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# Bioremediation of MEG, DEG, and TEG: Potential of Burhead Plant and Soil Microorganisms

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***Abstract*—**The aim of this work was to investigate the potential of soil microorganisms and the burhead plant, as well as the combination of soil microorganisms and plants to remediate monoethylene glycol (MEG), diethylene glycol (DEG), and triethylene glycol (TEG) in synthetic wastewater. The result showed that a system containing both burhead plant and soil microorganisms had the highest efficiency in EGs removal. Around 100% of MEG and DEG and 85% of TEG were removed within 15 days of the experiments. However, the burhead plant had higher removal efficiency than soil microorganisms for MEG and DEG but the same for TEG in the study systems. The removal rate of EGs in the study system related to the molecular weight of the compounds and MEG, the smallest glycol, was removed faster than DEG and TEG by both the burhead plant and soil microorganisms in the study system.

***Keywords*—**Ethylene glycol, burhead plant, soil microorganisms, phytoremediation

## I. Introduction

GLYCOL is an organic chemical widely used both in household and industrial applications [1]. Discharge of the glycol to water sources and groundwater is a potential hazard to the environment [2-4]. As it is very soluble in water, glycol is easily distributed in the environment and hard to remediate. Recovery systems have been applied in processes with high purity and high amounts of glycol, but it is not economical to use in processes with low concentrations of glycol. Generally, physical and chemical precipitations are used for the treatment of wastewater before discharging to the environment. However, these two methods cannot remove water-soluble compounds. Bioremediation is a choice usually used in processes with low concentrations of organic contaminants [5]. Bioremediation has been applied in processes of organic contamination [6]. Previously, the potential of the burhead plant was studied in glycol removal in soil-less conditions [7]. In the application, the remediation system contains plants, soils, and soil microorganisms, all of which affect the remediation's potential.

The objective of this research is to compare the potential of the burhead plant and soil microorganisms, as well as the potential of the combination of plant and soil microorganisms, in glycol removal, focusing on monoethylene glycol (MEG), diethylene glycol (DEG), and triethylene glycol (TEG).

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## II. Materials and Methods

### A. Plant and Culture Conditions

Burhead plant *(Echinodorus cordifolius* L.) was grown in the greenhouse of King Mongkut's University of Technology Thonburi, Bangkhuntein campus under 12 hr light/dark cycles and an average temperature of 30.5±3.0 °C. Three-month-old plants (7-8 leaves, 300-400 g in weight) were cleaned with tap and distilled water to disperse soil particles, algae, and insect larvae that were attached on the plant stem and roots. After that, the burhead plant was pre-cultured in a half-strength Hoagland nutrient solution for 15 days prior to starting the experiment.

### B. Experimental Design

The experiment was randomized with three treatments and one control set for each EGs to study the removal efficiency. The three treatments were soil+synthetic MEG wastewater, burhead plant+synthetic MEG wastewater, and burhead plant+soil+synthetic MEG wastewater. The control set was synthetic MEG wastewater without plants and soil. In DEG and TEG, the treatment was the same but synthetic DEG and TEG wastewater were used instead of synthetic MEG wastewater. The synthetic MEG, DEG, and TEG wastewater used in the experiment had initial MEG, DEG, and TEG concentrations of around 2,000 mg/l at a volume of 3,000 ml per pot. The soil used in this study is a kind of soil that was appropriate for aquatic plants from local markets. Five hundreds gram of soil per pot were used under soil conditions. The experiments were performed under caustic conditions at the initial pH 7-8.

### C. EGs Removal

The remaining MEG, DEG and TEG in the solutions were measured every three days by gas chromatography (GC). The system comprised of a Shimadzu model GC 17A with an Rtx-200 capillary column (30 m x 0.32 mm, 0.32 μm film thickness), an FID detector, and an auto injector (Shimadzu 20i long auto injector). Helium was used as a carrier gas. The GC conditions were as follows: the injection temperature was 250 °C, the oven temperature was 220 °C, and the detector temperature was 280 °C.

To study the effects of soil in the MEG, DEG, and TEG removal, 50 g of autoclaved soil were used and 200 ml of synthetic MEG, DEG, and TEG wastewater were added at a initial concentration 2,000 mg/l each. The experiment was carried out under sterile conditions. In all treatments, all flasks were covered with aluminium foil to prevent water evaporation and other microorganisms from entering. The analysis of the remaining EGs was as stated above.

