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Feasibility of 2,4,6-Trichlorophenol and Malonic Acid as Metabolic Uncoupler for Sludge Reduction in the Sequence Batch Reactor for Treating Organic Wastewater

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Abstract The activated sludge process generates a large amount of excess sludge as a byproduct, which is one of the most serious challenges in biological wastewater treatment. In the present study, the feasibility of 2,4,6-trichlorophenol (TCP) and malonic acid (MA) as metabolic uncouplers to reduce sludge generation in the sequence batch reactor (SBR) for treating organic wastewater for a long period was studied. The results showed that 2 mg/L TCP could reduce sludge generation by about 47%, while chemical oxygen demand (COD) removal efficiency and sludge settlability were not obviously influenced. Although 10 mg/L MA could also reduce excess sludge production by about 30% while slightly affecting COD removal, it seriously deteriorated sludge settlability. Accordingly, TCP is a better uncoupler for sludge reduction for a longer period in the SBR for treating organic wastewater, and MA can only be used as a short-term or transitional uncoupler. Microscopic and 16S ribosomal deoxyribonucleic acid analyses showed that the microbial population of sludge varied when uncouplers were fed to the activated sludge system. Occurrence of large amounts of filament and the disappearance of protozoa may be the main reason for the aggravation of sludge settlability under uncoupled metabolic conditions caused by MA.

Keywords COD removal · Microbial population · Sludge reduction · Sludge settling · Uncoupling metabolism

Introduction

Activated sludge is the most widely used biological treatment process for both domestic and industrial wastewater in the world. In the process, dissolved and suspended organic

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contaminants are transferred to biomass, bio-gas, and H_2O. However, the 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. Treatment and disposal of sewage sludge from wastewater treatment plants account for about 30% and sometimes up to 60% of the total cost of wastewater treatment [1, 2]. Furthermore, ultimate disposal of excess sludge by landfill creates environmental challenges because of the difficulty of locating suitable sites in densely populated urban areas such as Shanghai and Hong Kong in China. As environmental and legislative constraints increase, thus limiting disposal options, there is considerable impetus to reduce the amount of biomass produced during the activated sludge process. Hence, some technologies to minimize sludge generation in the activated sludge process have been explored and developed, such as prolonging aeration to promote conversion of organic pollutants to respiration products, inducing cell lysis to form autochthonous substrate on which cryptic growth occurs, or encouraging microbial predation by bacteriovores [3, 4]. However, these methods all have some disadvantages namely, they are difficult to control, have a low efficiency, or have higher costs [5]. During the last decade, uncoupling metabolism was developed as an alternative to reduce excess sludge generation in the activated sludge process because of its cost effectiveness and easy control [5].

Under normal conditions, catabolism of microbes is tightly coupled with anabolism in the light of energy requirements. However, when microbes are grown under some abnormal conditions such as high temperatures, nutrient limitation, and the presence of metabolic uncouplers, energy uncoupling can be induced because adenosine triphosphate (ATP) synthesis is inhibited by these abnormal conditions [6, 7]. Uncoupling metabolism occurs in such as manner that catabolism of the substrate can continue unhindered, while anabolism of biomass is restricted because of the lack of ATP, and this would achieve a reduction in the biomass yield [8, 9].

Many chemicals, such as chlorinated and nitrated phenols and 3,3′,4′,5-tetrachlorosalicylanilide, have been identified as uncoupling factors for sludge metabolism. These chemical uncouplers could reduce sludge generation by 20–80% in the activated sludge process but only with a slight effect on organic pollutant degradation [10–12]. However, in previous studies primarily focused on the evaluation of the effects of the uncoupler on sludge reduction and chemical oxygen demand (COD) removal in the short term, there have been few studies on long-term evaluation of the activated sludge process under uncoupling metabolic conditions. In addition, some other aspects such as the effect of residence uncouplers in the effluent on the eco-environment, effect of uncoupling metabolism on sludge settling properties, and the sludge ecosystem also have not been studied in detail. Evaluating long-term performance of the activated sludge process under uncoupling metabolic conditions, clarifying the effect of uncouplers on the sludge settling property and sludge ecosystem will provide some useful aids in controlling and improving activated sludge process under uncoupling metabolic conditions.

