

Understanding the alteration of organic pollutants and microbiome profiles in a petrochemical wastewater biotreatment process

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Abstract A better understanding of the alteration of organic pollutants and microorganisms in wastewater biotreatment processes is essential for the elucidation of pollutant removal mechanisms. This study investigated the alteration of organic pollutants, microbial communities and functional genes in a petrochemical wastewater treatment plant in China. Results show that the dominant organic components are phenols and aromatic amines in the influent of the biotreatment process, while linear hydrocarbons remained in the effluent. Microorganisms in the activated sludge samples were mainly bacteria, with Rhodocyclales and Burkholderiales dominating in the biotreatment process, but their relative abundances decreased dramatically during the biotreatment process, along with the removal of organic compounds. Based on functional gene annotations from the eggNOG database, the microbial community possessed high numbers of genes associated with amino acid transport and metabolism, energy production and conversion, replication, recombination and repair, and cell wall/membrane/envelope biogenesis. Generally, the relative abundances of KEGG orthologous genes involved in alkane and aromatic degradation initially increased, then dropped during the biotreatment process. This study gives insights into the mechanisms of petrochemical biodegradation processes in wastewater treatment plants.

Keywords: activated sludge; microbial community; organic compounds; functional metagenomics

1. Introduction

Petrochemical refining industry has been reported to use a significant amount of water due to the requirements of unit operations and refining processes (Thorat & Sonwani, 2022). The characteristics of the wastewater released from the petrochemical industry are varied and depend on the crude oil quality and refining process (Aljuboury et al., 2017). Usually, petrochemical wastewater contains highly toxic chemicals such as polycyclic aromatic hydrocarbons (PAHs) and phenolic compounds (Thorat & Sonwani, 2022; Yang et al., 2015).

Cost-effective biological processes (e.g., activated sludge process and biofilm process) are commonly used in petrochemical wastewater treatment plants for pollutant removal (Wang et al., 2020), within which microorganisms play a crucial role. Understanding the microbiome profiles in wastewater biotreatment processes is essential for the elucidation of pollutant removal mechanisms.

In this study, the microbial communities and the functional genes in a petrochemical wastewater biotreatment system (sequential anoxic/aerobic/aerobic processes) were analyzed by the shotgun metagenomic sequencing approach, and the organic compounds in the wastewater were also examined.

This work aims to: (i) understand the composition of the organic compounds in each tank; and (ii) identify the microbial community structures, as well as the shift of the functional genes during the biotreatment process.

2. Materials and methods

2.1. Sample collection

Activated sludge samples were taken from the anoxic, first and second aerobic tanks in a petrochemical wastewater treatment plant in China (Figure 1). Wastewater samples were taken from the inflow pipes of the tanks (W1, W2 and W3), as well as the outflow pipe of the second aerobic tank (W4).

2.2. Analytical methods

Organic compounds in the wastewater samples were analyzed by gas chromatography-mass spectrometry after liquid-liquid extraction by *n*-hexane. DNA was extracted from the activated sludge samples taken from the tanks to analyze the microbiome profiles, including microbial taxonomies and functional genes, by shotgun metagenomic sequencing. Microbial taxonomies and function genes information were annotated by comparing

the gene sets across different databases such as Evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG) and Kyoto Encyclopaedia of Genes and Genomes (KEGG). All samples were analyzed in triplicate and data are presented as mean.

3. Results and discussion

3.1. Organic compound composition

Organic compounds detected by gas chromatography-mass spectrometry were classified in to seven main categories, including monoaromatic hydrocarbons, PAHs, linear hydrocarbons, phenols, aromatic amines, N-heterocyclic aromatic compounds, and others. The organic compound composition and concentration of each wastewater sample are shown in Table 1.

Table 1. Organic compound composition and concentration of the four wastewater samples.

