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Removal of Methylene Blue Using UV-C Pretreated *Citrobacter freundii* JH 11-2 and *Bacillus pseudomycoides* JH 2-2 Biomass

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ABSTRACT

In this study, we evaluated the methylene blue (MB) adsorption potential of non-treated and UV-C pretreated bacterial biomass from aqueous solution. The UV-C pretreatment denature the biomass and has increased overall functional groups when compared to non-treated biomass. The biosorbent was exposed to various pH, biomass dose, and contact time. The results showed that the dried and UV-C pretreated biomass effectively removed MB within 30 min. Dried and UV-C pretreated biomass of *Bacillus pseudomycoides* JH 2-2 showed a adsorption of 858.2 and 1072.4 mg/g at optimum conditions (pH: 9.0, contact time: 30 min, biomass dose: 1 g/L). Similarly, dried and UV-C pretreated biomass of *Citrobacter freundii* JH 11-2 showed an adsorption 868.3 and 954 mg/g at optimum conditions (pH: 9.0, contact time: 10 min, biomass dose: 1.5 g/L). The changes in the functional groups of UV-C pretreated biomass could be responsible for enhanced adsorption of MB. The results obtained have shown that non-treated and UV-C pretreated biomass has a high adsorption capacity for MB dye and can be used as a low-cost biosorbent in wastewater treatments.

Key words : UV-C, Biomass, Methylene blue, Adsorption, Biosorbent

1. Introduction

Synthetic dyes have been widely used in several industries like textile, carpet, rubber, paper, food, leather, plastics and cosmetic to color the product and attract the people for business. Over several thousands of commercially available dyes exist and are produced in tons per year (McMullan et al., 2001; Pearce et al., 2003; Crini, 2006). However, a rough estimation indicating that 10,000 tons of dyes are produced and 7×10^8 kg are used for several purposes per annum (Bayramoglu and Arica, 2007; Ip et al., 2009). Due to their good solubility, synthetic dyes are common water pollutants and they may frequently be found in trace quantities in industrial wastewater. The presence of very small amounts of dyes in water leads to several problems such as increasing the toxicity and COD (Chemical Oxygen Demand) of the effluent, reducing the light penetration, which are harmful to fish and other aquatic organisms (Arami, 2005; Crini, 2006). Several problems such as vomiting, profuse sweating, mental confusion, painful micturition, and methemoglobinemia are arising due to ingesting of dye contaminated water (Ghosh and Bhattacharyya, 2002; Avom et al., 1997). When the dye concentration reaches its saturation level in particular ecosystem, it become highly toxic and leads to a lethal effect (Eccles, 1995).

Numerous conventional physico-chemical treatments such as coagulation, precipitation, filtration, electrodialysis, membrane separation, electrocoagulation, reverse osmosis, nano filtration, titania photocatalyst and oxidation have been used to remove the dye from contaminated water (Morais et al., 1999; Chiou and Li, 2003; Guillard et al., 2003; Aleboyeh et al., 2008; Nataraj et al., 2009). However, these processes are too expensive and adsorption is one of effective methods to remove dyes from wastewater. Thus, the present study made an attempt to remove the dye from wastewater, by an effective and low-cost biophysical method (living or dead microorganisms and ultraviolet

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pretreated). Earlier several pretreatment processes have been made to improve adsorption potential of the adsorbent (Aksu and Tezer, 2005; Vijayaraghavan and Yun, 2007). However, in our study an attempt using UV-C was used for pretreatment of bacterial biomass in order to improve adsorption capability. UV-C generally used for inactivating pathogenic microorganisms in sewage and drinking water (Zimmer and Slawson, 2002; Song et al., 2009). Since UV-C has a short wavelength of 245 nm it generates oxygen radical and induces RNA/DNA damage (Widmann et al., 1998). The principal inactivation of UV-C is the formation of pyrimidine dimer between adjacent pyrimidine molecules on the same strand of DNA/RNA (Franz et al., 2009), also the sludge contains high concentration of guanine and adenosine (Sies et al., 1996).

The objectives of the present study were to (i) to assess the methylene blue (MB) adsorption potential of UV-C pretreated *Citrobacter freundii* JH 11-2, and *Bacillus pseudomycoides* JH 2-2, (ii) assess the physico-chemical variables affecting optimal removal of MB, and (iii) identify the functional groups of the bacteria before and after UV-C pretreatment in order to have a better understanding on the adsorption and desorption of MB.

