



## Studies on Flocculating Activity of Bioflocculant from Closed Drainage System (CDS) and Its Application in Reactive Dye Removal

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### Abstract

Technological production processes of organic dyes soluble in water, as well as the processes for their application in textile industries, may heavily pollute natural waters, particularly from the point of view of their pronounced colored wastewaters. Reactive dyes are prominent among numerous groups of water-soluble dyes. The bioflocculant was effective in flocculating a kind of reactive soluble dyes (Cibacron yellow FN\_2R) in aqueous solution. A bioflocculant-producing bacterium were isolated from wastewater and sediments of Close Drainage Systems (CDS) located at the Prai industrial area.. Compared with conventional chemical flocculants, bioflocculants are biodegradable and nontoxic, and produce no secondary pollution. *Sphingomonas paucimobilis* was found to produce a bioflocculant with high flocculating activity for Kaolin suspension and water-soluble dyes. The best temperature flocculation performance was 35°C and shaking speed of 160 rpm. The highest flocculating efficiency achieved for Kaolin suspension was 98.4% at 35°C after 48 hours cultivation. Various culture temperatures were tested between 2 hours in order to investigate their effect on the bioflocculant production when the culture temperature was 35°C which the flocculating activity of *Sphingomonas paucimobilis* was up to 98.4%. It was found that, flocculating rate depends on time and temperatures. Determination flocculating activity was shown *Sphingomonas paucimobilis* is biodegradable and increase in number of bacteria during the time will confirm that. This study was conducted to biologically treat wastewater discharged from the textile industry using sequencing batch reactor (SBR) technology biological flocculation on COD removal and effects of solids detention times and MLVSS on EPS production.

**Keywords:** Bioflocculant, Reactive dye, *Sphingomonas paucimobilis*, Flocculating activity

### 1. Introduction

Rapid development of industrialization and human activities has lead to increase the discharge of waste and wastewater containing organic and inorganic pollutants. Bioflocculant is a kind of biodegradable macromolecular flocculants created by microorganisms. Because of their biodegradability, harmlessness and lack of secondary pollution bioflocculants have gained much wider attention and research to date (Li Y., 1999). Flocculation is an essential process in the treatment of wastewater, tap water production and dredging or downstream processing techniques in a variety of industrial fields (Fujita M., 2000). Dye wastewaters are recognized as one of the difficult pollutants to be treated. They are discharged to the environment from a wide variety of sources, including textiles, printing, dyeing, dyestuff manufacturing, and food plants (Lee C., 2008). The annual worldwide production of dyes is approximated at 800,000 tones and about 50% of these are azo-dyes. Hence, the treatment of such wastewaters is quickly becoming a matter of great concern and it is urgent to develop sound and cost-effective treatment technologies in order to comply with environmental regulations (M. Zhou, 2007) and (A.S. Özcan, 2005). Coagulation and flocculation have their place among the conventional processes that are frequently cited for treating dye-containing effluents (W. Zhu, 2001), (D. Georgiou, 2003) and (C. Allegre, 2004). The main advantage of coagulation and flocculation is the decolorize the waste stream by the removal of dye molecules from the dye bath effluents, and not by a partial decomposition of the dye, which can lead to even more harmful and toxic aromatic compounds (V. Golob, 2005).

The removal of dyes from wastewater presents a formidable challenge, as most dyes are completely soluble in aqueous solutions. Some cationic flocculants, such as the cationic polyaluminum chloride and polyacrylamide have been found to be effective in dye removal, and charge neutralization between anionic sulfuric groups in the dye molecules and cationic flocculants molecules have been found to play an important role in the flocculation process (J.H. Choi, 2001) and (B.H. Tan, 2000). At present, several methods have been developed to treat dye wastewater. Physicochemical treatments such as coagulation/flocculation, flotation, membrane processes or activated carbon adsorption are common

practices, but they are quite inefficient and result in a phase transfer of pollutants, leaving the problem unsolved. On the other hand, single biological treatments, the most economical and environmentally friendly ones, are not a suitable alternative when working with toxic and/or non-biodegradable wastewaters. In fact, most of disposed dyes are of non-biodegradable nature and standard biological treatment of their colored effluents is not effective (Garcia-Montano J., 2006). The application of SBR to color removal is rather a new approach compared to anaerobic-aerobic sequential treatment (Kapdan, I. K., 2005). Recent research has focused on the use of bio-coagulants and bio-flocculants (J. Roussy, 2005). This paper aims to examine the best culture conditions for the production of a bioflocculant by *Sphingomonas paucimobilis* using dye wastewaters as feeding substrate. The reactive dye (Cibacron yellow FN\_2R) was treated by sequencing batch reactor, where the dye concentration influenced the system.

