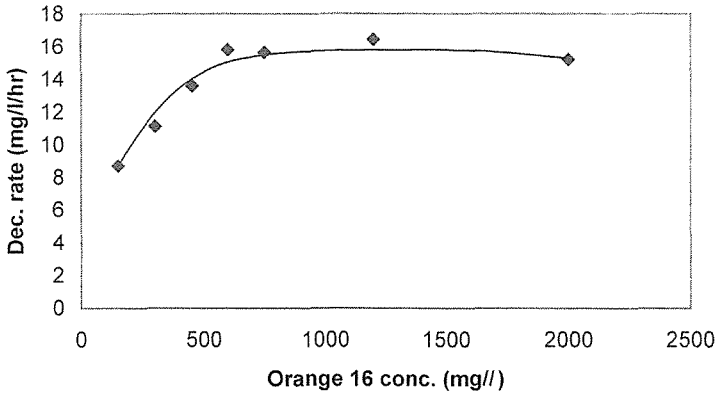

Figure 7. Decolorization rate with Orange 16 concentrations.

CONCLUSIONS

Bacterial strain Aer2-1, identified as *Aeromonas* sp., has ability to Orange 16 decolorizing potential.

By shifting the culture condition from the aerobic growth to the static condition, which is an oxygen limiting or anaerobic condition, Orange16 was efficiently decolorized. Complete decolorization of Orange 16 was observed at a concentration of lower than 750 mg/*l* for 55 hr with decolozation rate of 15.6 mg/*l*/hr.

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