A STUDY ON EFFECTS OF 1,3-BUTADIENE EXPOSURE ON BACTERIAL COMMUNITY IN ACTIVATED SLUDGE USING NEXT-GENERATION SEQUENCING

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Abstract: This study investigated effects of 1,3-butadiene (1,3-BD) exposure on bacterial community in activated sludge samples (designated as SL2 and SL4) collected from 2 different wastewater treatment facilities of petrochemical industry. While SL2 represented a bacterial community with no prior exposure to 1,3-BD and/or toluene, SL4 represented a bacterial community with prior exposure to both compounds. The sludge samples were enriched with 100 ppm of 1,3-BD (contributing 8,600 ppm of toluene as a solvent) for 6 weeks. The sludge samples exposed with only toluene were also conducted in parallel. Changes in microbial community profile was monitored based on 16s rDNA using next-generation sequencing platform. The sequences were assigned to the phylum as well as lower levels to represent the relative microbial abundance in each sample. Before an enrichment (Week 0), Proteobacteria (Alpha- and Beta-proteobacteria), Planctomycetes and Chloroflexi were the dominant bacterial populations in both samples whereas Firmicutes was found only in SL2. After an enrichment with toluene, Planctomycetes, Chloroflexi and Chlorobi tended to decrease over time while Actinobacteria and Candidatus Saccharibacteria (Candidate division TM7) increased continuously. In SL2, Firmicutes (Bacilli) was found to be the dominated taxa during toluene exposure while Proteobacteria (Alpha- and Beta-proteobacteria) decreased over time. In case of 1,3-BD exposure, Proteobacteria increased in both samples. Taxonomic classification into class level indicated that Alapha-proteobacteria (mainly Rhizobiales) and Beta-proteobacteria (mainly Burkholderiales) were the most abundant classes in both samples while a significant increase in Gamma-proteobacteria (Xanthomonadales and Pseudomonadales) was mainly observed in SL4. The fact that Beta-proteobacteria (mainly Burkholderiales) was the highest dominated bacterial populations in both samples exposed with 1,3-BD suggested that Burkholderiales may involve with degradation of 1,3-BD or its metabolites. An understanding on the bacterial community in toxic waste as well as how they evolve after an exposure can be applied further for selection of remediation solutions and environmental management.

Keywords- 1,3-Butadiene, Activated sludge, Bacterial community, Next-generation sequencing

I. INTRODUCTION

1,3-butadiene (1,3-BD) is one of the important synthetic chemicals. It has been widely used in a chemical industry as a building block of synthetic rubbers production. This compound is highly volatile and quickly broken down in the atmosphere. It can be emitted into the atmosphere by various sources including combustion of fuels, during the production, storage, use, transportation as well as released from the waste treatment process. 1,3-BD is considered as a one of the 189 hazardous air pollutants under the 1990 U.S. Clean Air Act Amendments (CAAA). As 1,3-BD and its degradation products are considered as carcinogens, the Occupational Safety and Health Administration (OSHA) has set an exposure limit of 1,3-BD at 5 ppm for the short-term exposure.

Microbial communities in the environment are an important resource and many of them perform important functions for improving bioprocess such as wastewater treatment and soil remediation (Saunders et al., 2016). Mixed bacterial consortium in activated sludge plays important roles in degrading various organic pollutants, toxicants and xenobiotics (Zhao et al., 2014). Chou and Lu (1998) used biotrickling filter and biofilter. 1,3-BD can be eliminated by using with pig manure compost and activated sludge as a media for elimination of 1,3-BD in air stream. There was, however, no report on the bacterial profile of the system. A study on bacterial community in activated sludge is useful for improving treatment efficiency as well as to understand the role of each bacterial group involving in waste treatment process. This knowledge can be applied further for remediation solutions and environmental management (Keshri et al., 2015).

The conventional molecular technique such as denaturing gradient gel electrophoresis (DGGE) has been used to study microbial communities in waste treatment systems (Zhao et al., 2014). Due to the high bacterial diversity, this conventional method canno fully represent the complexity and diversity of the community. Next-generation sequencing is the recent advance molecular technique widely used for exploring microbial structure with higher efficiency and resolutions among different microbial populations in several habitats (Geol Na et al, 2016). It also clearly reveals complicated interactions among different microbial populations (Kim et al., 2014). For example, Proteobacteria and unclassified bacterial sequences, Firmicutes, Bacteroidetes, Actinobacteria, Candidate phylum OD1, Cyanobacteria, Verrucomicrobia and Deinococcus-Thermus were identified in the study of bacterial community in two different mine waters of South

Africa: vanadium and gold mine water (Keshri et al., 2015). Proteobacteria, Bacteroidetes and unclassified bacteria were found to be the majority in activated sludge samples from different regions in China (Zhao et al, 2014). Investigation of the microbial communities in activated sludge provides useful information on bacterial population and possibly how to utilize them in the remediation.

