Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.0146

60 (2017) 114–121
January

Comparison of bioremediation and phytoremediation in treatment of diethylene glycol from stationery industry

Pattrarat Teamkao[a], Somkiet Techkarnjanaruk[b], Pratin Kullavanijaya[c], Paitip Thiravetyan[d,*]

[a]*Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand, email: pattrarat.te@kmitl.ac.th*
[b]*National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok 10150, Thailand, email: somkiet@biotec.or.th*
[c]*Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand, email: pratin@pdti.kmutt.ac.th*
[d]*Division of Biotechnology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand, Tel. +66 24707535; Fax: +66 24523455; email: paitip.thi@kmutt.ac.th*

Received 2 February 2016; Accepted 18 July 2016

ABSTRACT

Diethylene glycol (DEG) was found to contaminate wastewater from the stationery industry. The wastewater, after chemical precipitation, still had high DEG and Chemical oxygen demand (COD) resulting in undischarge according to the standard wastewater policy. Bioremediation and phytoremediation were chosen to solve this problem. Comparison of bioremediation and phytoremediation revealed that adding nutrient rich microorganisms removed more DEG, but less COD than using plants grown under hydroponic conditions. However, the bioremediation system had lower potential than plant + soil conditions. The application of a plant-soil system as a constructed wetland was able to remove all DEG (1,500 mg/L) in the wastewater within 8 d. The potential of a constructed wetland system can be enhanced by the addition of microorganisms and nutrients, reducing the time of remediation from 8 to 5 d. In addition, COD reduction to 110 mg/L was reduced from 14 to 11 d, which was lower than the acceptable level (COD ≤ 120 mg/L). However, the use of microorganisms alone cannot reduce COD in contaminated wastewater to the acceptable level. Using constructed wetland with the addition of microorganisms and nutrients is an effective way to remediate DEG and COD from stationery industry wastewater.

Keywords: Bioremediation; Phytoremediation; Constructed wetland; Diethylene glycol; *Echinodorus cordifolius* L. Griseb

1. Introduction

Diethylene glycol (DEG) is an organic solvent used in the stationery, textile and ink industries, including oil and gas production [1]. Moreover, DEG is used as a coolant and as a building block in organic synthesis [2]. DEG is used in many industries and is associated with contamination in the surrounding environment where large amounts of wastewater are discharged. The toxicity of DEG is relatively low, lethal dose response is in the range of 0.014–0.170 mg DEG/kg body weight for humans, and the toxic effect is similar to the toxicity from alcohol. DEG is the cause of metabolic acidosis and renal failure [3]. Additionally, DEG contamination in aquatic environments deteriorates water quality because it causes high levels of COD.

Conventional wastewater treatment methods, physical and chemical precipitation, are ineffective in reducing of DEG to an acceptable level. Further treatments are necessary

* Corresponding author.

to handle highly soluble properties of DEG. Both the Fenton system [4,5] and nano-filtration [6] have been applied to treat DEG and although these methods are effective, a high operation cost is required. An alternative and promising operation is the biological remediation approach. This approach is usually applied in the final wastewater treatment step due to a capacity to remove low concentrations of contaminants. Moreover, the biological method is environmentally friendly and easy to operate [7].

The biological remediation approach exploits living organism's activities to remove contaminants from contaminated environments. The biological remediation approach comprises two main systems: the microbial remediation system called "bioremediation", and the plant remediation system called "phytoremediation". Phytoremediation is a green technology based on using plants to clean up polluted sites. This technology is applicable to remove a broad range of contaminants, consisting of explosives, metals, radionuclides, organic compounds, and surfactants [8]. The burhead plant (*Echinodorus cordifolius* L. Griseb), an aquatic plant, has been reported to have potential for DEG removal [9]. However, phytoremediation technology has some limitations, including rate of DEG removal, as well as being climate dependent, and requiring a remediation site for planting.

Bioremediation has received increasing attention as an economical, eco-friendly, and effective biological approach in cleaning up polluted environments [10]. There are two common approaches for *in situ* bioremediation, bioaugmentation and biostimulation. Bioaugmentation is the addition of pre-grown microorganisms to degrade contaminated compounds [11], while biostimulation is the addition of nutrients and other supplementary components to induce native microbial growth, which affects degradation rate [10]. The use of microorganisms in a remediation system is believed to have a higher rate of removal and be easier to manage when applied to treat wastewater in the reactor scale or industrial sector.

Mechanisms of glycol degradation by plant and microorganisms, including effective species, are well studied [9,12–14]. However, a comparison of remediation potential of bio- and phytoremediation of industrial effluent is not known. This study firstly, investigated the effects of three bioremediation techniques (bioaugmentation, biostimulation, and combined bioaugmentation-biostimulation) on DEG remediation in stationery industry wastewater. Secondly, it compared of the efficiency of DEG removal in microbe systems, plants under hydroponic systems, and plant-soil systems.