In this study, the chemicals 2,4,6-trichlorophenol (TCP) and malonic acid (MA), which were found to be efficient uncouplers in reducing sludge generation but with only a slight effect on organic degradation in flasks experiment [13], were used as metabolic uncouplers to evaluate the effect on the sludge generation rate, substrate removal rate, and sludge settling property for a long period in the sequence batch reactor (SBR) for treating organic wastewater. In addition, polymerase chain reaction (PCR) plus denaturing gradient gel electrophoresis (DGGE), supplemented with microscopic examination, were used to investigate the variation of the sludge ecosystem for clarifying the cause of the changes in sludge settling characteristics under uncoupling metabolic conditions in the SBR.

Materials and Methods

Activated Sludge Process

Three laboratory-scale SBRs with a working volume of 2 L were used to evaluate long-term performance of the activated sludge process for treating organic wastewater under uncoupling metabolic conditions. The systems were incubated with aerobic-activated sludge from the Quyang municipal wastewater treatment plant (Shanghai, China). Municipal wastewater also from the Quyang municipal wastewater treatment plant was fed into three aeration tanks with COD strength between 600 and 700 mg/L. The metabolic uncouplers TCP and MA were fed to two of the tanks, and their concentration in the system was kept at about 2 and 10 mg/L respectively [13]. One operating cycle was 8 h, including 10 min for the fill phase, 6 h for the aeration phase, 1.5 h for the settling phase, and 20 min for the drawing phase. The dissolved oxygen level in the aeration tanks was maintained at about 4 mg/L by aerating, and the mixed liquid suspended solid (MLSS) level was maintained at about 2,000 mg/L by withdrawing the excess sludge. The three reactors were operated for 70 days at mean room temperature.

Routine Analysis

MLSS, COD, and the sludge volume index (SVI) were determined according to standard methods [14].

Microscopic Examination

About 0.1-mL culture distributed on a glass slide was observed under an Olympus microscope (CX31) at ×1,000 magnification.

Molecular Analysis of the Microbial Population

Separation of Total DNA from Sludge

Activated sludge from the 2-mL culture was treated by lysozyme, and total deoxyribonucleic acid (DNA) was separated according to the instruction of a 3S DNA Isolation Kit for Environmental Samples V2.2 (Shenerg Biocolor, Shanghai, China).

PCR Amplification of 16S rDNA of Sludge

PCR primers for amplification of 16S ribosomal DNA (rDNA) of the sludge was general bacterial primers BSF338GC (5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G ACT CCT ACG GGA GGC AGC AG-3′) and BSR534 (5′-ATT ACC GCG GCT GCT GGC-3′). The total 100-μL amplification solution contained 1 μg genomic DNA, 0.2 mmol/L primers, 200 μmol/L of deoxyribonucleotide triphosphate, 10 μL 10× PCR buffer (with $MgCl_2$), and 2.5 U *Taq* DNA polymerase (Shenerg Biocolor). The amplification program was as follows: 94 °C for 10 min for denaturalization, then 30 cycles of 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 45 s, followed by 72 °C for 5 min.

DGGE Analysis

The denaturing gel consisted of 8% w/v of acrylamide/bisacrylamide (37.5:1) to form a gel with denaturant deionized formamide 7 M urea having a concentration gradient varying from 30% v/v at the top, to 60% v/v at the bottom. Gels were polymerized with ammonia persulfate and *N*,*N*,*N*′,*N*′-etramethylethylenediamine according to the manufacturer's instructions and run at a constant voltage of 150 V at 60 °C for 5 h in 1× Tris–acetate–ethylenediamine tetraacetic acid buffer.

