Decolorization of Orange 16 by Mixed Culture of Bacteria

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Summary

A bacterial mixed culture MTT to decolorize Orange 16 was isolated from a sample from ditch receiving effluent from staining factories. MTT could decolorize up to 120 ppm of Orange 16 in 6 hr and up to 250 ppm in a prolonged periods in a static culture. MTT could decolorized 40 ppm of Orange 16 in 11 cycles of repeated addition at every 6 hr

Introduction

In developing countries waster-water treatment system is not prevailed well and many small factories of fabric staining discharge the effluent to rivers, creeks, ditches, or ponds without waste-water treatment. So those streams are stained colorfully. Azo dyes are the most popular dyes used much in the factories, but are quite tough to microbial degradation. We isolated a mixed culture of bacteria, MMT from one of ditch to decolorize active Orange 16 well. In here we report the preliminary decolorization of Orange 16 by mixed culture of bacteria MTT.

Materials and methods

The mixed culture of bacteria MTT was selected after sequential cultures-decolorizations at 40 ppm of Orange 16 by shaking and standing culture of flask. more than 5 kinds bacteria in the mixed culture MTT were contained, and 5 bacterial strains were isolated. None of isolated strain could decolorize 40 ppm of Orange 16 in a few days, nor the mixture of 5 strains. In this experiments we tried to decolorize Orange 16 by mixed culture MTT without isolating organisms.

The bacteria were cultured in a 300-ml conical flask containing 100-ml of the medium, which composed of 4.0 (g/*l*) yeast extract, 0.5 ammonium sulfate, 0.5 potassium dihydrogen phosphate, 1.5 dipotassium hydrogen phosphate, 0.2 magnesium sulfate heptahydrates, and 0.01 Orange 16 (pH 7). The Orange 16 concentrations were varied in experiments as indicated in the text.

The growth of bacteria was measured as suspending solid or by OD_{700}.

The decolorization was determined by A_{490}.

Orange 16 was purchased from Nissey Kasei in Nagoya, whose structure is shown in Fig. 1. Other chemicals were commercially available.

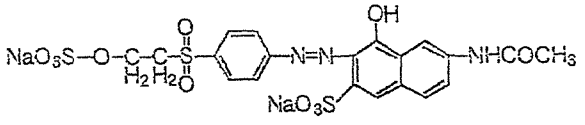

Fig. 1. Chemical structure of Reactive Orange 16.

Results and Discussion

Decolorization of Orange 16 in different aerobic conditions

Bacteria MTT were grown by shaking culture for 12 hr and three cultured flasks were mixed as a seed culture. 20-ml each of the culture was seeded into 300-ml conical flasks containing 180-ml of fresh medium containing different levels of Orange 16 (40, 60, 80, 100, and 120 ppm). The first set of flasks were air-bubbled and mixed by a magnetic stirrer, the second set of flasks were nitrogen-bubbled, and the third set of the flasks left stood at room temperature. Periodically samples were taken, and centrifuged at 10,000 rpm for 10 min. The COD and color of the supernatants were analyzed and the pellets were washed two times and dried (suspending solid). Those results are shown in Fig. 2.

The growths of MTT at different concentrations of Orange 16 were far much better in the aerobic condition than those in the anaerobic and static conditions. For example, the growth at 80 ppm Orange 16 in the aerobic condition was 5 to 6 folds higher than those in the anaerobic and static conditions.

The decreases of COD in the aerobic condition were almost linear, but that at 80 ppm Orange 16 was faster than that at 120 ppm. In the anaerobic condition the CODs at 100 and 120 ppm Orange 16 started to decrease after 9 hr, but that at 80 ppm decreased without any time-lag. In the static condition the decrease of COD at 120 ppm showed a time-lag of 9 hr, but the time-lag at 100 and 80 ppm were not significant.

The decolorizations in the aerobic condition were almost negligible, even though at 40 ppm. But the declorizations in the anaerobic and static conditions were almost complete at 6 hr except for that at 80 ppm in the static culture.