2. Materials and methods

2.1 Microbial inoculum

A microbial consortium capable of degrading DEG was isolated from the soil in a wetland system with a history of DEG contamination by the enrichment technique. Five grams of soil was inoculated into 100 mL of modified medium-C yeast base containing 1.5 g/L DEG in a 250 mL Erlenmeyer flask. The medium consisted of 4.5 g/L Na_2SO_4, 1.0 g/L NH_4Cl, 1.0 g/L yeast extract, 0.5 g/L KH_2PO_4, 0.3 g/L sodium citrate. $2H_2O$, 0.06 g/L $CaCl_2.6H_2O$, 0.06 g/L $MgSO_4.7H_2O$, 0.016 g/L $FeSO_4.7H_2O$, 0.2 g/L ascorbic acid, and 0.2 g/L sodium thioglycolate, pH adjusted to pH 7 then autoclaved at 121°C for 15 min. The DEG solution in water was added to the medium at the final concentration of 1.5 g/L before the medium was sterilized. The microbial culture was incubated at 30°C and shaken at 150 rpm for 5 d. The subculture was conducted by transferring 10 mL of the enriched culture to 90 mL of fresh medium (medium-C yeast base with 1.5 g/L DEG), incubated again at 30°C and shaken at 150 rpm for 5 d. Anaerobic enrichment was carried out following the aerobic subculture method but a 100 mL glass bottle with a rubber stopper was used instead of a 250 mL Erlenmeyer flask, and shaking was not required. Three subcultures were conducted to obtain a soil-free enrichment. This enriched-mixed culture was used as seed inoculum in the DEG remediation study.

2.2. DEG contaminated wastewater

DEG contaminated wastewater was obtained from the effluent of a stationery industry site at Samut Prakan, Thailand. The wastewater had been pre-treated by coagulation, but it was still high in COD content and approximately 1,500 mg/L DEG remained in the solution.

2.3. Bioremediation system

Wastewater treatment by microorganisms was divided into 4 treatments: wastewater alone (control), wastewater + microorganisms (bioaugmentation), wastewater + nutrients (biostimulation), and wastewater + nutrients + microorganisms (combined bioaugmentation-biostimulation), with three replicates for each treatment. The nutrients used in the treatments were half-strength modified yeast base medium-C, consisting of 2.25 g/L Na_2SO_4, 0.5 g/L NH_4Cl, 0.5 g/L yeast extract, 0.25 g/L KH_2PO_4, 0.15 g/L $Na_3C_6H_5O_7.2H_2O$, 0.03 g/L $CaCl_2.6H_2O$, 0.03 g/L $MgSO_4.7H_2O$, 0.008 g/L $FeSO_4.7H_2O$, 0.1 g/L ascorbic acid, and 0.1 g/L sodium thioglycolate. The study was performed in two scale sizes: a shake flask and a continuously stirred tank reactor (CSTR). The shake flask contained 90 mL of the DEG contaminated wastewater and 10 mL of inoculation (approximately 4.1×10^6 cells/mL) in a 250 mL Erlenmeyer flask, incubated at 30°C and shaken at 150 rpm. The CSTR reactor contained 1,800 mL of DEG contaminated wastewater, 200 mL of inoculation (approximately 4.1×10^6 cells/mL) in a 2,200 mL working volume CSTR reactor, and was incubated at room temperature (30°C ± 5°C) under a rotation speed of 150 rpm. A sample was taken every day and measured for remaining COD and DEG in the solution. The system pH was adjusted to pH 7 before the experiment.

2.4. Phytoremediation system

Burhead plant (*Echinodorus cordifolius* L. Griseb) was grown in a greenhouse at the Remediation Laboratory of King Mongkut's University of Technology Thonburi (KMUTT), Bangkhuntien Campus. Plants at the same growth stage (8 leaves, 500 g of fresh weight) were selected and cultured in glass cylinders (30 × 50 cm) containing Hoagland's solution for a week prior to the start of the experiments.

The experimental design was random with three treatments to study the efficiency of plants in the treatment system: wastewater (control), wastewater + burhead plants,

and wastewater + burhead plants + soil (500 g). All treatments were carried out in triplicate in separate glass cylinders under a static system, in which the 2,000 mL of DEG contaminated wastewater was not refreshed during the experimental period, but water was added to maintain a constant volume despite evaporation. The experiment was conducted at an average temperature of 32°C ± 5°C, with 60% ± 8% relative humidity and 12-h light/dark cycles.

2.5. Constructed wetland system

A constructed-wetland was used to represent the plant-soil system. The system was performed in two plastic square pots (96 × 62 × 29 cm) with a 0.8 cm high rock, 20 kg of clay soil, 16 burhead plants, and 120 L of DEG-contaminated wastewater for each pot. The wastewater was circulated between the two tanks at a flow rate of 150 mL/min. The wastewater treatment by plant-microbe system was set into two treatments with two controls: wastewater alone (control), wetland + tap water (control), wetland + wastewater, and wetland + wastewater + combined bioaugmentation-biostimulation. The experiment was performed for 14 d.

2.6. Wastewater analysis

The concentration of DEG in the solution was measured using gas chromatography with a flame ionization detector (GC-FID). The solutions were filtered through a 0.45 μm cellulose acetate filter before analysis. A CP-Volamine capillary column with 30 m × 0.32 μm diameter was used to analyze the sample. The injection temperature was 250°C, the oven temperature was 200°C, and the detector temperature was 280°C. Helium was used as the carrier gas at a flow rate of 1.8 mL/min. For analysis, the external standard technique was used. DEG (GC grade, Sigma-Aldrich) concentrations in the standard solutions for the calibration curve were 0, 100, 500, 1,000, 1,500, 2,000, 2,500, and 3,000 mg/L. A percentage of DEG removal efficiency was calculated as: DEG removal efficiency (%) = $(C_i–C_f)/C_i \times 100$, where C_i is the initial DEG concentration (mg/L) and C_f is the final DEG concentration (mg/L).