*D. Chemical Oxygen Demand (COD) Removal*

The remaining COD in synthetic MEG, DEG, and TEG wastewaters were measured every three days. The measurement of COD was done according to the standard method [8].

*E. Organic Acids Study*

Organic acids (acetic acid, propionic acid, butyric acid, and steric acid) and ethanol were studied with the GC technique using the DEG treatment as an example. The solutions on day 0, 3, 6, 9, 12 and 15 of the experiments were collected and analyzed by the Shimadzu model GC 14B with Carbo-pack B-DA column and FID detector. The carrier gas was nitrogen. Injection and detector temperature were 230 ºC and the oven temperature was 170 ºC.

## III. Results and Discussion

*A. EGs Removal Efficiency*

Burhead plant and soil microorganisms had the potential for MEG, DEG, and TEG removal from synthetic wastewaters (Fig.1). The removal rates under plant+soil conditions were the highest for all EGs. After 15 days of the experiment, 100% of MEG was removed under plant+soil conditions, while 90% and 60% were removed using burhead plant- alone and soil-alone conditions, respectively (Fig. 1A).

The removal of DEG was in the same trend to MEG (Fig. 1B). At the end of the experiment, approximately 100% of DEG was removed under plant+soil conditions, while 86% and 41% were removed using burhead plant-alone and using soil-alone conditions, respectively.

The removal efficiency of TEG is shown in Fig.1C. The rate of TEG removal was faster under the plant+soil condition, as approximately 85% of TEG was removed. The potential of burhead plant-alone and soil microorganisms-alone was the same as 24% of TEG was removed.

The system with both burhead plant and soil microorganisms had the highest removal rate. The plant, soil, and soil microorganisms supported each other, as the plants could get nutrients from the soil and also stimulate root zone microbial growth [9, 10]. Soil microorganisms transformed nutrients in the soil to a viable form for the plant [11]. However, using burhead plant-alone showed a higher potential than using soil-alone for MEG and DEG, but not for TEG. If the system was run longer, the difference in TEG removal may be observed.

Fig. 2 shows the effect of plant and soil conditions to EGs removal. The removal rate of EGs related to the molecular weight of the compounds. The lowest molecular weight glycol, MEG, was removed the fastest by both the burhead plant and soil microorganisms. The previous work reported the effect of the molecular size to EGs removal by the plant [7]. However, the uptake of EGs by microorganisms depends on the transport system that rerated to type of microorganisms [12], as different bacterium prefer different molecular weight glycols [12,13]. It is suggested that the removal of glycol by microorganisms depends more on the type of microorganisms in the remediation system than on the molecular weight of the compounds.

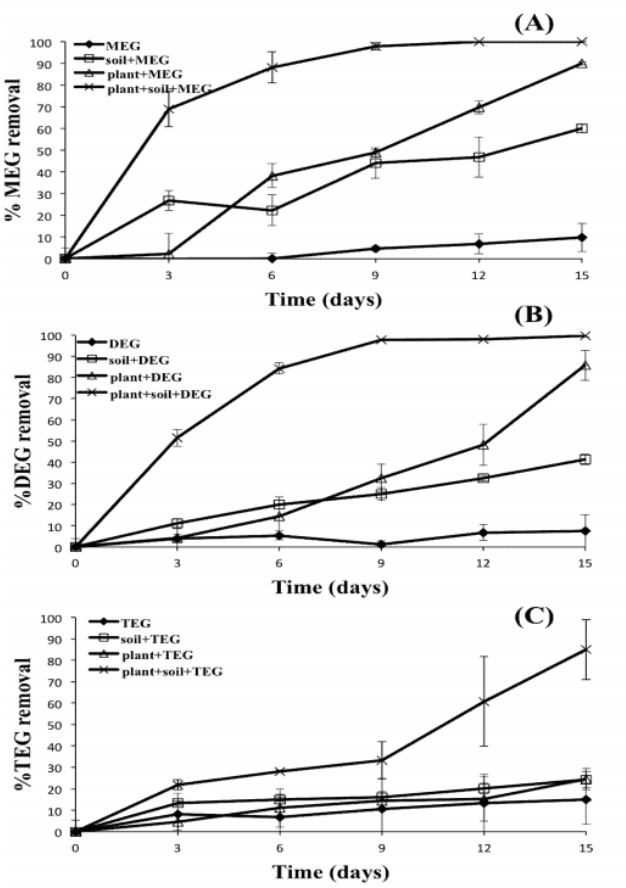


Fig. 1 EGs removal of synthetic MEG (A), DEG (B), and TEG (C) wastewater under various conditions

