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Succession of microbial community and enhanced mechanism of a ZVI-based anaerobic granular sludge process treating chloronitrobenzenes wastewater

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HIGHLIGHTS

- The combined ZVI–UASB process was established for the degradation of chloronitrobenzenes.
- There were the better shock resistance and buffering capacity for anaerobic acidification in the combined process.
- Novel ZVI-based anaerobic granular sludge (ZVI–AGS) was successfully developed.
- Adaptive shift of microbial community was significant in ZVI-based anaerobic granular sludge system.

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ABSTRACT

combined zero-valent iron (ZVI) and upflow anaerobic sludge blanket (UASB) process is established for the treatment of chloronitrobenzenes (ClNBs) wastewater, and the succession of microbial community and its enhanced mechanism are investigated in the study. Results showed that compared with the control UASB (*R*1), the stable COD removal, ClNBs transformation, and dechlorination occurred in the combined system (*R*2) when operated at influent COD and 3,4-Dichloronitrobenzene (3,4-DClNB) loading rates of 4200–7700 g m^{-3} d^{-1} and 6.0–70.0 g m^{-3} d^{-1}, and *R*2 had the better shock resistance and buffering capacity for the anaerobic acidification. The dechlorination for the intermediate products of *p*-chloroanaline (*p*-ClAn) to analine (AN) occurred in *R*2 reactor after 45 days, whereas it did not occur in *R*1 after a long-term operation. The novel ZVI-based anaerobic granular sludge (ZVI–AGS) was successfully developed in the combined system, and higher microbial activities including ClNB transformation and H_2/CH_4 production were achieved simultaneously. The dominant bacteria were closely related to the groups of *Megasphaera*, *Chloroflexi*, and *Clostridium*, and the majority of archaea were correlated with the groups of *Methanosarcinalesarchaeon*, *Methanosaetaconcilii*, and *Methanothrixsoehngenii*, which are capable of reductively dechlorinating PCB, HCB, and TCE in anaerobic niche and EPS secretion.

1. Introduction

Chloronitrobenzenes (ClNBs) are abundantly used as intermediates in the production of dyes, herbicides, pesticides, wood preservatives, and other industrial chemicals. A broad range of ClNBs have been discharged into the environment through agricultural use, industrial production, and improper handling, storage, and waste disposal [1]. As a result, they are widely present in nature and can be found as contaminants in the waters and soils. Because of their toxicity and carcinogenicity, ClNBs in nature pose a serious threat to the safety of humans and animals [2]. Biological treatment is an economic and effective treatment method, but ClNBs are degraded only to a limited extent in aerobic biological processes due to the electrophilic effect of the nitro- and chloric- substituents on the electron density of benzene [3,4]. Under anoxic and anaerobic conditions, the nitro-group of ClNBs can be transformed, and the corresponding chloroaniline (ClAn) can be subsequently dechlorinated via the *ortho*- and *para*-pathways by the co-metabolic process [5]. To date, several strains have been successfully cultivated during the ClNBs reduction process [6,7]. However, most microorganisms can only accomplish the productive catabolism of ClNBs to ClAn via various metabolic enzymes [8], and the pseudo-first-order rate constants of further dechlorination reactions were very low [9].

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Zero-valent iron (ZVI), as an emerging reductive material, has increasingly been used in permeable reactive barriers (PRBs) since Gillham and O'Hannesin first revealed its applicability to situ groundwater remediation of halogenated methanes, ethanes, and ethenes [10,11]. Thereafter, ZVI, bimetallic ZVI and nanoscale ZVI have been widely employed in the reductive transformation and dechlorination of recalcitrant contaminants, such as chlorinated ethylenes, azo dyes, nitrobenzene, heavy metals, DDT, pharmaceuticals, and diagnostic agents [12–15]. They have also been used for odor control in biosolids treatment and activated sludge digestion [16]. Numerous studies have proposed different reductive pathways in the ZVI–H_2O system as follows: (i) direct dechlorination on the ZVI surface, (ii) Fe (II) reductive dechlorination via the surface bond, and (iii) reduction by the H_2 produced from ZVI corrosion [13,17].

It is generally accepted that many microbes can use ClNBs as the terminal electron acceptor in dehalorespiration coupling with the oxidation of volatile fatty acids, simple organic substrates, or H_2 [18]. H_2 has thus been considered a preferred electron donor for many bacteria, such as sulfate reducers, methanogens, and reductive dehalogenators [19–23]. Therefore, the evolution of H_2 during ZVI corrosion provides the potential enhancement of the transformation and dechlorination of ClNBs by microorganisms. Many studies have combined the use of ZVI and anaerobic microbes for the treatment and remediation of various pollutants, including perchlorate, azo, polychlorinated biphenyl, and nitrate [24–26]. In general, the reactive sites provided by the available amount of ZVI were the determining factor that affects H_2 generation and pollutant reduction [27,28]. Nevertheless, a high level of ZVI has been observed to be inhibitory to microorganisms due to the corrosion-induced increase in pH, Fe^{2+}, HS^-, and iron precipitates beyond the optimum range of the microorganisms [11,29]. Therefore, the dose of ZVI should be optimized to satisfy the requirement of available active sites and H_2 production during the ZVI dissolution for the degradation of target contaminants. Microorganisms, as another important participator, also significantly affected the performance of the combined system. Therefore, it is critical to further understand the microbial population dynamics in the combined system.

