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Uncoupled Metabolism Stimulated by Chemical Uncoupler and Oxic-Settling-Anaerobic Combined Process to Reduce Excess Sludge Production

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Abstract

The effects of three uncoupled metabolic systems (conventional activated sludge process with the addition of 3,3', 4',5-tetrachlorosalicylanilide [TCS], oxic-settling-anaerobic [OSA] process modified by insertion of a sludge-holding tank in the sludge return line, and TCS and OSA combined process) on reducing excess sludge production were studied. Compared with the control conventional activated sludge process, the most effective system was the combined process, which could reduce excess sludge production by 46.90%. The 180-d operation results confirmed that TCS is an effective chemical uncoupler in reducing the sludge yield but that it had an adverse effect on substrate removal capability, effluent nitrogen concentration, and sludge settleability. The OSA process decreased excess sludge production by only 26% but had less adverse effect on effluent quality and could improve sludge settleability. The effluent total phosphorous concentration of the three systems was slightly lower than of the control unit. Microbial populations were monitored by both microscopic and molecular biologic analysis method (polymerase chain reaction [PCR]-denaturing gradient gel electrophoresis [DGGE]). The presence of TCS caused metazoans to disappear and decreased the number and activity of protozoa. PCR amplification of 16S rRNA and sequent DGGE analysis found a shift in the diversity of the predominant species. The results imply that OSA combined with the chemical uncoupler process may effectively reduce excess sludge yield and not affect process performance significantly.

Index Entries: Activated sludge process; excess sludge reduction; oxic-settling-anaerobic process; 3,3', 4', 5-tetrachlorosalicylanilide; uncoupler.

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Introduction

Biologic treatment mainly represented by the activated sludge process has become the major technology for treating both municipal and industrial wastewaters worldwide. Previously, the volume of wastewater turnover, maximum substrate removal, and effluent quality were the main criteria for the activated sludge process. However, recently the rising cost for the treatment and disposal of excess sludge produced from activated sludge operation and restrictive legislation have emphasized the need to minimize wasted sludge.

To solve excess sludge-associated environmental and economic problems, a few approaches have recently been proposed to reduce excess sludge production using either chemical or physical pretreatment to disintegrate and mineralize sludge, such as sludge alkaline heat treatment *(1)*, ozonation *(2,3)* and chlorination *(4)*, or mechanical pretreatment *(5)*, and then return pretreated sludge to the aeration tank, or by limiting sludge growth within the activated sludge process using metabolic uncoupler *(3,4,6–9)*. The sludge growth limitation approach seems more attractive than the pretreatment approach in terms of cost-effectiveness. Chemicals are available that can uncouple intracellular phosphorylation from the oxidation of organic matter, limiting cells' ability to capture energy from substrate oxidation, thereby inhibiting cell growth *(10)*. Uncouplers of oxidative phosphorylation act by short-circuiting the proton gradient that drives production of adenosine triphosphate (ATP) by adenosine triphosphatase in the cell membrane *(8)*. The chemiosmotic mechanism of oxidative phosphorylation (by which ATP is produced during catabolism *[11]*) can be uncoupled by some chemical uncouplers and under some abnormal circumstances; therefore, energy is dissipated. Dissipating energy intended for anabolism of cell mass without reducing the rate of removal of organics from aqueous waste provides a direct mechanism for reducing the yield of sludge. Oxidation of the substrate still occurs but the phosphorylation of adenosine 5'-monophosphate ADP to ATP is reduced and, consequently, less energy is available for the formation of biomass *(7)*. Uncoupled metabolism promoted by some abnormal circumstances such as oxic and anaerobic cycling, changing of process temperature, and limitation of nutrient has been applied to the activated sludge process to reduce sludge production, but the two latter methods either increased operation cost or were not practical in view of environmental engineering. Again, the two latter methods did not show a significant effect on reducing sludge production according to some investigators *(12–17)*. The introduction of metabolic uncouplers seems to be feasible provided that low-price and nontoxic uncouplers can be found.

It has been reported that 3,3',4',5-tetrachlorosalicylanilide (TCS), a component in the formulation of soaps, rinses, shampoos, and so on, can stimulate uncoupled metabolism in activated sludge cultures *(18,19)*. Cook and Russell *(20)* originally reported the use of this chemical uncoupler in promoting the energy uncoupling in a pure culture of *Streptococcus bovis*.