Results and Discussion

Long-term Performance of the Activated Sludge Process with TCP and MA

As previous experiments in flasks indicated, considering both sludge reduction and organic degradation, 2 mg/L TCP and 10 mg/L MA were suitable concentrations, respectively, for the uncoupling metabolism of activated sludge [13]. Therefore, in this experiment, the reactors were first run for 10 days, in which the system reached a steady state, then 2 mg/L of TCP and 10 mg/L of MA as uncouplers were fed into reactors 2 and 3, respectively. The COD removal efficiency, sludge generation rate, and sludge settling properties were evaluated for a long period. Figure 1 shows the accumulated excess sludge (including withdrawn sludge) in the three SBRs over 70 days. Obviously, the sludge accumulative rate of the three reactors was maintained at about 1.0 g/day over the initial 10 days. However, after feeding the uncouplers, accumulated sludge yields in the three reactors exhibited obvious differences. In the control reactor, average sludge generation rate remained at about 1.0 g/day, and the total accumulated sludge amount after 70 days was about 70 g. However, in the reactor with 2 mg/L TCP, the average sludge generation rate dropped to about 0.53 g/day (a reduction of 47%), and the total accumulated sludge after 70 days was

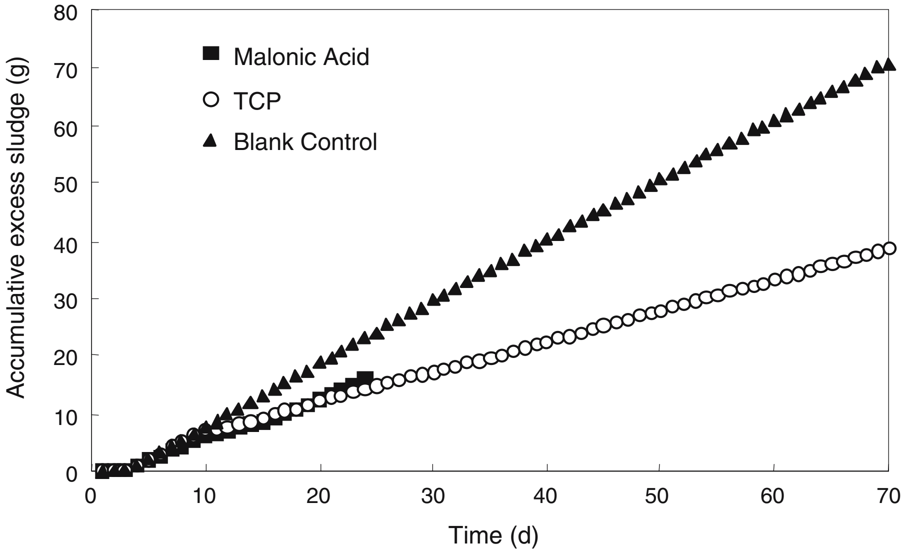

Fig. 1 Accumulative excess sludge in the three reactors during the operation period

reduced to about 37 g. Although the reactor with 10 mg/L MA was terminated after running 25 days because of the occurrence of sludge bulking, the average sludge generation rate during the period also reduced to about 0.7 g /day, and it decreased by about 30% compared to the control.

The COD removal efficiency of the three SBRs during the 70 days is shown in Fig. 2. It is apparent that the initial addition of either TCP or MA resulted in a fast drop of COD removal efficiency. This could be due to the death of some microbes supersensitive to TCP or MA in the sludge. However, in several days, the sludge had adapted to the presence of uncouplers, and the COD removal efficiency in the uncoupling metabolic reactors recovered to a higher level again. During 70 days, the average COD removal efficiency of the reactor with 2 mg/L TCP reached about 84% or rather only reduced by 8% compared to the control reactor. Owing to the occurrence of serious sludge bulking, the reactor with 10 mg/L MA was terminated after running for 25 days, but the average COD removal efficiency during the period reached about 86%.

Analysis result showed that in the TCP reactor, average specific substrate removal rate (SSRR) of the activated sludge during the aeration phase at day 60 was about 0.0165 g COD g^{-1} MLSS h^{-1}, while in the control reactor was about 0.013 g COD g^{-1} MLSS h^{-1}. This indicated that 2 mg/L TCP not only did not inhibit but also slightly enhanced the catabolism capability of the sludge. A higher SSRR and a lower sludge generation rate confirmed that catabolism and anabolism of the sludge from the TCP reactor was uncoupled. A slight decrease in COD removal efficiency during the treatment process in the reactor with TCP was mainly caused by the reduction in sludge biomass.

