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Full Length Article

Prospects of Bacterial Granule for Treatment of Real Textile Industrial Wastewater

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ABSTRACT

The Mk-8 isolated bacterial granule was selected due to its ability of decolorizing textile dye and COD removal. Study on the efficiency of this bacterial granule on real textile wastewater treatment from Batik textile industry was performed under anaerobic condition at room temperature for 14 days. The bacteria culture was mixed with fine sand, soybean bran and rice flour followed by granularization process to obtain pellets measuring 1 cm in length with a diameter of 0.5 cm. Decolorization and filter chemical oxygen demand (FCOD) reducing efficiency was 96.9% and 66.7%, respectively. Morpho-physiological characteristics and biochemical properties as well as DNA sequencing of 16SrRNA indicated that the bacterium was *Bacillus subtilis*, while Mk-8 was identified as *Bacillus* Mk-8.

Key Words: Decolorization; Bacterial granule; Real textile wastewater; Filter chemical oxygen demand

INTRODUCTION

Currently, large textile dyeing industry in Thailand utilizes a considerable amount of water in its production process that eventually results into wastewater with a large amount of dye particulates/molecules. This wastewater discarded to the water sources causes a drastic decrease in oxygen concentration due to the presence of hydrosulfides in certain dyes that can react with oxygen. It also blocks the passage of light to the water body by increasing the turbidity, which is detrimental to water ecosystem (Jirawat, 1998). In fact, many dyes employed by the textile industry are synthetic azo types (Pearce *et al*., 2003). Some dyes may also disintegrate into toxic amine compounds (Jirawat, 1998). Physical and chemical treatments of dye wastewater envisage high initial investment cost on equipment construction and a large number of remnant hazardous substances (Pearce *et al.,* 2003). Based on these reasons, efforts are underway on the identification of microorganisms that are capable of converting the dye residues into digestible organic materials for further biological treatment process. The capability of microorganisms to decolorize or reduce various kinds of dye residues depends mainly on the characteristics of each microbe (Kuo *et al.*, 2003a). This includes many species of bacteria such as *Pseudomonas* KF46 and *Kurthia* sp. (Zimmerman *et al.,* 2003), *Aeromonas hydrophila* (Chen *et al.,* 2008), *Pseudomonas luteola* (Hsueh & Chen, 2008), bacterial consortium (*Aeromonas caviae, Proteus mirabilis & Rhodococcus globerulus* (Joshi *et al*., 2008), white rot fungi such as *Phanerochaete chrysosporium* and *Plurotus ostreatus* (Swamy & Ramsay, 1999), actinomycetes and blue-green algae (Dilek *et al.,* 1999). However, a number of researches focused primarily on the digestion of azo dyes by bacteria and fungi rather than other microorganisms (Kuo *et al.,* 2003b). The objective of this research was to identify and test the efficiency of bacteria with dyes-decolorizing capability in the granulate form such that the potential use of this bacteria in textile industry could be established. Various types of materials, for example, soybean residue, rice bran and other agricultural byproducts, were investigated to select the most suitable granularization medium in the treatment of wastewater. Once the polluted components were removed, the treated water could be returned subsequently to the original water source with minimum impact to the aquatic life. In addition, the preliminary data obtained from this study might be beneficial to the design of economical wastewater treatment process with minimization of energy loss and serve as a case study of an alternative treatment that could be implemented to other types of wastewater.

MATERIALS AND METHODS

Measurement of maximum absorbance of wastewater from textile industry. A sample of wastewater from textile industry was collected from Batik textile industry, Lamphun province and its maximum absorbance by UV spectrophotometer (SPECTRONIC® GENESYS™) was measured.

Bacterial identification. The isolated bacteria Mk-8 were examined microscopically for their morphological and

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physiological characteristics. The biochemical properties were also compared based on the instruction of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) and DNA sequencing technique to identify the bacteria type.

DNA extraction and purification. Total genomic DNA was extracted from overnight NB cultures by an Isoplant DNA extraction kid (No. 314-02731) Nippon Gene, Japan.