2. Materials & Methods

2.1. Bacteria biomass preparation

C. freundii JH 11-2 and *B. pseudomycoides* JH 2-2 were isolated from abandoned metal mine soil from Gyeongsang-buk-do. The culture medium was composed of (g/L) beef extract, 1.0; yeast extract, 2.0; peptone, 5.0; and sodium chloride, 5.0 g. The pH of the medium was adjusted to 6.8 before autoclaving. The bacterial cultures were inoculated into the flasks, incubated at 30°C for 24 hr with agitation speed at 130 rpm. After incubation, the biomass was harvested from the medium by centrifugation at 6359 xg for 15 min. The separated biomass was washed three times with distilled water and stored at 4°C for further study.

2.2. Preparation of adsorbate

MB used in this study was purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solution (100,000 mg/L) was prepared by dissolving 1.0 g MB in 100 ml distilled water; other concentrations were obtained by successive dilutions. The concentration of MB in the solution was analyzed by using a UV-Vis spectrophotometer (Agilent 8453 spectrophotometer, Agilent Technologies, Germany) with the absorption maxima at 668 nm (Hameed et al., 2007).

2.3. Pretreatment of bacterial biomass by using UV light

The bacterial biomass was dispersed in distilled water (800 ml) and the optical density of the solution was adjusted to 0.9 at 600 nm. A 4W low-pressure lamp was used to emitting mainly at 254 nm. The UV-C radiation was varied between 0.4 and 0.6 mW/cm^2 extending the exposure time up to 1 h. Later, biomass was harvested from water by centrifugation at 6000 rpm for 15 min and then dried at 70°C.

2.4. Biosorption experiment

Batch studies were carried out by shaking 100 ml of MB solution with different concentrations of biomass (0.01, 0.05, 0.1, 0.15, 0.2 g). The effect of pH on the adsorption of MB was determined at different initial pH levels (3, 5, 7, 9 and 11). The pH of the solution was adjusted using basic acid and alkali before mixing the biomass. Samples were collected at predetermined time intervals (0, 10, 20, 30, 60, 90 min) and centrifuged at 4000 rpm for 3 min, and the supernatant was analyzed for the dye concentration.

The biosorption capacities at equilibrium, q_e (mg/g), were calculated using the following equation

$$q_e = (C_o - C_e) / M^*V \quad (1)$$

where C_o (mg/L) and C_e (mg/L) are the initial and equilibrium concentrations of the dye, respectively. V is the volume of the dye solution (L) and M is the amount of biomass used (g).

All the experiments were done in triplicates and the results were interpreted statistically using Sigma Plot Software (Ver. 10.0).

2.5. FT-IR analysis of control and UV treated biomass

The Fourier transform infrared spectra (FTIR) of the control and UV-C pretreated biomass were obtained on a Perkin-Elmer FTIR spectrophotometer (USA) in the diffuse

