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Removal of Malachite Green Employing Physical and Biological Processes

Subuntith Nimrat,[1*] Pilanya Sawangchit[2] and Verapong Vuthiphandchai[3]

[1] Department of Microbiology and Environmental Science Program, Faculty of Science, Burapha University, Chonburi 20131, Thailand.
[2] Department of Microbiology, Faculty of Science, Burapha University, Chonburi 20131, Thailand.
[3] Department of Aquatic Science, Faculty of Science, Burapha University, Chonburi 20131, Thailand.

[*] Corresponding author, E-mail: subunti@buu.ac.th

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ABSTRACT: This work involved the treatment of malachite green, a basic dye, extensively used in industries and aquaculture. A two-step technique associated with physicochemical and biological treatments was established to remove malachite green in terms of color and chemical oxygen demand (COD) reduction by the use of various adsorbents. Activated carbon and soil represented the best adsorbents for removal of the green color of malachite green and COD reduction. The results showed that activated carbon and soil can decolorize the green color of malachite green more efficiently than the use of ground shrimp skeleton, ground oyster shell and ground charcoal. However, all tested adsorbents, except ground shrimp skeleton, could reduce the COD level to similar extents. One millimolar malachite green (1,690 mg/L COD) was passed through activated carbon and soil and then to either aerobic or aerobic denitrifying activated sludge for COD reduction. The results demonstrated that the adsorption of malachite green using activated carbon or soil and treating the filtrate with either aerobic or aerobic denitrifying activated sludge could efficiently decrease the COD level by 77.5% to 86.0%. When a higher concentration of malachite green (2.0 mM) was tested using the same technique, the efficiency for COD reduction improved to 85.8-87.7%. Therefore, the adsorption efficiency with the use of physical and biological systems for COD reduction of malachite green appeared to increase with increasing concentration of the dye.

KEYWORDS: malachite green, adsorption, activated sludge, aerobic denitrification.

INTRODUCTION

Malachite green is one of the basic dyes used extensively in the textile industry for dyeing silk, leather, cotton, wool and jute [1,2], and has been extensively used as a bactericide, fungicide and parasiticide in aquaculture industries worldwide.[1,3] Malachite green is highly toxic to mammalian cells and causes kidney tumors in mice and reproductive problems in rabbit and fish.[4,5] The potential carcinogenic, genotoxic, mutagenic and teratogenic properties of malachite green have been reported in many animal species and cell lines.[6]

Industrial wastewater containing dyes is a growing concern to the environment due to their color and accumulated toxic chemicals.[7] These dyes can be removed by physical and chemical treatments, but those methods require more energy and chemicals than biological processes.[8,9] Additionally, those processes leave more sludge for further treatment.[7] Activated carbon is the most commonly used adsorbent for organic matter removal, but its application is limited due to its high cost.[10] It is, therefore, reasonable to find other low-cost adsorbents to replace the commercially expensive activated carbon. Biological techniques are attractive treatments from the industry standpoint because they are usually less expensive, compared to physical and chemical treatments. However, the conventional process of activated sludge alone is not effective to treat wastewater containing dyes to meet the effluent standards, since those dyes are not biodegraded but sometimes only adsorbed to activated sludge biomass.[8,11]

Aerobic denitrifying bacteria are an interesting group of bacteria because they have the capacity to co-utilize two electron acceptors: oxygen and nitrate.[12] As a consequence, aerobic denitrifying bacteria could provide more advantages than aerobic bacteria. First, bacteria can utilize and degrade nitrate as the electron acceptor through a denitrifying reaction. Second, the aerobic denitrifying process causes less sludge production compared to aerobic processes, because denitrifying bacteria convert the carbon source to less biomass than aerobic bacteria. Finally, aerobic activated sludge or conventional activated sludge processes generate high amounts of sludge.[13] Aerobic denitrifying processes could produces less sludge, because this reaction is a combination of aerobic and denitrifying processes.[12,14] Lukow and Diekmann[15] found that aerobic denitrifying bacteria were widely distributed in sewage treatment plants. These bacteria can be used to degrade water pollutants in an innovative activated sludge acclimated for aerobic denitrifying conditions where aerobic denitrifying bacteria dominated over aerobic bacteria. This system can decrease the operation cost because it utilizes less oxygen and eliminates nitrate simultaneously in the aerobic denitrifying tank.

