Removal of pentachlorophenol from water by a hydrogen-based membrane biofilm reactor

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Introduction

Pentachlorophenol (PCP) is used as a pesticide, disinfectant, and wood preservative [1]. It is a widespread environmental contaminant in soils, surface water, and groundwater [2]. Due to its probable carcinogenesis [3], a great deal of concern has been raised about adverse ecosystem effects.

Microbial dechlorination is an economic and efficient way for detoxifying PCP [4]. Under anaerobic conditions, bacteria can dechlorinate PCP and produce various less- or even non-chlorinated phenol [5]. Hydrogen (H_2) is a universal yet nontoxic electron donor for autotrophic dechlorination with lower biomass yield [6]. The challenge lies in supplying H_2 in demand (i.e. ideally 100% utilization) to avoid H_2 explosion. The H_2-based membrane biofilm reactor (MBfR) provides accurate and secure H_2 delivery as it enables controllable H_2 diffusion through the micropores of a bubbleless gas-transfer membrane [7].

In this study, we tested removal of PCP in a H_2-based MBfR for the first time, and identified intermediate products of PCP dichlorination and functional microbial communities.

Materials and methods

The MBfR used in this study was made up of a transparent plastic cylinder (28 cm in height and 10 cm in inner diameter) sealed with the plastic ring, silicone pipelines and peristaltic pumps. Pure H_2 was supplied to the fiber bundles through a H_2 gas tank regulated by a metering valve.

The basic synthetic water contained (mg/L): $CaCl_2 \cdot 2H_2O$, 1; $MgCl_2$ 10; $FeSO_4 \cdot 7H_2O$ 1; $ZnSO_4 \cdot 7H_2O$ 0.013; H_3BO_3 0.038; $CuCl_2 \cdot 2H_2O$ 0.001; $Na_2MoO_4 \cdot 2H_2O$ 0.004; $MnCl_2 \cdot 4H_2O$ 0.004; $CoCl_2 \cdot 6H_2O$ 0.025; $NiCl_2 \cdot 6H_2O$ 0.001; and Na_2SeO_3 0.003. $NaNO_3$ and $NaHCO_3$ were added as nitrogen and carbon sources for the growth of autotrophic microorganisms, respectively. 2 mM Phosphate was added into the feed water to stabilize the pH at 7.2±0.5 throughout the study.

The MBfR was continuously operated for multiple stages after the startup. 20 mg-N/L NO_3^- and 50 mg/L SO_4^{2-} were also supplied in the first 2 stages. The flow rate was constantly set at 1 mL/min, giving a hydraulic retention time (HRT) of 24 hours. In the following two stages, we removed SO_4^{2-} from the influent. After a steady state

was reached, the phenol (5 mg/L) instead of PCP was added to the influent to examine whether phenol could be degraded by the biofilm or not. All experiments were conducted at ambient temperature (25±1℃) controlled by the air conditioner. Actual concentrations were measured daily.

DNA samples were collected from the initial sludge and biofilm samples in the ends of Stages 1 (with SO_4^{2-}) and 3 (without SO_4^{2-}). Next generation sequencing (NGS) library preparations and Illumina MiSeq sequencing were conducted at GENEWIZ, Inc. (Beijing, China).

The products of PCP dichlorination were measured by Gas Chromatography-Mass Spectrometry (GC-MS, Trace DSQII-MS, Thermo Fisher, USA). The PCP and CPs were determined by a high-performance liquid chromatography (HPLC) (Shimadzu LC-20A, Japan) equipped with diode-array detector and a Agilent C18 column. The method of detection of PCP and TCP was: the mobile phase was a mixture of acetonitrile and ultrapure water (with 0.1% acetic acid) in the proportion of 70/30. The HPLC pump was controlled at the flow rate of 1.0 ml/min, and the UV detector was set at 220 nm. The method of detection of other CPs was: the mobile phase was a mixture of acetonitrile and ultrapure water (with 0.1% acetic acid) in the proportion of 55/45. The HPLC pump was controlled at the flow rate of 1.0 ml/min, and the UV detector was set at 272 nm. NO_3^-, NO_2^- and SO_4^{2-} were measured by an ion chromatograph (ICS-5000, Dionex, USA) using an AS-19 column. Coupon fibers were cut off, and the remaining fiber were then sealed with glue.

Results and discussion

Transformation paths of PCP

According to Gibbs free energy analysis, PCP can be reduced to phenol with H_2 as electron donor.[8] Bioreduction of PCP occurred in the H_2-based MBfR with H_2 as the electron donor when PCP was added to the influent. The possible degradation pathways of PCP are shown in Fig. 1. At the first step, PCP tended to be ortho-dechlorinated (2,3,4,5-tetrachlorophenol). And the next, 2,3,4,5-tetrachlorophenol tended to be ortho-dechlorinated to 3,4,5-trichlorophenol. Products 3,5-dichlorophenol, 3-chlorophenol and phenol showed that para-dechlorination, and meta-dechlorination happened. However, after 1 month, concentration of effluent phenol could not be detected, indicating the complete cleavage of all the PCP. And the result of addition of phenol instead of PCP showed that phenol alone could be degraded by the biofilm. The phenol removal capacity was further confirmed by the appearance of the genus *Xanthobacter* in the microbial community, which was able to anaerobically mineralize phenol, with the final products of CH_4 and CO_2 [9].