Organic compound	Concentration (mg/L)			
	W1	W2	W3	W4
Monoaromatic hydrocarbons	2.83	0.57	0	0
Polycyclic aromatic hydrocarbons	1.13	0.43	0	0
Linear hydrocarbons	0.51	0.63	1.15	1.21
Phenols	12.8	3.26	0	0
Aromatic amines	6.21	1.85	0.02	0.02
Aromatic N-heterocyclic compounds	2.44	1.03	0.02	0.02
Others	0.63	0.33	0.02	0.02

The organic components were more diverse in W1 and W2 than in W3 and W4. Phenols, commonly found in petrochemical wastewater (Wang et al., 2016; Ye et al., 2020), were predominant in W1 (12.8 mg/L). The amounts of monoaromatic hydrocarbons, PAHs and phenols substantially decreased after the anoxic tank (W1 and W2), and were completely removed in the 1st aerobic process (W3). Similar trends were observed for the aromatic amines, aromatic N-heterocyclic compounds, and the others, but with small amounts of residues remained in the effluent of the 1st and 2nd aerobic tanks (W3 and W4). However, the concentration of linear hydrocarbons increased during the biotreatment process.

3.2. Microbial taxonomy composition

Microorganisms in the activated sludges play a crucial role in the petrochemical wastewater biotreatment process. Bacteria were the majority (>98%) of the microbial community in the activated sludge samples. The microbial taxonomy compositions at order level in the activated sludge samples are shown in Figure 2. Rhodocyclales and

Burkholderiales, with the ability of aromatic compound degradation (Huang et al., 2017), were dominant in the anoxic and 1st aerobic tank, but both of their relative abundances dramatically dropped to around 8% in the 2nd aerobic tank. This observation matched with the trend of aromatic compound removal in the biotreatment process (Table 1). In contrast, the relative abundances of some orders significantly increased in the 2nd aerobic tank, such as Rhizobiales and Phycisphaerales which are involved in the nitrogen removal process (Fan et al., 2017).

3.3. Functional genes distribution based on the eggNOG annotation analysis

The eggNOG function classification includes 26 categories (Figure 2). A significant number of unigenes were annotated as being associated with metabolism, and cellular processes and signaling. Not considering the unknown function category, the highest number of genes were assigned to E (amino acid transport and metabolism), followed by L (replication, recombination and repair), C (energy production and conversion), M (cell wall/membrane/envelope biogenesis), P (inorganic ion transport and metabolism), and T (signal transduction mechanism).

3.4. Selected functional genes involved in organic compounds degradation based on KEGG Ontology annotation analysis

To further investigate the specific functional traits shifting along the biotreatment process, functional genes associated with organic compounds degradation, including alkane and aromatic degradation, were compared between the sludge samples from the anoxic, 1st and 2nd aerobic tanks (Figure 4). In general, the KEGG Ontology annotated functional genes in the anoxic and 1st aerobic tanks displayed a higher abundance than those in the 2nd aerobic tank, indicating that biodegradation of the organic compounds mainly occurred during the anoxic and 1st aerobic processes, which is consistent with the trends of aromatic compound removal in the wastewater samples.

4. Conclusions

Organic components, microbial compositions, and functional genes in a petrochemical wastewater biotreatment process were investigated. Phenols were the predominant organic matter in the wastewater, but were completely removed after the biotreatment process. Rhodocyclales and Burkholderiales dominated in the activated sludge samples, and were inferred to be responsible for aromatic compound degradation. Biodegradation of the organic compounds was inferred to have occurred in the anoxic and 1st aerobic tanks, based on functional genes shifting analysis. This work provides insights into the organic compounds, microbial community structure and metabolic functional genes in a petrochemical wastewater treatment system.

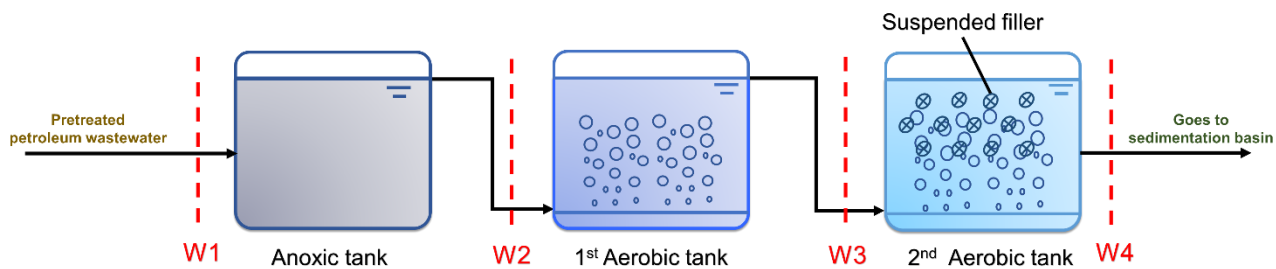


Figure 1. Schematic diagram of the petroleum wastewater biotreatment process.