The chemical oxygen demand (COD) and pH of the solution was also measured during the time of study. The COD was analyzed in accordance with method 508C of standard methods [15].

2.7. Statistical analysis

Data was analyzed by Minitab program Version 16.0. The significance of treatments was set at a *P*-value of less than or equal to 0.05.

3. Results and discussion

3.1. Soil microorganisms

Soil from the wetland system of DEG contaminated wastewater is a good source of effective microorganisms for DEG remediation. The target microorganisms from soil were cultivated using a medium containing DEG to select microorganisms with high potential for DEG degradation. The remaining DEG concentrations during the cultivation process are shown in Fig. 1. The culture under aerobic and anaerobic conditions showed that the cultivated microorganisms from both conditions were able to remove DEG from the wastewater. However, during microbial subculture it was observed that microbes growing in aerobic cultivation had a faster potential for DEG removal than the microbes growing in anaerobic cultivation (Fig. 1).

Ethylene glycol has been reported to biodegrade in aerobic and anaerobic conditions. The result of this study was similar to the study of Huang et al. which showed that the aerobic biodegradation of glycol was much higher than in the anaerobic process [16,17]. The main group of microorganisms in soil and roots associated with rhizosphere microorganisms of burhead plants grown in DEG-contaminated wastewater has been reported to be acid-producing bacteria and sulfate-reducing bacteria [9,18]. These microorganisms were able to degrade organic compounds which resulted in organic acids as products of degradation. However, other microorganisms have also been reported to possess DEG degradation potential, such as *Pseudomonas aeruginosa*, *Flavobacterium* sp., and *Pelobacter venetianus* sp. nov.

Cycle 1 of the subculture took a shorter time for DEG removal than the other cycles. The longer DEG removal time in cycle 2 and 3 compared to cycle 1 of the subculture process suggested that this might occur from the adsorption of soil particles to the DEG. Soil particles and soil organic material can adsorb many compounds depending on the properties of the compound and soil components [19]. However, after cycle 1, the cultures were soil-free and the potential of DEG removal occurred from only the microorganisms.

3.2. Potential of microorganisms in DEG removal

3.2.1. Flask scale study

The mixed microorganisms from aerobic isolation were selected to study the potential of DEG removal from stationery wastewater. Bioaugmentation, biostimulation, and combined biostimulation-bioaugmentation showed different DEG removal potentials from stationery wastewater. The combined bioaugmentation and biostimulation had the highest removal efficiency and took the shortest time for DEG removal (Fig. 2(a)). Also, the biostimulation technique showed higher removal potential than bioaugmentation. Within 7 d of the experiment, bioaugmentation removed 64% DEG in wastewater, the biostimulation removed 98% DEG, and the bioaugmentation combined with biostimulation enhanced DEG removal potential to 100%.

3.2.2. CSTR reactor study

The potential of microorganisms in DEG removal were studied in CSTR reactors with a working volume of 2,000 mL for possible application in factories. The scaling up of the study showed the same trend as the flask scale study (Figs. 2(c) and (d)). The combination of bioaugmentation and biostimulation increased the rate of DEG removal. The combined bioaugmentation-biostimulation removed 100% DEG, while the biostimulation removed 95% DEG and the bioaugmentation removed 40% DEG within 10 d of experiment.