The oxic-settling-anaerobic (OSA) activated sludge process is an uncoupled metabolism system based on oxic and anaerobic cycling. The OSA process is a system in which activated sludge is exposed to an anaerobic or anoxic zone under no food and low oxidation-reduction potential conditions periodically *(13–15)*. The OSA process is a modification of the conventional activated sludge process by inserting a sludge-holding tank in the sludge return line between the aeration tank and the secondary clarifier. It has been believed that the settler can minimize both substrate residues in the liquid and food storage in the sludge, which induces sludge starvation under anaerobic conditions. It should be pointed that this OSA process is different from the conventional biologic nitrogen removal process in which the anaerobic tank contains an adequate amount of substrate to proceed with denitrification or phosphorous release. Recirculation of activated sludge among an oxic (aeration tank), starvation (settling tank), and anaerobic environment (sludge-holding tank) can reduce 40–50% excess sludge *(21)*. The effect of the OSA process on reducing excess sludge production was not significant, compared with that of chemical uncoupler, but it can improve the sludge settleability and allow better substrate removal efficiency. Moreover, the activated sludge process with the addition of TCS influenced sludge settleability and effluent quality *(6,7,18)*.

To maximize excess sludge reduction efficiency in an activated sludge system coupled with a less adverse effect on process performance and sludge characteristics, we employed a TCS and OSA combined process and compared the combined process with a conventional activated sludge process, a conventional activated sludge process with the addition of TCS, and an OSA process in terms of excess sludge production, substrate removal capability, effluent quality, and sludge settleability. The present study was aimed at evaluating the feasibility of using a TCS and OSA combined process to minimize activated sludge growth with the least or no effect on process performance. It is hoped that the results generated from this investigation may provide useful information for future research on the feasibility of uncoupled metabolism approaches in the reduction of excess sludge.

Materials and Methods

Cultivation of Sludge

Four identical glass cylindrical (aeration tank) and four identical plastic conical vessels (settling tank) were used to construct four parallel, bench-scale, completely mixed activated sludge processes that were fed synthetic wastewater (Table 1). The volumes of the aeration tank and settling tank were 12.6 and 1.2 L, respectively. To start the cultivation, sludge taken from a local sewage treatment plant was seeded to all four experimental setups (Fig. 1). After 2 mo of cultivation, sludge production and treated water quality in these four processes became stable. Afterward one of them was used as the TCS process by adding 50 mg of TCS/d to the aeration tank during the first 3 mo and 100 mg of TCS/d during the latter 3 mo. One of

Table 1
Components and Concentrations of Synthetic Municipal Wastewater[a]

Component	Concentration (mg/L)
Starch	268
Glucose	200
Peptone	132
Yeast extract	68
Urea	8
$NaHCO_3$	80
$MgSO_4$	66
$CaCl_2$	6
KH_2PO_4	28.8
$(NH_4)_2SO_4$	112
$FeSO_4$	0.3
$MnSO_4$	6

[a]Mean influent quality: COD_{Cr} = 550 mg/L; biologic oxygen $demand_5$ = 260 mg/L; total nitrogen = 110 mg/L; NH_4^+-N = 60 mg/L; total phosphorus = 4.0 mg/L.

the setups was modified as an OSA process by inserting an 8.5-L sludge-holding tank between the aeration tank and settler. One of the setups was modified as the OSA and TCS combined process by adding 50 mg of TCS/d into the aeration tank and inserting the same sludge-holding tank. TCS was added into the aeration tank during aeration. A conventional activated sludge process only involved an aeration tank and a settling tank, which served as a control unit without any change. A schematic diagram of the models is shown in Fig. 1. Withdrawal of excess sludge did not start until the level of mixed-liquor suspended solids (MLSS) in the aeration tank exceeded 4500 mg/L. The MLSS level was then maintained at this level throughout the remaining period of the test. The sludge cultivation and subsequent experiments were all conducted at a room temperature of 25 ± 1°C.

Analytical Procedure

Chemical Analysis

MLSS, chemical oxygen demand (COD), NH_4^+-N, total nitrogen, total phosphorus, and sludge volume index (SVI) (mixed liquor was diluted to about 2 g/L prior to the SVI test) were determined according to *Standard Methods for the Examination of Water and Wastewater (22)*.

