DIOXINS BIODEGRADATION IN SYDNEY HARBOUR SEDIMENT

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Introduction

Sydney Harbour is a site of major dioxins contamination. Over the last century, organohalide manufacturing companies along Sydney harbor have produced extraordinary amounts of products, including pentachlorophenol and Agent Orange. Dioxins have entered Sydney Harbour with the discard of these chemical wastes, from a prior manufacturing site upstream- Homebush Bay (HBB) (Figure 1) [1]. The spread of dioxins throughout the estuary has led the government to completely ban commercial fishing in Sydney Harbour[2], however, the risk of human exposure remained. Therefore, an effective and inexpensive technology for dioxins remediation in Sydney Harbour sediment is required.

Bioremediation of organohalide pollutants has been developed over decades and is considered an ideal option for extensive contaminated sites such as Sydney Harbour[3]. Previous literature has identified that dioxins are also subject to biodegradation by microorganism and suggested that dioxin-degrading microorganisms could be discovered in contaminated sites[4-5]. Therefore, this project aims to survey for indigenous dioxin-degrading microorganisms and assess the feasibility of bioremediation in Sydney Harbour sediment.

Materials and methods

The research project can be divided into two phases: 1) analysing dioxins levels and microbial communities around the harbour, seeking indigenous dioxin-degrading microbes; 2) enriching potential dioxins-degrading microbes and investigating their capacity for dioxins detoxification (Figure 2).

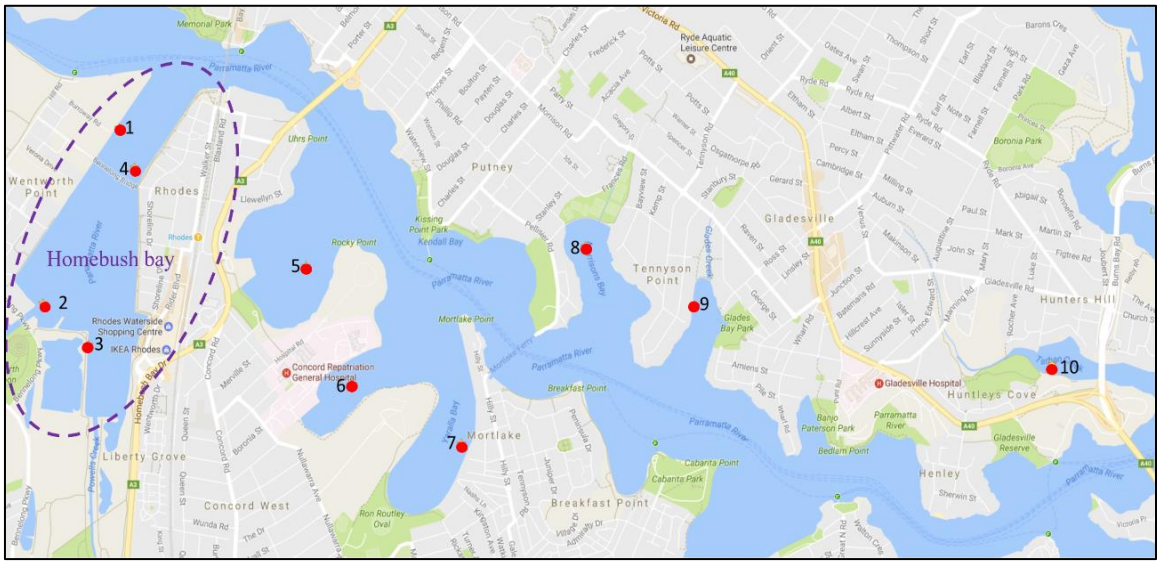

Figure 1. The location of sediment samples in Sydney harbour

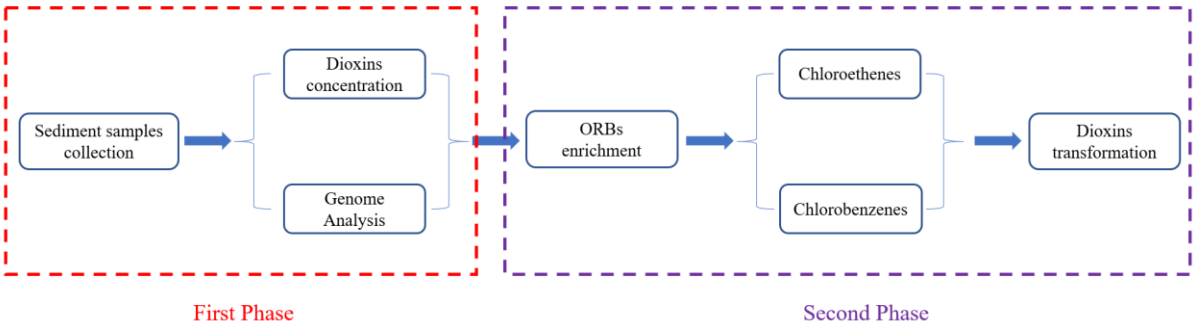

Figure 2. Flow chart of research project

In 2017, sediment samples were taken from 10 locations in Sydney Harbour (Figure 1). Samples from each location were divided into upper layer (0.25 m depth) and lower layer (0.25 to 0.5 m depth) sediment. Dioxins in sediment samples were extracted by solvents and analysed by gas chromatography-mass spectrometry.