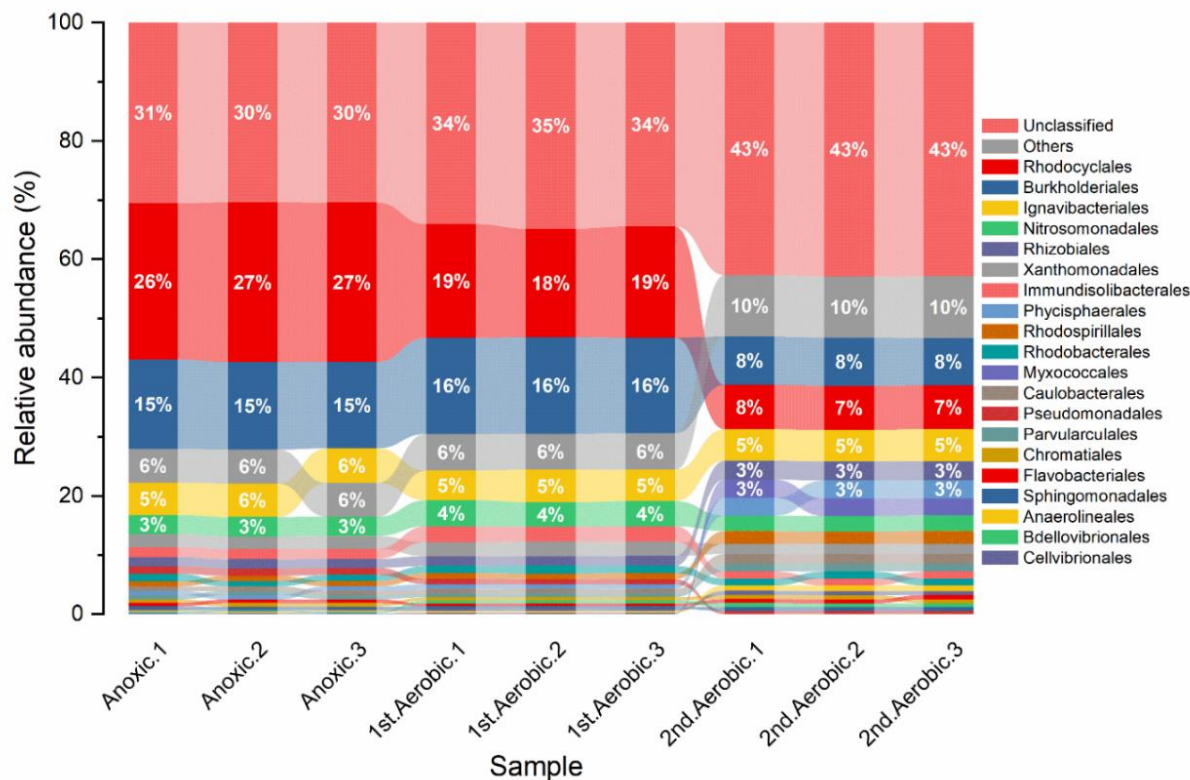


Figure 2. Barplot of the 20 most abundant orders in the activated sludge samples.