In order to remove the contaminated dyes effectively from industrial wastewater, a proper treatment process with high quality effluent and low operation cost is required. In this present study, malachite green was chosen to study its fate by physical and biological treatments. The principal aim of this work was to remove malachite green by a proposed two-step technique using a combination of physical and biological treatments. The first step compared the adsorption of malachite green by a commonly used adsorbent (activated carbon) with other low cost adsorbents (soil, ground shrimp skeleton, ground oyster shell and ground charcoal). The supernatant from the first step was treated further with two different biological treatments (conventional aerobic and aerobic denitrifying activated sludge). The effect of dye concentration on removal efficiency was also investigated with the best performance adsorbents.

Materials and Methods

Acclimation and Characterization of Activated Sludge

Activated sludge used in this study was collected from an aeration tank (oxidation ditch) from South Saen Suk Wastewater Treatment Plant, Chonburi Province, Thailand. Collected activated sludge was placed in two 5-L glass jars. One jar each was subjected to aerobic and aerobic denitrifying conditions. In each condition, sodium benzoate solution (0.5 M) was added daily as a carbon source for activated sludge microorganisms for twenty days. Aerobic and aerobic denitrifying jars were equipped with air pumps to provide enough oxygen for aerobic microorganisms. To obtain aerobic denitrifying conditions, potassium nitrate (final concentration 5 mM) was provided as an electron acceptor, along with oxygen as previously described. Sludge Volume Index (SVI) and Mixed Liquor Suspended Solid (MLSS) of acclimated activated sludge were determined by standard methods.[16] The pH was monitored by pH meter (Orion Model 520A, USA).

Adsorbents

Activated carbon, soil, ground shrimp skeleton, ground oyster shell and ground charcoal were utilized as the adsorbents in this study. Activated carbon was purchased from a local distributor. This local activated carbon was selected because it is inexpensive and produced in Thailand. Soil samples were collected from an organic rich soil area in Chonburi Province, Thailand. Ground shrimp skeleton and ground oyster shells were collected from agricultural processing industries, washed repeatedly in tap water, dried overnight at 60°C, then ground and sieved for 2.0 mm size. Ground charcoal purchased from a local distributor was ground, washed with distilled water to remove fines, air-dried for 24 hrs. and then sieved for 2.0 mm size.

Chemicals

Malachite green (Ajax Finechem, Auburn, Australia) was dissolved in dimethyl sulfoxide (DMSO) prior to diluting in 7% NaCl to a final concentration of 100 mM as a stock solution.

Media

Defined mineral salt media was modified from Makekhayai.[17] The following was dissolved in 1.0 L of distilled water: 1.3 g KCl, 0.2 g KH_2PO_4, 23 g NaCl, 0.5 g NH_4Cl, 0.1 g $CaCl_2.2H_2O$ and 1.0 g $MgCl_2.6H_2O$. This defined medium was supplemented with 1 mL of vitamin solution (containing 1.0 mg/L vitamin B_{12}, 20 mg/L biotin, 20 mg/L folic acid, 50 mg/L nicotinic acid, 50 mg/L *p*-aminobenzoic acid, 50 mg/L pantothenic acid, 100 mg/L pyridoxine HCl, 50 mg/L riboflavin, 50 mg/L thiamin, and 50 mg/L thiotic acid), 5 mL of trace salt solution (consisting of 2 g/L $CoCl_2.6H_2O$, 0.01 g/L $CuCl_2$, 0.38 g/L H_3BO_3, 1.33 g/L $MnCl_2.4H_2O$, 0.17 $Na_2MoO_4.2H_2O$, 0.1 g/L $NiCl_2.6H_2O$, and 0.14 g/L $ZnCl_2$) and 29.8 mL of bicarbonate solution (84 g/L) utilized as the buffer system in the enrichment culture.[18] To obtain aerobic denitrifying conditions, nitrate (5 mM at the final concentration) was added for another electron acceptor in the cultures.