DNA sequencing of 16S rRNA. Three universal primer pairs (27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 520R (5'-ACCGCGGCKGCTGGC-3')), (357F (5'-CTACGGGAGGCAAG-3') and 1080R (5'-CCCAACATCTCACAC-3')) as well as (920F (5'-AAACTCAAAGGAATGACGG-3') and 1522R (5'-AAGGAGGTGATCCRCCGCA-3')) were used in the amplification of small subunit rRNA (16S rRNA) gene sequences of the *Bacillus* isolates. Computer analysis of the 16S rRNA sequences was performed by comparison with sequences of those in the Gen Bank non-redundant nucleotide database with BLAST program (http://www.ncbi.nlm.nih.gov).

Bacterial granularization. The bacteria isolate were cultured for 24 h in Nutrient Broth (NB) and the optical density was measured at 660 nm (Banat *et al.,* 1996) by UV spectrophotometer (SPECTRONIC® GENESYS™). The bacterial granule was made by uniform mixing of bacteria culture with determined level of optical density to other granulate components in a meat grinder. The drying of granulate was performed in the oven (Standard Lab Oven (Bender), Model No. 01-25103) at 45°C.

Testing of bacterial granule efficiency in the treatment of textile dye industry wastewater. The test was initiated by pouring 500 mL of wastewater into the Erlenmeyer flask. The pH value was in the range of 7.2-7.5. The bacterial granule 5% (w/v) and culture were later added and maintained under anaerobic condition at room temperature for 14 days. The sampling was done every two days for the analysis of filter chemical oxygen demand (FCOD) and dye removal.

RESULTS AND DISSCUSION

Maximum absorbance of wastewater from textile dye industry. The maximum absorbance of a dark blue wastewater sample for 620–750 nm wavelength scan was at 715 nm, which was in accordance with the theory of color substance. As the light with red color wavelength was absorbed by the dyes, the reflected light to the eyes of observer was thus blue and green. The variation of dyes used for each finishing product and substances aiding reaction between the dyes and color structure had resulted in large fluctuation of absorbance varying from one sample to another.

Bacterial identification. Mk-8 bacteria was selected for this study, because it showed an ability to decolorize textile dyes. The morphological and physiological characteristics were investigated through microscope, while the biochemical analysis was also applied to assist in the identification of the bacteria to genus level (Table I). The morphological analysis on nutrient agar indicated a flat white-cream bacteria colony (Fig. 1). The threshold

Table I. Comparison of bacteria isolate Mk-8 with the Bergey's Manual of Determinative Bacteriology

Characteristic	*Bacillus* sp.	Mk-8
Rod-Shaped in Young Culture	+	+
Diameter Over 2.5 µm	-	-
Filaments	-	ND
Rods or Filaments Curved	-	-
Cocci in Tetrads or Packets	-	-
Endospores Produced	+	+
Motile	+	+
Stain Gram Positive at Least in Young Cultures	+	+
Strict Aerobes	D	-
Facultative Anaerobes or Microaerophiles	D	+
Strict Anaerobes	-	+
Product of Carbohydrate Fermentation is Almost all Lactate	D	+
Sulfate Actively Reduced to Sulfide	-	-
Catalase	+	+
Oxidase	D	-
Marked Acidity from Glucose	+	+
Nitrate Reduced to Nitrite	D	+
Requires 3-12% NaCl for Growth	D	-

Fig. 1. Bacteria isolate Mk-8

Fig. 2. *Bacillus* Mk-8 (width of 0.5-0.8 µm and length of 2.5-5.0 µm)