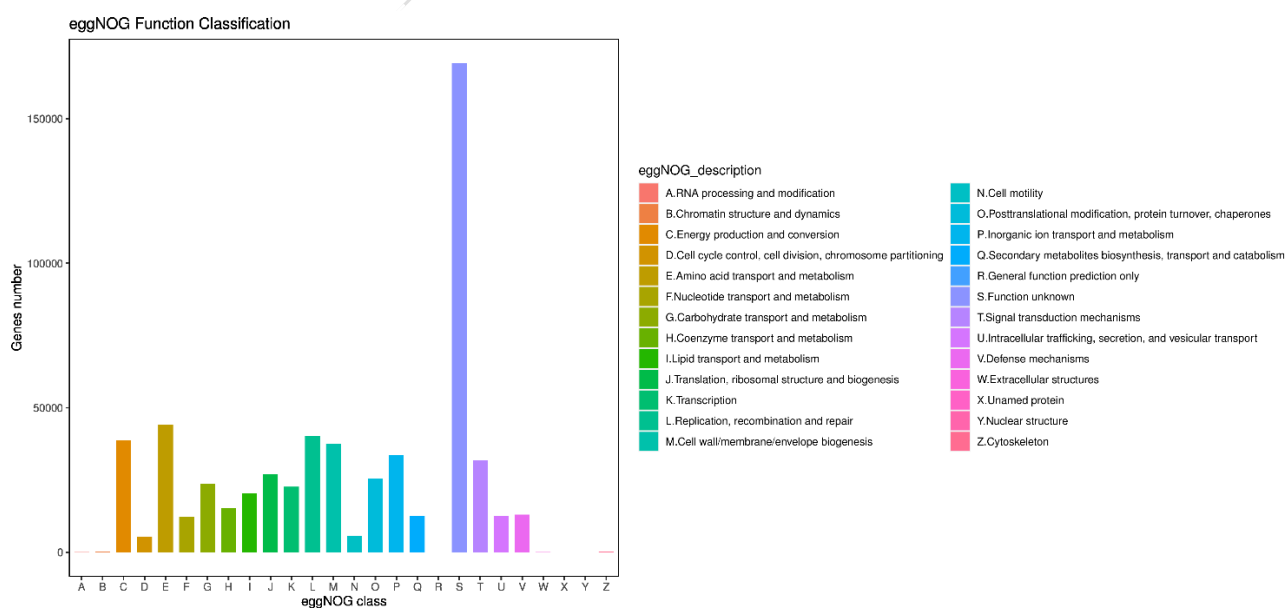


Figure 3. The gene number distribution based on eggNOG function classification. eggNOG: Evolutionary genealogy of genes: Non-supervised Orthologous Groups.

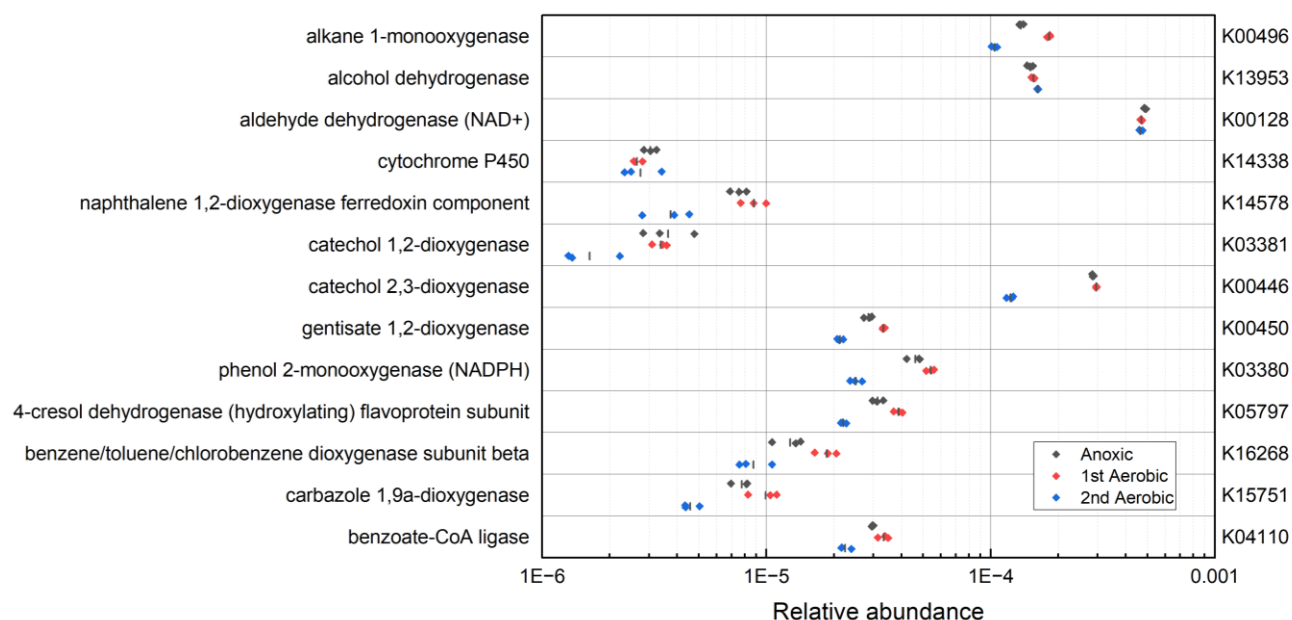


Figure 4. Shifts of functional genes by KEGG Orthology annotation in the activated sludge samples. Selected molecular functions related to organic compound degradation are listed with the K number labeled on the right.

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