Journal of Industrial and Engineering Chemistry 18 (2012) 882–887

Contents lists available at SciVerse ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

Short communication

Bench-scale *ex situ* diesel removal process using a biobarrier and surfactant flushing

Young-Chul Lee [a], Sung Geun Woo [b,e], Eun-Sil Choi [c], Yeonghee Ahn [d], Joonhong Park [e], Myungjin Lee [b,*], Ji-Won Yang [a,**]

[a] Department of Chemical and Biomolecular Engineering (BK21 program), KAIST, 335 Gwahak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Research and Development Division, H-Plus Eco Ltd., BVC #301, KRIBB, Eoeun-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
[c] Department of Biological Sciences, College of Natural Sciences, ChonnamNational University, Gwangju 500-757, Korea
[d] Department of Environmental Engineering, Dong-A University, 840 Hadan-dong, Saha-gu, Busan 604-714, Republic of Korea
[e] School of Civil and Environmental Engineering, Yonsei University, Seoul 120-749, Republic of Korea

ARTICLE INFO

Article history:
Received 14 October 2011
Accepted 11 January 2012
Available online 21 January 2012

Keywords:
Ex situ biodegradation
Diesel oil
DGGE
T-RFLP
Bench-scale system

ABSTRACT

Ex situ diesel removal was demonstrated using a biobarrier with immobilized cells and surfactant flushing in a bench-scale system. Four strains (two *Acinetobacter* sp., one *Gordonia* sp., and one *Rhodococcus* sp.) isolated from a diesel-contaminated site were immobilized onto a matrix to act as a biofiller. Peat moss, bentonite, and alginate were used as a hybrid support, and a procedure for the use of a bench-scale biobarrier was also employed. According to a microbial counting assay used for the biobarrier, the total amount of bacterial cells increased from approximately 2×10^9 to 8×10^9 (colony forming unit (CFU)/g) and the amount of inoculated diesel-degrading bacteria slightly increased from $\sim 2 \times 10^6$ to $\sim 5 \times 10^6$ (CFU/g) in the same period (over 30 days). This increase resulted in the reduction of diesel from 6000 ± 45 mg/kg to below 5 mg/kg. While 99.9%, i.e. up to below 5 mg/L of the diesel in soil was treated during 30 days of operation, diesel biodegradation accounted for 24.3% of the reduction of diesel.

1. Introduction

Diesel oil leaks from underground storage tanks, army depots, and oil-contaminated sandy beaches pose a serious threat to aqueous bodies of water, soil ecosystems, and human beings [1]. Physical and chemical processes, such as adsorption, thermal treatment, soil washing [2], and many biological treatments, have been reported to treat diesel-contaminated sites [3–7]. Among them, bioaugmentation by mixed microbial cultures has appeared to be effective [4,5].

Especially, physical immobilization of cells has been suggested although some releasing of cells is emerged. As a candidate of matrix for immobilization of cells, peat moss is promising due to a high and fast removal of hydrophobic contaminants [2]. The hybrid support (here, denoted as PBA) consisting of Peat moss combined with bentonite and alginate provides higher diesel adsorption and promotes cell immobilization due to a higher surface area [9,10].

Herein, diesel removal has been demonstrated using immobilized cells on the PBA as a biofiller in the biobarrier for the treatment of diesel oil-contaminated soil, where the biobarrier was supported by flushing with a biodegradable surfactant solution. This biobarrier and surfactant flushing system offers the possibility to use the biobarrier until achieving the clean-up goal without spreading of diesel as an *in situ* remediation technology in diesel-contaminated sites, becoming a cost efficient process. Moreover, the clean-up aim by immobilized microorganisms in the diesel-contaminated soil ends up remediating sites without exchange of biofiller to below 5 mg/kg of target diesel. If remediation project has been done, the biobarrier can function to avoid problems for additional events of diesel spreading. When the biobarrier is necessitated no more, biofiller materials can be treated easily such as burning or use of fertilizer, and so forth.

Prior to pilot experiment, a bench-scale study grants important information because applications from the pilot-scale to field-scale have led to different results, meaning that prediction is difficult in scale-up case studies [8]. This information includes the amount of flushing agent for diesel treatment, biofiller loading (biobarrier thickness), and the microorganism injection period. We have a goal to do *ex situ* bench and pilot systems by using diesel-contaminated soil to be excavated diesel, but the ultimate remediation of field application is focused on *in situ* system. Therefore, the specific

* Corresponding author. Tel.: +82 42 863 7494; fax: +82 42 863 7424.
** Corresponding author. Tel.: +82 42 350 3924; fax: +82 42 350 3910.
E-mail addresses: mjlee@hpluseco.co.kr (M. Lee), jiwonyang@kaist.ac.kr, jwyang@kaist.ac.kr (J.-W. Yang).

1226-086X/$ – see front matter © 2012 The Korean Society of Industrial and Engineering Chemistry. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jiec.2012.01.020

purposes of this work in the bench-scale system were the following: (1) evaluation of the *ex situ* diesel oil degradation, (2) counts of the total and diesel-degrading bacteria in the biobarrier, and (3) qualitative analyses of the cells in the biobarrier by denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) during 30 days of operation.

