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Cometabolic degradation of low-strength coking wastewater and the bacterial community revealed by high-throughput sequencing

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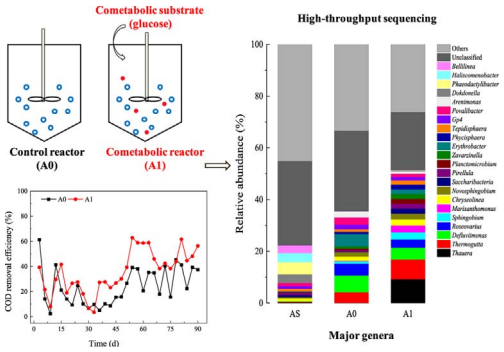
GRAPHICAL ABSTRACT



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ABSTRACT

Cometabolism technology was employed to degrade low-strength coking wastewater (CWW) in Sequencing Batch Reactor (SBR). The bacterial community compositions were monitored by high-throughput sequencing. Cometabolic substrate effectively improved the chemical oxygen demand (COD) removal efficiency in glucose-added system (A1) compared to glucose-free system (A0). Meanwhile, A1 exhibited larger biomass, better settlement performance, and higher dehydrogenase activity (DHA). More importantly, high-throughput sequencing revealed that dominant populations in A1 were quite different with A0. *Thauera* (9.27%), *Thermogutta* (7.58%), and *Defluviimonas* (4.6%) began to enrich in A1 after cometabolic substrate supplement. Especially, *Thauera*, as the most dominant populations in Al, could degrade a wide spectrum of aromatic compounds, which may contribute to the better system performance. This work would provide a novel option to treat low-strength CWW, discern the relationship between bacterial community and CWW quality, and further explore the cometabolic degradation through bacterial community structures.

1. Introduction

Coking wastewater (CWW) is a typical industrial wastewater, which generated from the processes of coal coking, coal gas purification, coke recovery, and coal liquefaction (Shi et al., 2015). It is characterized by a lot of refractory and toxic pollutants, such as phenols, cyanide,

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thiocyanate, ammonia, naphthalenes, pyridines, quinolines, indoles, carbazole, etc. (Wu et al., 2016a,b), which may pose a potential risk to the environment and human health (Zhou et al., 2015). Therefore, it is necessary to find effective methods to improve CWW treatment, especially the removal of refractory organics.

Physical, chemical and biological methods have been employed for CWW treatment up to now. Among which, biological treatment has the highest removal efficiency and lowest operational cost. Microorganisms are prevalent biological techniques after CWW pre-treatment. Previous works were mainly conducted with single bacterial strains or activated sludge to target one or a few specific organic contaminants in CWW (Zhang et al., 2014; Jemaat et al., 2013). Pure cultures are always not competent to remove a wide range of refractory contaminants in industrial CWW, whereas activated sludge is more widely applied to CWW treatment plant. However, traditional activated sludge process faces difficult to meet the new *Emission standard of pollutants for coking chemical industry* in China (GB 16171-2012, COD_{cr} < 80 mg/L, NH_3-N < 10 mg/L) (Ma et al., 2015; Wei et al., 2017).

In recent years, it had been found that cometabolic degradation could economically and environmental-friendly remove recalcitrant compounds (Lv et al., 2016), and several mechanisms have been proposed. Cometabolic substrates such as methyl alcohol and glucose can provide sufficient carbon and energy sources for microbial growth; induce the synthesis and/or secretion of corresponding enzymes that involve in growth and non-growth substrates metabolism; produce the cofactors such as NADH as electron donor in metabolic pathways; their metabolites also could take part in the degradation process of refractory compounds (Wang et al., 2017a,b). But, little attention has been devoted to the cometabolic degradation in CWW treatment.

Additionally, cometabolism may be synergistically caused by different functional dominant microorganisms. The microbial community of activated sludge in CWW treatment has always been regarded as a "black box". Understanding on relationship between microbial community and system performance is still limited and even contradictory. Thus, it is essential to gain a detailed insight into the abundance, diversity, and distribution of microorganisms in activated sludge during CWW treatment, and explore its effect on the system performance. In this context, high-throughput sequencing technology which could identify the minority bacteria that were hardly detected by the conventional molecular biology methods, has become a highly efficient tool for revealing the entire profile of microbial community.

In this study, activated sludge was cultivated to treat the low-strength CWW in SBRs, one of which was added glucose as cometabolic substrate. Bacterial compositions in activated sludge were revealed by high-throughput sequencing technology. The objectives of this work were to (1) evaluate the feasibility of CWW treatment by activated sludge combined with cometabolic substrate; (2) reveal the abundance and diversity of bacteria in the activated sludge at different operating conditions; (3) discern the relationships between bacterial community and system performance; (4) explore the cometabolism degradation phenomenon in relation to the changes of bacterial community in CWW treatment. This work will provide useful information for the biological process of CWW treatment.