The effect of TCP and MA on the sludge settling was also studied, and the results are shown in Fig. 3. It is clear that after feeding TCP, the SVI value of the sludge showed a slight increase, but after 1–2 weeks, the SVI value generally recovered to normal levels and remained at about 90 during the later stages. However, after feeding MA, the SVI values of the sludge increased greatly over 6 days, and resulted in failure of the system. This implied that MA affected the sludge structure hugely.

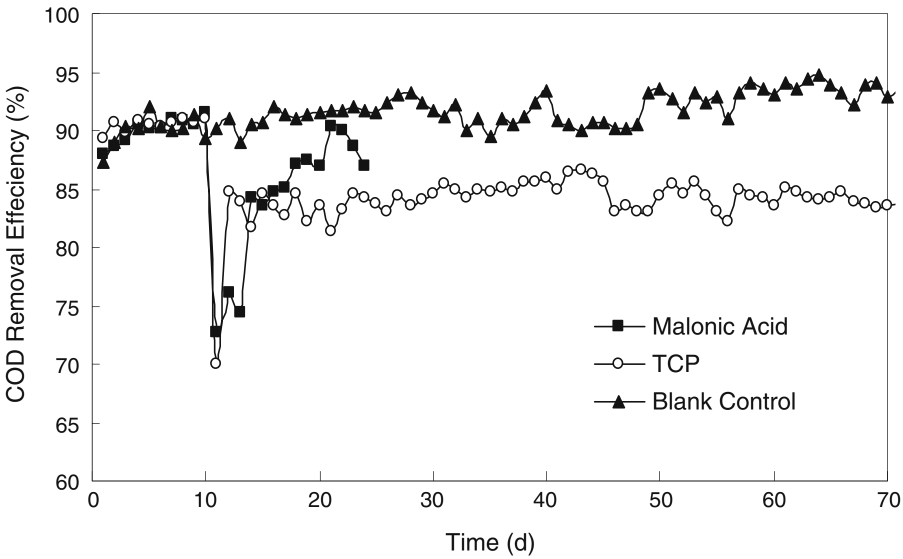

Fig. 2 COD removal efficiency of the three reactors during the operation period

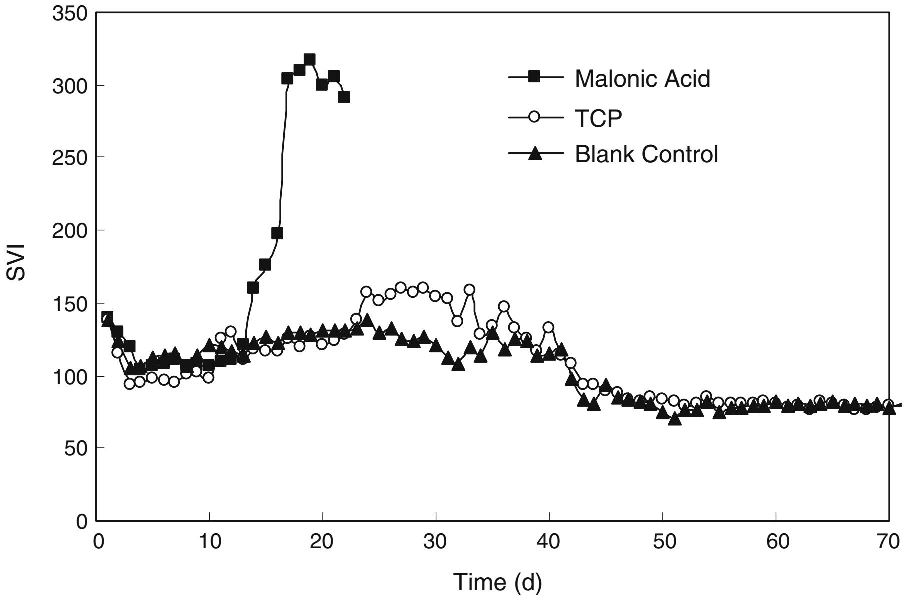

Fig. 3 SVI values of sludge in the three reactors during the operating period

These results indicated that from the view of COD removal efficiency, sludge reduction ratio, sludge settling properties, dosage, and operation cost, 2 mg/L TCP was a better uncoupler for sludge reduction in the SBR for treating organic wastewater for a long period. MA can only be used as a short-term or transitional uncoupler because of its resulting obvious sludge bulking. However, before using chemicals as a metabolic uncoupler to reduce sludge generation in practical operation, the fate of the metabolic uncoupler in the sludge system, the bio-safety of the remaining uncoupler in the effluent should be studied. Our preliminary experimental results showed that a large amount of TCP was adsorbed on the sludge surface during the operation periods, and the remaining TCP in the effluent did not have obvious cyto-toxicity to VERO cells. Although the details are being studied in our laboratory, the obvious accumulation of TCP on the sludge surface indicates that the generated excess sludge should be disposed safely.