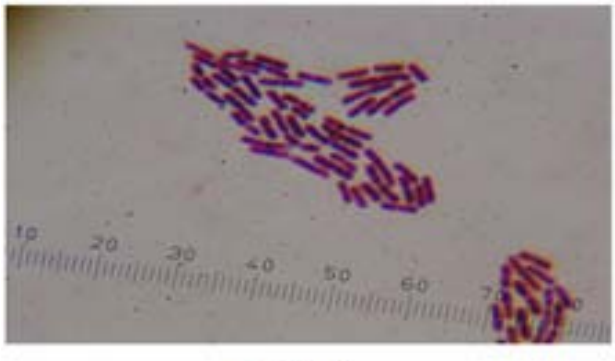


Fig. 3. Result of PCR amplification of genomic DNA from *Bacillus subtilis* isolate using the universal 27F and 520R = Lane 1 , 357F and 1080R = Lane 2 , 920F and 1522R = Lane 3. The position of size makers (~500 bp, ~600 bp, ~700 bp)

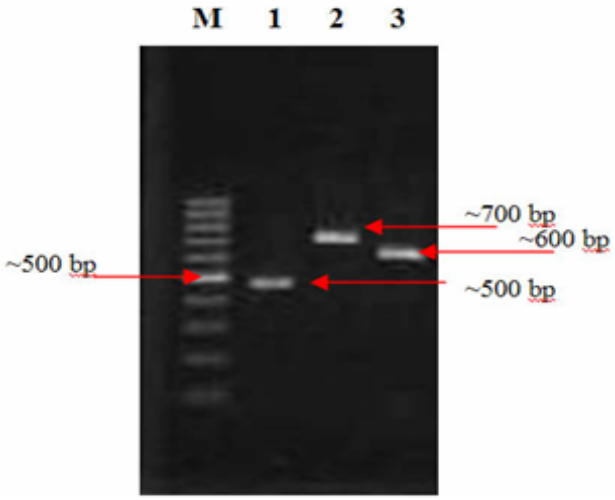


Fig. 4. Bacterial granule with size of 1 cm length and 0.5cm diameter

temperature of this bacterial was between 35-45°C. The observed shape of bacterial under 1000× magnification was extended lump with 0.5-0.8 µm width and 2.5-5.0 µm length as shown in Fig. 2. The positive stained bacterial also appeared to be motile and able to form endospores between the anterior and middle of the cell. All these morphological and biochemical analyses led to the conclusion that this bacterial was *Bacillus* sp. The classification of Mk-8 bacteria isolate through comparison of the genetic sequence 16S rRNA gene with that of genetic database (Gen Bank), indicated that Mk-8 isolate was *B. subtilis* and Mk-8 was identified as *Bacillus* Mk-8 (Fig. 3).

Bacterial granularization. The identified *Bacillus* Mk-8 was cultured in NB and the OD_{660} was regularly monitored for 24 h, after which the bacteria was granularized aseptically with other components by meat grinder in the laminar flow chamber. The drying of granule was performed in the oven with temperature setting of 45°C to avoid the granule deactivation at the temperature above the threshold level determined from the previous section. At the end of granularization process, the light brown granule length of 1 cm and diameter of 0.5 cm (Fig. 4) was obtained. It should be noted that the dimension of granule may vary depending on the specific application.