Analysis of Sludge Microbial Population

Variations in sludge settleability have been correlated to the ratios of floc-forming and filamentous microorganisms. Exocellular polymer production and cation concentration have also been correlated with settleability *(23,24)*. Uncoupled metabolism may affect growth rates of individual

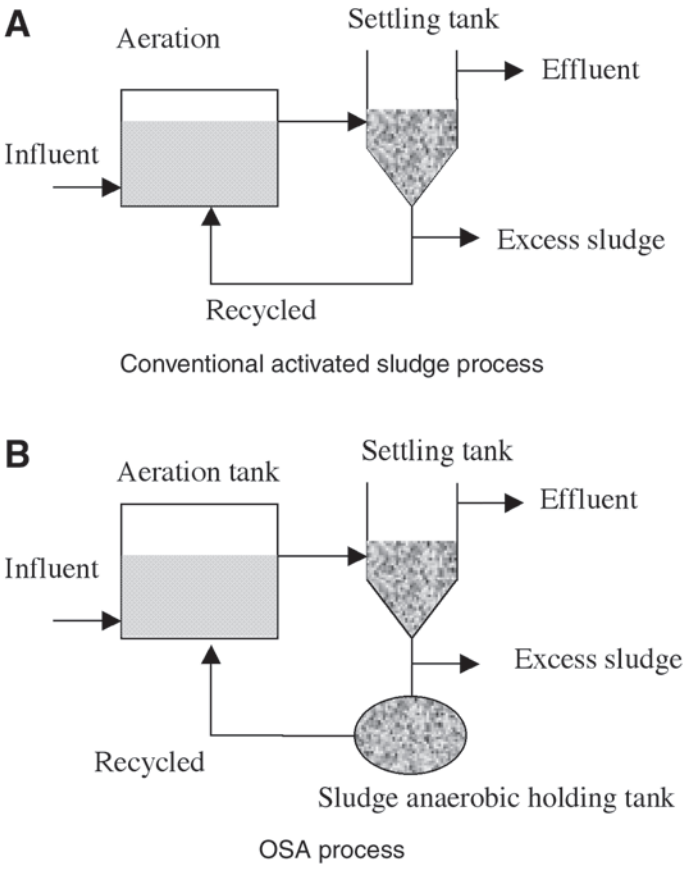

Fig. 1. Schematic diagram of four experimental systems: **(A)** conventional activated sludge process; **(B)** OSA process.

species in the sludge population differently. Therefore, sludge population dynamics may change, which may, in turn, alter sludge settleability. A diverse microbial population is required to effectively remove nutrients and achieve sludge flocculation. Metabolic uncoupling in a mixed population may shift the sludge population dynamics. Traditional methods of studying sludge population dynamics often involved enumeration and morphologically based identification using microscopy in conjunction with biologic strains and selective isolation procedures.

Molecular biologic methods, such as polymerase chain reaction (PCR) to amplify selected fragments of DNA, offer greatly increased sensitivity and are more representative of the microbial population *in situ*. In microbial community analysis, it is usually the 16S rRNA genes that are examined. These have been shown to contain sections of DNA sequence that are conserved throughout all bacteria and other sections that are unique to individual bacterial strains.

Bacterial 16S rRNA genes are amplified from a DNA extract obtained from the environmental sample. These products are of mixed sequence

composition. Exploitation of DNA sequence differences from a mixed population would provide a fingerprint of the microbial community composition contained within an environmental sample. This may be achieved by denaturing gradient gel electrophoresis (DGGE). DNA fragments from PCR products are electrophoresed through a polyacrylamide gel that contains an increasing concentration gradient of a denaturant, usually urea. At a certain denaturant concentration, the fragment unzips or becomes denatured and electrophoretic migration ceases. The point of denaturation is dependent on the DNA sequence, which is a function of the bacterial species. A mixed population that contains a mixed number of DNA types will produce a "ladder." However, these DNA analysis techniques do not distinguish active cells from those that are dead or dormant. Analysis of small molecular weight rRNA directly has been used to determine the active population of bacteria.

The aim of population investigation is to discern whether a shift in population occurs and identify any resulting loss in the efficacy of the process. Therefore, microscopic and molecular analysis of sludge was conducted to detect reliably any changes in the microbial population of the process caused by uncoupled metabolism.

