Textile Wastewater Treatment by Mutant *Enterobacter* sp.

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Abstract

The current study aimed to enhance the ability of *Enterobacter* to decolorize textile wastewater (TW) by sequential mutation using the UV irradiation method. The Enterobacter sp., wild type, was exposed to UV for 60 sec at a distance of 60 cm. The mutant strain showed the highest decolorization efficiency at 87.52±0.11% after using the UV irradiation for 60 sec. While, only 81.15% was observed by wild type. Afterward, the effects of environmental conditions were optimized to enhance the decolorization of TW. The mutant strain showed the highest decolorization efficiency at 90.88% using 100% of TW under pH 7, 100 rpm, 35 °C and 12 h of incubation. Moreover, mutant not only decolorize dye in TW but also improved the quality of the water under optimal conditions. The treated TW met the criteria of the Water Quality Standard, Thailand.

Keywords: *Enterobacter* sp., Mutation, Optimization, Textile wastewater, Ultraviolet

1. Introduction

The problem of wastewater is becoming more and more severe today. The discharge of waste into the water from various factories tends to have a high rate, making the water rotten easily. Most wastewater treatment physical and chemical treatments are used. Physical methods have increased sludge volume generation (management, treatment, cost), high initial capital cost, energy costs, maintenance operation costs and low removal of arsenic [1]. The disadvantages of chemical methods include chemicals required, chemical consumption, ineffective in the removal of the metal ions at a low concentration high sludge production, handling and disposal, the release of volatile compounds and aromatic amines [2]. Therefore, the biological method gains much more interest due to the advantages of this method are as a very clean, inexpensive, and sufficient alternative and efficiently eliminates biodegradable organic matter. Moreover, the application of microorganisms for the biodegradation of organic contaminants is simple, economically attractive and well accepted by the public [3]. *Enterobacter* strain TS1L showed good ability to decolorize textile wastewater at 81.15% and the quality of the TW after treatment was improved dramatically after biological degradation using TS1L [4].

There are many reports of increased the ability of bacteria through genetic improvements using ultraviolet (UV). IIn the current study, UV radiation was selected as it is a standard mutagen and its mutability has been studied extensively due to its ubiquity in nature and convenience of handling. A major benefit of UV radiation is that the circulation and use of such mutants created by this approach are not subjected to the restrictions as those mutants genetically generated and modified. In addition, UV radiation is also applied in many fields, such as UV disinfection technology in clinical microbiology and evolution engineering in biotechnology. Irradiation with UV radiation induces thymine dimer lesions in DNA sequences. The unrepaired lesions increase the rate of replication errors, i.e., mutations. The UV-induced mutation rates can be kept consistent regardless of the differences in the UV dose, viabilities against UV and/or spontaneous mutation rates. The broad spectrum and high upper limit of the speed in the mutability of UV suggest ubiquitous roles of UV radiation in accelerating the evolutionary process. This current study aimed to increase the possibility of *Enterobacter* sp. to decolorize textile wastewater using ultraviolet mutation.

2. Materials and Methods

2.1 Textile wastewater

Textile wastewater was collected from the Ban Phraek Weaving Group, Phatthalung, Thailand. The parameters were determined including pH, total dissolved solids (TDS), total Kjeldahl nitrogen (TKN), oil and grease (O&G), sulfide, total suspended solids (TSS), settleable solids (SS), COD, biochemical oxygen demand (BOD) and color. In addition, the textile after treated with mutant strain of Enterobacter sp. was also characterized under the similar parameters.

2.2 Ultraviolet mutation

The strain *Enterobacter* sp. TS1L (wild type), was inoculated in a PHA-producing medium and incubated at 35 °C, 150 rpm for 24 h. Then, cell was harvested at the logarithmic phase and centrifuged at 8,000 rpm for 10 min at 4 °C. After this, the cells washed using sodium chloride and then spread on PHA detection agar. The plates were placed under a UV lamp at a distance of 60 cm for various periods of time from 0 to 70 sec. After UV radiation, the plates were kept in the dark for 1 h and then incubated at 35 °C for 3 days. The mutagenesis strain was inoculated and incubated at 35 °C and 150 rpm for 24 h. After that, the mutant was analyzed the decolorization efficiency under the fixed conditions, incubated at 35 °C for 12 h at an agitation speed of 150 rpm [5]. The highest decolorization efficiency of the mutant was selected for the optimization study.

2.3 Optimization study

The effects of environmental conditions on decolorization efficiency were analyzed in 100% textile wastewater using mutant strain. Ten percent of inoculum containing $1x10^6$ cells/ml was incubated at 35 °C for 12 h at an agitation speed of 150 rpm. Subsequently, the pH (6-9), temperature (20-40 °C) and agitation speed (100-300 rpm) were evaluated. After 12 h, the samples were tested for cell dry mass (CDM) and decolorization efficiency. Then, the effect of the time of cultivation for up to 12 h on CDM and decolorization efficiency was determined every 6 h. The optimization conditions were selected to TW characterization comparing between before and after decolorization.

2.4 Determination of decolorization efficiency

The decolorization efficiency was measured by the method of Rakkan and Sangklarak, [5]. Decolorization efficiency (expressed as % of decolorization) was calculated as follows:

% Decolorization = [(Initialabsorbance - Finalabsorbance) / Initialabsorbance] × 100

2.5 Statistical analysis

All experiments were run in triplicate. A completely randomized design was used throughout this study. Data were subjected to analysis of variance (ANOVA), and mean comparison was carried out using Duncan's multiple range test. All analyses were performed using the statistical package for social science, SPSS (SPSS 24 for windows, SPSS Inc., Chicago, IL, USA) [5].