From the above experiments we concluded that the decolorization of Orange 16 by mixed culture of bacteria MTT in the anaerobic and static conditions were better than that in the aerobic conditions. In further experiment we employed the static condition.

Diazo-dye Reactive Red 3 at 40 to 120 ppm was also decolorized in the anaerobic and static conditions as well as Orange 16.

Decolorization of Orange 16 by repeated addition

In order to make the decolorization more efficient, Orange 16 was added intermittently at every 6 hr after the decolorization. The results are shown in Fig. 3. The bacteria were grown at 60 (A) and 80 (B) ppm Orange 16 in shaking culture for 12 hr, which were shifted to the static condition. The growth in both culture was attained to the maximum at 9 hr and they did not increase in further incubation. The decolorization did not proceed any in the shaking culture, but proceeded in both static cultures as expected from the previous results, and it was almost complete at 18 hr, that is, 6-hr decolorization. Then, Orange 16 was added to give 80 (A) and 100 (B) ppm at 6-hr interval. The decolorization in each addition of the dye were quite well in both culture.

Decolorization of Orange 16 at higher concentration

In each repeated addition of Orange 16, the concentration of the dye was increased as shown in Fig. 4. The culture was started with 80 ppm Orange 16 in the shaking culture and at 12 hr it was shifted to the static culture. The growth