For molecular analysis, samples (2 mL) were removed from the culture and frozen in liquid nitrogen for 15 s and stored at –70°C for subsequent analysis. Cells were disrupted and total DNA was extracted using a 3S DNA isolation Kit V2.1 for environmental samples (Shenergy Biocolor BioScience & Technology, China). PCR amplification was performed with F_{357}GC and R_{518} primers. The sequences of F_{357}GC and R_{518} primers were 5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3' and 5'-ATT ACC GCG GCT GCT GG-3', respectively. The final concentrations of the different components in the master mix were 25 pmol of each primer, 5 mL of 10X PCR reaction buffer (with $MgCl_2$), 200 μ*M* deoxynucleoside triphosphates, 2.5 U of *Taq* DNA polymerase (Shenergy Biocolor BioScience & Technology). DNA amplification was performed using a Mastercycle gradient thermal cycler (Eppendorf) with the following program: 94°C for 5 min; 35 cycles of 94°C for 1 min, 50°C for 50 s, and 72°C for 50 s; and 72°C for 7 min. Amplified DNA was verified by electrophoresis aliquots of PCR reaction (5 mL) in 1.0% agarose 1X TAE buffer. The product of PCR amplification was about 200 bp.

DGGE was performed using a Bio-Rad Dcode™ (Bio-Rad, Hercules, CA) system. The PCR products were loaded onto 10% (w/v) polyacrylamide gels in 1X TAE (20 m*M* Tris, 10 m*M* acetate, 0.5 m*M* EDTA, pH 8.0). The polyacrylamide gels were made with denaturing gradient ranging from 35 to 60% (where 100% denaturant contained 7 *M* urea and 40% formamide). Gels were polymerized with ammonia persulfate and *N*,*N*,*N*',*N*'-tetramethylethylenediamine according to the manufacturer's instructions. Electrophoresis was run for 6 h at a constant 160 V under 60°C. Gels were silver stained after being fixed.

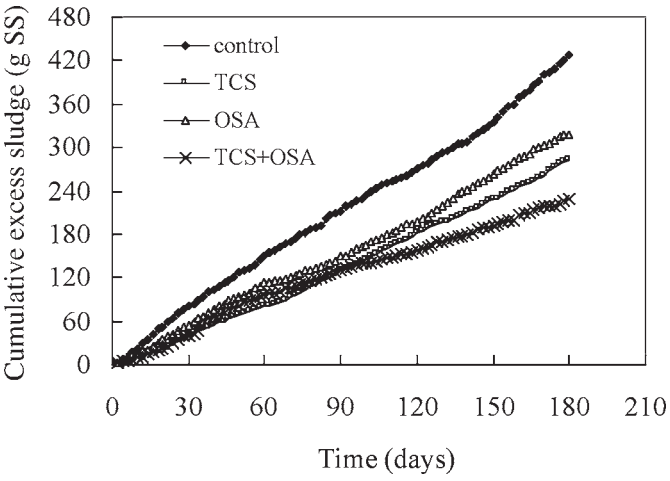

Fig. 2. Cumulative excess sludge production in control system, TCS system, OSA process, and combined process during 180 d of continuous operation.

Results and Discussion

Effect of Combined Process on Sludge Yield

Figure 2 shows the accumulated amount of excess sludge during the 180-d operation experiment in four processes (one was the control). In the control process, the excess sludge production rate was 2.386 g of suspended solid/d, and in the TCS process, OSA process, and combined process, the rates dropped to 1.575, 1.767, and 1.267 g of SS/d or reduced by 33.99, 25.94, and 46.90%, respectively. During the latter 90 d of operation, the sludge production rate was higher than that of the first 90 d of operation in the TCS process, suggesting that the microbial population became resistant to the uncoupling effect of TCS at this concentration. Afterward the dosage of TCS was increased to 100 mg/d in the TCS process but did not change in the combined process. The 180-d operation data further confirmed that TCS can effectively reduce excess sludge production, and that the TCS and OSA combined process is the best technology, which can alleviate microbial resistance and reduce sludge production more effectively owing to the cooperative effect. There are several reports on the TCS process and OSA process, respectively, and the results here are consistent with the results of those reports. However, the TCS and OSA combined process was first reported and offered a new approach to achieve excess sludge reduction in activated sludge process.

