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Comparison of Excess Sludge Reduction Effect of Dichlorophenol Isomers

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Abstract

Differences in sludge reduction effect due to chlorinated sites of dichlorophenol (DCP) isomers used in an uncoupling method were investigated. The study involved two different experiments. A batch experiment conducted to examine the effect of DCPs on SOUR clearly indicated that 3,5-DCP exhibited the strongest uncoupling activity. In the second experiment, in which six different sequence batch reactors were amended with 100 mM DCPs and operated for 2 months, 3,5-DCP also exhibited the strongest sludge reducing effect. The operations examined in this research maintained the concentration of DCPs at 100 mM, and reactors added with 3,5-DCP produced a COD removal rate of >80%, with almost no excess sludge generated in 2 months of continual operation. These results were not obtained with other DCPs. This is the first study to report differences in the sludge reduction effect associated with the chlorination site in the DCP isomer. The results indicate that 3,5-DCP is the most effective option for use in uncoupling methods involving DCPs.

Keywords: Activated sludge process; Excess sludge reduction; metabolic uncoupler; Dichlorophenol isomers

Introduction

Disposal of excess sludge is one of the most significant unsolved issues in activated sludge processes [1]. Physical, chemical, and biological methods are being studied for the treatment of excess sludge, and although each has been successful to some degree, all of the methods have advantages and disadvantages [2,3]. Biological sludge reduction methods utilize the metabolic function of microorganisms. Such methods can lower the sludge yield through the multistage sludge food chain [4]. Uncoupling methods are also biological treatment techniques that have the advantage of simplicity, requiring only the addition of an uncoupler to a sludge aeration tank, with no need for sophisticated processing facilities. To date, a variety of uncouplers have been validated, and many of these are aromatic compounds (e.g., 3,3,4,5-Tetrachlorosalicylanilide) [5]. However, because these compounds are biodegraded in activated sludge, it is difficult to maintain their effect. Therefore, it is desirable that the uncoupler be recalcitrant to degradation. Several dichlorophenol isomers (2,3-DCP, 2,4-DCP, 2,5-DCP, 2,6-DCP, 3,4-DCP, and 3,5-DCP) are currently used as uncoupling agents [6]. These DCPs are generally poorly biodegradable. In addition, dichlorophenols specifically adsorb to biodegradable plastics (e.g., poly-[ε-caprolactone]) [7]. Therefore, DCPs can be re-used by recovering them from treated water via adsorption. Because dichlorophenols are poorly biodegradable, adsorptive, and readily removable from water, they are suitable uncouplers for excess sludge reduction.

These agents are all known to exert uncoupling activity [8], but studies of their effects in laboratory-scale reactor operations have generally involved the addition of a single isomer, and no studies have investigated which of the six isomers exhibits the strongest excess-sludge reducing effect. However, it is likely that the chlorination position significantly affects the excess-sludge reducing effect of the dichlorophenols, in view of the analogous effect of chlorination position on the toxicity of trichlorobenzene [9]. Identifying which dichlorophenol is most effective in reducing excess sludge could enhance the efficiency of uncoupling methods.

In the present study, we constructed sequence-batch-reactors and then added one of the six dichlorophenol isomers to activated sludge in each reactor. Subsequently, chemical and biological analyses were performed to determine which of the isomers provided a superior excess-sludge reducing effect.

Materials and Methods

Activated sludge used in the study was collected from a wastewater treatment plant in Kure City, Hiroshima Prefecture, Japan. The activated sludge used in the experiments consisted of artificial wastewater (SWA) [10], which was acclimatized within a continuously operated laboratory-scale reactor for approximately 3 months to stabilize the microbial community. The concentration of dichlorophenol added was determined by measuring the specific oxygen uptake rate (SOUR) of the acclimatized activated sludge. After adding DCPs, SWA, and activated sludge to a incubator bottle, the oxygen uptake rate (OUR) was calculated by measuring the oxygen concentration at the oxygen electrode. In this case, the COD load of SWA was set at 700 mg/L, and the sludge concentration was set at 2 g-MLVSS/L. The DCP concentration was set at 50, 100, 200, or 500 μM. Furthermore, the SOUR was calculated from the sludge concentration. For details of the experimental method, refer to Çokgör et al. [11].