In this study, two activated sludges collected from different wastewater treatment systems. One of them is a synthetic rubber manufacturing facility where 1,3-BD was used in the production process. Changes in microbial community profile was monitored after exposing the activated sludge samples with 1,3-BD.

II. MATERIALS AND METHODS

2.1 Chemical and activated sludge samples collection

An analytical grade 1,3-BD (20% w/v in toluene) was purchased from Sigma-Aldrich (Queenstown, Singapore). Activated sludge samples (designated as SL2 and SL4) were collected from 2 different wastewater treatment facilities of petrochemical industry.

2.2 Analysis of VOCs in the activated sludge samples

Activated sludge samples were analyzed for VOCs composition by using GC-MS/MS (Agilent 7890A/5975C). Sludge samples were centrifuged at 7,000 rpm for 5 min and 2 mL of the supernatants were incubated at 70°C for 10 min. The head space (250 µL) was then analyzed by GC-MS/MS with a split mode (ratio 1:2). The column used was a HP-5MS (30 m-length, 0.25 mm-inner diameter, 0.25 µm-film thickness). The injector temperature was maintained at 250°C. The pressure of the carrier gas (helium) was kept at 95 kPa. The oven temperature was set at 40°C (5 min) then raised to 180°C at 8°C/min before being raised to 250°C (held for 0.17 min) at 30°C/min. Identification of the compounds was done based on a comparison with library spectra data bases of National Institute of Standard and Technology (NIST).

2.2 An exposure with 1,3-BD

Changes in microbial community profile was monitored after exposing the activated sludge samples to 1,3-BD. Twelve grams of activated sludge samples were placed in a serum bottle containing 18 mL of sterile mineral salts medium (KH_2PO_4, 0.91g/L; K_2HPO_4, 0.40 g/L, $Na_2HPO_4\,H_2O$; 2.39 g/L; KNO_3, 2.96 g/L; $(NH_4)_2SO_4$, 1.97 g/L; $MgSO_4\,7H_2O$, 2.00 g/L; $FeSO_4\,7H_2O$, 0.20 g/L; $NaHCO_3$. 0.50 g/L; $MnSO_4\,H_2O$, 0.54 g/L; $ZnSO_4\,7H_2O$, 0.04 g/L; $CaCl_2$, 2.26 g/L; $Na_2MoO_4\,2H_2O$, 1.00 g/L; $CoCl_2\,6H_2O$, 0.04 g/L; $Na_2EDTA\,2H_2O$, 8.86 g/L) supplemented with 100 ppm of 1,3-BD (contributing 8,600 ppm of toluene as a solvent) for enrichment cultivation. The samples were incubated at 30°C, 200 rpm for 6 weeks. Similar experiment set up without supplementation of 1,3-BD (supplemented with only toluene) were also conducted for comparison.

2.3 Genomic DNA extraction, amplification and sequencing

During an enrichment of activated sludges (SL2 and SL4) with 1,3-BD for 6 weeks, genomic DNA from samples at week 0, 2, and 6 were extracted by using a commercial kit (Soil DNA Isolation Kit, Norgen Biotek, Canada). Purified genomic DNA was used for constructing of PCR library of the 16S V3–V4 region by using 16 amplicon forward primer (5'-TCGTC GGCAGCGTCAGATGTGTATAAGAGACAGCCT ACGGGNGGCWGCAG-3') and 16 amplicon reverse primer (5'-GTCTCGTGGGCTCGGAGATGTGTAT AAGAGACAGGACTACHVGGGTATCTAATCC-3') (Klindworth et al., 2013). The amplification was performed in 80 µL reaction volume with the initial denaturation at 98°C for 2 min 30 s, 25 cycles of denaturation, annealing and elongation at 98°C for 10 s, at 55°C for 30 s and 72°C for 20 s, respectively, with a final extension step at 72°C for 2 min. The PCR product (550 bp) was and then purified using PCR DNA fragments extraction kit (HiYield™ Gel/PCR DNA Fragments Extraction Kit, RBC, Taiwan). Genomic DNA concentration as well as the amplified PCR fragment were quantified using a Nanodrop2000c spectrophotometer (ThermoScientific, Wilmington, USA). The libraries were sequenced using MiSeq sequencer (Illumina) and the nucleotide sequence reads were aligned to a reference sequence with bioinformatics software.

2.4 Sequencing analysis

Operational taxonomic units (OTUs) were clustered at the 0.03 dissimilarity cut-off levels. The relative abundances (%) of single OTUs were calculated for each sample by comparing the number of total sequences assigned to a specific taxon against the number of total obtained sequences and were used to generate bar plots of bacterial diversity. BLAST database searching on NCBI was used to analyzed the bacterial sequences before and after the enrichment cultivation.