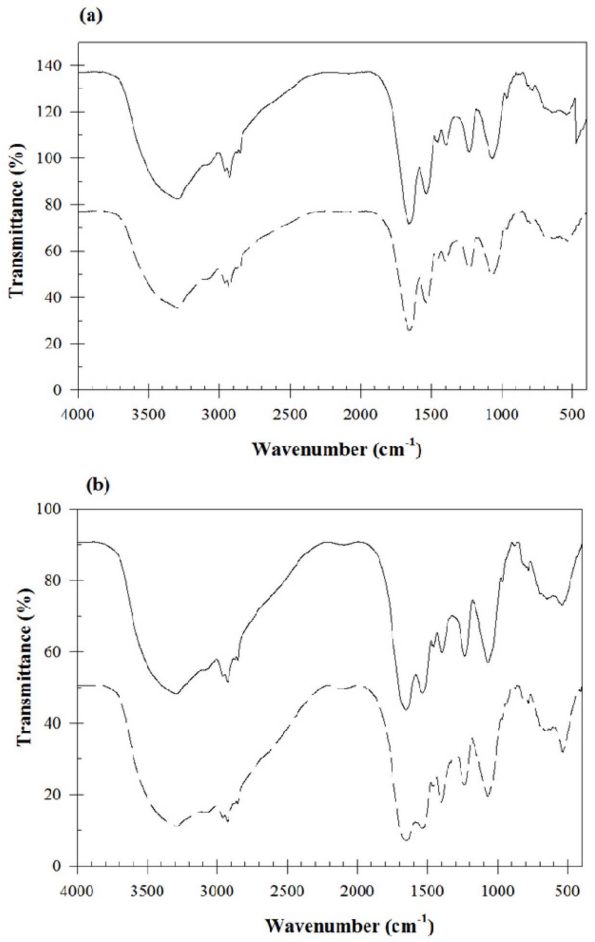

Fig. 1. (a) FT-IR spectrum of *B. pseudomycoides* JH 2-2 dried (⋯) and UV-C treated (–) (b) *C. freundii* JH 11-2 dried (⋯) and UV-C treated (–).

reflectance mode at a resolution of 4 cm^{-1} in KBr pellets.

3. Results and discussion

3.1. Properties of biomass

In order to determine the functional groups present in the biomass, the FT-IR spectra of the native and UV-C pretreated biomass was obtained and the results are shown in Fig. 1. FT-IR results showed the presence of various functional groups on *B. pusedomycoides* JH 2-2 and *C. freundii* JH 11-2 cell membrane. The broad peaks at 3,000 to 3500 cm^{-1} revealed hydroxyl groups (-OH) and 3,000 to 2850 cm^{-1} were C-H stretching vibration (Pavia et al., 2008). The peaks at 1725 to 1500 cm^{-1} could be C = O groups in carboxyl and N-H groups in amine, respectively. These results indicated that the presence of various functional

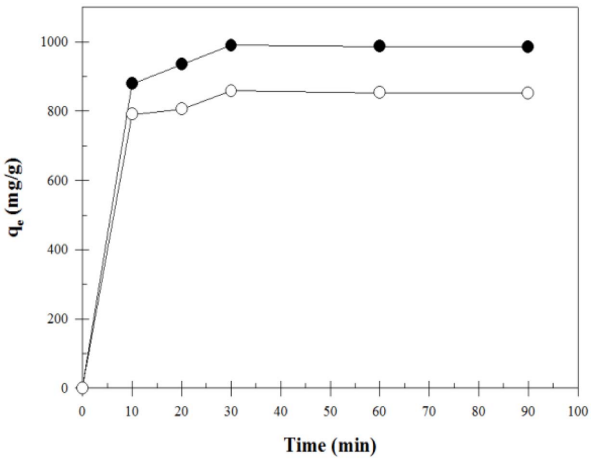

Fig. 2. Effect of the contact time on removal of MB by dried (○) and UV-C treated (●) *B. pseudomycoides* JH 2-2.

groups such as hydroxyl, carboxyl, and amine groups on surface of cell membrane which is potentially known to bind with dyes (Colak et al., 2009). When comparing with native biomass, UV-C pretreated biomass showed some functional group changes. A significant shift can be found in UV-C pretreated biomass (Fig. 1). For example the peaks around 1650, 1500 and 1400 cm^{-1} were shifted and/or broadened and which confirms the effective modification of the biomass.

3.2. Effect of contact time

Dye adsorption rapidly increased during the first 10 min, and gradually increased with laps of time until it reaches the equilibrium (30 min) (Fig. 2). The dye adsorption during the first 30 min was impressive for all the initial concentrations and attained 90 and 78% removal for UV-C pretreated and dead cells, respectively. After 60 min of initial contact time, a minor decrease in adsorption was observed. In general, UV-C pretreated biomass exhibited high adsorption rate than the dried biomass which could be attributed to the increase of binding sites on the biomass surface. The maximum rate of adsorption increased gradually within 10 min (878.9 mg/g), followed by 20 min (934.5 mg/g). After 20 minutes the rate of adsorption remained constant (990.25 mg/g).