The combined zero-valent iron (ZVI) and up-flow anaerobic sludge blanket (UASB) process is established for the treatment of ClNBs wastewater, and the objectives are: (i) to study the synergetic effect of ZVI on ClNBs removal by anaerobic microorganism, (ii) to characterize the formation of anaerobic granular sludge and the succession of microbial community in response to different levels of ClNBs, and (iii) to demonstrate the enhanced mechanism of combined ZVI and UASB system.

2. Materials and methods

2.1. Operation of UASB reactor

Two identical UASB reactors were built with an internal diameter of 7 cm, height of 85 cm, and an effective volume of 3.6 L. The reactors had a settling chamber with the peripheral three-phase separator on the top. Two reactors were initially operated with a COD loading of 2500–3000 $g\,m^{-3}\,d^{-1}$ at ambient temperature ($30 \pm 2\,^{\circ}C$) for 2 months. After the COD removal efficiency achieved over 90%, one of the reactors (hereafter referred to *R*2) was dosed with 30 $g\,L^{-1}$ of ZVI powder once, and the other (*R*1) was taken as a control reactor. The study was divided into five phases, and the experimental parameters are shown in Table 1. In the first phase, both reactors were continuously fed with 3,4-DClNB at a flow-rate of 4.32 L d^{-1}, resulting in a hydraulic retention time (HRT) of 20 h. During phases 2, 3, and 4, different influent 3,4-DClNB loadings were obtained by adjusting HRT (10–20 h) and 3,4-DClNB concentration, in order to evaluate the reactors performance at different conditions. In phase 5, *p*-ClNB was added in the influent as another target pollutant to strengthen dechlorination of the intermediate product *p*-ClAn.

2.2. Inoculum and synthetic wastewater

Seed sludge was obtained from an anaerobic digester at a local municipal wastewater treatment plant in Hangzhou, China. The biomass concentration was approximately 38 g MLSS L^{-1}, and the inoculum volume was 3.0 L.

The synthetic wastewater consisted of 3,4-DClNB or *p*-ClNB as target compounds, sucrose (90; all units in $g\,L^{-1}$) as the carbon and energy source to facilitate biomass growth, NH_4HCO_3 (14.1), KH_2PO_4 (0.97), and $K_2HPO_4{\cdot}3H_2O$ (1.62) as nitrogen and phosphorous source, with C/N/P ratio of 200/5/1 and $NaHCO_3$ (33.3) as pH buffer. Additionally, necessary trace elements were added to the influent with the composition described in [30].

2.3. Analytical methods

2.3.1. Traditional physicochemical index

The concentrations of 3,4-DClNB, *p*-ClNB, and the metabolites (*p*-ClAn and AN) were analyzed using a waters 2587 HPLC (Milford, MA, USA). The biogas yield in the UASB reactors was determined with a wet gas flowmeter (Keluomu, Co., Ltd., Shanghai, China). The fraction of gaseous H_2 and CH_4 were analyzed by head space injection into a gas chromatograph (Agilent 7890A, USA).

The oxidation–reduction potential (ORP), the concentration of ferrous and ferric irons, the mixed liquor suspended solid (MLSS), the mixed liquor volatile suspended solid (MLVSS), and the sludge volume index (SVI) were determined according to standard methods for the examination of water and wastewater [31]. The iron concentration in the granular sludge was determined using the hydrochloric acid extraction method.

2.3.2. Sludge characteristics

The size distribution of granular sludge was analyzed using the wet sieve method. Sludge samples obtained from different parts of the reactors at each stage were washed with PBS (pH 7.6) and sieved through stainless steel standard screens of 4, 3.2, 2.5, 2, 1.25, 0.9, and 0.45 mm, respectively. Thereafter, different parts of granular sludge were dried until the constant weights and their mass fractions were calculated.

The morphology of granular sludge was determined by scanning electron microscopy/energy-dispersive X-ray spectroscopy (SEM/EDX, FEI SIRON 308947Q, The Netherlands) and transmission

Table 1
Operational Conditions of UASB reactors.

Phase	Time (day)	COD ($g\,m^{-3}\,d^{-1}$)		3,4-DClNB ($g\,m^{-3}\,d^{-1}$)		4-ClNB ($g\,m^{-3}\,d^{-1}$)	
		*R*1	*R*2	*R*1	*R*2	*R*1	*R*2
1: Start-up	0–40	4200	4200	6	6	–	–
2: 3,4-DClNB loading increase	41–117	4200–7500	4200–7500	10–70	10–70	–	–
3: Recovery	118–150	2800	4000	5–30	25–35	–	–
4: Stable operation	151–255	4200	4200	20–30	20–30	–	–
5: 4-ClNB loading increase	256–425	4500	4500	30–50	30–50	8.5–50	8.5–50