Enumeration of Aerobic, Aerobic Denitrifying, Denitrifying and Methanogenic Bacteria by Most Probable Number Determination

Aerobic, aerobic denitrifying, denitrifying and methanogenic bacteria were enumerated using a modification of a five-tube MPN method [16] as follows:

Aerobic and Aerobic Denitrifying Bacteria

One milliliter of acclimated activated sludge either under aerobic or aerobic denitrifying conditions was inoculated into 10 mL lactose broth. Three sets of tubes with 5 tubes in each set of most probable number determinations were used. All of tubes were incubated at 37°C and visual appearance of growth by the occurrence of gas in the Durham tube after 2 days was used to score positive tubes. The lactose broth had the following composition : 5.0 g/L lactose , 5.0 g/L gelatin, 3.0 g/L beef extract. For aerobic denitrifying conditions of acclimated activated sludge, 5.0 mM nitrate was added in lactose broth as the other choice of electron acceptor.

Denitrifying Bacteria

The denitrifying mineral medium was prepared and flushed with nitrogen gas for 30 min. The denitrifying medium consisted of 1.2 g/L KNO_3, 0.5 g/L $K_2HPO_4.3H_2O$, 0.5 g/L KH_2PO_4, 3.0 g/L beef extract, 5.0 g/L peptone, and 1.0 g/L NH_4Cl. Three sets of tubes with 5 tubes in each set of most probable number determinations were used. One milliliter of acclimated activated sludge either under aerobic or aerobic denitrifying conditions was inoculated into 10 mL of denitrifying mineral medium. All tubes were degassed with nitrogen gas for 30 min, and incubated at 37°C. Visual appearance of growth by the occurrence of gas in Durham tubes after 14 days of incubations was used to score positive tubes.

Methanogenic Bacteria

The methanogenic medium containing the following: 20.0 g/L $CaCO_3$, 1.0 g/L NH_4Cl, 0.4 g/L K_2HPO_4 .$3H_2O$, 0.1 g/L $MgCl_2$ .6 H_2O, and 20.0 g/L methanol, was prepared, degassed for 30 min. and autoclaved. Three sets of tubes with 5 tubes in each set of most probable number determinations were used. One milliliter of acclimated activated sludge either under aerobic or aerobic denitrifying conditions was inoculated into 10 mL methanogenic medium. All tubes were degassed with nitrogen gas for 30 min. and incubated at 37 C. Visual appearance of growth by occurrence of gas in the Durham tubes after 14 days was used to score positive tubes.

Isolation and Identification of Bacteria

Isolation of bacteria was conducted by spread plating dilutions in plate count agar and incubating the plates at 30°C for 48 hrs. The method used for aerobic denitrifying bacteria was established, similar to aerobic plate count technique, except nitrate was added as the second choice for an electron acceptor in the plate count agar. The isolated colonies on plate count agars under both conditions were identified by Gram staining and standard biochemical tests according to Bergey's manual of determinative bacteriology.[19,20]

Column Setup and Experimental Design

The experimental column was filled with adsorbent, 400 g of either activated carbon, soil, ground shrimp skeleton, ground oyster shell or ground charcoal. After the adsorbent was saturated with water, malachite green (1 mM) was loaded onto the column at a flow rate of 2.40 mL/min. For each adsorbent, removal of malachite green was tested with three runs to determine the removal efficiency of malachite green using 120 mL of malachite green without any material to elute the material for each run. Plastic columns (height, 17 cm; length, 12 cm; width, 5 cm) were used. All experiments were conducted at room temperature (approximately 25°C).

Biodegradation of Malachite Green and Its Metabolites under Aerobic and Aerobic Denitrifying Conditions

Two different enrichment cultures under aerobic and aerobic denitrifying conditions were prepared to evaluate biodegradation of malachite green. Ten milliliters of acclimated activated sludge were added to 60-mL serum bottles containing 40 mL of minimal salt media. After the media and inocula were added, each bottle was capped with a butyl rubber stopper and aluminum crimp. Then, 1.0 mL of the filtrate from the previously described experimental column was injected with a 1-mL syringe to get the final concentration of 1.0 mM malachite green in the cultures and incubated in the dark at 30°C. Background (with no added substrate) and sterile bottles (with autoclaved and acclimated activated sludge) were designed as the controls. To obtain aerobic denitrifying conditions, 5 mM potassium nitrate was added as another electron acceptor in the cultures. Enrichments were monitored for substrate loss at different time points.