Effect of Uncoupling Metabolism on the Microbial Population of Sludge

Clarifying the variation of the sludge ecosystem after feeding uncouplers will provide some useful aids in overcoming sludge bulking and improving the sludge settlability. Therefore, the microbial population structure of sludge in the three reactors was studied by microscopy and DGGE plus PCR technology. Microscopic examination showed that in the control reactor, microbial flocs were dense and contained both stalked and free-swimming ciliated protozoa devoid of filaments (Fig. 4a), while the microbial flocs were loose but also contained some protozoa in the TCP reactor (Fig. 4b). However, in the MA reactor, few flocs were observed, and microbes were seen to be predominantly filamentous almost without protozoa (Fig. 4c). This indicated that MA resulted in an obvious variation of the sludge's microbial population, while the sludge's ecosystem of the TCP reactor only varied slightly from the control. Occurrence of large amounts of filaments might be the main cause of the sludge bulking and system failure in the reactor with MA.

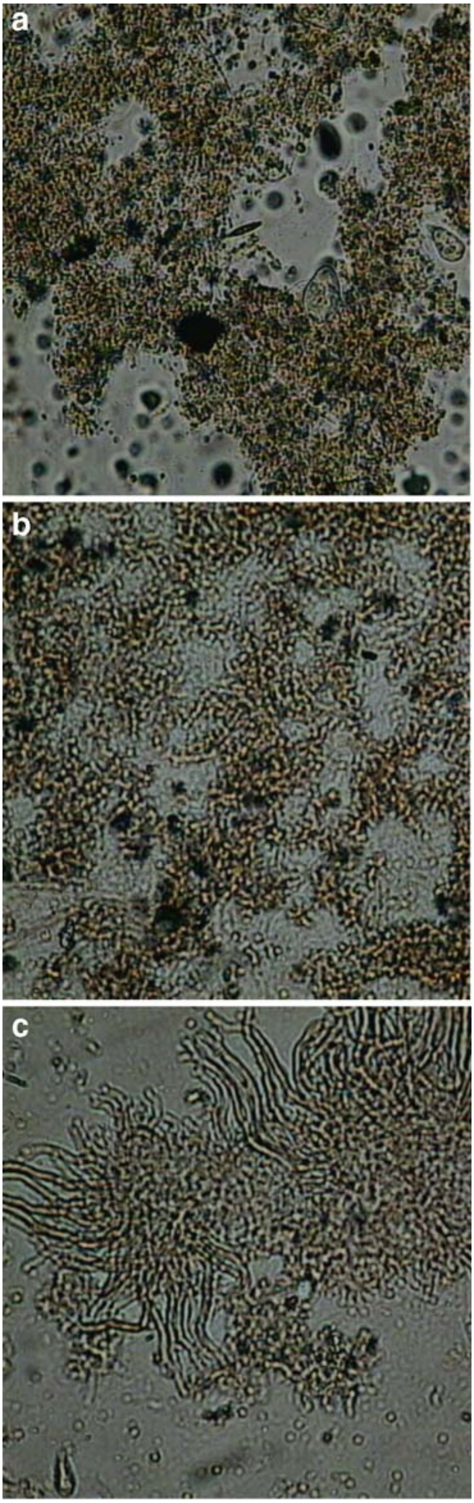

Fig. 4 Microscopic images of activated sludge (magnification of 1,000 times). **a** Sludge in the control reactor, **b** sludge in the reactor with TCP, **c** sludge in the reactor with MA