The activated sludge process was carried out in a sequence batch reactor at room temperature (25°C) for 2 months, from September to November 2015. For comparisons, 100 mL of acclimatized sludge was collected in a conical flask, added to each reactor, and the dichlorophenol isomer was added to a final concentration of 100 μM. The control system consisted of a reactor to which no DCP was added. Fed-batch reactor operation was employed. Half of the sludge (50 mL) was removed from the reactor twice each week and centrifuged at 6000 *g*, after which the

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resulting sludge pellet was returned to the reactor and the supernatant was discarded. SWA and DCP were again added in a quantity equal to the quantity disposed of (thus obtaining a final concentration of 100 μM).

We constructed a calibration curve of sludge turbidity (OD_{660}) versus MLVSS and used the curve to determine the MLVSS. Sludge samples were placed in microtubes and diluted 10 times with distilled water, and the OD_{660} was measured on a Bio Spec spectrophotometer (Shimadzu Corp.), MLVSS was measured with reference to the previous report [12]. The calibration curve shown in Figure 1 was constructed for OD_{660} and MLVSS. The MLVSS value was calculated using the following approximate expression:

MLVSS concentration (g-MLVSS/L)=0.6921 × OD_{660}(–).

Determination of the COD removal capacity was measured based on the TOC concentration after 0, 16, 43, and 63 days of operation. A calibration curve of TOC concentration versus COD concentration of SWA was constructed. TOC and COD concentrations were analyzed using a TOC meter (TOC-V CSH, Shimadzu Corp.) and HACH COD analysis kit (HACH Corp.), respectively. As this study used the same component (SWA) as the substrate, the COD value could be uniquely determined from the TOC value. The calibration curve shown in Figure 2 was then constructed, and the COD was calculated using the following approximate expression: COD (mg/L)=2.0773 × TOC (mg/L).

The amount of COD removed was then determined from the measured TOC value. The COD addition was 700 mg·L^{-1}. The COD removal ratio and removal rate were determined from the COD concentration immediately after and 4 h after SWA addition, respectively. Sludge yield was determined from the increase in MLVSS concentration and the amount of COD removal for an additional 4 h after SWA addition.

The number of DCP-resistant bacteria was determined after 63 days of operation by the dilution plate technique for counting live bacteria in medium consisting of R2A gellan-gum with 100 μM DCPs. Count data were compared with counts of live bacteria in R2A medium to which no DCPs were added. Microorganisms grown in R2A medium containing 100 μM DCP in this experiment were defined as having resistance to DCPs. For details, refer to the previous report [12].

Results and Discussion

The relationship between the concentration of DCPs and SOUR was investigated using activated sludge acclimated with SWA (Figure 3). With the addition of DCPs, the SOUR of sludge decreased. This decrease was greater for 3,5-DCP than 3,4-DCP. No differences were observed with 2,3-DCP, 2.4-DCP, 2,5-DCP and 2,6-DCP examined. Based on the decrease in the SOUR, 100 μM DCP was used in the sequence batch reactor operation in the next experiment. The sequence batch reactor with DCPs at final concentration of 100 μM was maintained for 2 months. Figure 4 shows the measured sludge weight over a period of approximately 2 months. The results showed that compared with the system to which no DCP was added, each of the added DCPs inhibited proliferation during the 63 days of fed-batch reactor operation. In addition, the turbidity of the system containing 3,5-DCP was substantially lower than that of the other systems.

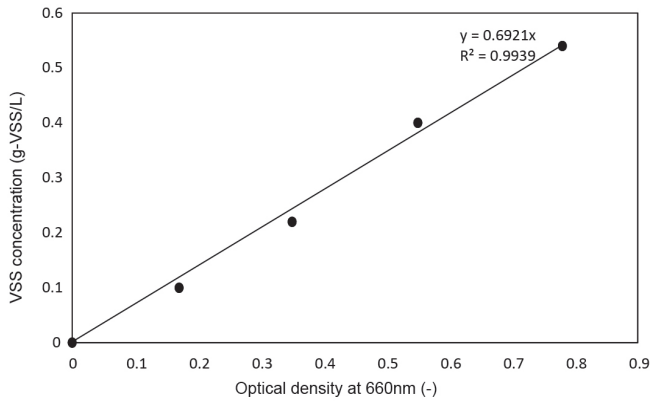

Figure 1: Calibration curve of VSS concentration and sludge optical density.