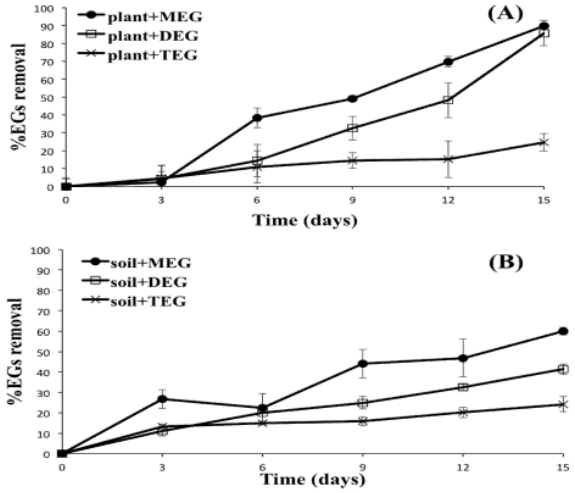


Fig. 2 EGs removal from synthetic MEG, DEG, and TEG wastewater under plant (A) and soil (B) conditions

*B. COD Removal*

Biochemical and chemical oxygen demand (BOD and COD) are parameters usually used to indicate water quality. COD is a parameter used to measure organic compounds that can oxidize by a chemical oxidizing agent. The COD removal efficiency of soil, plant, and plant+soil conditions is shown in Table I. Generally, COD removal was higher than EGs removal except under DEG removal by burhead plant and TEG removal by soil+plant conditions. In this study COD was used for the indirect measurement of EGs concentration in synthetic wastewaters. One aspect of using COD is the interfererence in the measurement. In this case COD removal was no different from synthetic TEG wastewater (Fig.3) but was totally different if the measured concentration of TEG used the GC technique (Fig.1C). The EGs in the solution may change to other compounds like acetic acid and ethanol that still give COD to the solution but they are not EGs. Moreover, decomposition of plant tissues and compounds in the soil can also interfere in the measurement. Then the direct measurement of EGs concentration using the GC technique is given a more reliable result.

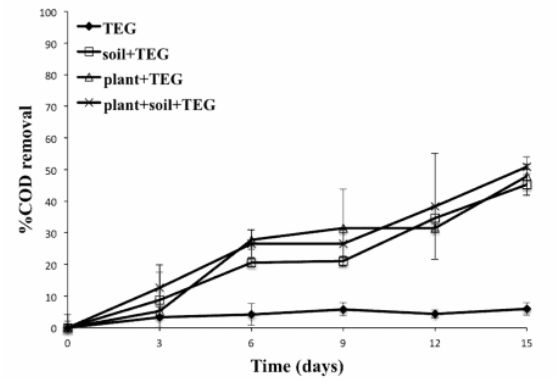


Fig. 3 COD removal from synthetic TEG wastewater under various conditions

*C. EGs Adsorption by Sterile Soil*

In a soil system, not only soil microorganisms can reduce EGs concentration in synthetic wastewater. Soil particles also had an effect on the removal efficiency of the remediation system. Soil particles also have potential in organic chemicals adsorption [14]. The ability of sterile soil particles used in this study to remove MEG, DEG, and TEG were 1.62±0.43, 0.81±0.32, and 0.48±0.20 mg EG/g soil, respectively.

Efficiency of each parameter to MEG and DEG removal was shown in Fig.4. Burhead plant is the main factor in MEG and DEG removal in remediation systems. The efficiency of each parameter for TEG removal could not be calculated on day 15 of the experiment because the remediation system was in an adaptation period and the potential of the plant and soil were no different from the control set. However, after running the experiment longer, burhead is still the main factor for TEG removal (Fig.4).