## 2. Materials and methods

### 2.1 Nomenclature

CDS: close drainage systems

COD: chemical oxygen demand

EPS: exocellular biopolymers substance

HRT: hydraulic retention time

MLVSS: mixed liquor suspended solids

OD: optical density

PGA: Polyglutamic Acid

R: reactor

SBR: sequencing batch reactor

### 2.2 Preparation of biopolymer flocculants

One loop, full of bio-flocculants producing bacteria isolated from Closed Drainage System, (CDS) located at the Prai area and grown on slant agar at 35° C for 2 days in oven, (K. Kakii, 1986) and (T. Endo, 1976).

### 2.3 Bioflocculant-producing medium and culture conditions

The composition of the basic culture medium for the production of bioflocculant Compositions of medium were as follows: 20(g/L) glucose, 0.5(g/L) yeast extract, 50(g/L) L-glutamic acid and 0.5(g/L) magnesium sulphate heptahydrate (MgSO<sub>4</sub>.7H<sub>2</sub>O) and the medium was adjusted to pH 7.0. The medium was solidified by addition of 15 g bacteriological agar powder in 1.0 L deionized water. Polyglutamic Acid (PGA) was used as a medium for cultivation of bioflocculant producing bacteria. After autoclaving and inoculating the medium, the bacteria were cultured in 250 mL conical flasks on a rotary shaker at 160 rpm and 35°C for 2 days. Samples were taken at various time intervals to determine the pH, flocculating activity. At the same conditions used to culture the single colonies that were selected in PGA broth to measure their flocculating activities.

### 2.4 Flocculating Rate Measurement

The flocculating rate was measured using a previous method with a slight modification, in which Kaolin clay was chosen as the suspended solid (Kurane R., 1986). Kaolin clay was suspended in distilled water at the concentration of 5000 mg/L. 4.50 mL of 1% CaCl<sub>2</sub> and 0.5 mL of culture broth were added to 45 mL Kaolin suspended solution in 100 mL beaker in turn. The mixture was vigorously stirred and was allowed to stand for 5 minutes. The optical density (OD) of the clarifying solution (A) was measured with a spectrophotometer at 550 nm. A control experiment was prepared using the same method, but the culture broth was replaced by distilled water (B). The flocculating activity was calculated according to the equation:

$$\text{Flocculating activity (\%)} = \{(B - A)/B\} \times 100 \quad (\text{H. Yokoi, 1997; S. B. Deng, 2003})$$

Where:

A: is the optical density of the sample experiment at 550 nm

B: is the optical density of control experiment at 550 nm

Effects of pH, flocculant's concentration, and various actions on flocculation were investigated. Two sets (A and B) of 5 conical flasks were taken. 10 ml of dye wastewater was taken in each flask of set A whereas 10 ml distilled water was added to each flask of set B. A control flask was prepared using the same method but the sample was replaced by distilled water flocculating activity was measured by 5 conical flask as controlling group and another 5 conical flask as dye wastewater which was taken every 2 hours at different temperatures

### 2.5 Preparation of dye wastewater

The dye concentrations were adjusted to be 20, 50, 100 and 150 mg/L in the reactors R1, R2, R3 and R4 and were determined for COD and MLVSS every day.

## 3. Results and discussion

### 3.1 Biofloculant-producing properties

The bacteria was in the highest flocculation of Kaolin suspension attained was 98%. The same phenomenon happens where the flocculating activity paralleled the growth of the microorganism.

### 3.2 Effect of time on the flocculating rate

Based on the preceding results, the flocculants activity measurements at after every 2 hours and at different temperatures are employed. Durations of incubation period are number1: first two hours, number 2: second two hours, number 3: third two hours and etc. For example at 35°C, 40°C and 45°C as illustrated below in figure 1, will show that flocculants activity will increase along the time periods.

Figure1: the effect of different incubation periods on flocculent rates at temperature 35°C

Figure 1, shows the flocculants activity with different time percentage of absorbance for dye wastewater more than control the flocculation mirrored the growth of the biomass and increased with time every two hours, the bacteria was in its exponential growth phase, because control include bacteria and distilled water and dye wastewater include Cibacron yellow FN\_2R and bacteria result shows that bacteria more growth in dye wastewater and the flocculation also rapidly increased the highest flocculation of kaolin suspension attained was 98.4% at 35°C as shown in Figure 1.