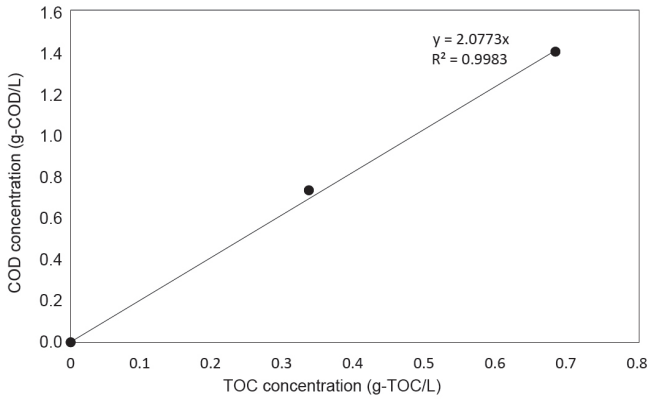

Figure 2: Calibration curve of COD and TOC concentrations.

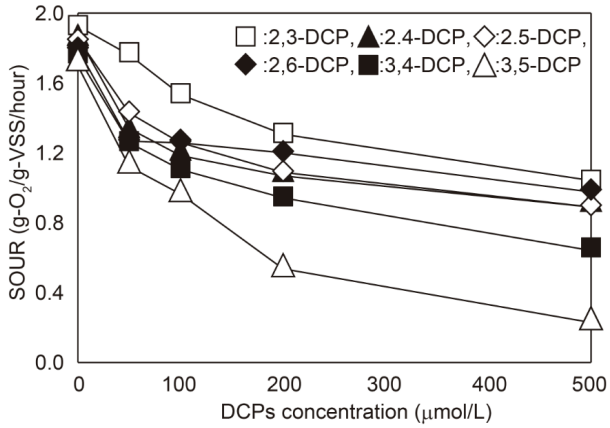

Figure 3: Effect of DCP concentration on SOUR.

Figure 5 shows the COD processing capacity for each of the reactors after 0, 16, 42, and 63 days of operation. As shown, all of the reactors removed 70% of the COD in the first 4 h following COD addition. In addition, the consumption of organic matter with 3,5-DCP was on average approximately 5% lower than that of the system to which no DCP was added, with no serious adverse effects observed. In continuous operation, the MLVSS processing rate, as determined from the COD removal rate per MLVSS unit, declined for all reactors other than the reactor to which 3,5-DCP was added, but the rate was maintained without substantial decline in the 3,5-DCP reactor. These findings indicate that the 3,5-DCP uncoupling agent enhanced the respiratory activity of the sludge cells. As shown in Figure 5, moreover, only 3,5-DCP maintained a sludge yield of less than 20%, a value

that was clearly lower than that of all other reactors (20 to 40%). This finding indicates that after 63 days of operation, 3,5-DCP continued to significantly affect the microbial population in the reactor. With regard to the number of resistant bacteria (Figure 6), only the system to which 3,5-DCP was added showed clear indication of inhibited proliferation. Proliferation of resistant bacteria is related to a decline in the sludge-reducing effect, and the inhibition observed for 3,5-DCP indicates that it is an uncoupling agent with a superior sludge-reducing effect.

Of the reactors examined in this study, only the reactor to which

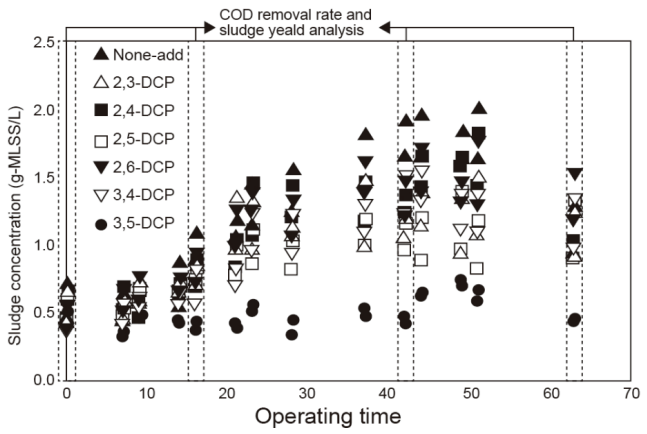

Figure 4: Results of fed-batch reactor operation with dichlorophenol addition.