Fig. 5. The efficiency of bacterial granule on dye removal

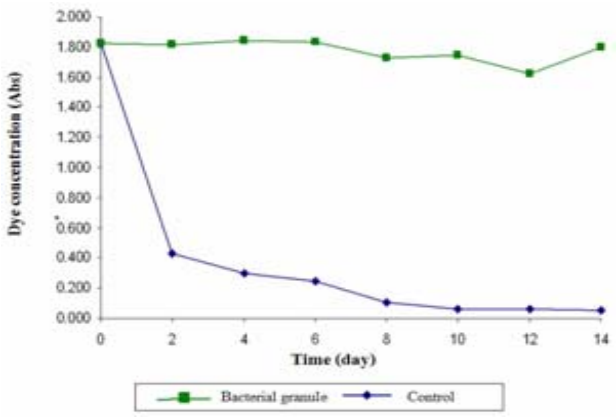


Fig. 6. Percentage of dye removal by bacterial granule

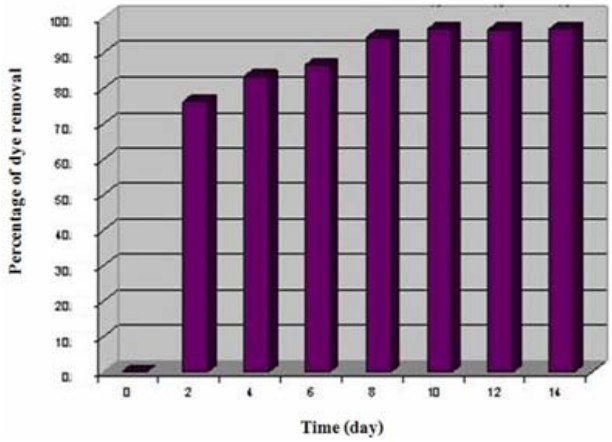


Fig. 7. The efficiency of bacterial granule on FCOD removal

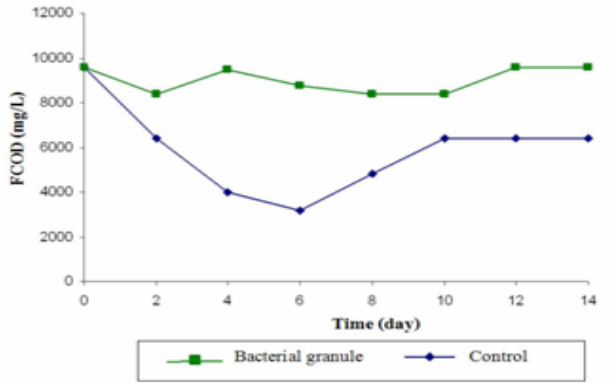
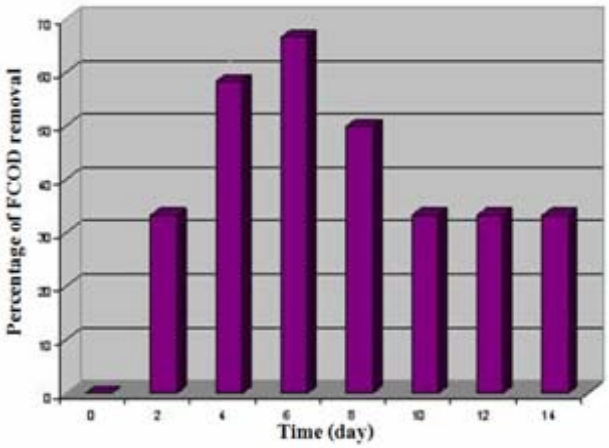


Fig. 8. Percentage of FCOD removal by bacterial granule



Testing of the efficiency of bacterial granule on textile wastewater treatment from textile industry. The efficiency of bacterial granule was examined for 14 days under anaerobic condition at room temperature. The absorbance at 715 nm was decreased during the first day which was followed by the slight increase on day 2 until day 10. The absorbance began to stabilize until the last day of the experiment (Fig. 5). This result was in agreement with Wuhrmann (1980) who reported *B. cereus*, *Sphaerotilus naan* and *Arthobacter* sp. to be efficient eliminators of azo dyes under anaerobic conditions.

Results (Fig. 6) indicated that the percentage of dissolving textile dyes of the bacterial granule increased from day 2 until the last day of the experiment, giving 96.8%, suggesting that granular bacteria had the ability to dissolve substances in the textile dye wastewater under these experiment conditions.

The comparison of dye decolorization efficiency under anaerobic conditions observed from *Bacillus* Mk-8 with other bacteria indicated the similarity to *Aeromonas hydrophila* that decolorized 90% of RED RBN within 8 days (Kuo *et al*., 2003b) as well as *E. coli* and *Pseudomonas* sp. that were able to degrade Congo Red and Direct Black 38 at 80-98% level (Mustafa *et al*., 2002).

The capability of bacterial granule to reduce FCOD in textile wastewater decreased from day 1 to 6 (Fig. 7). The subsequent increase was observed from day 8 until the stable period was reached during the last day of the experiment. Such results indicated that the bacteria had the capacity to reduce the organic substances in the wastewater. High COD in decolorized effluent was due to the recalcitrant nature of amines, which form during the azo bond reduction by bacteria under anaerobic conditions (Singh *et al.,* 2007). The increase in the FCOD value on the later days might be the evidence of inappropriate mixing ratio between bacterial granule and organic materials.