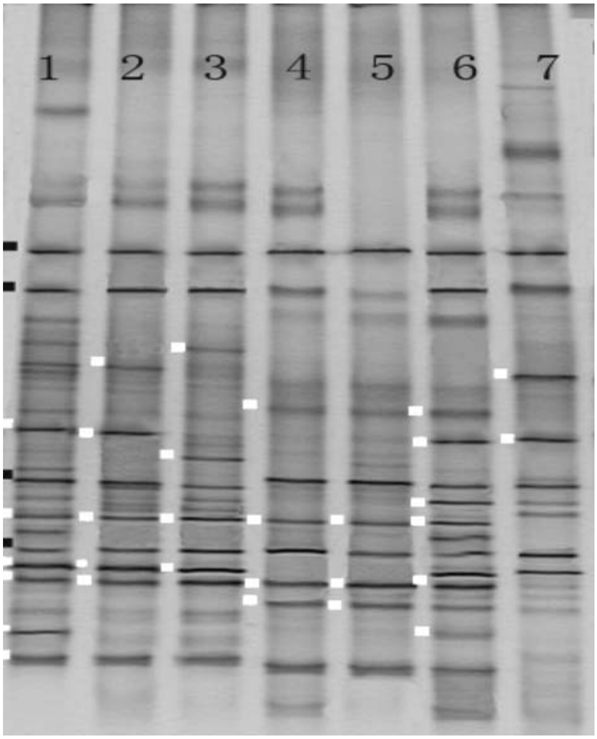

Fig. 5 DGGE analysis in total 16S rDNA of different sludge amplified by PCR. *Lane 1* represents the microbial population of sludge in the control reactor; *lanes 2 to 5* represent the microbial population of sludge with TCP on days 13, 25, 40, and 70; *lanes 6 and 7* represent the microbial population of sludge with MA on days 13 and 17. *filled squares*, mutual bands, *empty squares*, representative bands

DGGE analytic results in 16S rDNA of sludge amplified by PCR is shown in Fig. 5. Many distinguishable bands in the separating pattern indicated that sludge from different reactors sampled at different times contained more than 20 species. The difference in banding patterns between before and after feeding the uncouplers demonstrated that the microbial population diversity varied in accordance with the presence of uncouplers. Some bands existed in the samples from the control reactor and the reactors with uncouplers (bands marked by a black dot in Fig. 5), illustrating that some microbes in the sludge were resistant to the uncouplers and could exist stably in the sludge system containing the uncouplers. However, as is clearly shown in Fig. 5, some bands disappeared, and some new bands occurred after feeding uncouplers. This implied that after feeding of the uncouplers, some more sensitive microbes in the sludge system were replaced by some new microbes that might be more resistant to TCP or MA. Comparing these results with the findings of the microscopic examination, some newly occurring bands in lanes 6–7 might represent the filaments observed by microscopy (bands marked by a white dot in lanes 6–7). The DNA sequences of the mutual bands and newly occurring bands after feeding uncouplers are currently being analyzed to determine the genera of stable and newly occurring microbes in the sludge systems under uncoupling metabolic conditions.

Conclusions

Long-term experimental results indicated that 2 mg/L TCP and 10 mg/L MA could reduce sludge generation by about 47 and 30%, respectively, while only slightly affecting COD removal. This was because catabolism and anabolism of the activated sludge in the SBR was uncoupled. However, MA seriously deteriorated the sludge settlability after 6 days of feeding. Consequently, from the views of COD removal, sludge reduction, and sludge settling, TCP is a better uncoupler for sludge reduction in the activated sludge process for treating organic wastewater for a long period of time, while MA can only be used as a short-term or transitional uncoupler. Microscopic examination and DGGE analyses found that adding uncouplers resulted in variation in the microbial population of sludge. Occurrence of large amounts of filaments and disappearance of protozoa might be the main cause for the aggravation of sludge settlability under uncoupling metabolic conditions caused by MA.

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