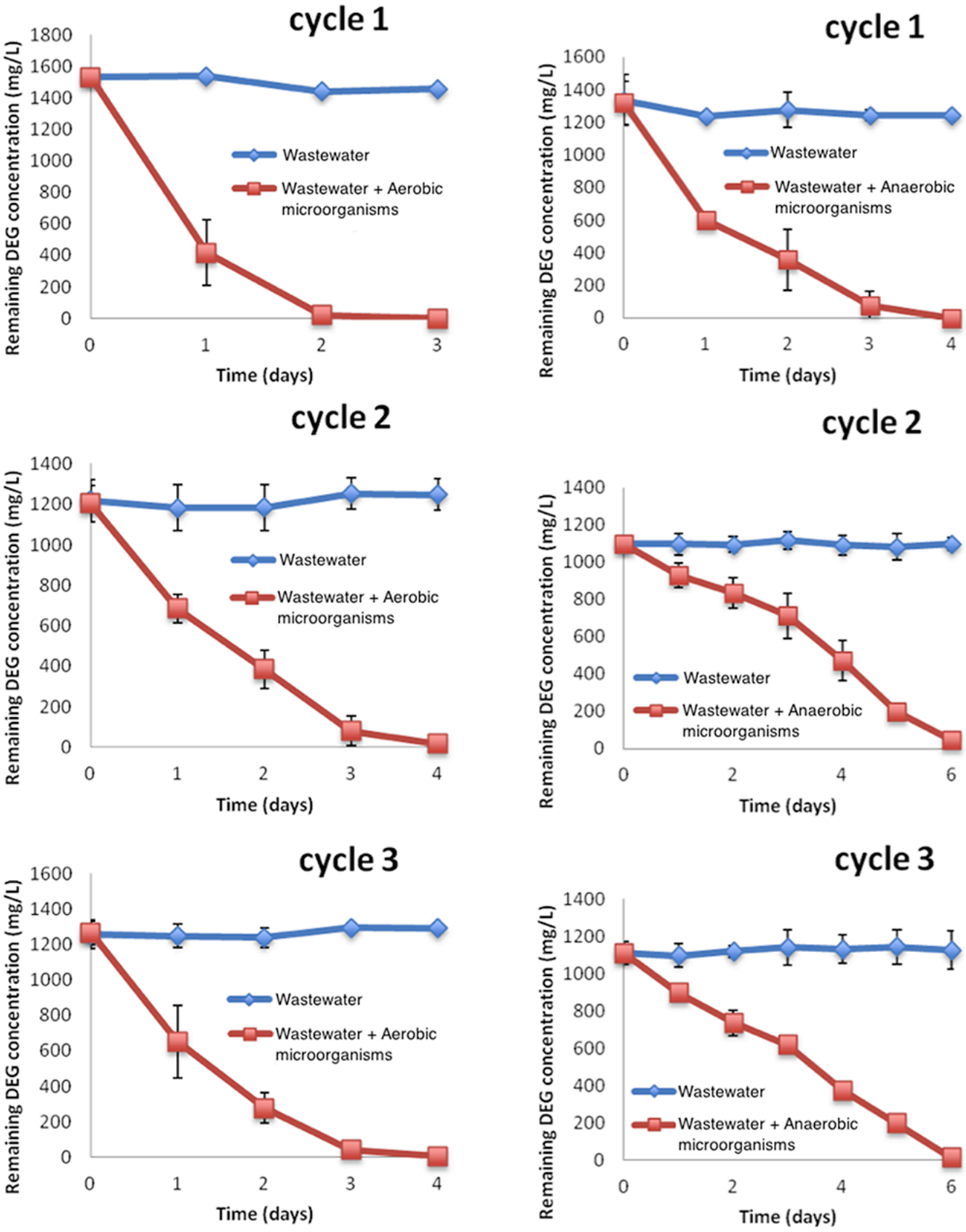

Fig. 1. DEG concentrations in the solution during cultivation of microorganisms under aerobic (left) and anaerobic (right) conditions.