The increase in FCOD removal on the second day of experimental period of two weeks with the highest level observed on the 6th day (Fig. 8). The highest FCOD reduction percentage during the experiment was found to be 66.7%.

CONCLUSION

Present studies revealed that the wastewater from textile industry had maximum absorbance of 715 nm and morpho-physiological characteristics including biochemical tests and DNA sequencing of 16S rRNA revealed that Mk-8 bacteria isolate revealed it to be a *B. subtilis* and Mk-8 was identified as *Bacillus* Mk-8. Bacterial granule was able to decolorize 96.9% of the dye with 66.7% reduction of FCOD.

REFERENCES

Banat, I.M., P. Nigam, D. Singh and R. Marchant, 1996. Microbial decolorization of textile dye-containing effluents: a review. *Biores. Technol.,* 58: 217–27

Chen, B.Y., W.M. Chen, F.L. Wu, P.K. Chen and C.Y. Yen, 2008. Revealing azo-dye decolorization of indigenous *Aeromonas hydrophila* from fountain spring in Northeast Taiwan. *J. Chin. Inst. Chem. Engrs.,* 39: 495–501

Dilek, F.B., H.M. Taplamacioglu and E. Tarlan, 1999. Colour and AOX removal from pluping effluents by algae. *Appl. Microbiol. Biotechnol.,* 52: 585–91

Holt, J.C., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Willians, 1994. *Bergey's Manual of Determinative Bacteriology,* 9th edition

Hsueh, C.C. and B.Y. Chen, 2008. Exploring effects of chemical structure on azo dye decolorization characteristics by *Pseudomonas luteola*. *J. Hazard. Mater.,* 154: 703–10

Jirawat, C., 1998. *Comparison of Reactive Dye Adsorption Capacity by Activated Carbon and Rice Husk Char*. Department of Environmental Engineering, Faculty of Engineering Chiangmai University

Joshi, T., L. Iyengar, K. Singh and S. Garg, 2008. Isolation, identification and application of novel bacterial consortium TJ-1 for the decolourization of structurally different azo dyes. *Biores. Technol.,* 99: 7115–21

Kuo, C.C., Y.W. Jane, C.C. Huang, Y.M. Liang and J.H. Sz-Chuwun, 2003a. Decolorization of azo dye using PVA- immobilized microorganisms. *J. Biotechnol*., 101: 241–52

Kuo, C.C., Y.W. Jane, J.L. Dar and J.H. Sz-Chuwun, 2003b. Decolorization of the textile dyes by newly isolated bacterial strains. *J. Biotechnol*., 101: 57–68

Mustafa, I. and T.S. Delia, 2002. Effect of oxygen on Decolorization of azo dyes by *Escherichia coli.* and *Pseudomonas* sp. and fate of aromatic amines. *Process Biochem.,* 38: 1183–92

Pearce, C.I., J.R. Lloyd and J.G. Guthrie, 2003. The removal of colour from textile wastewater using whole Bacterial cells: a review. *Dye and Pigments,* 58: 179–96

Singh, P., R. Sanghi, P. Anjali and I. Leela, 2007. Decolorization and partial degradation of monoazo dyes in sequential fixed-film anaerobic batch reactor (SFABR). *Biores. Technol*., 98: 2053–56

Swamy, J. and J.A. Ramsay, 1999. The evaluation of white rot fungi in the decolorization of textile dyes. *Enzyme Microb. Technol*., 24: 130–7

Wuhrmann, K., K. Mechsner and T. Kappereler, 1980. Investigations on rate determining factors in the microbial reduction of azo dye. *European J. Appl. Microbiol. Biotechnol*., 9: 25–38

Zimmermann, T., H.G. Kulla and T. Leisinger, 1982. Properties of purified orange II azoreductase, The enzyme initiating azo dye degradation by *Pseudomonas* KF46. *J. Biochem.,* 129: 197–203

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