Analysis of Substrate Loss

All samples were monitored for substrate loss by 2 criteria: color intensity and COD. Firstly, malachite green removal was determined by the change of color intensity, compared to the standard dilutions. At the beginning, 1.0 mM malachite green (score 0) was diluted by a factor of 0.5 until colorless (score 10). As a result, the concentrations of malachite green were 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078, 0.0039, and 0.00195 mM, assigned the scoring system from 0-10, respectively. Those standards were used for comparison with the change in color of malachite green after treated with different types of adsorbents by visual comparison and spectrophotometer measurement at the 616.9 nm. Secondly, the change of COD value was determined for the malachite green and its metabolites during the experiment. The COD of samples was measured by colorimetry using a Hach COD incubator (DR 200, Loveland, Colorado, USA). The reaction proceeded for 2 hrs. at 150°C. The substrate loss during the experiment was determined by comparison with standard solutions.

Results

Removal of 1.0 mM Malachite Green with Five Different Adsorbents

The COD value for 1.0 mM of malachite green was 1,690 mg/L. Table 1 shows removal of 1.0 mM malachite green in terms of color and COD reduction by different adsorbents (activated carbon, soil, ground shrimp skeleton, ground oyster shell and ground charcoal) for three runs. Although there was no significant difference ($P > 0.05$) in color reduction between activated carbon and soil, their efficiency in color reduction was significantly higher ($P < 0.05$) than the other three adsorbents. Activated carbon and soil were able to remove the green color of 1.0 mM malachite green with 100% efficiency. Ground shrimp skeleton and ground charcoal had moderate capacity to remove the green color of malachite green, indicated by the color reduction scoring of between 5-8. Activated carbon, soil, ground oyster shell and ground charcoal had high capacity to remove COD with the values ranging from 49.5%-72.4%, which were not significantly different ($P > 0.05$) among these adsorbents but were significantly higher ($P < 0.05$) than that of ground shrimp skeleton (3.1 % - 31.1 %). Ground shrimp skeleton showed superior efficiency on color removal with a color reduction score of 8, but not for COD value reduction. In addition, bad odor occurred with the ground shrimp skeleton treatment during the second and third runs when ground shrimp skeleton was reused to study on the adsorption capacity. Therefore, activated carbon and soil can remove 1.0 mM malachite green in terms of color removal and COD reduction more efficiently than ground shrimp skeleton, ground oyster shell and ground charcoal.

Comparison of Aerobic, Aerobic Denitrifying, Denitrifying and Methanogenic Bacteria in Acclimated Activated Sludge under Aerobic and Aerobic Denitrifying Conditions Using MPN Method

The change in characteristics of acclimated activated sludge under aerobic and aerobic denitrifying conditions was monitored as shown in Table 2. Acclimated activated sludge under aerobic conditions showed more variety of cultivable bacteria than those from aerobic denitrifying activated sludge. *Micrococcus* sp., *Flavobacterium* sp., *Acinetobacter* sp., *Bacillus* sp., *Staphylococcus* nonaureus and unidentified species (4 isolates) identified by Bergey's Manual Systems, were isolated from the aerobic activated sludge, while *Micrococcus* sp., *Desulfotomaculum* sp., *Bacillus* and unidentified species (4 isolates) were identified from activated sludge under the aerobic denitrifying conditions. During the course of acclimation of microorganisms, biodiversity of the bacterial populations (aerobic, aerobic denitrifying, denitrifying and methanogenic bacteria) in the activated sludge was clarified using the modification of MPN methods. Fig 1 shows the number of aerobic, aerobic denitrifying, denitrifying and methanogenic bacteria obtaining from

Table 2. Characteristics of acclimated activated sludge under aerobic and aerobic denitrifying conditions.

Parameters	Acclimated activated sludge	
	Aerobic conditions	Aerobic denitrifying conditions
pH	9	10
SVI (mL/g)	86.65	69.53
MLSS (mL/g)	5449	5207
Isolated bacteria	*Micrococcus* sp. *Bacillus* sp. *Staphylococcus* nonaureus *Flavobacterium* sp. *Acinetobacter* sp. Four different unidentified species	*Micrococcus* sp. *Desulfotomaculum* sp. *Bacillus* sp. Four different unidentified species

Table 1. Reduction of 1.0 mM malachite green by different adsorbents.