3.3. Effect of optimum pH

pH is one of the most important factor to influence dye

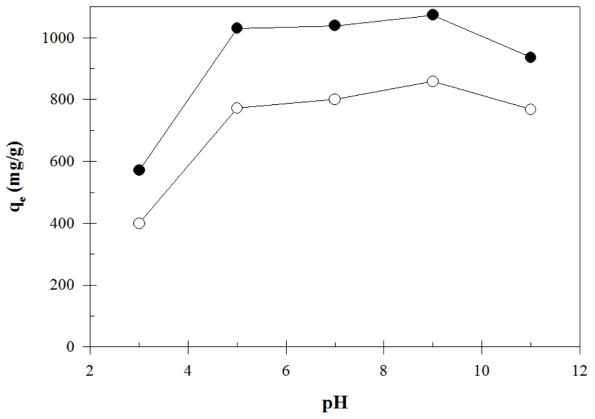

Fig. 3. Effect of initial pH on removal of MB by dried (○) and UV-C treated (●) *B. pseudomycoides* JH 2-2.

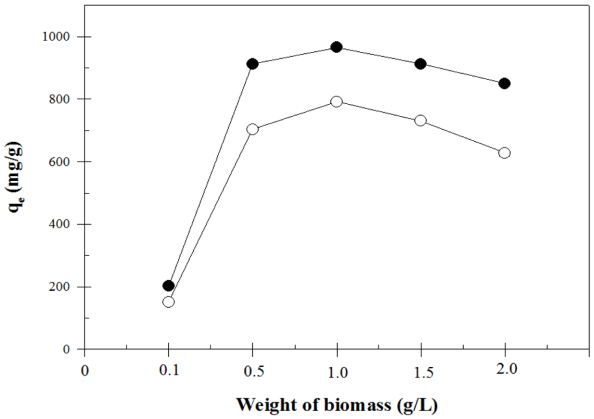

Fig. 4. Effect of biomass dose on removal of MB by dried (○) and UV-C treated (●) *B. pseudomycoides* JH 2-2.

adsorption. According to pH, functional groups located on the surface of the adsorbent were dissociated or protonated. The adsorption of MB by *B. pseudomycoides* JH 2-2 biomass was determined as a function of pH and the results are shown in Fig. 3. The removal rate was low at acidic pH (pH 2.0), gradually increases and reaches the maximum adsorption at pH 9. The high alkaline pH (9.0) generates negatively charged absorption sites (OH-) in the surface of the bacteria which enhances the adsorption of positively charged MB. However, a further increase in pH (10.0) decreased the adsorption rate of MB. The results are consistent with previous studies reported the enhanced adsorption of MB at pH 9.0 (Gupta et al., 2004; Janos, 2003).

The cell wall of the Gram-positive bacteria is different from Gram-negative bacteria. The Gram-positive bacteria consist of peptidoglycan layer connected by amino acid bridges (Mera et al., 1992). If Gram-positive bacteria exposed to nearly neutral or alkaline pH condition, the cells may get negative charge (Vijayaraghavan et al., 2008). However, MB is a cationic dye which may electrostatically attracted towards the negatively chared cell surface. Carboxyl group present in the cell wall of *B. pseudomycoides* JH 2-2 was also responsible for adsorption of MB. The P_{ka} value of the carboxyl group remains in the range of 3.6 - 4.5 (Romero González et al., 2001). The carboxyl group has negative charge at pH 5 and electrostatically attracts MB towards bacterial biomass surface. At pH values below 5, the carboxyl group was in positive charge which reduces the adsorption of MB onto biomass surface.

3.4. Effect of biosorbent dose

The effect of adsorbent dosage for both native and UV-C pretreated biomass was evaluated and the results are shown in Fig. 4. The removal rate was increased with increase in adsorbent dosage up to 0.1 g. However, a further increase in dosage to 0.2 g decreased the removal rate. Thus, the optimum dose of biomass for maximal removal of MB was determined as 1 g/L with removal efficiency of 1072.39 mg/g. Electrostatic interaction between biosorbent and MB could be responsible for adsorption. The poor adsorption rate at high adsorbent dose could be due to aggregation between biomass (Kumar and Porkodi, 2007). A higher adsorption obtained with a small amount of UV-C pretreated biosorbent could be attributed to an increase in the negative charged functional groups on the biosorbent surface.