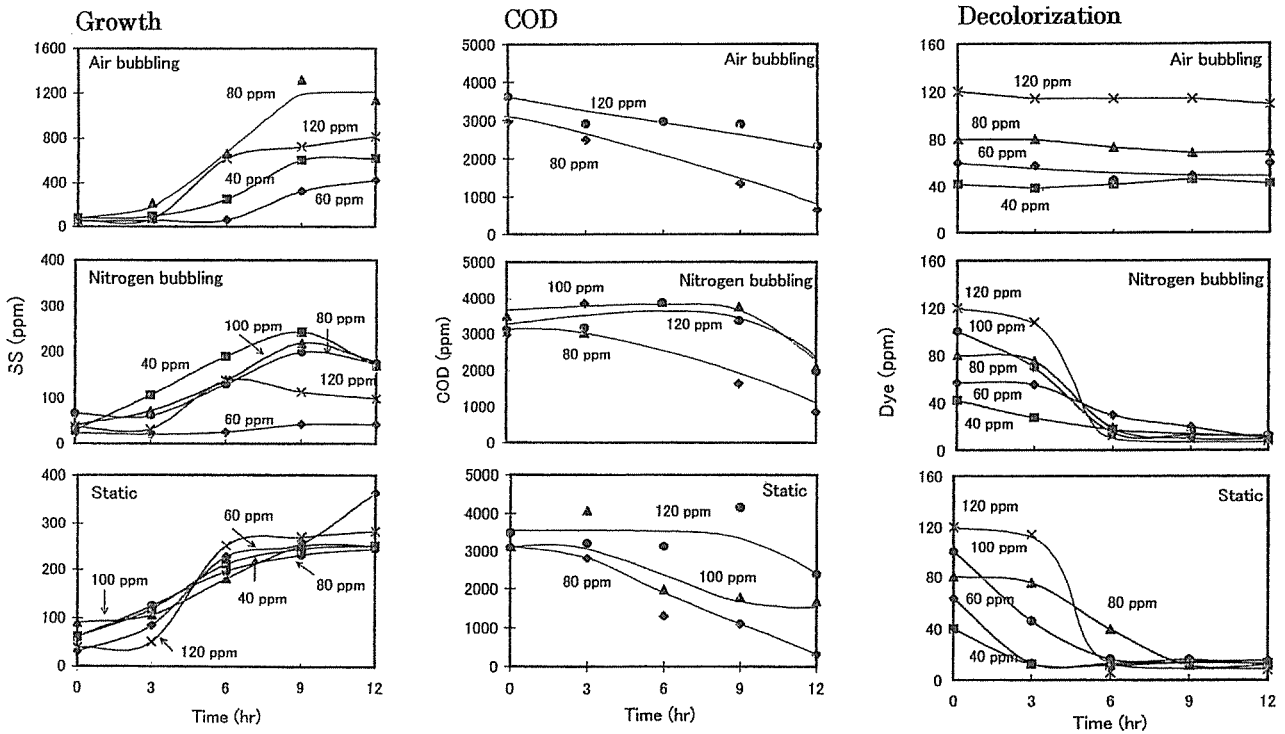

Fig. 2. Time courses of decolorization of Orange 16 at various concentrations by mixed culture of bacteria MTT under air bubbling, nitrogen bubbling, or static conditions

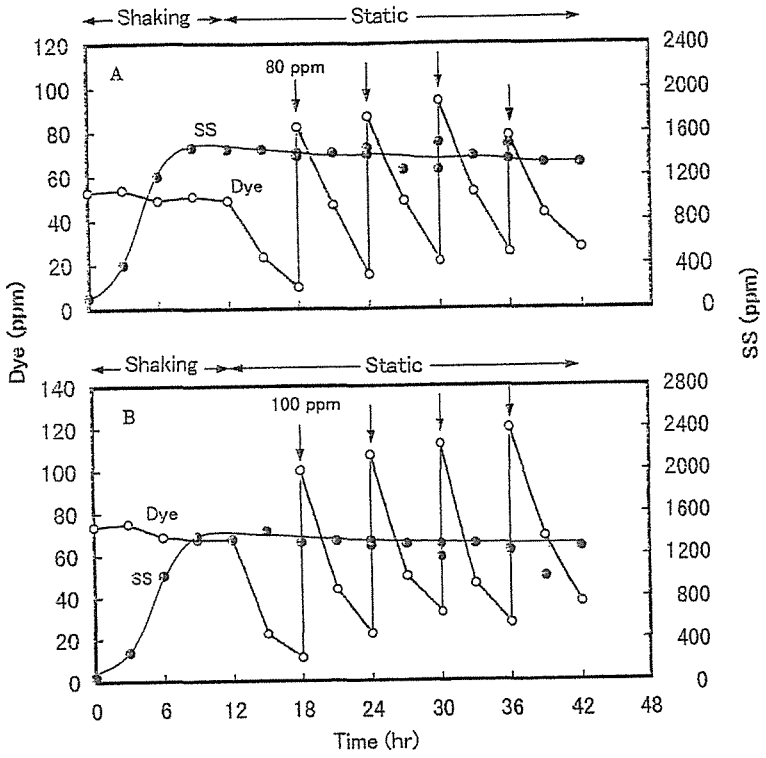

Fig. 3. Decolorization of Orange 16 by mixed bacteria MTT with repeated addition

attained to the maximum at 12 hr. Orange 16 was not decolorized until 12 hr and it was decolorized rapidly after the shift. The dye was added at 15 hr to give 150 ppm and it was decolorized at 27 hr. Then the dye was added to give 250 ppm at 45 hr and to give 300 ppm at 63 hr. The decolorization proceeded well at 200 and 250 ppm, whose period were 18 and 13 hr, respectively. The degradation rate at 250 ppm was exceptionally and that at 300 ppm very low. MTT might not degrade Orange 16 at higher than 300 ppm. The bacterial cell mass decreased at higher than 150 ppm dye. Orange 16 at 250 to 300 ppm might have very toxic effect on bacteria MTT.