Type of adsorbents	% Reduction					
	Run 1		Run 2		Run 3	
	Color reduction	% COD reduction	Color reduction	% COD reduction	Color reduction	% COD reduction
Activated carbon [a,1]	10	49.5	10	65.7	10	56.5
Soil [a,1]	10	62.7	10	59.7	10	57.9
Ground shrimp skeleton [b,2]	8	31.1	8	3.1	8	5.1
Ground oyster shell [a,4]	3	72.4	3	54.0	3	51.4
Ground charcoal [a,3]	5	58.3	5	54.0	5	59.7

Values of color reduction range from 0-10 in which 0: for the green color of malachite green at the beginning and 10: for no green color of malachite green remaining.
[a,b]Different superscript letters indicate significant differences ($P < 0.05$) in percentage of COD reduction between treatments.
[1,2,3]Different superscript numbers indicate significant differences ($P < 0.05$) in color reduction between treatments.

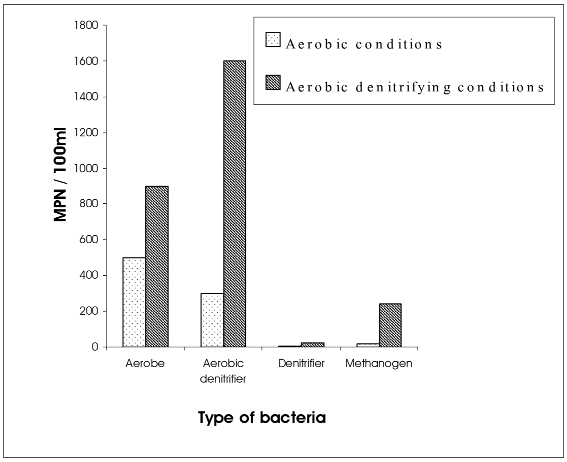

Fig 1. Comparison of aerobic, aerobic denitrifying, denitrifying and methanogenic bacteria in acclimated activated sludge under aerobic and aerobic denitrifying conditions using MPN method.

the acclimation of activated sludge under aerobic conditions via the MPN method, which averaged 500, 300, 4 and 17 MPN/100 mL, respectively. From this result, aerobic bacteria were found with the highest number followed by aerobic denitrifying bacteria, while anaerobic bacteria had relatively low number in the activated sludge under aerobic conditions. In contrast, aerobic denitrifying bacteria were found with the highest value (1600 MPN/10 mL.) in the acclimated activated sludge under aerobic denitrifying conditions (Fig 1). Aerobic bacteria were the next largest group in acclimated activated sludge under aerobic denitrifying conditions. Anaerobic bacteria (denitrifying and methanogenic bacteria) were detected in activated sludge with relatively low number, similar to those found in acclimated activated sludge under aerobic conditions. As a consequence, the change in biodiversity and the number of microbial population in the acclimated activated sludge under both conditions were correlated with the enrichment nutrients.

Table 3. Reduction of 1.0 mM malachite green by two different adsorbents and then by two types of biological treatment.

Physical treatment	% COD reduction	Biological treatment	% COD reduction	% Total COD reduction
Activated carbon [a]	52.0 ± 9.8	Aerobic [1]	53.2 ± 4.8	77.5 ± 6.4
		Aerobic denitrifying [1]	55.9 ± 3.9	78.8 ± 7.1
Soil [a]	66.6 ± 4.2	Aerobic [1]	54.9 ± 3.5	84.9 ± 3.9
		Aerobic denitrifying [1]	58.2 ± 4.1	86.0 ± 4.1

[a]The same superscript letter indicates the mean of percentage of COD reduction and total COD reduction was not significantly different ($P > 0.05$) between the two physical treatments.
[1]The same superscript number indicates the mean of percentage of COD reduction and total COD reduction was not significantly different ($P > 0.05$) between the two biological treatments.