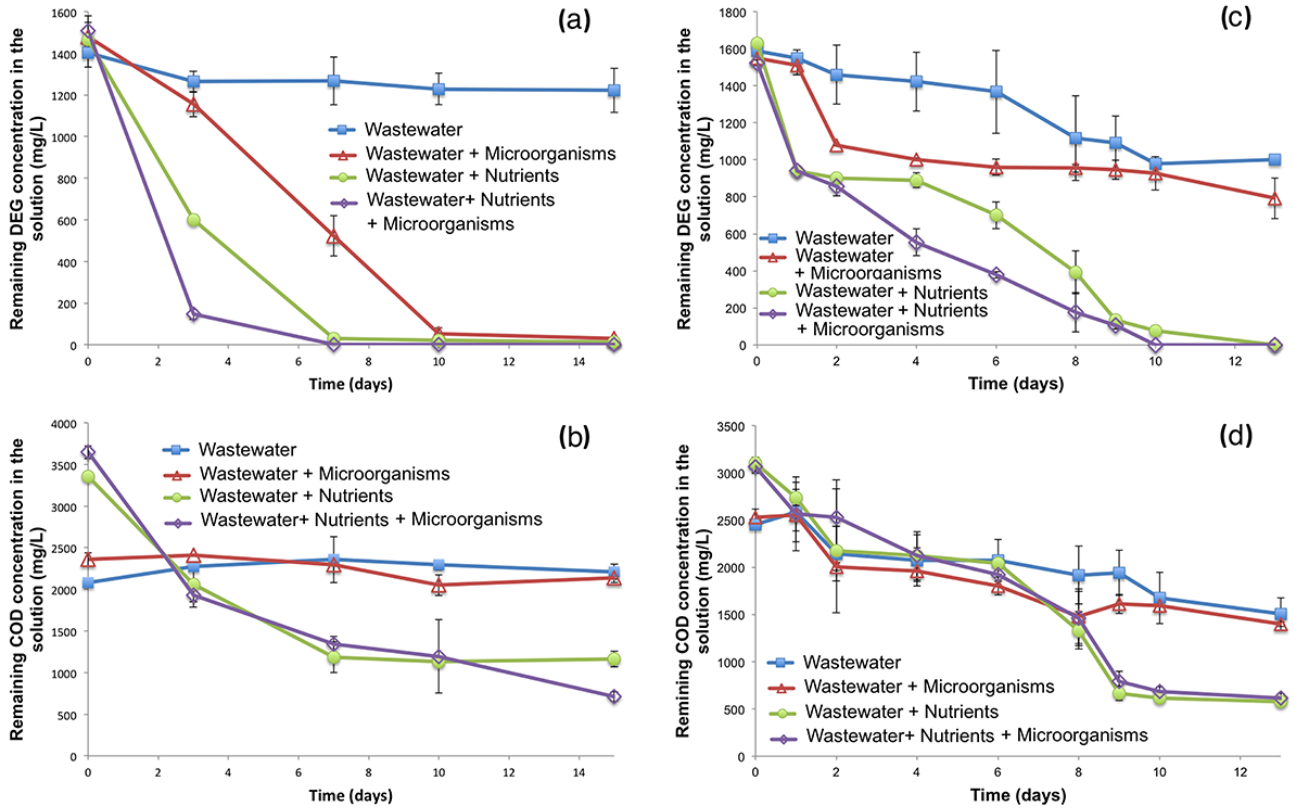

Fig. 2. Remaining DEG ((a) and (c)) and COD ((b) and (d)) concentrations in the solution under small scale remediation ((a) and (b)) and in the CSTR reactor ((c) and (d)) under various conditions.

The bioremediation potential could be heightened by increasing microbial activity. The microbial activity is enhanced by adjusting the appropiate conditions for microbial growth such as moisture, pH, oxygen, and nutrients. The nutrients that are usually added to elevate the potential of remediation are carbon, nitrogen, phosphorus, ready metabolite compounds, and some trace elements [20–22]. The yeast extract, which contains metal ions and required macronutrients, is an excellent nitrogen source for bacterial growth. The addition of nutrients, or biostimulation, has been the most practiced bioremediation strategy and is also well-documented [23]. Biostimulation enhanced activity of indigenous microorganisms in the wastewater, whereas bioaugmentation added effective microorganisms to the remediation system. The potential of added microorganims depends on environmental conditions. The remediation may not be successful due to the lack of appropriate nutrients for the microbe as in enriched media, and/or competition of native microorganisms and inoculum.

Bioaugmentation had a lower potential than biostimulation, which may be from eventual cell death after inoculation by limiting nutrients [24]. Although the wastewater has a full carbon source from DEG, the essential nutrients such as nitrogen are limited. The reduction of DEG by microorganisms in CSTR reactors was lower than in shake flasks (Fig. 2). The culture potential usually drops when scaling up from shake flasks to fermenters [25]. This may have occurred from the different supplies and limitations of oxygen. Furthermore, temperatures between the two studies were different. The removal of DEG by microorganisms under the screening process was higher than under flask scale and CSTR reactor experiments (Figs. 1 and 2). This might be due to the effect of nutrients. The flask scale and CSTR reactor experiments used only half-strength medium, while the screening process experiments used full-strength medium.

Although, microorganisms had a potential for DEG removal, the use of microorganisms or enhancing microbial activity could not reduce the COD of the wastewater, and also the addition of nutrients and microorganisms into the wastewater increased the COD of the solution (Figs. 2(b) and (d)). After 15 d of the experiment, the COD remained at 710.62 ± 55.61 mg/L under bioaugmentation combined with biostimulation in the flask scale study. The use of microorganisms in remediation is a method that can reduce DEG from wastewater, and an awareness of using microorganisms is increasing COD concentrations in wastewater.

3.3. Comparison of bioremediation and phytoremediation

Burhead plant has been studied for its potential in DEG remediation. The potential of the plant under hydroponic conditions compared to microorganisms is shown in Table 1. The plants in hydroponic solution and microorganisms (bioaugmentation) were not successful in DEG remediation. The use of microorganisms and added nutrients (combined bioaugmentation-biostimulation) had a greater DEG removal

Table 1
Treatment of DEG and COD from stationery industry wastewater under various conditions for 10 d

Parameter	DEG (mg/L)		COD (mg/L)	
	Initial	Final	Initial	Final
Wastewater (Control)	1,587.77 ± 2.53^{AB}	978.28 ± 7.50^{C}	2,453.09 ± 42.61^{b}	1,675.57 ± 272.76^{c}
Wasterwater + microorganisms (Bioaugmentation)	1,547.74 ± 31.69^{AB}	925.98 ± 90.35^{C}	2,525.41 ± 93.76^{b}	1,591.19 ± 61.37^{c}
Wasterwater + nutrients (Biostimulation)	1,630.28 ± 20.77^{A}	76.86 ± 11.98^{E}	3,104.03 ± 59.66^{a}	614.77 ± 51.14^{d}
Wasterwater + nutrients + microorganisms (Bioaugmentation + Biostimulation)	1,523.99 ± 14.09^{B}	0^{E}	3,061.84 ± 68.19^{a}	687.10 ± 51.14^{d}
Wasterwater + Plant	1,586.66 ± 23.09^{AB}	185.22 ± 0.02^{D}	2,424.68 ± 48.36^{b}	512.48 ± 20.88^{d}
Wasterwater + Plant + soil	1,611.00 ± 25.35^{AB}	0^{E}	2,340.56 ± 50.12^{b}	127.07 ± 16.12^{e}

The data is presented as the means ± SD of three individual experiments. Values in the same column with the same letter are not significantly different (α = 0.05).

potential than using plants under hydroponic solution. However, plants grown in the soil system gave the best removal potential for both DEG and COD (Table 1). Not only did the soil support plant growth but it also enhanced DEG adsorption. The native microorganisms in soil also aid DEG removal. Phytoremediation in hydroponic conditions had microbial association, nevertheless the plant played a major role in remediation [26]. The best bioremediation method (combined bioaugmentation-biostimulation) still had a lower potential than the plant-soil system (Table 1). Soil has a variety of microbial communities that are enhanced in the bioremediation process [27], suggesting that soil adsorption and native soil microorganisms enhanced DEG and COD removal.