Figure 2: the effect of different incubation periods on flocculent rates at temperature 40°C

Figure 3: the effect of different incubation periods on flocculent rates at temperature 45°C

Figure 2 and 3, show that at 40°C and 45°C flocculating efficiency was lowest amount and the flocculation of kaolin suspension attained was 82 % at 45°C. The decreasing in flocculation rate could be due to thermal effect (Wu J. Y., 2007). Structure of biofloculant would be destroyed while heating, hence the biofloculants are considered thermally unstable.

### 3.3 Effect of HRT on COD and MLVSS

Figure 4 and 5, show that between different concentration of dye wastewater for 20, 50, 100 and 150 mg/L every 24h, COD decrease and MLVSS increase.

Figure 4: Effect of HRT on COD (HRT=1day)

Determination of MLVSS in different concentration of dye (figure 5) show that during period as time pass, we have increase in MLVSS (HRT =24h). This results shows that bacteria growth during the time (Ilgi Karapinar Kapdan, 2006).

Figure 5: Effect of HRT on MLVSS (HRT=1day)

In figure 5, the first MLVSS was varied from low concentration to high concentration. This behavior could be result of the saturation of available colloidal surface with the attached EPS chemical bridge. Furthermore, production of EPS and bacteria gradually increase with time.

## 4. Conclusion

Flocculation processes gave satisfactory results. The results show that the optimal flocculant quantity has to be used. The biofloculant production was found to considerably depend on the culture temperature. Various culture temperatures were tested between 2 hours in order to investigate their effect on the biofloculant production when the culture temperature was 35°C where the flocculating activity of *Sphingomonas paucimobilis* was up to 98.4% and when the culture temperature was 45°C the flocculating activity was in lower amount. The best temperature was found be for production this bacteria is 35°C. A biofloculant produced by *Sphingomonas paucimobilis* was effective for the removal of dye. Cibacron yellow FN\_2R is one of the main dyes that are used in textile industries in Malaysia. Therefore, industrial effluents containing dyes must be treated before their discharge into the environment (Wong Pei Wen, 2003) and Closed Drainage System (CDS) located in Prai industrial areas was selected as microbial source. CDS served a major industrial zone in Prai where heavy industries such as chemical process, petrochemical and heavy metal are located. Selected *Sphingomonas paucimobilis* isolated from (CDS) and Cibacron yellow FN\_2R as dye wastewater because it was reactive dye wastewater. Reactive wastewater and reactive dyes are more frequently used in dyeing processes due to their good solubility in water. Counting bacteria and determination flocculating activity was shown *Sphingomonas paucimobilis* is biodegradable and increase in number of bacteria during the time will confirm that. Results show that time and a temperature effect on growth of bacteria is effective. This study was using sequencing batch reactor (SBR) technology at different concentrations of dye wastewater between every day (HRT = 1 day)

bacteria growth and produce biopolymer every day and decrease COD, increase MLVSS. Its practical application in industry would also be developed in further progress.

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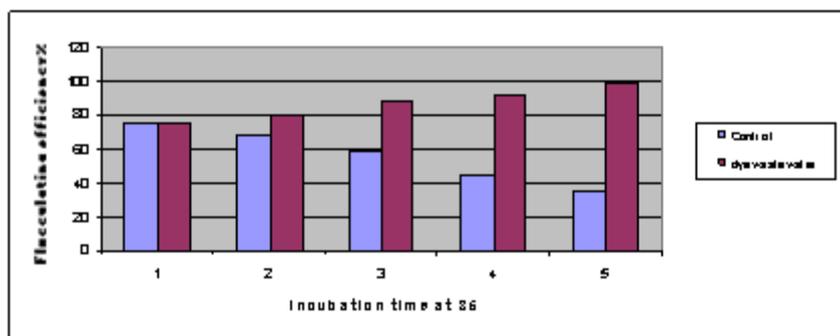


Figure 1. The effect of different incubation periods on flocculent rates at temperature 35°C

