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Distribution variation of a metabolic uncoupler, 2,6-dichlorophenol (2,6-DCP) in long-term sludge culture and their effects on sludge reduction and biological inhibition

Yu Tian [a,b,*], Jun Zhang [b], Di Wu [b], Zhipeng Li [b], Yanni Cui [b]

[a] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), Harbin 150090, China
[b] School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China

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ABSTRACT

Distribution variation of a metabolic uncoupler, 2,6-dichlorophenol (2,6-DCP), in long-term sludge culture was studied, and the effects on sludge reduction and biological inhibition of this chemical during the 90-day operation were established. The extracellular polymeric substance (EPS) matrix functioned as a protective barrier for the bacteria inside sludge flocs to 2,6-DCP, resulting in the transfer of 2,6-DCP from the liquid phase to the activated sludge fraction. Significant sludge reduction (about 40%) was observed after the addition of 2,6-DCP in the first 40 days, while the ineffective function of 2,6-DCP in sludge reduction (days 70–90) might be correlated to the EPS protection mechanism. The inhibitory effect of 2,6-DCP on the COD removal was extremely lower than on the nitrification performance due to the fact that 2,6-DCP was much more toxic to autotrophic microorganisms than heterotrophic microorganisms. Moreover, both of them recovered to a higher level again with the transfer potential of 2,6-DCP to sludge. Thus, the application of metabolic uncoupler for excess sludge reduction should be cautious.

1. Introduction

The conventional activated sludge process generates a large quantity of excess sludge as a byproduct, which is one of the most serious challenges in biological wastewater treatment (Wei et al., 2003). An ideal way to solve the problem is to reduce sludge production through treatment process control rather than the post-treatment of excess sludge produced (Mahmood and Elliott, 2006). Intensive attention has been received about the technologies applied in the wastewater purification processes based on the following mechanisms: lysis-cryptic growth (Kamiya and Hirotsuji, 1998; Yan et al., 2008), maintenance metabolism (Wagner and Rosenwinkel, 2000), predation on bacteria (Elissen et al., 2006; Song and Chen, 2009) and uncoupling metabolism (Chen et al., 2000, 2002; Liu, 2003; Saby et al., 2003).

Addition of chemical uncoupler to the aeration tank can reduce excess sludge production effectively without modification of conventional wastewater treatment processes (Liu and Tay, 2001), which is the main advantage of chemical uncoupler over the other process control methods. The idea of metabolic uncoupling reduction is to dissociate the energy coupling between catabolism and anabolism, thereby a part of energy extracted from substrates is wasted through futile cycles, which leads to less production of bacterial cell mass (Rho et al., 2007). Increased attention has focused on the feasibility of using chemical uncouplers, such as chlorinated, nitrated phenols and TCS, to reduce excess sludge production

* *Corresponding author*. State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), Harbin 150090, China. Tel./fax: +86 451 8628 3077.
E-mail address: hittianyu@163.com (Y. Tian).

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from the wastewater biological treatment process (Aragon et al., 2009; Chen et al., 2006; Liu, 2000; Low et al., 2000; Saini and Wood, 2008; Yang et al., 2003). The effectiveness of para-nitrophenol (pNP) on reducing biomass production was investigated in a bench-scale activated sludge process (Low et al., 2000). The results revealed that the biomass reduction was 49% but the total substrate removal efficiency was also decreased by 25%. In addition, twelve chemical uncouplers effects on sludge reduction were compared in batch cultures and 2,4,5-trichlorophenol (TCP) was screened as the most effective uncoupler which reduced average biomass yield by about 50% (Strand et al., 1999). There have been few reports on sludge reduction induced by 2,6-dichlorophenol (2,6-DCP). In this study, 2,6-DCP was selected as the metabolic uncoupler for sludge reduction.

Previous studies have revealed chemical uncouplers' positive effects on excess sludge reduction. However, most of the studies were conducted in the batch tests for a short-term operation, while metabolic uncoupler was expected to be applied in the full-scale activated sludge facilities. Besides, most of the chemical uncouplers tested were xenobiotic and potentially harmful to the active microorganisms in the activated sludge treatment plants and environment in practical application. Therefore, a more comprehensive understanding of the metabolic uncoupler transformation behavior, sludge reduction and inhibitory effects in the activated sludge system for a long term is of great importance and deserves special attention. In a previous publication Qiao et al. (2012) reported the distribution and acute cytotoxicity of a chemical uncoupler, 2,4,6-trichlorophenol (TCP), in the activated sludge system. In the literature TCP concentrations in the effluent, sludge surface and cell interior were investigated to elucidate the distribution of TCP in the sludge system, while the degradation fraction of TCP was not evaluated during the 54 days of operation. Vero cells were used to assess the biotoxicity of TCP in the effluent to humans, while the exact toxic effect of TCP on the microbial bacteria of activated sludge (including the heterotrophic and autotrophic microorganisms) has not been reported yet. Toxicants might inhibit the activity of the microbial population in activated sludge, resulting in lower treatment efficiency or break down of the treatment system. In addition, the important role of EPS in the sludge reduction function of a chemical uncoupler has not been widely studied in the literature.

