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# Effects of metabolic uncouplers on excess sludge reduction and microbial products of activated sludge

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## HIGHLIGHTS

- Three uncouplers all resulted in significant sludge reduction.
- The EPS content and composition were changed with three uncouplers.
- The PHB production was stimulated by three uncouplers.

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## ABSTRACT

The present study investigated the influences of three metabolic uncouplers (pCP, oCP and oNP) on excess activated sludge reduction and microbial products of extracellular polymeric substances (EPS) and intracellular storage product (polyhydroxybutyrate, PHB) in short-term tests. Results showed sludge was reduced 58.2%, 59.8% and 80.8%, respectively, at pCP, oCP and oNP concentrations of 20 mg/L. The dosage of three uncouplers had no obviously influences on COD removal and sludge settleability, but had significant inhibition effect on ammonia removal, especially for oNP. Low concentration of pCP and oNP (5 mg/L) dosing resulted in protein and polysaccharide content increased in EPS, however, they were decreased at high pCP and oNP concentrations (>5 mg/L). To oCP, the protein content in EPS was increased linearly with oCP concentration. Furthermore, metabolic uncouplers addition stimulated the production of PHB. Among three uncouplers, oCP could be an alternative uncoupler for sludge reduction in activated sludge process.



## 1. Introduction

Conventional activated sludge process which is widely used in municipal and industry sewage treatment plants (WWTPs), will produce a large quantity of excess sludge. These excess sludge should be treated before discharge and the treatment costs of the excess sludge were high, which may be accounted for 50–60% of the total operation costs of wastewater treatment plants (Horan, 1999; Campos et al., 2009; Feng et al., 2013). Therefore, effective strategies should be developed to reduce sludge production through either sludge post treatment or sludge in-situ treatment. Due to the high operational complexity and expenses of the post treatment methods, in-situ excess sludge reduction processes have drawn great attentions (Guo et al., 2013). Different approaches of excess sludge in-situ reduction have been applied and the mechanism of these methods can be categorized as cell lysis–cryptic growth, maintenance metabolism, predation on bacteria, and uncoupling metabolism (Liu, 2003; Wei et al., 2003; Chen et al., 2004; Ye and Li, 2005; Tian et al., 2013). Because of the advantages of convenience, high-efficiency and easy operation, uncoupling metabolism achieved by adding metabolic uncouplers has been proven to be a highly useful approach (Li et al., 2012; Qiao et al., 2012; Feng et al., 2013).

The mechanism of metabolic uncoupling reduction is to dissociate the energy coupling between catabolism and anabolism. Thus, a part of energy extracted from substrates is wasted through futile cycles and energy supply for anabolism is limited, leading to a decrease in biomass production (Chen et al., 2000; Tian et al., 2013). Different metabolic uncouplers, such as 2,4-dinitrophenol (dNP), para-nitrophenol (pNP), 2,4-dichlorophenol (dCP) and 3,3′,4′,5-tetrachlorosalicylanilide (TCS) addition led to excess

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sludge production reduced (Aragon et al., 2009; Chen et al., 2006; Ye and Li, 2005; Tian et al., 2013). Chen et al. (2002) reported by adding 0.8–1.0 mg/L TCS, 40.0% of the excess sludge yield was reduced. Tian et al. (2013) and Zhang et al. (2013) had proved the effectiveness of 2,6-DCP on biomass growth in long-term sludge culture and found the sludge yield was decreased about 40%.

In these studies, the sludge production was decreased with metabolic uncouplers addition. However, what the other part substrate is used for and where the other energy goes are still unknown yet. In recent years, some studies investigated the link between microbial products and sludge reduction with metabolic uncoupler addition. Extracellular polymeric substances (EPS), an extracellular microbial product, consisted of proteins, polysaccharides, humic acid, DNA and lipids, were demonstrated to provide a protective barrier for the bacteria inside activated sludge (Frølund et al., 1996; Henriques and Love, 2007; Sheng et al., 2010). It is reported adding 2,6-DCP in activated sludge system resulted in the significant increase in EPS content (Tian et al., 2013; Zhang et al., 2013). When dosing TCS or TCP to sequencing batch reactors, the contents of tryptophan, tyrosine protein-like substances and tryptophan, tyrosine amino-like substances were increased (Feng et al., 2013). Furthermore, other microbial product, such as intracellular storage product of polyhydroxybutyrate (PHB) which is important in nutrient removal is usually found in activated sludge systems (Majone et al., 1999). However, the investigations of the influences of the metabolic uncouplers on intracellular storage product of PHB were still scarce.

Therefore, in this study the influences of different metabolic uncouplers on excess sludge reduction and nutrient removal were investigated. In addition, the effects of metabolic uncouplers on microbial products of EPS and PHB production were also evaluated. Hopefully, the results would provide a better understanding of metabolic uncouplers on activated sludge in the biological wastewater treatment systems.