3.4. Application of plant-microbe system for DEG removal

This study showed that phytoremediation of stationery wastewater with plants in a soil system had the highest removal efficiency (Table 1). Using the plant and soil system for DEG remediation as a constructed wetland system with the flow of wastewater (Fig. 3) was suitable to remove DEG and COD from wastewater. The wetland system took 8 d to remove DEG from wastewater (Fig. 4). During 2 weeks of the experiment, the COD of wastewater remained at 250 ± 17 mg/L. However, the use of bioaugmentation-biostimulation combined with wetland system showed the DEG and COD removal was reduced to 5 and 11 d, respectively (Fig. 4). Plant and microorganisms were able to enhance each other's growth, which made this system successful in stationery wastewater remediation. The wetland system had a higher rate of removal than the use of microorganisms alone (Fig. 2) because the wetland system had soil, the rhizosphere and root-associated microorganisms. Soil can adsorb many compounds and supply nutrients to the plant and microorganisms. In addition, microorganisms associated with plant roots, rhizosphere and soil microorganisms also enhanced the remediation.

The combination of microorganisms and plants enhanced the remediation potential of organic compounds [28] and also the number of microorganisms [18]. Plants and their associated microorganisms interacted with each other. Plants supplied the microorganisms with a special carbon source that stimulates the microorganisms in order to degrade organic

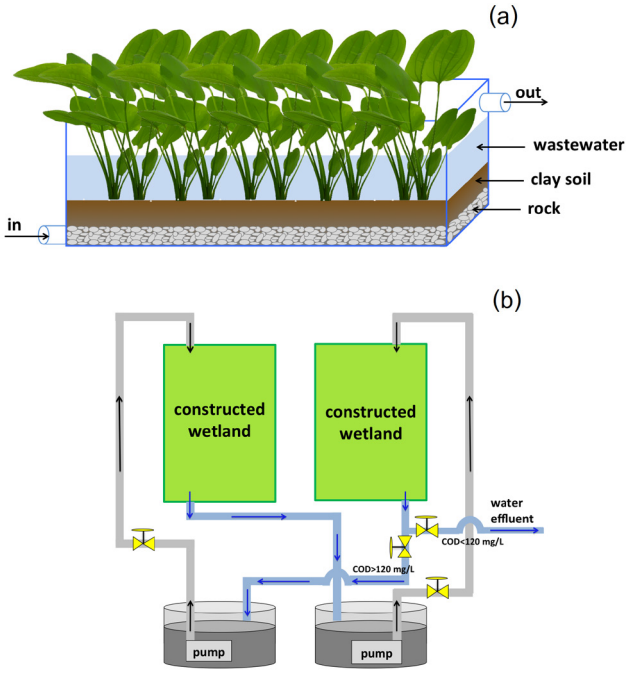

Fig. 3. Constructed wetland model (a) and the flow of wastewater in the system (b).

contaminants. Microorganisms can support their host plant to become tolerant to stress from contaminants, and improve plant growth and development in return. A better understanding of plant-microorganisms partnerships could be exploited to enhance the remediation of contaminants [29]. The combined use of plants and effective microorganisms for the remediation of DEG-contaminated wastewater was more effective than the use of microorganisms and the use of plants alone.

Another benefit of wetland systems is the reduction of wastewater pH. Most organisms grow well in neutral pH, except for some species that can grow in extreme conditions. The pH of stationery wastewater was pH 12, which is extremely basic and is not appropriate for burhead plant and soil microorganisms to grow. The recommended pH for burhead plant is between pH 6.2–7.1 and it can grow under

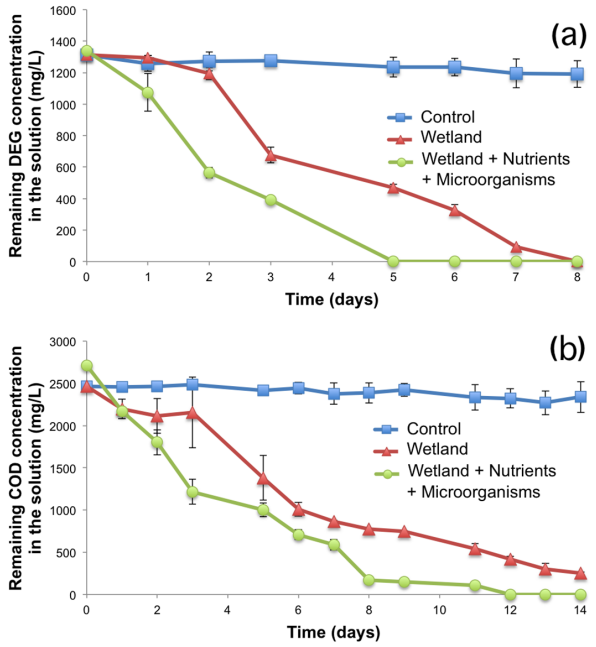

Fig. 4. Remaining DEG (a) and COD (b) concentrations in the plant-soil system under various conditions.

