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Characteristics of microbial community functional structure of a biological coking wastewater treatment system

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ABSTRACT

Nitrogenous heterocyclic compounds are key pollutants in coking wastewater; however, the functional potential of microbial communities for biodegradation of such contaminants during biological treatment is still elusive. Herein, a high throughput functional gene array (GeoChip 5.0) in combination with Illumina HiSeq2500 sequencing was used to compare and characterize the microbial community functional structure in a long run (500 days) bench scale bioreactor treating coking wastewater, with a control system treating synthetic wastewater. Despite the inhibitory toxic pollutants, GeoChip 5.0 detected almost all key functional gene (average 61,940 genes) categories in the coking wastewater sludge. With higher abundance, aromatic ring cleavage dioxygenase genes including *multi ring1,2diox*; *one ring2,3diox*; *catechol* represented significant functional potential for degradation of aromatic pollutants which was further confirmed by Illumina HiSeq2500 analysis results. Response ratio analysis revealed that three nitrogenous compound degrading genes- *nbzA* (nitro-aromatics), *tdnB* (aniline), and *scnABC* (thiocyanate) were unique for coking wastewater treatment, which might be strong cause to increase ammonia level during the aerobic process. Additionally, HiSeq2500 elucidated carbozole and isoquinoline degradation genes in the system. These findings expanded our understanding on functional potential of microbial communities to remove organic nitrogenous pollutants; hence it will be useful in optimization strategies for biological treatment of coking wastewater.

Introduction

The coking wastewaters (CWWs) carry large amount of phenolics and complex mixture of heterocyclic compounds, and many other contaminants (Zhang et al., 2012) which may induce genotoxicity to humans (Zhu et al., 2013). As biological treatment has become most reliable option to remove pollutants, understanding the microbial communities and their metabolic potential for biodegradation would provide meaningful control over the detoxification and purification of the CWW (Manefield et al., 2005).

Microbial diversity and its extent in CWW treatment plants has been extensively studied (Huang et al., 2016; Joshi et al., 2016; Ma et al., 2015a; Meng et al., 2016; D. Zhang et al., 2015; Zhu et al., 2015) showing comparatively slightly lower but unique community composition which has high coherence with wastewater characteristics and environmental variables (Jia et al., 2016). These studies focused only on microbial taxonomic composition. Although many abundant microbial genera including *Thiobacillus*, *Comamonas*, *Burkholderiales*, *Pseudomonas*, *Ottowia*, *Corynebacterium etc.* were speculated to remediate major pollutants like phenol, thiocyanate, and

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NHCs or PAHs (Joshi et al., 2016; Ma et al., 2015b; Meng et al., 2016; X. Zhang et al., 2015; Zhu et al., 2015), but the biodegradation potential has not been clearly elucidated. Nevertheless, the knowledge on key functional genes responsible for treatment bioprocesses during the treatment is still elusive. So, one of the objectives of this study was to unveil uniqueness of overall microbial community functional structure in CWW treatment plants.

While total phenol (about 600 mg/L) constitute 60%–80% of total organic fraction in CWW, nitrogenous compounds (about 200 mg/L) like aniline, quinoline, isoquinoline, indole, pyridine, carbazole, cyanides, thiocyanates which represent 20%–40% are second most abundant contaminants of CWW (Joshi et al., 2016; Li et al., 2003; Meng et al., 2016; Shen et al., 2014). The removal of nitrogenous heterocycles and other related compounds is an indication of detoxification of CWW (Liu et al., 2016). During aerobic process, these compounds are degraded releasing ammonia nitrogen in effluent (Hung and Pavlostathis, 1997; Sun et al., 2009). In our previous study, we achieved almost complete removal of six nitrogenous heterocyclic compounds and thiocyanate and cyanide in a bench scale bio-reactor with diverse microbial community (Joshi et al., 2016). Although the degradation pathways for nitrogenous compounds were described (Fetzner, 1998; Watts and Moreau, 2016; Xu et al., 2006), but the studies either used pure cultures or examined single substrate or focused on particular compounds like cyanide (Wang et al., 2015). So, another aim of the present study was to explore key functional genes and pathways related to the nitrogen compound degradation during the aerobic treatment of CWW.