## 2. Methods

### 2.1. Activated sludge, reactor and wastewater

The activated sludge used for experiments was collected from Jiangning municipal wastewater treatment plant in Nanjing, China. Before experiments, the sludge was cultivated in a sequencing batch reactor (SBR) with the working volume of 3.6 L at room temperatures. The reactor was operated sequentially within a 6-h cycle, including 7 min of influent filling, 328 min of aeration, 10 min of settling, and 15 min of effluent discharging. The hydraulic retention time was 12 h and the sludge retention time was maintained at 15 d. The dissolved oxygen (DO) concentration was controlled above 4 mg/L and the mixed liquid suspended solid (MLSS) level was kept at about 2500 mg/L by withdrawing the excess sludge. The cultivation continued for 2 months without the addition of metabolic uncouplers.

A synthetic wastewater was used for cultivation, which composed of sodium acetate (770 mg/L) as a sole carbon source for bacterial growth, $NH_4Cl$ (153 mg/L), $KH_2PO_4$ (43.8 mg/L), $MgSO_4$ (20 mg/L), and $CaCl_2$ (20 mg/L). In addition, other microelement solution of 1.0 ml/L was added, which contained (unit in mg/L): EDTA 50; $ZnSO_4{\cdot}7H_2O$ 22; $MnCl_2{\cdot}4H_2O$ 5.1; $FeSO_4{\cdot}4H_2O$ 5.0; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 1.1; $CuSO_4{\cdot}5H_2O$ 1.8; and $CoCl_2{\cdot}6H_2O$ 1.6. HCl or NaOH was applied to adjust pH around 7.0.

### 2.2. Batch experiments

Batch experiments were performed in triplicate to investigate the effect of different metabolic uncouplers on sludge growth and microbial products. In this study, o-chlorophenol (oCP), p-chlorophenol (pCP) and o-nitrophenol (oNP) were chosen as metabolic uncouplers, and their concentrations were 5, 10, 15 and 20 mg/L, respectively. Meanwhile, control tests without the addition of uncouplers were also conducted in parallel. For all batch experiments, initial biomass and influent substrate concentrations were fixed at about 2500 mg MLSS/L and 300 mg COD/L, respectively. All batch tests were carried out for 6 h, and the beakers were aerated with air pumps to maintain a DO concentration above 4 mg/L.

### 2.3. EPS extraction

The EPS of activated sludge were extracted using the cation exchange resin (CER) technique according to Frølund et al. (1996). The sludge samples were harvested by centrifugation at 5000 rpm for 15 min, and then the pellets were washed twice with 100 mmol/L NaCl solution. The sludge pellets were re-suspended to its original volume with a phosphate buffer. After that, the sludge suspension was mixed with CER in sodium form (Dowex Marathon C, 20–50 mesh, Sigma–Aldrich) with a dosing of 60 g/g VSS and followed by stirring at 200 rpm for 6 h at 4 °C. The samples were then centrifuged at 10,000 rpm and 4 °C for 30 min and filtered through 0.45-μm cellulose acetate membrane.

### 2.4. Analytic methods

COD, $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, MLSS, mixed liquor volatile suspended solids (MLVSS), and sludge volume index (SVI) were measured according to the Standard Methods (APHA et al., 2005). The observed sludge yield ($Y_{obs}$) was defined as the excess biomass generated per COD consumed. The efficiency for excess sludge reduction is evaluated by the reduction percentage of $Y_{obs}$ compared with the control test (Li et al., 2012).

The content of protein was determined using the modified Lowry method using bovine serum albumin as the standard (Frølund et al., 1996), and the content of polysaccharide was measured with the anthrone method utilizing glucose as the standard (Raunkjaer et al., 1994). The intracellular storage product of PHB was measured according to Fang et al. (2009).

Three-dimensional excitation–emission-matrix (EEM) fluorescence spectrometry was used to characterize the EPS of activated sludge under metabolic uncouplers dosing. All EEM spectra were measured using a luminescence spectrometry (F7000, Hitachi, Japan). The EEM spectra were collected with subsequent scanning emission spectra from 200 to 450 nm at 0.5 nm increments by varying the excitation wavelength from 200 to 600 nm at 10 nm increments. Excitation and emission slits were both maintained at 5 nm, and the scanning speed was set at 1200 nm/min for all measurements. The spectrum of the deionized water was recorded as the blank (Sheng and Yu, 2006). The software MatLab 7.0 (MathWorks Inc., USA) was employed for handling EEM data.

## 3. Results and discussion

### 3.1. Influences of metabolic uncouplers on sludge reduction

The influences of pCP, oCP and oNP at different concentrations on sludge yield and sludge reduction are shown in Fig. 1. When pCP concentration was increased from 0 to 20 mg/L, sludge production in terms of $Y_{obs}$ was declined from 0.45 to 0.18 mg VSS/mg COD. In the presence of 20 mg/L pCP, the sludge reduction reached about 60.0% compared to the control test without uncouplers addition. Increase in oCP concentration also led to a decrease in $Y_{obs}$. At an oCP concentration of 20 mg/L, the $Y_{obs}$ was 0.19 mg