Removal of 1.0 mM Malachite Green by Physical and Biological Processes

Table 3 shows the removal of 1.0 mM malachite green by physical treatment (activated carbon and soil) and biological treatment (aerobic and aerobic denitrifying conditions). The percentage of COD removal by soil (66.6 $\pm$ 4.2%) was higher than that of activated carbon (52 $\pm$ 9.8%), although there was no significant difference ($P > 0.05$) between the two adsorbents. Then the filtrate from either soil or activated carbon was treated with two types of biological activated sludge (aerobic or aerobic denitrifying conditions). The two types of biological treatments were able to decrease COD value with similar performance (53.2-58.2%) without significant difference ($P > 0.05$). As a consequence, the proposed two-step technique using soil as an adsorbent and subsequently treating with either aerobic or aerobic denitrifying conditions showed the best capacity for removal of 1.0 mM malachite green (84.9-86.0%).

Removal of 2.0 mM Malachite Green by Physical and Biological Processes

Table 4 shows the percent removal of 2.0 mM malachite green by activated carbon and soil from three consecutive loadings of 2.0 mM malachite green. There was a significant difference ($P < 0.05$) in terms of color removal between activated carbon and soil. Soil

Table 4. Reduction of 2.0 mM malachite green by activated carbon and soil.

Type of adsorbents	% Reduction					
	Run 1		Run 2		Run 3	
	Color reduction	% COD reduction	Color reduction	% COD reduction	Color reduction	% COD reduction
Activated carbon [a,1]	9	70.0	8	74.4	7	80.0
Soil [a,2]	10	68.2	10	70.8	10	73.8

Values of color reduction range from 0-10 in which 0: for the green color of malachite green at the beginning and 10: for no green color of malachite green remaining.
[a]The same superscript letter indicates the percentage of COD reduction was not significantly different ($P > 0.05$) between the two adsorbents.
[1,2]Different superscript numbers indicate significant difference ($P < 0.05$) in color reduction between the two adsorbents.

Table 5. Reduction of 2.0 mM malachite green by two different adsorbents and then by biological treatment.

Physical treatment	% COD reduction	Biological treatment	% COD reduction	% Total COD reduction
Activated carbon [a]	74.8 ± 4.5	Aerobic [1]	80.1 ± 3.2	85.8 ± 3.8
		Aerobic denitrifying [1]	81.8 ± 3.7	86.4 ± 4.1
Soil [a]	70.9 ± 5.8	Aerobic [1]	80.7 ± 5.2	86.3 ± 5.5
		Aerobic denitrifying [1]	82.7 ± 4.5	87.7 ± 5.2

[a]The same superscript letter indicates the mean of percentage of COD reduction and total COD reduction was not significantly different ($P > 0.05$) between the two physical treatments.
[1]The same superscript number indicates the mean of percentage of COD reduction and total COD reduction was not significantly different ($P > 0.05$) between the two biological treatments.

can completely adsorb the green color of 2.0 mM malachite green. In contrast, partial absorption with high efficiency removal of the green color of added malachite green was observed with activated carbon. However, there was no significant difference ($P > 0.05$) in the percentage of COD removal between the treatments with activated carbon (70-80%) and soil (68.2-73.8%) as shown in Table 4.

Table 5 shows the removal of 2 mM malachite green in terms of COD value with physical (activated carbon and soil) and biological treatments (aerobic and aerobic denitrifying activated sludges). Activated carbon and soil were capable of removing malachite green at 74.8 7.4% and 70.9±5.7%, respectively, which were not significantly different ($P > 0.05$). Moreover, both biological treatments can equally reduce the COD values by 80.1 – 87.7%, with no significant difference ($P > 0.05$) (Table 5). Therefore, the percentage of total COD removal was not affected when the filtrate from activated carbon and soil were subsequently treated with aerobic or aerobic denitrifying conditions.

Discussion

High chemical oxygen demand (COD) and color intensity are the major parameters of concern for release of wastewater from textile industry treatment plants.[21] The proposed two-step process for malachite green removal was studied with low (1.0 mM) and higher (2.0 mM) concentrations of malachite green. The first step was to treat with physical process using five different types of adsorbents (activated carbon, soil, ground shrimp skeleton, ground oyster shell and ground charcoal). The criteria for selection of the appropriate adsorbent for the removal of malachite green were color reduction and percentage of COD removal. The constituents of the different adsorbents make different contributions toward the color and COD reduction of tested malachite green, as shown in Table 1. The obtained results showed that soil and activated carbon were capable of complete removal of the color of 1.0 mM malachite green in three successive loadings and could decrease the COD from 49.5% to 65.7%.