2. Materials and methods

2.1. Characteristics of CWW and operational parameters

Raw wastewater was obtained from the secondary sedimentation tank of CWW treatment plant of the Yankuang Group (Shandong, China). The quality indicators of CWW were presented in Table 1 (the COD value increased to 240 mg/L from day 52 due to the change of CWW treatment plant process conditions or technical operation). Two identical SBRs (3 L) were seeded with same activated sludge, which gathered from the secondary sedimentation tank of Wulongkou Municipal Wastewater Treatment Plant (MWTP) operated with A^2/O (anaerobic–anaerobic–anoxic) process. The mixed liquor suspended solids (MLSS) of activated sludge in SBRs were 6.0 g/L, and both SBRs operated under room temperature (15–25 °C). The values of dissolved oxygen (DO) were maintained in the range of 2.5–3.0 mg/L throughout the experiments. A0 was blank control reactor; A1 with glucose was cometabolic reactor. Small amount of urea was added into A1 in order to obtain the desired C: N: P ratio (100:5:1). Around 1 L supernatant of A0 and A1 were decanted and replaced by fresh raw CWW at 3 days intervals; quantitative glucose and urea were added into A1 at the same time. During phase I (days 1–30), the concentration of glucose and urea were 0.7 g/L and 0.086 g/L, respectively; then reduced to 0.4 g/L and 0.049 g/L in phase II (days 31–60), and 0.1 g/L and 0.012 g/L in phase III (days 61–90).

2.2. Analytical methods

Activated sludge samples were collected everyday, and centrifuged at 8000 rpm for 5 min to be further analyzed. The COD, NH_3–N, MLSS, mixed liquor volatile suspended solids (MLVSS), Sludge Volume Index (SVI), total phosphorus (TP), cyanides and volatile phenol were analyzed according to Standard Methods for Water and Wastewater Examination (APHA, 2012). COD produced by additional glucose was subtracted when calculating the COD removal efficiency in A1. The pH was measured with a Hach Q30d. The DO concentration was measured using a DO meter (YSI 550A, USA). TOC and TN content were determined by a TOC analyzer (TMM-1, SHIMADZU, Japan). Nitrate was measured using an UV-spectrophotometric screening method (Persee TU-1900, China), nitrite was measured using the colorimetric method. Supernatants were filtered through a 0.45 μm PTFE membrane, then element concentrations were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES 5100, Agilent, USA). Extracellular polymeric substances (EPS) can be divided into loosely-bound EPS (LB-EPS) and tightly-bound EPS (TB-EPS). Protein (PN) and polysaccharide (PS) as the main components were measured to explore the sludge characteristics. The extraction of EPS was based on previous research (Yu et al., 2008); PN was measured by the modified Lowry method (Frølund et al., 1995); PS was determined by the anthrone method (Gerhardt et al., 1994), respectively. All statistics were conducted by Statistical Product and Service Solutions (SPSS).

2.3. Comparison of enzyme activity

Dehydrogenase was involved in all energy metabolic pathways of microorganisms and played a key role in biological oxidation of organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang et al., 2010). Moreover, Meng et al. (2017) suggested that the increase of dehydrogenase activity (DHA) may contribute to the reduction of COD. The DHA was determinated at the end of each experimental phase. The 2,3,5-triphenyl tetrazolium chloride (TTC) salt was used as the hydrogen acceptor, since the colorless TTC once accept hydrogen atoms in dehydrogenation will change into red triphenyl formazen (TF) (Jafari et al., 2015). The collected activated sludge samples were suspended and added to tubes containing 2 mL Tris-HCl (pH = 7.6), 2 mL glucose (0.1 M), 2 mL TTC (0.5%), and then incubated in water bath of 37 °C for 24 h. After the incubation, the reaction was stopped by adding 2 mL of sodium dithionate ($Na_2S_2O_4$), then 5 mL toluene was added, and tubes were centrifuged at 5000 rpm for 10 min. Finally, the supernatant was withdrawn, and the absorbance was measured at 492 nm. The DHA was calculated according to the calibration curve of TF and expressed in terms of l mg TF/g VSS·h.

2.4. DNA extraction and PCR procedure

The seed activated sludge, sludge samples of the 85th day in A0 and A1 (denoted as AS, A0 and A1, respectively), were collected for the bacterial community analysis. Total genomic DNA was extracted using