Effect of Combined Process on Process Performance

Figure 3 shows the COD removal rates in the four continuous operations of the activated sludge cultures. The mean COD removal rates in the control unit, TCS process, OSA process, and combined process were 89.52,

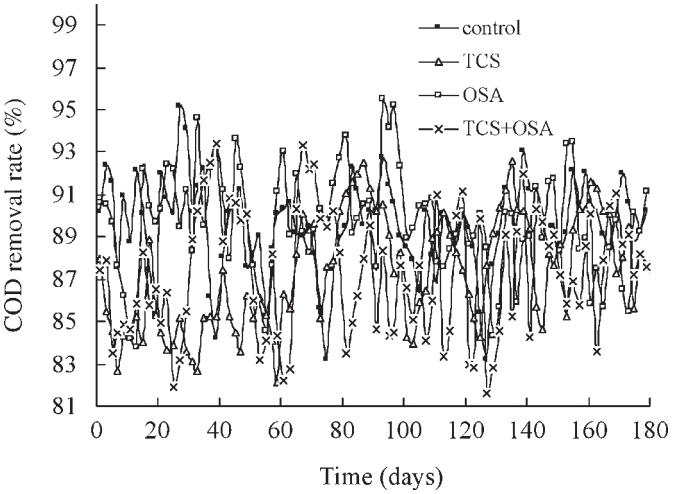

Fig. 3. Variations in COD removal efficiencies in control system, TCS system, OSA process, and combined process during 180 d of continuous operation.

87.32, 89.72, and 87.29%, respectively. It was apparent that the substrate removal efficiencies in these continuous processes were not affected significantly by the presence of TCS and not at all by the insertion of the sludge anaerobic tank. The good effluent quality demonstrated in these experiments was comparable with that reported for 2,4,6-trichlorophenol *(8)*, *p*-nitrophenol (*p*NP) *(21)*, 2,4-dinitrophenol *(25)*, and TCS *(18,19)*, except for the conclusion of Low et al. *(6)*, who found that the introduction of an uncoupler (pNP) caused substrate removal efficiency to decrease, but they considered that this decline was attributed to a surmountable effect arising from the design of the apparatus. It has been reported that the OSA process is able to improve the COD removal efficiency, which may be due to the longer retention time of sludge in anaerobic holding tank than that in our test *(16)*.

Figures 4 and 5 summarize the variations in the effluent NH_4^+-N concentrations and total nitrogen removal rates in four processes. The mean effluent NH_4^+-N concentrations in the control unit, TCS process, OSA process, and combined process were 25.84, 31.91, 26.96, and 36.23 mg/L, respectively. The mean total nitrogen removal rates in the control process, TCS process, OSA process, and combined process were 42.42, 36.21, 41.33, and 34.39%, respectively. Figures 4 and 5 demonstrate that effluent NH_4^+-N concentrations and total nitrogen removal rates were affected by the addition of TCS, but not by the insertion of a sludge-holding tank, which possibly was owing to oxic-anaerobic recycle of sludge to cause a nitrification-denitrification effect. The results confirmed the views of Low and colleagues, who thought effluent nitrogen concentration would increase by uncoupled metabolism, although in their investigation with pNP, they did not test the effluent nitrogen content *(6,21)*.

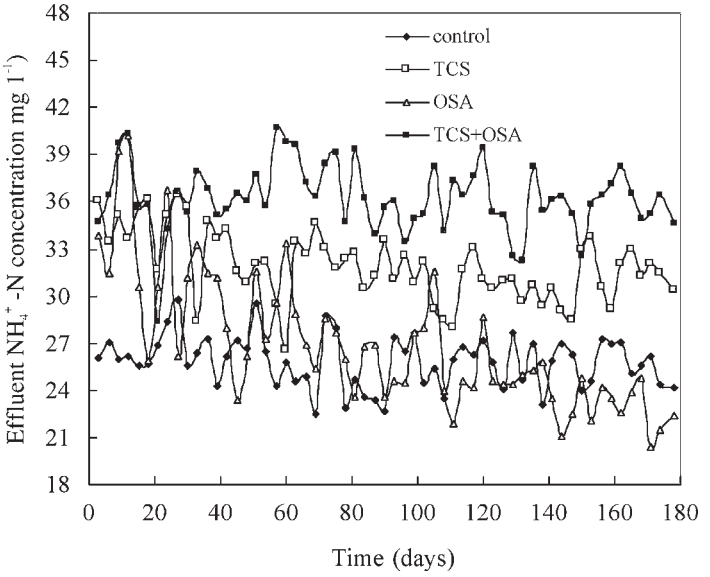

Fig. 4. Variations in effluent NH_4^+-N concentrations in control system, TCS system, OSA process, and combined process during 180 d of continuous operation.