3. Results and Discussion

3.1 The characteristic of textile wastewater before treatment

The characteristics of the textile wastewater before treatment as follows: pH 6.92, TDS 407 mg/l, TKN 40 mg/l, O&G 2 mg/l, Sulfide 0.02 mg/l, TSS 20 mg/l, SS 3 mg/l, COD 5,600 mg/l, BOD 240 mg/l and a black red color. The results found that the characteristics of textile wastewater such as pH, BOD and nitrogen concentration (TKN) exceeded the threshold value in the Water Quality Standard, Thailand, resulting in eutrophication and effect on the environment [4].

3.2 Ultraviolet mutation

The strain TS1L were placed under a UV lamp at a distance of 60 cm for various periods from 0 to 70 sec. All survival cell at each time was collected and studied for the decolorization efficiency (Fig. 1).

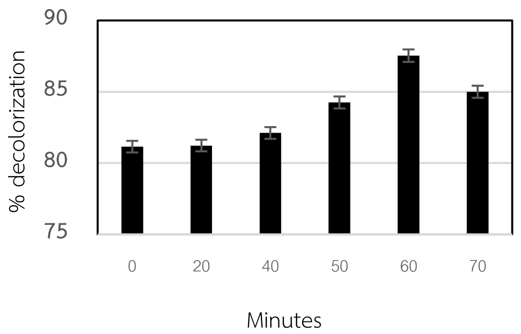

Fig. 1 The effect of UV expose on % decolorization

The mutant gave the best result after 60 sec of UV exposure. The high percentage of decolorization at 87.53±0.10% was observed. Therefore, this mutant strain was selected and used for optimization experiment.

3.3 Optimization study

The decolorization efficiency of the textile wastewater by mutant strain was found to be better in the at neutral and mildly alkaline pH levels (pH at 7-8). In addition, the suitable cultivation temperature was observed at 35 °C. The percentage of decolorization at 87.53±0.10% was obtained. Interestingly, the decolorization efficiency increase (90.88±0 . 52%) when the agitation speed was reduced from 150 to 100 rpm. The optimal cultivation time for mutant strain was observed at 12 h (Fig. 2). The efficiency of mutant strain was also compared with wild type *Enterobacter* sp. TS1L. Lower decolorization efficiency was obtained.

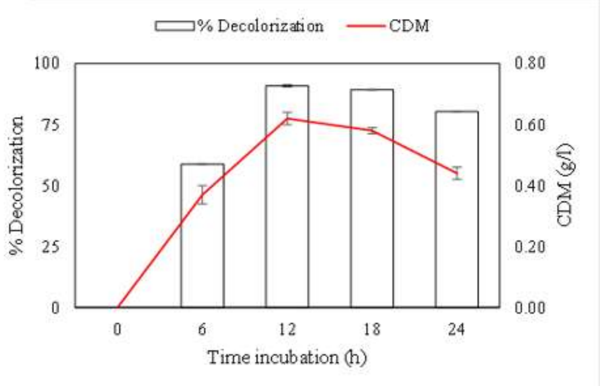

Fig. 2 Effect of time incubation on decolourization of TW by mutant strain

3.4 The characteristic of textile wastewater after treatment

The characteristics of the textile wastewater after treatment as follows: pH 6.45, TDS 69 mg/l, TKN <10 mg/l, O&G 4 mg/l, Sulfide 0.00 mg/l, TSS 4 mg/l, SS <0.1 mg/l, COD 160 mg/l, BOD 19 mg/l and a less red color (Table 1). The results found that the characteristics of textile wastewater after treatment passed the threshold value in the Water Quality Standard, Thailand [4].

Table 1 The characteristic of textile wastewater before and after treatment by *Enterobacter* mutant strain

Parameters	Textile wastewater	
	Before	After
pH	6.92	6.45
TDS (mg/l)	407	69
TKN (mg/l)	40	<10
O&G (mg/l)	2	4
Sulfide (mg/l)	0.02	0.00
TSS (mg/l)	20	4
SS (mg/l)	3	<0.1
COD (mg/l)	5,600	160
BOD (mg/l)	240	19
Color	Black red	Less red

The ability of Enterobacter to remove dye in textile wastewater was also compared with other studies (Table 2). This current study showed the possibility to increase textile wastewater treatment using mutant strain of *Enterobacter* sp. This strain able to remove not only dye but also organic content in TW. Table 2 indicated that the highest percentage of dye removal is ultrafiltration method. However, this method is expensive which not suitable for large scale. This method gave several benefits over chemical and physical methods and able scale up in industrial scale.

Table 2 The comparison of dye removal efficiency using various techniques

Method	Dye removal (%)	Ref
Enterobacter sp. (mutant)	90.88	This study
Microfiltration	26	[6]
Ultrafiltration	100	
Nanofiltration	98	
Reverse osmosis	85.1	

4. Conclusions

The decolorization efficiency was enhanced by UV mutation method. Random mutagenesis of *Enterobacter* strain TS1L by UV radiation showed enhanced decolorization of TW. The decolorization efficiency of TW was observed at 90.88±0.52 using 100% of TW under pH 7, 100 rpm, 35 °C and 12 h of incubation and the treated wastewater met the requirements of the Water Quality Standard, Thailand. Positive outcomes achieved in this study for decolorization of textile wastewater by the mutant strain can effectively be utilized for implementing biological decolorization of industrial wastewater.

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