Genomic DNA was extracted from sediment samples for microbial community analysis. Several potential organohalide respiring bacteria (ORBs) were abundant in sediment, so enrichment cultures were set up with chloroethenes and chlorobenzenes. These compounds are known to be dechlorinated by ORB in an energy yielding process, and so were used to enrich potential dioxins-degradaing ORB from sediment samples. Following this, enriched cultures were inoculated into 2,3,7,8-tetrachloro- and octachlorodibenzo-*p*-dioxin (TCDD and OCDD) spiked medium respectively and incubated over 3 months.

Results and discussion

Dioxin congeners concentration was transferred to WHO-TEQ concentration (Figure 3). Homebush Bay location 4 (HBB4) had the highest levels of dioxins at both upper and lower layer sediment. HBB4 is the area closest to where the organohalide manufacturing company was located. Dioxins could be found at various levels at all 10 locations, suggesting that the tidal current has assisted the spread of dioxins throughout Sydney Harbour. Unfortunately, compared with the results of Birch et al. (2007)[1], the TEQ concentrations have not significantly decreased over the last decade, suggesting an effective remediation strategy is needed.

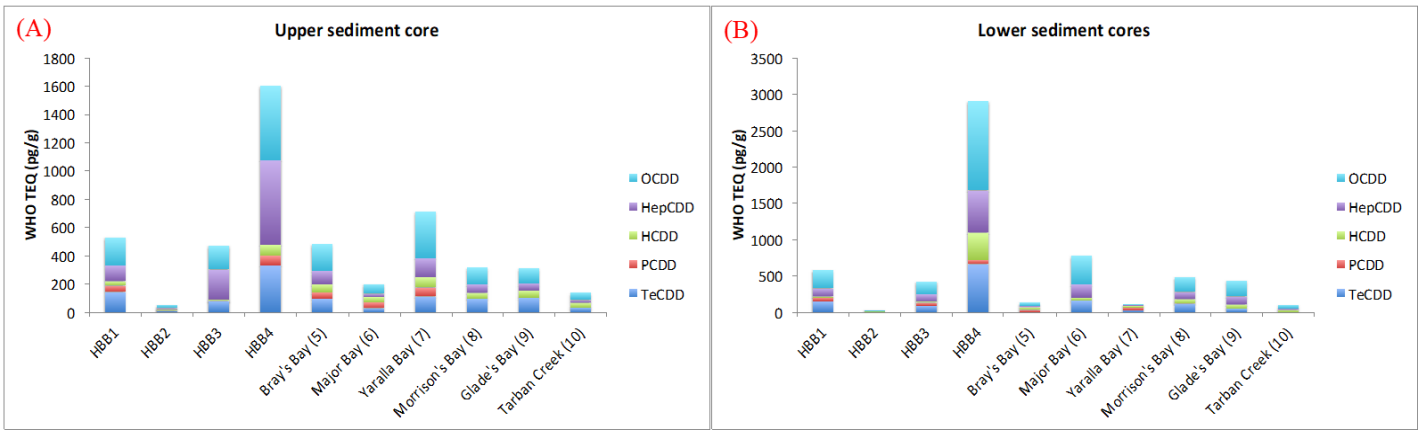

Figure 3. WHO-TEQ concentration of dioxins at 10 locations. (A) upper layer sediment and (B) lower layer sediment

Through genome technologies, several known ORB genera were identified in the sediment, including *Dehalococcoides*, *Desulfovibrio* and *Desulfitobacterium*. The obligate ORB *Dehalococcoides*, which have previously been reported to dechlorinate dioxins[6-7], was found in each sampling location (Figure 4). Therefore, the next phase was to enrich potential dioxin-degrading ORB from Sydney Harbour sediment samples.