3.5. Biosorption potentials of *C. freundii* JH 11-2 and *B. pseudomycoides* JH 2-2

The study bacteria *C. freundii* JH 11-2 and *B. pseudomycoides* JH 2-2 showed effective MB removal compared with other biomass (Table 1). The UV pretreated bacterial biomass showed maximum removal at optimum conditions (pH 9.0; constant time 10 min; biosorbent dose, 0.15 g). The maximum biosorption q_e of dried and UV-C pretreated biomass were 868.3 mg/g and 954 mg/g, respectively (Fig. 5). The differences in the adsorption could be attributed to the differences in cell wall composition of the bacteria. The presence of high amount of peptidoglycon and low amount of lipid may enhance the

Table 1. Comparison of MB adsorption rate by *B. pseudomycoides* JH 2-2, *C. freundii* JH 11-2 and other reported bacteria

Content	Dye	Operating conditions				q_e (mg/g)	Ref.
		pH	M*	V**	Equilibrium		
P. notatum,	MB	8.0	0.01 g	30 ml	6 h	33.6	Kumar and Porkodi, 2007
P. oceanica		6.0	0.4 g	50 ml	3 h	4.64	Ncibi et al., 2007
C. glutamicum		9.0	0.1 g	40 ml	12 h	207.3	Vijayaraghavan et al., 2008
E. spp.		6.0	0.05 g	50 ml	3 h	273.73	Ncibi et al., 2009
C. freundii		9.0	0.15 g	100 ml	10 min	954	This work
B. pseudomycoides		9.0	0.1 g	100 ml	30 min	1072.4	This work

*M-biomass dose
**V- Volume

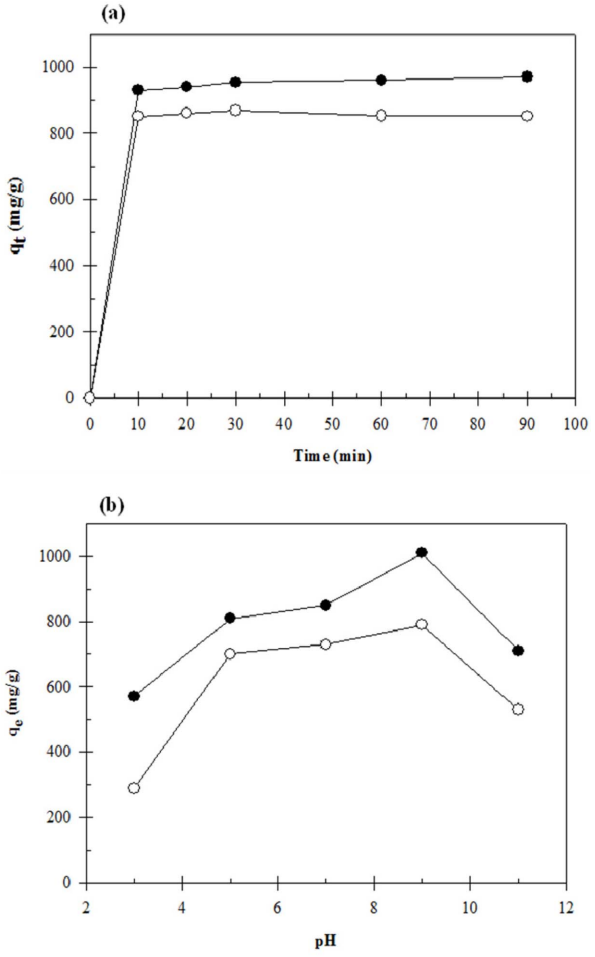

Fig. 5. (a) MB adsorption onto *C. freundii* JH 11-2 dried and UV-C treated biomass under optimized condition (b) Effect of initial pH on removal of MB by *C. freundii* JH 11-2 (dye concentration, 1100 mg/L; pH, 9; amount of biomass, 0.15 g; dried biomass (○); UV-C treatment biomass (●)).

adsorption rate of MB in Gram-positive bacteria *B. pseudomycoides* JH 2-2.

4. Conclusion

C. freundii JH 11-2 and *B. pseudomycoides* JH 2-2 biomass were used for the removal of MB in aqueous solution under optimum conditions. Maximum biosorption of MB was observed in the UV pretreated *C. freundii* JH 11-2 and *B. pseudomycoides* JH 2-2 biomass were 954 and 1072.4 mg/g, respectively. The physico-chemical variables greatly influence the biosorption. This study indicated the scope of the wastewater recycling using bacterial biomass.

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