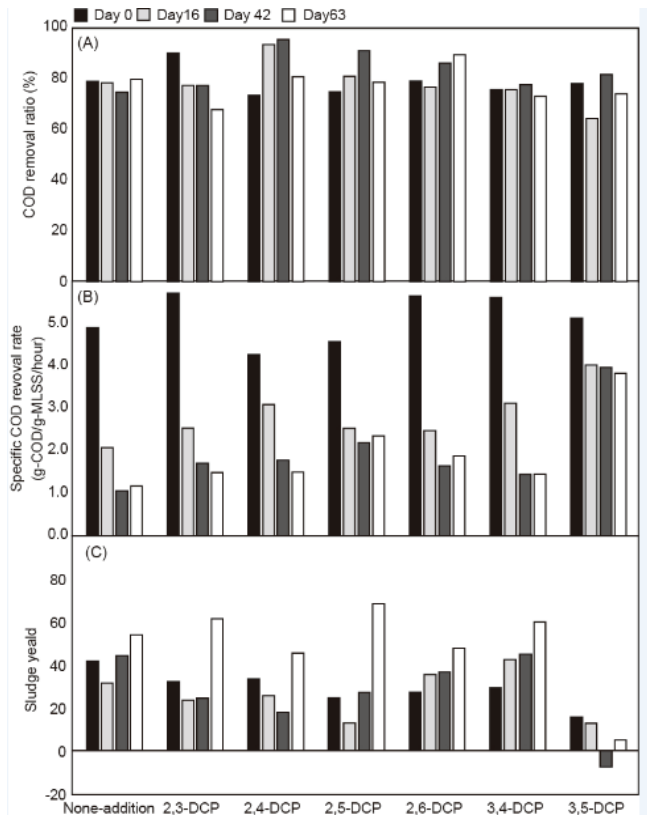

Figure 5: Organic processing characteristics of activated sludge after 0, 16, 42, and 63 days of operation. (A) COD removal ratio; (B) COD removal rate; and (C) sludge yield.

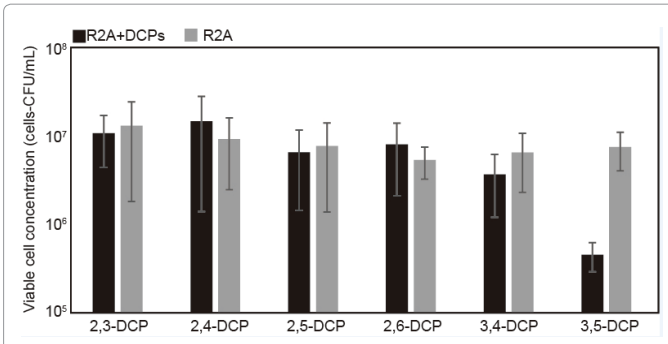

Figure 6: Number of dichlorophenol-resistant bacteria after 63 days of reactor operation with different dichlorophenol isomers.

3,5-DCP was added exhibited a substantial difference from the others in terms of sludge increase, thus indicating that this isomer is the most effective for reducing excess sludge. At the final concentration of 100 μM used in this study, 3,5-DCP had no substantial effect on organic matter decomposition. In summary, the results indicate that the reactor to which 3,5-DCP was added effectively reduced sludge production without affecting the removal of organic matter.

Prior studies have generally focused on the effects of spiked addition of uncoupling agents to sludge [13], and no studies have examined the extended addition of uncoupling agents. Previous studies by the present authors on spiked addition of 3,5-DCP to similar fed-batch reactors also showed that the capacity of 3,5,-DCP to reduce the quantity of sludge produced was lost in approximately 3 weeks [12].

In the present study, unlike any prior study, the concentration of the uncoupling agent was maintained over an extended period. This is presumably the reason for its effectiveness in reducing excess sludge generation throughout the trial period. The method used in this 3,5-DCP addition system resulted in the only reported reduction of excess sludge by an uncoupling technique for a period of 2 months and may therefore have a major effect on the future direction of studies on uncoupling techniques. It has also been reported that the pharmacologic activity of uncoupling agents tends to result in extracellular polysaccharide production [14]. This can reduce occlusion (bio-fouling) of solid-liquid filtration membranes due to polysaccharide adsorption and thereby resolve what represents the greatest challenge to membrane separation with membrane bioreactor processes. Combining the uncoupling technique using 3,5-DCP with activated-sludge membrane separation could lead to the development of a water treatment process that simultaneously meets the three objectives of (1) freedom from membrane fouling, (2) excess sludge production, and (3) effective wastewater treatment.

It must also be noted that 3,5-DCP exhibits strong toxicity, and its use will require effective measures for the prevention of external runoff and for recovery and *in situ* biodegradation in the event of such runoff.

Conclusion

Among the six types of DCP isomers, it was clarified that 3,5-DCP has the strongest effect on respiratory activity and excess sludge reduction. The effect of the other five isomers on the reduction of excess sludge was slight compared with 3,5-DCP. Therefore, the effectiveness of 3,5-DCP as an uncoupler for use in reducing excess sludge has been elucidated.

Acknowledgment

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