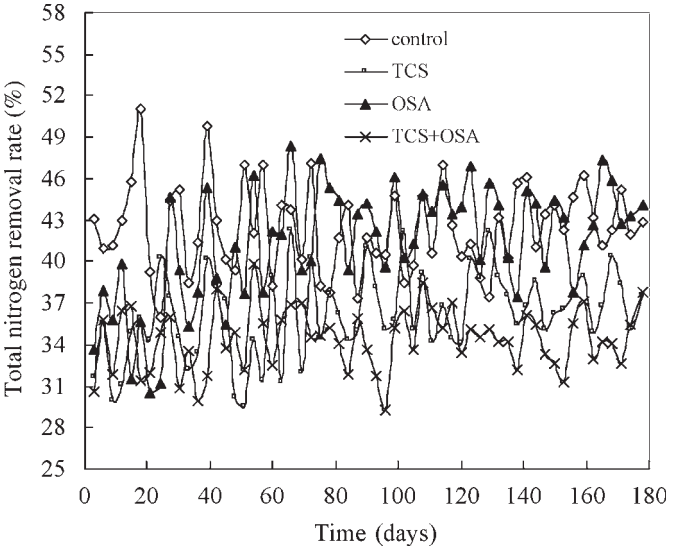

Fig. 5. Variations in effluent total nitrogen removal rates in control system, TCS system, OSA process, and combined process during 180 d of continuous operation.

Figure 6 shows the effluent total phosphorous concentrations in the four processes. The mean effluent total phosphorous concentrations in the control unit, TCS process, OSA process, and combined process were 1.63, 1.45, 1.50, and 1.37 mg/L, respectively. The introduction of TCS and a sludge anaerobic tank decreased the effluent total phosphorous concentra-

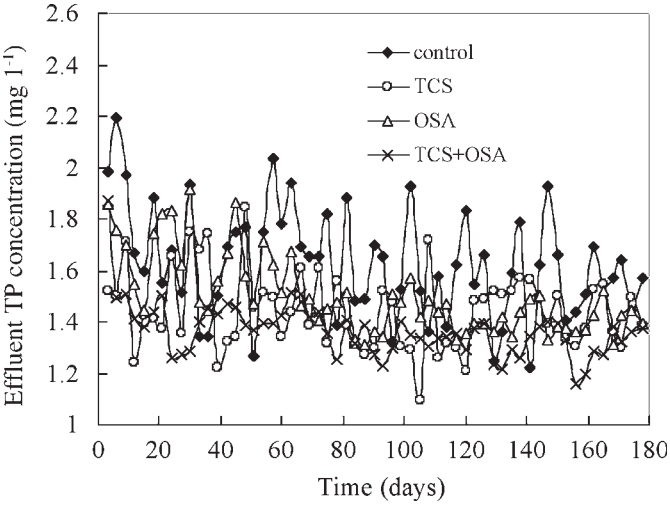

Fig. 6. Variations in effluent total phosphorous (TP) concentrations in control system, TCS system, OSA process, and combined process during 180 d of continuous operation.

tion, which possibly resulted from the higher organic substrate in the sludge of the TCS process, OSA process, and combined process (higher SVI values of sludge) and more phosphorus being absorbed. However, until now there has been no report about the effect of uncoupling metabolism on the effluent nitrogen and phosphorous concentrations. Owing to limited literature on uncoupled metabolism, it is quite difficult to compare the results obtained from our study with those of the previous relevant studies. The detailed information should be investigated thoroughly in the future.

Effect of Uncoupled Metabolism on Sludge Characteristics

Figure 7 shows that sludge settleability of the TCS process was worse than that of the reference system, but it is also obvious that SVI values remained less than 100 in the OSA process over the entire operation during the 180-d operation in our study, which was consistent with the results of Chen et al. *(18,19)*. These findings may be attributed to the release of intracellular polymers under anaerobic condition, because they can act as flocbridging agents to improve sludge settleability. SVI values in the combined process were higher than those in the control unit, but lower than those of processes with TCS, which implied that insertion of a sludge anaerobic tank can counteract the adverse effect of TCS on sludge settleability.