Long term decolorization of Orange16 by repeated addition of dye in a 2-*l* iar-fermentor

Long term decolorization of Orange 16 was carried out by repeated addition of 40 ppm dye using a 2-*l* jar-fermentor. To operate the long term, the dye concentration should not be harmful to bacterial cells and 40 ppm Orange 16 was selected. The bacteria MTT was grown with 10 ppm dye ina 300-ml flask in the shaking condition for 12 hr, it was seeded to the jar-fermentor, it was cultured at 300 rpm of agitation rate and 0.5 vvm of aeration rate untill the maximum cell mass (12 hr), and then it was shifted to the slow agitation (50 rpm) under nitrogen gas. The incubation was continued for 6 hr, but buring the period the dye was not decolorized well as before and the cell mass decreased in the presence of only

10 ppm dye, which was quite strange that the cell decrease was not expected, though the anaerobic condition was employed instead of the static condition. However, here the dye was added to give 40 ppm at every 6 hr (Fig. 5). The decolorization was carried out 11 cycle of the addition (until 90 hr) with almost same decolorization rate. But the decolorization of 40 ppm dye took more than 6 hr, which was quite slower than that expected from previous experiments. The low activity in jar-fermentor might not only be the anaerobic condition but also mis-setting in the fermentor.

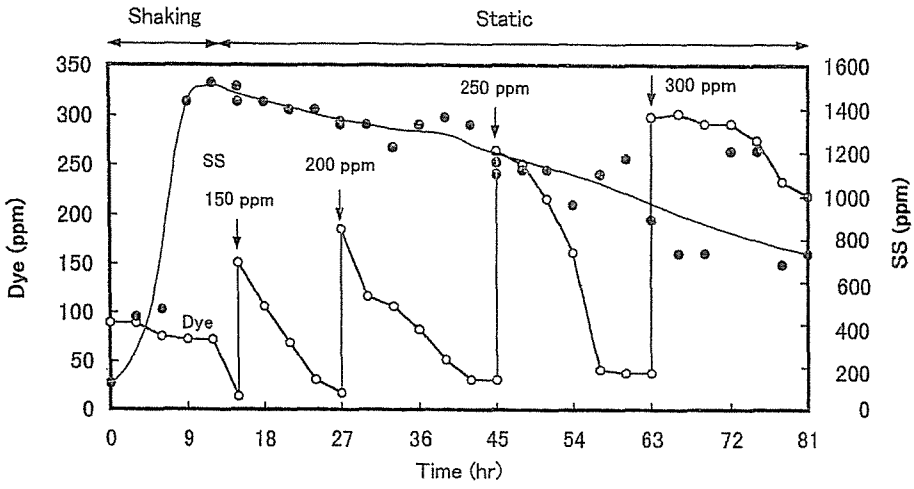

Fig. 4. Decolorization of higher concentration of Orange 16 by repeated culture

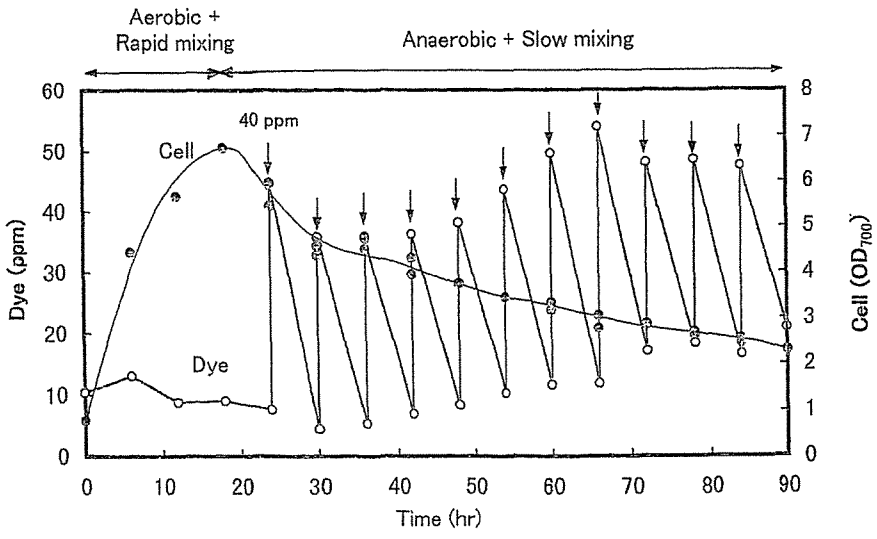

Fig. 5. Long term decolorization of Orange 16 by repeated culture in a 2-/ jar fermentor