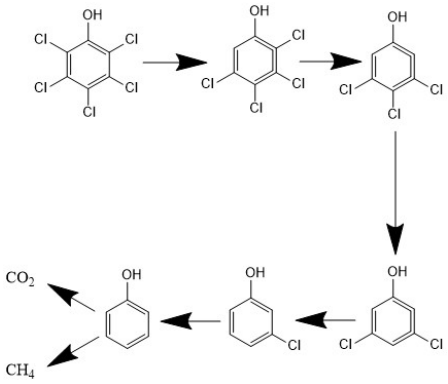


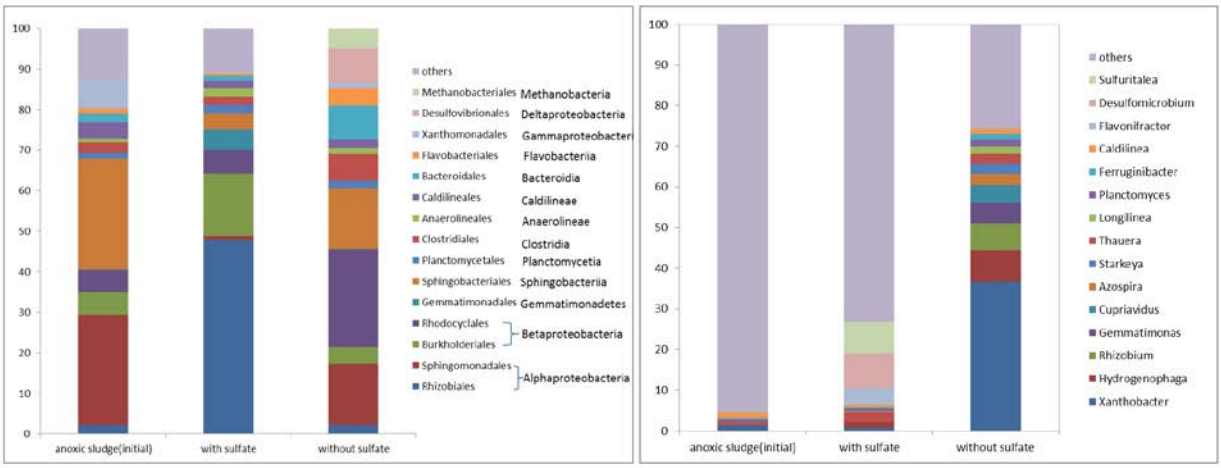
Fig. 1: The possible degradation pathways of PCP in H_2-based MBfR

PCP dichlorination promoted by sulfur cycling

In these stages, the effluent of SO_4^{2-} fluctuated in the reactor due to the sulfur cycle: SO_4^{2-} was reduced to sulfide (HS^-/S^{2-}) or elemental sulfur (S^0) which then were re-oxidized to SO_4^{2-} [10]. Though neither HS^-/S^{2-} nor S^0 was detected, the occurrence of the sulfur cycle was indicated by the incomplete SO_4^{2-} reduction and further supported by the abundance *Desulfomicrobium* (8.5%) and *Sulfuritalea* (7.7%) that dominated the microbial community of the biofilm in presence of SO_4^{2-}. *Desulfomicrobium* was a group of SO_4^{2-}-reducing bacteria which reduces SO_4^{2-} to HS^-/S^{2-} nor S^0 [11]. On the other hand, *Sulfuritalea*, a group of facultatively autotrophic bacteria, was reported to oxidize elemental S^0 as sole energy sources for autotrophic growth by respiring NO_3^- [12] and possibly PCP. And the end product of sulfur oxidation was SO_4^{2-}, which could be utilized by *Desulfomicrobium.* Therefore, the function of *Desulfomicrobium* and *Sulfuritalea* were interacted, providing supplementary electron sinks (HS^-/S^{2-} or S^0) for denitrification and dechlorination.

Complete degradation of PCP with NO_3^-

When SO_4^{2-} was not added to the reactor, and after 3 months, another biofilm sample showed that microbial community structure changed. Genus *Xanthobacter*, which had the ability of reducing organic chlorine and autohydrogenotrophic denitrification, became dominated in the reactor. [13] Other genus, like *Hydrogenophaga*[14], *Cupriavidus*[15], *Starkeya*[16], *Thauera* [17] were able to reduce organic chlorine and NO_3^- simultaneously. *Rhizobium* [18] and *Longilinea*[19] were reported to dechlorinate and *Planctomyces* [20] was a kind of denitrifiers. *Azospira* was said to reduce perchlorate and NO_3^-.[21] However, perchlorate was not added to the reactor. As a consequence, it could be informed that *Azospira* could reduce organic chlorine. Genus *Ferruginibacter*, capable of hydrolyzing some organic matter [22], were enriched from 0.29% in anoxic sludge to 1.45% in the reactor. The shown up of *Ferruginibacter* indicated that phenol were degraded to micromolecule organics. And SO_4^{2-}-reduction bacteria (*Desulfomicrobium*, *Sulfuritalea*) decreased to 0. From the analysis of microbial community, SO_4^{2-}-reduction bacteria might be able to dechlorinate.



(A) (B)

Fig. 2: Relative abundance of bacterial community composition in three samples: a) the relative abundance of total bacteria grouped by class, b) the relative abundance of the total bacteria grouped by genus

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