Removal of a higher concentration (2.0 mM) of malachite green using activated carbon and soil was also assessed. The ability of activated carbon to remove the color of 2.0 mM malachite green was slightly decreased during successive runs from run 1 to 3, compared to that with 1.0 mM malachite green. This finding is in agreement with the work done by Aitcheson *et al.*[22] They reported that activated carbon efficiently removed malachite green present in a low concentration in aqueous solution. Interestingly, in the present work, activated carbon showed higher capacity for reduction of 2.0 mM malachite green in terms of COD removal than for 1.0 mM malachite green. Activated carbon uses its developed porous structure comprised of hydrophobic graphene layers and hydrophilic surface functional groups to adsorb malachite green.[23] Activated carbons were also noted to be capable of removing malachite green (a cationic dye) due to its high degree of porosity and an extensive surface area for adsorption capacity.[24] For example, some activated carbons consist of weakly acidic functional groups and protonatable complexing groups at the surface of carbon, such as carboxylic, lactonic and phenolic groups,[19] which can adsorb malachite green. Due to these properties and its ability to remove malachite green, activated carbon may be considered to be an appropriate material from an environmental point of view, as it is economical, abundant and precursors of activated carbon are readily available in Thailand. Since all agricultural solid wastes used in the present study are freely, abundantly and locally available, the activated carbons are expected to be economically viable for wastewater treatment.

In contrast, soil had increased capacity to reduce the color of 2.0 mM malachite green, but the capacity of soil to remove COD (70.9 ± 5.7%) was similar to activated carbon (74.8 ± 7.4%). One reason that soil has the best ability to adsorb 1.0 and 2.0 mM malachite green may be due to the presence of dissolved humic substances (DHS), naturally occurring organic matter in soils. Soils have been shown to bind hydrophobic organic solutes forming humic solute complexes in the aqueous phase.[25]

The second step was to assay the impact of two different biological treatments (acclimated activated sludges under aerobic and aerobic denitrifying conditions) on removal of malachite green based on the same 2 criteria: color reduction and COD removal. The filtrate from soil or activated carbon of low (1.0

mM) and higher concentration (2.0 mM) of malachite green was transferred into two types of biological treatments. The results showed that the efficiency of two types of activated sludges for removing the filtrate from 1.0 mM malachite green was similar (53.2-58.2% COD reduction). The increase in malachite green concentration in both acclimated activated sludge conditions resulted in increased efficiency of dye removal in terms of COD value (80.1 – 82.7% COD removal). As a consequence, aerobic and aerobic denitrifying microorganisms in acclimated activated sludge had similar capacity for biodegradation of malachite green and its metabolites. This indicated that the second step for malachite green removal can be either conventional activated sludge or activated sludge under aerobic denitrifying conditions. Further study is required to improve the removal of malachite green, particularly on the reduction of COD level during the biological treatment based on the proposed two-step technique using different columns.

In conclusion, the application of a two-step technique for malachite green removal could be a prominent process to treat malachite green in a cost-effective manner. The first step or the adsorption process is the major step in malachite green removal. Soil, a natural material, or low-cost activated carbon could be the choices for the appropriate adsorbents, depending on availability. The second step is the method to remove the remaining of malachite green and its metabolites. Both conventional activated sludge and aerobic denitrifying activated sludge can be utilized during the second step with similar capacity. This proposed process is an environmentally friendly and cost efficient method for malachite green removal.