2. Experimental

2.1. Materials

Peat moss was purchased from TERRA-TORF, DEMETRA (Yuzhno-Sakhalinsk Timovskoye, Russia). Bentonite, alginate, and Tween 80® were obtained from Sigma–Aldrich (St. Louis, USA). Dichloromethane was purchased from Merck (Darmstadt, Germany). The biosurfactant used as a flushing agent in this study was used with SWA 1503® (H-Plus Eco., Daejeon, Korea; see Table S1 in supporting information). The frames of the bench-scale system were fabricated from stainless steel with mesh-type barrier fabrics.

2.2. Isolation, identification, and culturing of bacteria

Four strains of bacteria (*Acinetobacter* sp. MJ1, *Rhodococcus* sp. MJ2, *Gordonia* sp. MJ3, and *Acinetobacter* sp. MJ4) were isolated from a diesel oil-contaminated site in Daejeon, Korea [1]. The 16S rRNA gene sequences of the four strains were deposited in the NCBI GenBank with the accession numbers GU991530, GU991529, GU991528, and HQ650820, respectively. The phylogenetic tree (clustering) showing similarities within groups and between groups for the four strains is shown in Fig. S1 (supporting information). Detailed isolation and culture methods of the bacteria from the soil sites are included in Ex. S1 (supporting information).

2.3. Preparation of biofiller

Biofiller to pack inside of biobarrier was prepared: the each cultured bacteria (~10^9 CFU) was centrifuged, and then the collected biomass was redispersed with PBA. Afterwards all materials were mixed in the container vigorously by stirrer (3000 rpm and 30 min), resulting in a slurry, i.e. biofiller. The composition of the PBA was sterilized peat moss, bentonite, and alginate (ratio of 80/19/1, w/w/w) whose recipe was designed to give enough permeability for the biobarrier [1]. The ratio of the PBA and double-distilled (DI) water was 1:1 (w/w). Based on previously reported studies, the PBA was immobilized with the four bacterial strains prior to packing in the column. For the bacteria immobilized into the PBA, the cultured microorganisms for *Acinetobacter* sp. MJ1, *Rhodococcus* sp. MJ2, *Gordonia* sp. MJ3, and *Acinetobacter* sp. MJ4 were determined to be 3×10^9, 7×10^9, 5×10^9, and 2×10^9 CFU/g, respectively. To quantify the immobilized cells, a 1 mL aliquot of biofiller was placed into a 15 mL conical tube (BD Falcon™) with 9 mL sterilized DI water. After vigorous vortexing for 5 min to dislodge the immobilized cells, an aliquot of 100 μL was plated onto a R2A agar plate and serially diluted until colonies appeared at 37 °C. The number of colonies was expressed as CFU per 1 g of biofiller on a wet weight basis. The quantification experiments were conducted in triplicate.

2.4. Description of bench-scale set-up

As shown in Fig. 1, the volume of the equipment was 0.11 m^3 (45 kg) with dimensions 0.3 m × 0.6 m × 0.6 m (width/length/height). The volume of the barrier was 0.01 m^3 (1.6 kg) with dimensions 0.3 m × 0.05 m × 0.6 m; the barrier connected to each of the three injection and outlet points at the top, middle, and bottom of set-up. The biobarrier composed of mesh-type stainless steel is contacting the position (A) and (B) without a gap. The flushing flow rate was determined to be 1 mL/min and flushing

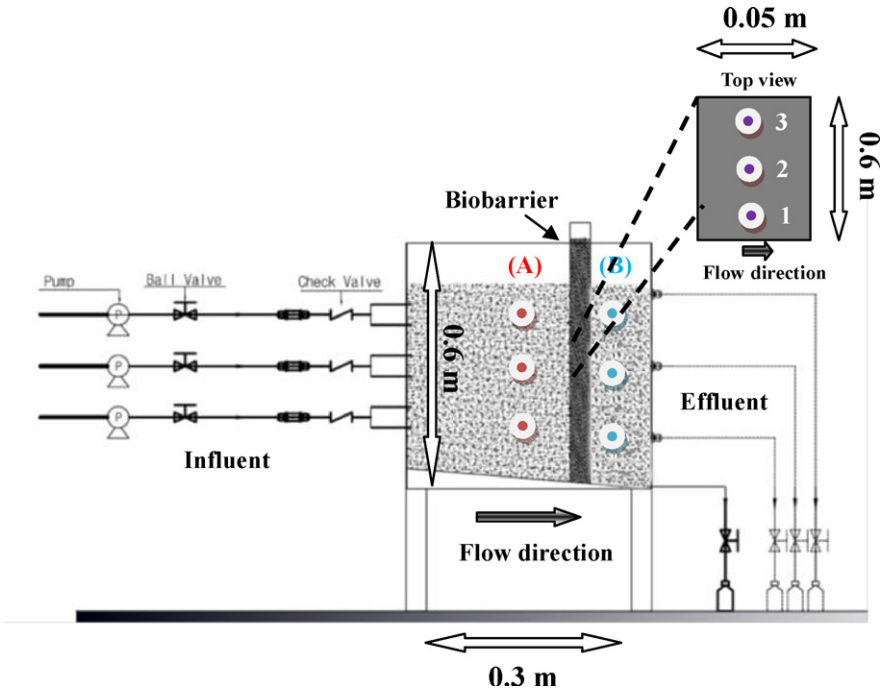

Fig. 1. Schematic presentation of the bench-scale set-up horizontally. *Note*: Three sampling points at positions (A) and (B) represent the diesel analysis at the left/right of the biobarrier. Three sampling points of position (1–3 points) in the biobarrier represent microbiological analysis.