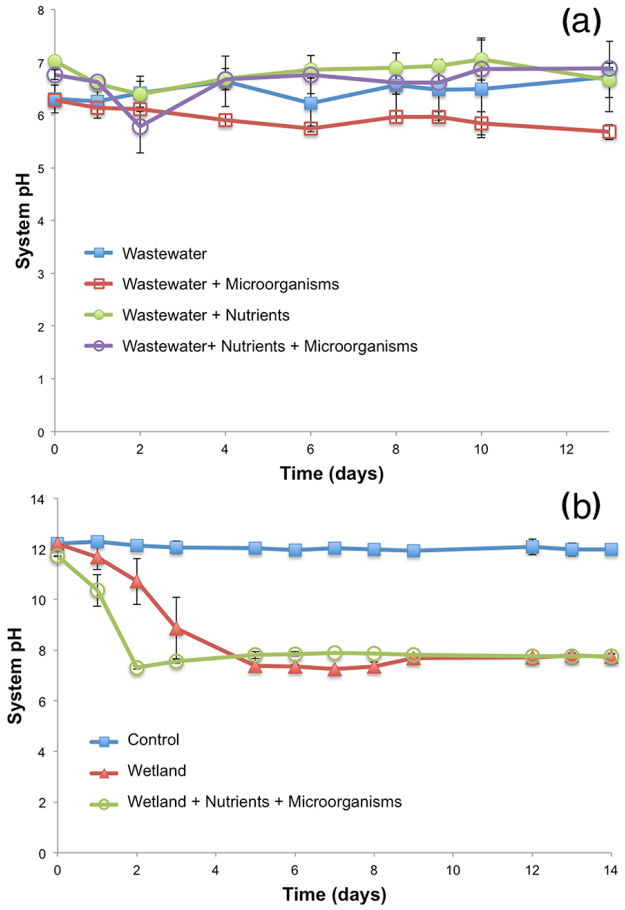

Fig. 5. System pH of wastewater during treatment by microorganisms in a CSTR reactor (a) and in the plant-microbe system (b).

pH 5.0–7.5. However, the application of wetland systems for wastewater treatment changed the pH from base to neutral (Fig. 5) because burhead plant released phosphate ions in the system (data not shown). The use of the plant and microorganisms in the remediation system of this wastewater was a suitable method because the wastewater did not require to pH adjusting before treatment, which reduced the cost of operations.

4. Conclusions

Rhizosphere microorganisms and soil microorganisms enhanced DEG contaminated wastewater from stationery industries. It was also found that aerobic microorganisms had a higher potential than that of anaerobic microorganisms. The addition of microorganisms (bioaugmentation), nutrients (biostimulation), and microorganisms + nutrients (combined bioaugmentation-biostimulation), decreased DEG in the wastewater. Among the use of microorganisms in the remediation system, the combined bioaugmentation-biostimulation was the best method. However, the addition of either microorganisms or nutrients into the wastewater increased the initial COD of the wastewater and the microorganisms were not able to decrease it to an acceptable level during the time of the remediation study. Burhead plant alone had lower DEG removal potential than the combined bioaugmentation-biostimulation, but the use of plant-soil systems had higher potential than that of the microbial remediation system. The application of the plant-soil system as a constructed wetland with a flow of wastewater enhanced remediation potential. Also, the combined wetland with bioaugmentation and biostimulation enhanced the potential of the system, and all DEG was removed within 5 d and COD was lower than the acceptable level within 11 d. Using the wetland system was easier in management because the pH of wastewater did not require adjusting before treatment as burhead plant adjusted the system pH to neutral by releasing phosphate ions in the system. However, the combined use of constructed wetland system and bioremediation method (combined bioaugmentation-biostimulation) had higher remediation efficiency than the use of wetland alone. This knowledge can be applied to treat DEG contaminated wastewater and the remediation system potential can be enhanced by the addition of nutrients and effective microorganisms.

Acknowledgements

This research was supported by King Mongkut's University of Technology Thonburi through the 'Kmutt 55th Anniversary Commenmorative fund'.

References

[1] S. Gorbi, M. Benedetti, C.V. Lamberti, B. Pisanelli, G. Moltedo, F. Regoli, Biological effects of diethylene glycol (DEG) and produced waters (PWs) released from offshore activities: A multi-biomarker approach with the sea bass *Dicentrarchus labrax*, Environ. Pollut., 157 (2009) 3166–3173.

[2] A. Vale, Ethylene and diethylene glycol, Medicine, 35 (2007) 617–618.

[3] L.A. Ferrari, L. Giannuzzi, Clinical parameters, postmortem analysis and estimation of lethal dose in victims of a massive intoxication with diethylene glycol, Forensic Sci. Int., 153 (2005) 45–51.