III. RESULTS AND DISCUSSIONS

3.1 VOCs profile of activated sludge samples

Activated sludge samples collected from two different wastewater treatment systems (referred as SL2 and SL4) as well as the commercial standard 1,3-BD (20% w/v in toluene) were analyzed for VOCs content using a static headspace gas chromatography technique. The result indicated that 1,3-BD and toluene could be detected only from SL4 sample. 1,3-BD presented in SL4 was estimated at approximately 20-30 ppm.

3.2 Bacterial community structure

The changes in the bacterial community was observed using 16S rDNA to distinguish bacteria at each taxa level. The classification at the phylum level for each activated sludge bacterial community was summarized in Fig. 1.

Before an exposure (Week 0), Proteobacteria was the dominant bacterial group, constituting between 30 to 45% of the bacterial population in both activated sludge samples. This is in agreement with the bacterial communities observed in activated sludges from various wastewater treatment systems (Xu et al., 2018; Isazadeh et al., 2016; Wang et al., 2012; Xia et al., 2010) which were dominated by the phylum Proteobacteria.

Planctomycetes and Chloroflexi were subdominant groups, comprising of approximately 3 to 16% and 3 to 11% of the detections, respectively. These subdominant groups were also reported as members of bacterial communities observed in activated sludges (Wagner et al., 2002). Although Firmicutes was detected only in SL2 in this study, they were found in high abundance in several activated sludge samples (Kwon et al., 2010; Xia et al., 2010; Ye et al., 2011).

After an enrichment with toluene (Fig. 1a), Planctomycetes, Chloroflexi and Chlorobi tended to decrease over time while Actinobacteria and Candidatus Saccharibacteria (Candidate division TM7) increased continuously. Previous studies have reported that Actinobacteria was the dominated member (accounting for 60%) in soil after an exposure to toluene (Olapade et al., 2015) whereas Candidatus Saccharibacteria (Candidate division TM7) was also identified as the dominant bacterium responsible for carbon uptake from toluene in agricultural soil (Luo et al., 2009). In SL2, Firmicutes (Bacilli) was found to be the dominated taxa during toluene exposure while Proteobacteria (Alpha- and Beta-proteobacteria) decreased over time (Fig. 1a). This observation is in agreement with the fact that several Bacillus strains were reported for their ability to degrade toluene (Olapade et al., 2015).

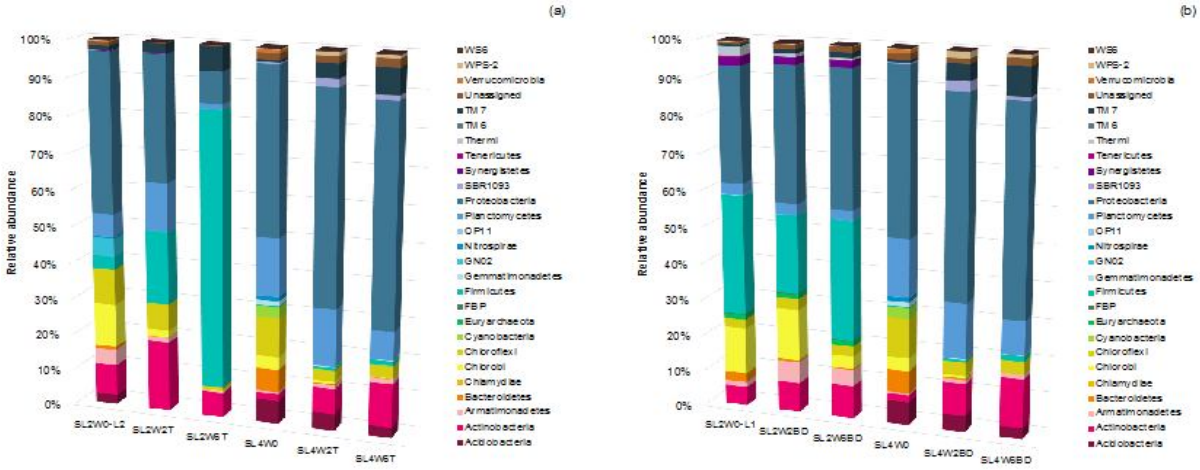


Fig.1 Bacterial community structures at phylum level of different activated sludge samples. (a) The relative abundance of activated sludge samples (SL2 and SL4) before (SL2WK0-L2 and SL4WK0) and after exposure to toluene for 2 (SL2WK2T and SL4WK2T) and 6 weeks (SL2WK6T and SL4WK6T). (b) The relative abundance of activated

sludge samples (SL2 and SL4) before (SL2WK0-L1 and SL4WK0) and after exposure to 1,3-BD for 2 (SL2WK2BD and SL4WK2BD) and 6 weeks (SL2WK6BD and SL4WK6BD). The relative abundance is presented in terms of a percentage of the total bacterial sequence in sample.