References

1. Culp SJ and Beland FA (1996) Malachite green: a toxicological review. *J Am Coll Toxicol* **15**, 219-38.
2. Culp SJ, Blankenship LR, Kusewitt DF, Doerge DR, Mulligan LT and Beland FA (1999) Toxicity and metabolism of malachite green and leucomalachite green during short-term feeding to Fischer 344 rats and B6C3F mice. *Chemico-Biological Interaction* **122**,153-70.
3. Fitzpatrick MS, Schreck CB, Chitwood RL and Marking LL (1995) Evaluation of three candidate fungicides for treatment of adult spring chinook salmon. *Progressive Fish-culturist* **57,** 153-5.
4. Fernandes C, Lalitha VS and Kao KVK (1991) Enhancing effect of malachite green on the development of hepatic pre-neo-plastic lesions induced by *N*-nitrosodiethylamine in rats. *Carcinogenesis* **12**, 839-45.
5. Henderson AL, Schmitt TC, Heinze TM and Cerniglia CE (1997) Reduction of malachite green to leucomalachite green by intestinal bacteria. *Applied Environmental Microbiology* **63**, 4099-101.
6. Hajee CAJ (1997) Residues of mebendazole and malachite green in eel and trout: analytical and pharmacokinetical aspects, pp 183. Dissertation at Utrecht University, Utrecht.
7. Chu W (2001) Dye removal from textile dye wastewater using recycled alum sludge. *Water Research* **35(13)**, 3147-52.
8. Churchley JH (1994) Removal of sewage effluent–the use of a full scale ozone plant. *Water Science and Technology* **30(3)**, 275-84.
9. Stern SR, Szpyrkowicz L and Rodighiero I (2003) Aerobic treatment of textile dyeing wastewater. *Water Science and Technology* **47(10)**, 55-9.
10. Kadirvel K, Kavipriya M, Karthika C, Radhika M, Vennilamani N and Pattabhi S (2003) Utilization of various agricultural wastes for activated carbon preparation and application for the removal of dyes and metal ions from aqueous solutions. *Bioresource Technology* **87**, 129-32.
11. Shaw CB, Carliell CM and Wheatley AD (2002) Anaerobic/aerobic treatment of coloured textile effluents using sequencing batch bioreactor. *Water Research* **36**, 1993-2001.
12. Patureau D, Zumstein E, Delgenes JP and Moletta R (2000) Aerobic denitrifiers isolated from diverse natural and managed ecosystems. *Microbial ecology* **39**, 145-52.
13. Ratsak CH, Maarsen KA and Kooijman SALM (1996) Effects of protozoa on carbon mineralization in activated sludge. *Water Research* **30(1),** 1-12.
14. Carter JP, Hsiao YH, Spiro S and Richardson DJ (1995) Soil and sediment bacteria capable of aerobic nitrate respiration. *Applied Environmental Microbiology* **61**, 2852-58.
15. Lukow T and Diekman H (1997) Aerobic denitrification by a newly isolated heterotrophic bacterium strain TL1. *Biotech Lett* **19**, 1157-9.
16. APHA (1998) Standard methods for the examination of water and wastewater, *20th ed.*, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.
17. Makekhayai S (2000) Biodegradation of methyl parathion, *p*-nitrophenol and *p*-aminophenol under anoxic conditions (Ph.D. in Environmental Sciences). Department of Environmental Sciences, Rutgers, The State University of New Jersey, New Brunswick, USA.
18. Owen WF, Stuckey DC, Healy JB, Young LY and McCarty PL (1979) Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research* **13**, 485-92.
19. Gerhardt PR, Murray GE, Wood WA and Krieg NR (1994) Methods for general and molecular microbiology. American Society for Microbiology, Washington, D.C.
20. Krieg NR and Holt JG (1984) Bergey's manual of systematic bacteriology vol 1. Williams and Wilkins Co, Baltimore, Maryland, USA.
21. Lee CK, Low KS and Gan PY (1999) Removal of some organic dyes by acid treat spent bleaching earth. *Environmental Technology* **20**, 99–104.
22. Aitcheson SJ, Arnett J and Murray KR (2001) Removal of aquaculture therapeutants by carbon adsorption. *Aquaculture* **192**, 249–64.
23. Guo Y, Zhang H, Tao N, Liu Y, Qi J, Wang Z and Xu H (2003) Adsorption of malachite green and iodine on rice husk-based porous carbon. *Materials Chemistry and Physics* **82(1),** 107-15.
24. Malik DJ, Strelko V, Streat M and Puziy AM (2002) Characterization of novel modified active carbons and marine algal biomass for the selective adsorption of lead. o:*Water Research* **36**, 1527-38.
25. Rebhun M, Smedt FD and Rwetabula J (1996) Dissolved humic substances for remediation of sites contaminated by organic pollutants. Binding-desorption model predictions. *Water Research* **30(9)**, 2027-38.