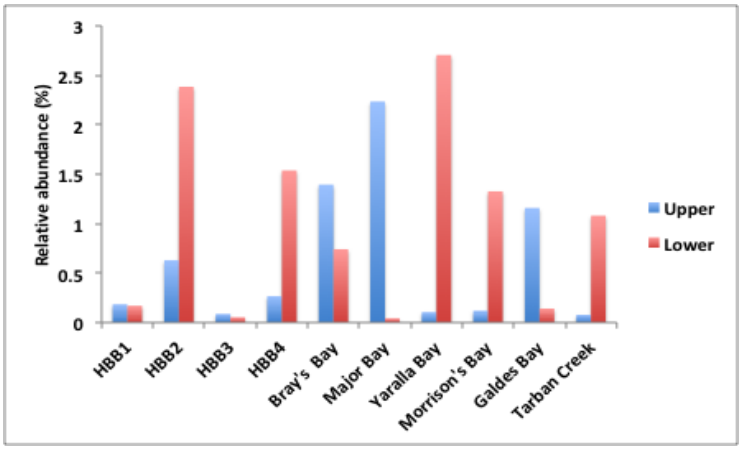

Figure 4. Relative abundance of *Dehalococcoides* at the 10 sampling locations

Two organohalides, tetrachloroethene (PCE) and trichlorobenzenes (TCBs), were used to enrich ORB from HBB4 sediment samples. PCE-enriched cultures could dechlorinate PCE to trichloroethene and sequentially to cis- and trans-dichloroethene, however, neither vinyl chloride nor ethene was generated. TCB-enriched cultures could dechlorinate 1,2,3- and 1,2,4-trichlorobenzene to 1,3- and 1,4- dichlorobenzene and sequentially to monochlorobenzene.

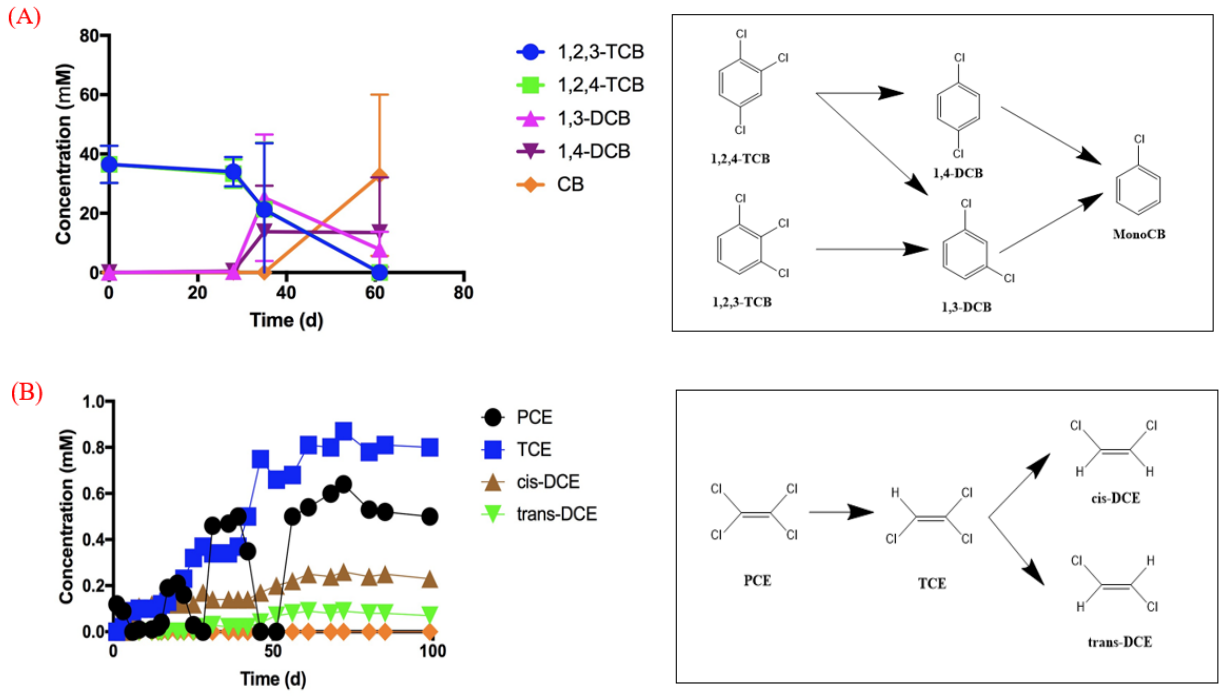

Figure 5. Transformation of 1,2,3- and 1,2,4-TCB to monochlorobenzene (A) or PCE to cis- and trans-DCE (B) with *in situ* (HBB4) microorganisms

However, when PCE enriched and TCBs enriched cultures were inoculated into 2,3,7,8-TCDD and OCDD spiked culture, no dechlorinated daughter product was generated from TCDD and OCDD over 3 months incubation. With the amendment of a biosurfactant- lecithin, the bioavailability of TCDD and OCDD has been improved, however, the dechlorination was not affected.

Conclusion and perspective

Even though the levels of dioxins in Sydney Harbour sediment have not significantly decreased over the last decade, the discovery of ORB in the sediment is a promising result. Sediment ORB that are able to dechlorinate chlorobenzenes and chloroethenes are being enriched with the aim of isolation. Unfortunately, enrichment cultures derived from the harbour currently cannot dechlorinate dioxins.

Future works include: 1) investigation on the ability of the PCE- and TCB-enriched cultures to dechlorinate dioxins precursors 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D) and pentachlorophenol (PCP); 2) investigation on the possibility of introducing exogenous dioxins-degrading microbes *Dehalococcoides mccartyi* CBDB1[6] into Sydney Harbour; and 3) isolation of the chlorobenzene- and chloroethene-dechlorinating ORB from these enrichment cultures.

Acknowledgements

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Reference

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