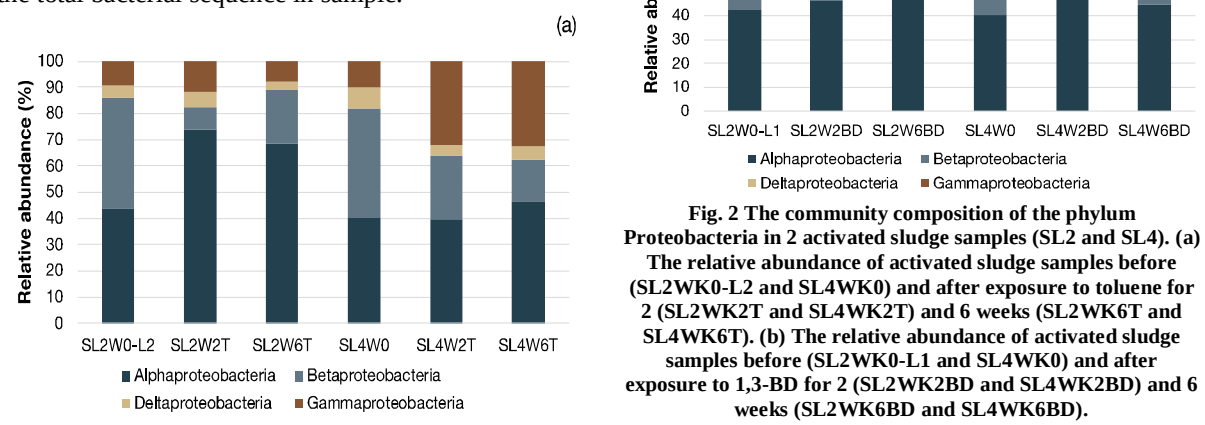


Fig. 2 The community composition of the phylum Proteobacteria in 2 activated sludge samples (SL2 and SL4). (a) The relative abundance of activated sludge samples before (SL2WK0-L2 and SL4WK0) and after exposure to toluene for 2 (SL2WK2T and SL4WK2T) and 6 weeks (SL2WK6T and SL4WK6T). (b) The relative abundance of activated sludge samples before (SL2WK0-L1 and SL4WK0) and after exposure to 1,3-BD for 2 (SL2WK2BD and SL4WK2BD) and 6 weeks (SL2WK6BD and SL4WK6BD).

In case of 1,3-BD exposure, Proteobacteria was increased in both samples (Fig. 1b). Taxonomic classification into class level of Proteobacteria indicated that Alapha-proteobacteria (mainly Rhizobiales)and Beta-proteobacteria (mainly Burkholderiales) were the most abundant classes in both samples (Fig. 2). It is interesting that there has been no report on the bacterial profile related to the 1,3-BD biodegradation.

Only a few works have described the degradation of 1,3-BD in animal metabolism (Henderson et al., 1996; Himmelstein et al., 1997) and reactions with O_3 under an atmospheric condition (Kramp and Paulson, 2000). 1,3-BD was only reported once in the biofilter using pig manure compost and activated sludge (Chou and Lu, 2011). The fact that Beta-proteobacteria (mainly Burkholderiales) was the highest dominated bacterial populations in both samples exposed with 1,3-BD (comparing to those exposed to toluene alone) suggested that Burkholderiales may involve with degradation of 1,3-BD or its metabolites.

CONCLUSIONS

The results from this study demonstrated that the microbial communities in activated sludges collected from 2 different wastewater treatment facilities responded differently towards an exposure to 1,3-BD and/or toluene. Proteobacteria was the dominant phylum in both samples (SL2 and SL4) whereas Firmicutes was found only in SL2. Actinobacteria and Candidatus Saccharibacteria (Candidate division TM7) were increased continuously in both samples when exposed to toluene. Firmicutes (Bacilli) was found to be the dominated taxa during toluene exposure only in SL2.

During 1,3-BD exposure, Proteobacteria (Alpha- and Beta-proteobacteria) was the most abundant phylum in both samples. Within Proteobateria, Beta-proteobacteria (mainly Burkholderiales) was the highest dominated bacterial populations in both samples exposed with 1,3-BD (comparing to those exposed to only toluene) suggested that this group of bacteria may associate with the degradation of 1,3-BD or its metabolites.

ACKNOWLEDGMENTS

This study was financially supported by the Research and Researchers for Industries (RRi) under Thailand Research Fund Organization (MSD6010039) and SCG Chemicals Company Limited.

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