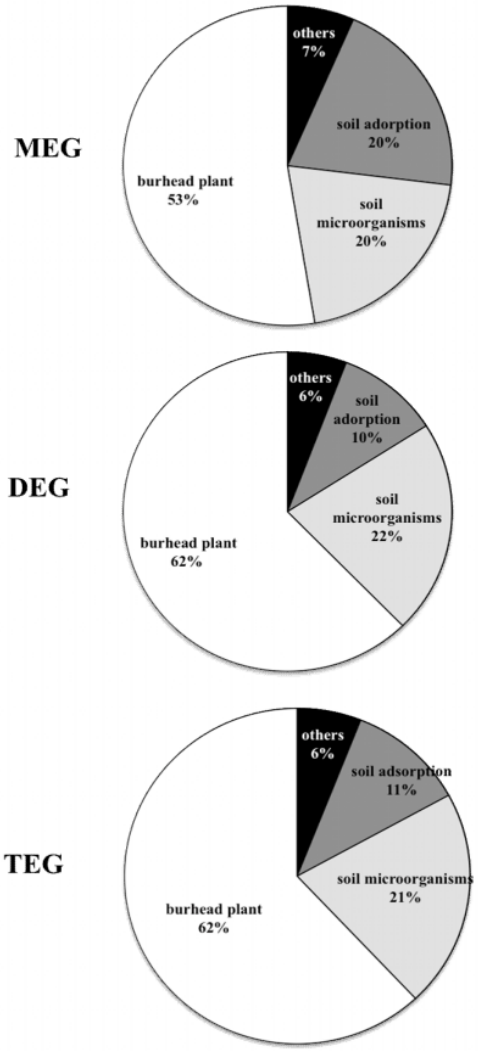


Fig. 4 Efficiency of plant, soil microorganisms, and sterile soil adsorption in MEG, DEG, and TEG removal

TABLE I
COD REMOVAL EFFICIENCY OF SYNTHETIC MEG, DEG, AND TEG WASTEWATER BY SOIL, PLANT, AND PLANT+SOIL CONDITIONS

| | MEG | DEG | TEG |
|---|---|---|---|
| soil | 71.36±2.27% | 59.87±5.19% | 45.18±3.20% |
| plant | 90.99±5.21% | 64.64±12.56% | 47.89±6.17% |
| plant+soil | 100.00±0.00% | 99.75±0.10% | 50.84±0.91% |

### *D.Organic Acids*

From the study, only acetic acid was found in the solutions. Acetic acid was found in only-soil+DEG and plant+soil+DEG conditions (Fig.5). Acetic acid occurred from the degradation of DEG by microorganisms in the soil. Many studies show the potential of microorganisms to degrade ethylene glycol [15,16] and the one degradation product is acetic acid [15]. Under the no-plant condition, all of DEG in the synthetic wastewater was used by soil microorganisms but under the plant condition, DEG in the synthetic wastewater was used by both soil microorganisms and plants. More available DEG used by soil microorganisms resulted in higher acetic acid production. After 12 days of the experiment, acetic acid disappeared from the solution. Acetic acid is a carbon source for microorganisms and can be converted into cell mass, resulting in a low acetic acid concentration in the solution [17,18].

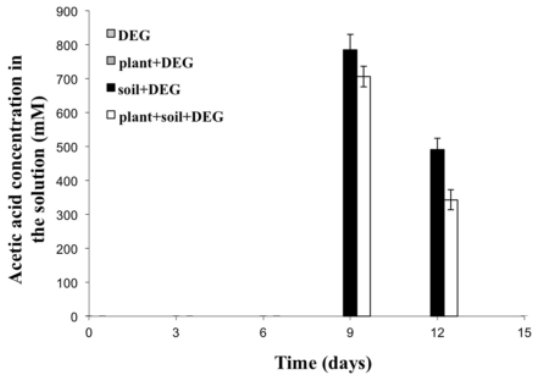


Fig. 5 Acetic acid concentration in the solution under various conditions

## IV. CONCLUSION

The results from this study show the potential of the burhead plant, soil microorganisms, and the combination of plant and soil microorganisms for EGs removal. The potential of the remediation system may enhance when the number of plants and the amount of soil used is increased (in the experiment one plant per pot and 500 g of soil was used). This knowledge can be applied to create a wetland system for treatment of real wastewater that is contaminated with EGs.