The present study aims to evaluate the relationship between the transformation, sludge reduction and inhibitory effects of 2,6-DCP in the sequence batch system during the 90-day operation periods, focusing on: (1) investigating the transformation of 2,6-DCP in the activated sludge system concerning persistence in the effluent, adsorption and biodegradation contributions; (2) evaluating the relationship between 2,6-DCP transformation behavior and its inhibitory effects on the sludge reduction, COD removal efficiency and nitrification performance in sewage treatment process; (3) comparing different inhibitory effects of 2,6-DCP on the heterotrophic and autotrophic microorganisms in the activated sludge cultures. This information will be useful to assess the environmental and ecological influences of utilization of metabolic uncoupler to reduce excess sludge production in the biological wastewater treatment processes.

2. Materials and methods

2.1. Cultivation of activated sludge

The activated sludge in the experiments was taken from the municipal wastewater treatment plant of Harbin, China. It was cultivated in a fill-and-draw reactor operated with an anoxic–oxic mode at room temperatures of 25 ± 1 °C. The dissolved oxygen (DO) level was kept above 5 mg L^{-1} and the mixed liquid suspended solid (MLSS) level was maintained at about 2000 mg L^{-1} by withdrawing the excess sludge. A synthetic wastewater was used for cultivation, which composed of glucose (200 mg L^{-1}), dissolved starch (200 mg L^{-1}) (final concentration equal to 400 mg COD L^{-1}), NH_4Cl (95.5 mg L^{-1}), $CO(NH_2)_2$ (32.1 mg L^{-1}), K_2HPO_4 (29.4 mg L^{-1}), KH_2PO_4 (17.6 mg L^{-1}), $MgSO_4$ (40 mg L^{-1}), $CaCl_2$ (5 mg L^{-1}). In addition, other minerals were added, which contained: $MnSO_4 \cdot H_2O$ (1.25 mg L^{-1}), $FeSO_4 \cdot 7H_2O$ (1.25 mg L^{-1}), $CoCl_2$ (0.3 mg L^{-1}), $ZnSO_4 \cdot 7H_2O$ (1.25 mg L^{-1}), $CuSO_4$ (0.25 mg L^{-1}). 300 mg L^{-1} of $NaHCO_3$ was introduced to reactors to maintain the pH around 7.0. The cultivation continued for one month without the addition of 2,6-DCP.

2.2. Batch experiments

Batch experiments were performed in triplicate to investigate the distribution of 2,6-DCP in activated sludge cultures. Distribution experiments were conducted in 2 L beakers at a 2,6-DCP concentration of 20 mg L^{-1} (the optimal dosage for sludge reduction observed in our previous study). For all the batch tests, initial biomass and influent substrate concentrations were fixed at 2000 mg MLSS L^{-1} and 400 mg COD L^{-1}, respectively. The control beakers were filled with water instead of activated sludge (Quan et al., 2003). Batch tests were carried out for 9 h at 25 ± 1 °C and pH 7.0 ± 0.3, and the beakers were aerated with air pumps to maintain a dissolved oxygen concentration above 5 mg L^{-1}. The diffused air was also used for stirring. Homogenized mixed liquor samples were taken from beakers at fixed intervals of time (3 h, 6 h and 9 h). Meanwhile, control tests without the addition of 2,6-DCP were also conducted in parallel. The removal percentage of 2,6-DCP by abiotic loss was calculated as:

$$R\ (\%) = \frac{100(M_{in} - M_{eff})}{M_{in}}\% \tag{1}$$

where M_{in} and M_{eff} are 2,6-DCP mass (mg) in influent and effluent wastewater at specific time intervals.

2.3. Long-term activated sludge experiments

Two 13 L SBR reactors filled with the activated sludge cultivated for one month were operated in parallel during this study. The reactor A served as the control system without the addition of 2,6-DCP while reactor B received a dose of 2,6-DCP at a 20 mg L^{-1} level three times per day. Before the long-term experiments, the reactors were first run for 10 days, during which the system reached a steady state, then 20 mg L^{-1} of 2,6-DCP as metabolic uncoupler was fed into reactor B. The same synthetic wastewater as that used in the activated sludge cultivation reactor was fed to the reactors A and B. The MLSS concentrations in