Therefore, a long run bench scale bioreactor treating CWW and a control bioreactor treating non-phenolic synthetic wastewater were compared for functional genes applying functional gene array (GeoChip 5.0) in combination with metagenomic sequencing (Illumina HiSeq2500 platform) to analyze microbial community functional structure. GeoChip 5.0 is a powerful metagenomic tool (He et al., 2010; Zhou et al., 2010) that has been widely used to examine microbial community functional structure in various wastewater treatment systems (Sun et al., 2014; Wang et al., 2014; Xia et al., 2014; Yu et al., 2014; Y. Zhang et al., 2013, 2015). Illumina high throughput sequencing provides detailed profile of functional genes and related metabolic pathways (Ye et al., 2012). In this study, we demonstrate a significant difference between functional structures of CWW treatment and control bioreactor; and further, present profile of aromatic pollutant degrading genes and pathways in CWW treatment system. Finally, we elucidate key functional genes possibly involved in biodegradation of selected nitrogenous compounds. The overall results of our study expanded the knowledge of functional profile and useful clues for bioremediation of nitrogenous heterocyclic pollutants of coking wastewater.

1. Materials and methods

1.1. Bench scale treatment of CWW and control bioreactor

The CWW obtained from coking facility in Tangshan City, Hebei Province, China was treated using a bench-scale system consisting of anaerobic and aerobic bioreactors (CWW bioreactor) as designed in our previous study (Joshi et al., 2016). The anaerobic pretreatment aimed to reduce toxicity and enhance the biodegradability in downstream treatment units (Li et al., 2005). Sequentially arranged up-flow anaerobic sludge blanket reactor (UASB) and a conventional aerobic bioreactor were operated for over 500 days at an ambient temperature of 20–25°C with hydraulic retention times (HRT) of 42 and 72 hr, respectively. The dissolved oxygen concentration in aerobic bioreactor was 2–6 mg/L. After pretreatment, average chemical oxygen demand (COD), total organic carbon (TOC), and ammonia nitrogen of influent wastewater were 1992.0 ± 440.0, 493.6 ± 102.1, and 137.6 ± 103.8 mg/L. The operational parameters were more or less consistent except the slight variation in pH (7.1–8.9) of the mixed liquor after 300 days onwards.

In parallel, the control bioreactor (15 × 10 × 29.5 cm) with an effective volume of 2 L was constructed and run for long period over 500 days. The reactor was inoculated with seed sludge from a municipal sewage treatment plant in Beijing, and then operated at HRT of 24 hr in dark by continuously feeding synthetic wastewater contained of glucose, starch, tryptone, and sodium carboxymethyl cellulose as mixed carbon sources. COD concentration of synthetic wastewater was maintained low approximately 400 mg/L. NH_4^+-N was 27 mg/L prepared from $(NH_4)_2SO_4$ (126 mg/L), PO_4^{3-}-P (7 mg/L) from KH_2PO_4 (31 mg/L), alkalinity (250 mg/L) from $NaHCO_3$, and the trace elements contained (mg/L): H_3BO_3 (0.5); $MnSO_4{\cdot}4H_2O$ (0.4); $ZnSO_4{\cdot}7H_2O$ (0.4); $Na_2MoO_4{\cdot}4H_2O$ (0.2); $CuSO_4{\cdot}5H_2O$ (0.1); $CoCl_2$ (0.1); KI (0.1); $NiCl_2$ (0.1).

1.2. Sampling and analytical measurements

The wastewater samples were collected with grab sampling strategy for continuous monitoring of the treatment performance of both CWW and control bioreactors. The standard methods of routine analysis for wastewater quality were followed as previously described (Joshi et al., 2016).

For microbial community functional gene analysis, the sludge sampling from CWW bioreactor was based on the performance of the bioreactors, and the aerobic samples were collected on ten different time points — days 125, 194, 228, 276, 325, 353, 385, 416, 454, and 484, and sample names were designated as CW1, CW2, CW3, CW4, CW5, CW6, CW7, CW8, CW9, and CW10, respectively. For control bioreactor, duplicate sludge samples were collected on day 100 (sample name: C1, C2), 300 (C3, C4), and 480 (C5, C6). In order to make each sample composite, three sub-samples of sludge collected on the same day were mixed together. The sludge samples were dewatered immediately by centrifuging at 9167 ×*g* for 10 min and stored under −80°C until DNA extraction.

1.3. Microbial community DNA extraction and quality control

For each sample, 0.25 g (wet weight) of the sludge was used to extract total community DNA using MO BIO PowerSoil® DNA Isolation Kit (MO BIO Laboratories, USA; Catalog no. 12888-100) in conjunction with bench-top vortex adaptor according to the manufacturer's directions. For each sample, DNA was extracted in triplicate to avoid bias, and extracts from the same sample were pooled together. The quality of DNA was evaluated using NanoDrop ND-1000 spectrophotometer

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