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## REFERENCES

[1] R. Dye, "Ethylene glycols technology," *Korean J. Chem. Eng.*, vol. 18, no. 5 pp. 571-579, 2001.
[2] B. Ballantyne and W.M. Snellings, "Developmental toxicity study with diethylene glycol dosed by gavage to CD rats and CD-1 mice," *Food Chem. Toxicol.*, vol. 43, no. 11, pp. 1637-1646, 2005.
[3] R. A. Corley, S. A. Saghir, M. J. Bartels, S. C. Hansen, J. Creim, K. E. McMartin and W. M. Snellings, "Extension of a PBPK model for ethylene glycol and glycolic acid to include the competitive formation and clearance of metabolites associated with kidney toxicity in rats and humans," *Toxicol. Appl. Pharmacol.*, vol. 250, no. 3, pp. 229-244, 2011.
[4] Center for the Evaluation of Risk to Human Reproduction, "NTP-CERHR Expert Panel report on the reproductive and developmental toxicity of ethylene glycol." *Reprod. Toxicol.*, vol. 18. no. 4, pp. 457-532, 2004.
[5] J. L. Bankston, D. L. Sola, A. T. Komor, and D. F. Dwyer, "Degradation of trichloroethylene in wetland microcosms containing broad-leaved cattail and eastern cottonwood," *Water Res.*, vol. 36, no. 6, pp. 1539-1546, 2002.
[6] K. E. Gerhardt, X. D. Huang, B. R. Glick and B. M. Greenberg, "Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges," *Plant Sci*., vol. 176, no. 1, pp. 20-30, 2009.
[7] P. Teamkao and P. Thiravetyan, "Phytoremediation of ethylene glycol and its derivatives by the burhead plant (*Echinodorus cordifolius* (L.)): Effect of molecular size." *Chemosphere*, vol. 81, no. 9, pp. 1069-1074, 2010.
[8] APHA, "*Standard methods for the examination of water and wastewater*," 20thed., American Public Health Association / American Water Works Association / Water Environment Federation, Washington DC, USA, 1998.
[9] J. Kozdrója and J.D. van Elsas, "Response of the bacterial community to root exudates in soil polluted with heavy metals assessed by molecular and cultural approaches," *Soil Biol. Biochem*., vol. 32, no. 10, pp. 1405-1417. 2000.
[10] L. A. Phillips, C. W. Greer, R. E. Farrell and J. J. Germida, "Plant root exudates impact the hydrocarbon degradation potential of a weathered-hydrocarbon contaminated soil," *Appl. Soil Ecol*., vol. 52, pp. 56-64, 2012.
[11] A. Zaidi, M.S. Khan and M. Amil, "Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.)," *Eur. J. Agron.*, vol. 19, no. 1, pp. 15-21. 2003.
[12] L.D.L. Jenkins, C. Maslen and R.B. Cain, "An active-transport mechanism for the uptake of ethylene glycol and its low-molecular-weight oligomers by *Pseudomonas fluorescens*," *604th Meeting, Cambridge*, vol. 11, pp. 739.
[13] B. Schink and M. Stieb, "Fermentative degradation of polyethylene glycol by strictly anaerobic, gram-negative, nonsporeforming bacterium, *Pelobacter venetianus* sp. nov.," *Appl. Environ. Microbiol*., vol. 45, no. 6, pp. 1905-1913, 1983.
[14] R. Calvet, "Adsorption of organic chemicals in soils," *Environ. Health Perspect.*, vol. 83, pp. 145-177, 1989.
[15] D.F. Dwyer, "Anaerobic biodegradation of ether compounds by ether bond-cleaving bacteria and methanogenic consortia," Michigan State University, Ph.D. thesis, 1989.
[16] O. Mrklas, A. Chu, S. Lunn and L. R. Bentley, "Biodegradation of monoethanolamine, ethylene glycol and triethylene glycol in laboratory bioreactors," *Water Air Soil Pollut*., vol. 159, no. 1, pp. 249-263, 2004.
[17] A. Mohseni-Bandpi, D.J. Elliott and A. Momeny-Mazdeh, "Denitrification of ground water using acetic acid as a carbon source," *Water Sci. Technol*., vol. 40, no. 2, pp. 53-59, 1999.
[18] C. Turner, M.E. Gregory and N.F. Thornhill, "Closed-loop control of fed-batch cultures of recombinant *Escherichia coli* using on-line HPLC," *Biotechnol. Bioeng*., vol. 44, no. 7, pp. 819-829, 1994.