[4] T. Turan-Ertas, M.D. Gurol, Oxidation of diethylene glycol with ozone and modified Fenton processes, Chemosphere, 47 (2002) 293–301.
[5] J. Araña, J.A. Ortega Méndez, J.A. Herrera Melián, J. M. Doña Rodríguez, O. González Díaz, J. Pérez Peña, Thermal effect of carboxylic acids in the degradation by photo-Fenton of high concentrations of ethylene glycol, Appl. Catal. B, 113–114 (2012) 107–115.
[6] A. Orecki, M. Tomaszewska, K. Karakulski, A.W. Morawski, Separation of ethylene glycol from model wastewater by nanofiltration, Desalination, 200 (2006) 358–360.
[7] Q. Zhou, T. Hua, Bioremediation: a review of applications and problems to be resolved, Prog. Nat. Sci., 14 (2004) 937–944.
[8] E. Pilon-Smits, Phytoremediation, Annu. Rev. Plant Biol., 56 (2005) 15–39.
[9] W. Sriprapat, S. Kullavanijaya, S. Techkarnjanaruk, P. Thiravetyan, Diethylene glycol removal by *Echinodorus cordifolius* (L.): the role of plant-microbe interactions, J. Hazard. Mater., 185 (2011) 1066–1072.
[10] T. Iwamoto, M. Nasu, Current bioremediation practice and perspective, J. Biosci. Bioeng., 92 (2001) 1–8.
[11] A. Mrozik, Z. Piotrowska-Seget, Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds, Microbiol. Res., 165 (2010) 363–375.
[12] G. Toscano, L. Cavalca, M.L. Colarieti, R. Scelza, R. Scotti, M.A. Rao, V. Andreoni, S. Ciccazzo, G. Greco, Aerobic biodegradation of propylene glycol by soil bacteria, Biodegradation, 24 (2013) 603–613.
[13] P. Teamkao, P. Thiravetyan, Phytoremediation of mono-, di-, and triethylene glycol by *Echinodorus cordifolius L.* Griseb, Int. J. Phytorem., 17 (2015) 93–100.
[14] O. Mrklas, A. Chu, S. Lunn, L.R. Bentley, Biodegradation of monoethanolamine, ethylene glycol and triethylene glycol in laboratory bioreactors, Water Air Soil Pollut., 159 (2004) 249–263.
[15] Standard Methods for the Examination of Water and Wastewater, Section 5210, 18th Edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA, 1992.
[16] C. McGahey, E.J. Bouwer, Biodegradation of ethylene glycol in simulated subsurface environments, Water Sci. Technol., 26 (1992) 41–49.
[17] Y.L. Huang, Q.B. Li, X. Deng, Y.H. Lu, X.K. Liao, M.Y. Hong, Y. Wang, Aerobic and anaerobic biodegradation of polyethylene glycols using sludge microbes, Process Biochem., 40 (2005) 207–211.
[18] J. Tang, R. Wang, X. Niu, Q. Zhou, Enhancement of soil petroleum remediation by using a combination of ryegrass (*Lolium perenne*) and different microorganisms, Soil Tillage Res., 110 (2010) 87–93.
[19] T. Kobayashi, Y. Shimizu, K. Urano, Estimation of adsorbed amounts of volatile chlorinated organic compounds to wet soil based on the properties of the compounds and soils, Sci. Total Environ., 301 (2003) 215–223.
[20] B. Jefferson, J.E. Burgess, A. Pichon, J. Harkness, S. J. Judd, Nutrient addition to enhance biological treatment of greywater, Water Res., 35 (2001) 2702–2710.
[21] Y.S. Oh, D.S. Sim, S.J. Kim, Effects of nutrients on crude oil biodegradation in the upper intertidal zone, Mar. Pollut. Bull., 42 (2001) 1367–1372.
[22] D. Chen, J. Chen, W. Zhong, Enhancement of methyl tert-butyl ether degradation by the addition of readily metabolizable organic substrates, J. Hazard. Mater., 167 (2009) 860–865.
[23] L. Huang, T. Ma, D. Li, F.L. Liang, R.L. Liu, G.Q. Li, Optimization of nutrient component for diesel oil degradation by *Rhodococcus erythropolis,* Mar. Pollut. Bull., 56 (2008) 1714–1718.
[24] M. Tyagi, M.M. da Fonseca, C.C. de Carvalho, Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes, Biodegradation, 22 (2011) 231–241.
[25] H. Wang, F. Wang, D. Wei, Impact of oxygen supply on rtPA expression in *Escherichia coli* BL21 (DE3): ammonia effects, Appl. Environ. Microb., 82 (2009) 249–259.
[26] Q. Wang, W. Zhang, C. Li, B. Xiao, Phytoremediation of atrazine by three emergent hydrophytes in a hydroponic system, Water Sci. Technol., 66 (2012) 1282–1288.
[27] M.T. Del Panno, I.S. Morelli, B. Engelen, L. Berthe-Corti, Effect of petrochemical sludge concentrations on microbial communities during soil bioremediation, FEMS Microbiol. Ecol., 53 (2005) 305–316.
[28] Y. Wang, H. Oyaizu, Enhanced remediation of dioxins-spiked soil by a plant-microbe system using a dibenzofuran-degrading *Comamonas sp.* and *Trifolium repens* L, Chemosphere, 85 (2011) 1109–1114.
[29] S. Khan, M. Afzal, S. Iqbal, Q.M. Khan, Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils, Chemosphere, 90 (2013) 1317–1332.