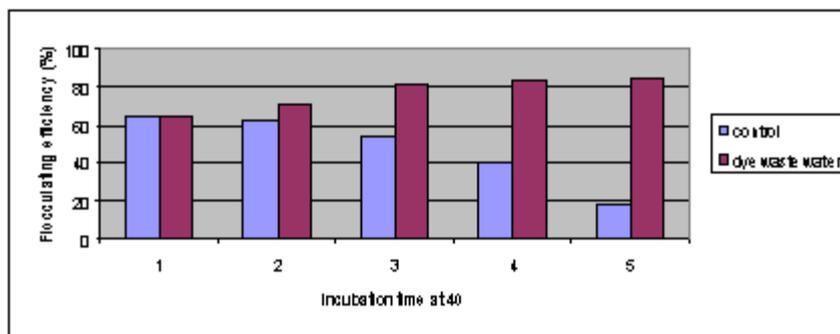


Figure 2. The effect of different incubation periods on flocculent rates at temperature 40 °C

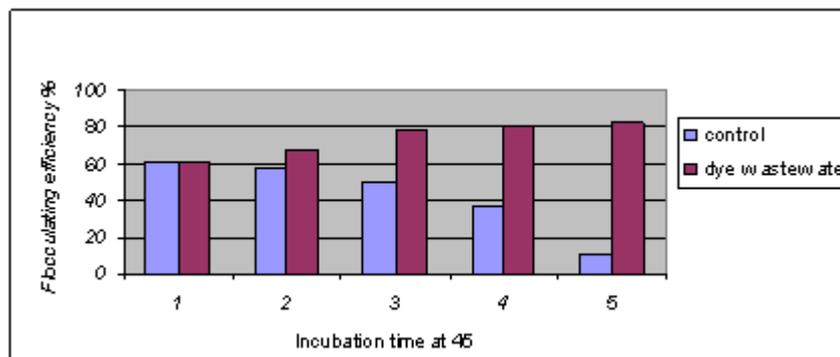


Figure 3. The effect of different incubation periods on flocculent rates at temperature 45°C

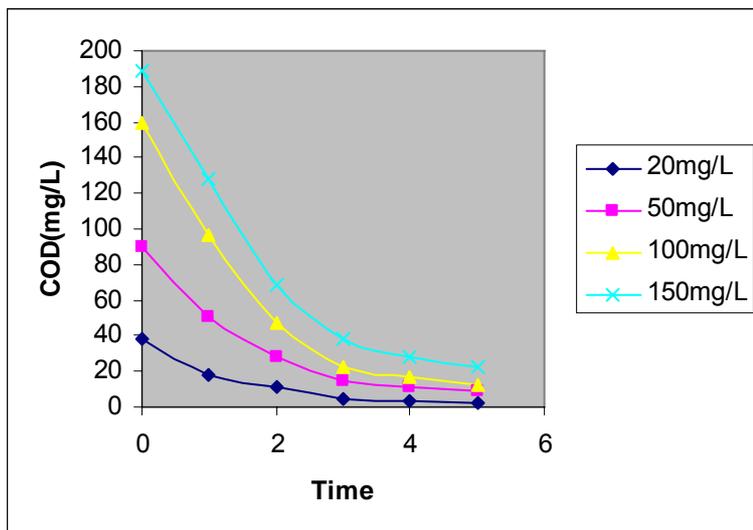


Figure 4. Effect of HRT on COD (HRT=1day)

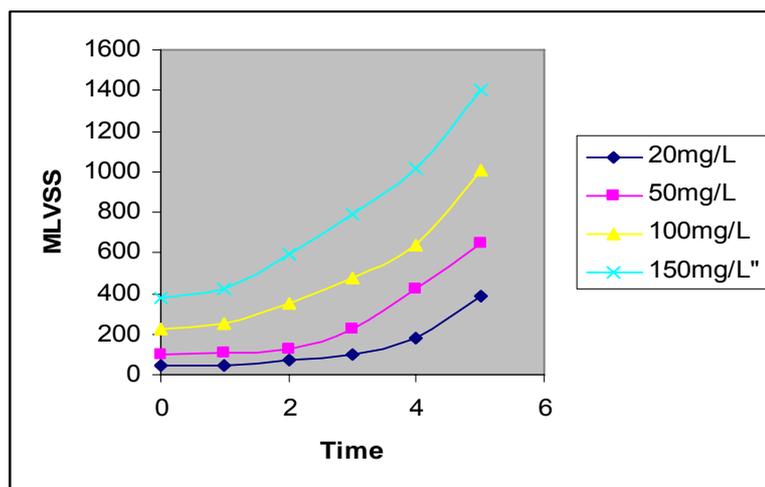


Figure 5. Effect of HRT on MLVSS (HRT=1day)