Microscopic examination showed that after 180 d of operation, microbes agglomerated in dense flocs and predominantly cocci and short bacilli. Sludge contained both stalked and free-swimming ciliated protozoa, such as *Paramecium candatum* and *Vorticella*, and metazoan such as *Rotifera* and *Nematoda* in the control unit and OSA process. However, in the TCS process and combined process, metazoans were not observed, and the

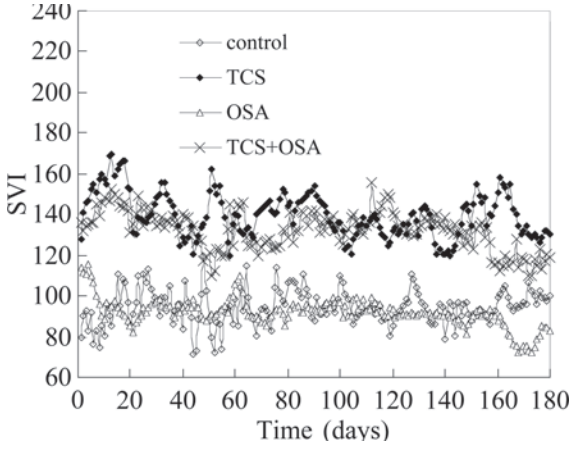

Fig. 7. Variations in SVI in control system, TCS system, OSA process, and combined process during 180 d of continuous operation.

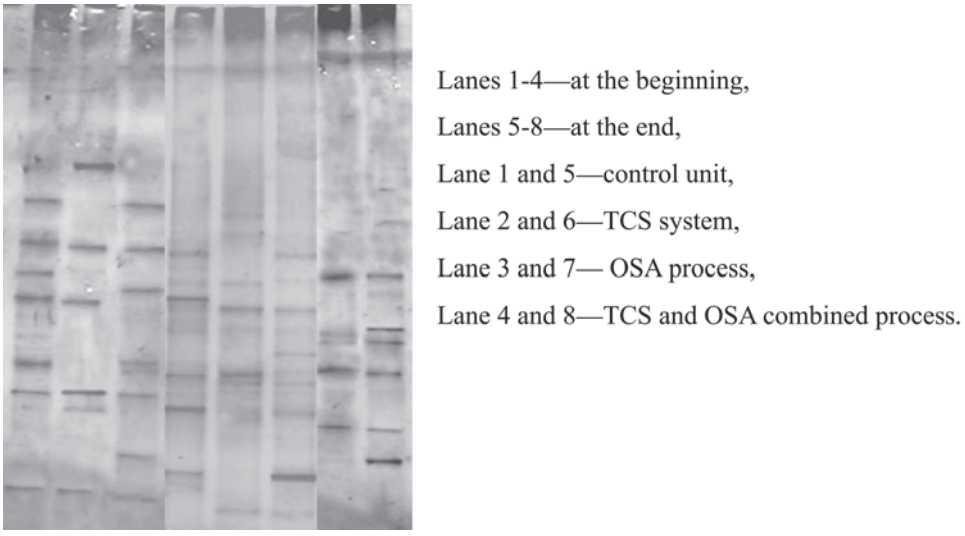

Fig. 8. DGGE profiles of activated sludge samples from four systems at beginning and end of experiment.

number and activity of protozoa also were reduced, indicating that TCS more strongly influenced higher animal in the sludge. To investigate the effect of TCS and sludge anaerobic tank on bacteria, a PCR-DGGE method was used to study the microbial population. Figure 8 shows that changes in banding patterns were observed in metabolic uncoupling systems after 180 d of operation, but band pattern did not change much in the control unit. PCR-DGGE is likely to represent the dominant species in the population and, thus, any loss of bands may signify the relegation of dominant species rather than their loss from the sludge.

Conclusion

Our results indicated that TCS is an effective metabolic uncoupler to reduce sludge production rate in activated sludge process, and the insertion of a sludge-holding tank into the returned sludge circuit to form an OSA process can result in a significant decrease in excess sludge production. In particular, the TCS and OSA combined process minimized excess sludge yield most heavily. Compared with the control unit, COD removal rates were not affected by the uncoupled metabolism in the TCS process, OSA process, and combined process. The effluent nitrogen content was adversely influenced, but the three uncoupled metabolism processes did not influence effluent total phosphorous concentrations. Sludge settleability was unfavorably affected by the addition of TCS but was improved with the insertion of a sludge anaerobic tank. Microscopic examination and the PCR-DGGE technique both identified a shift in the predominant microbial species after the occurrence of uncoupled metabolism after 180 d of operation. These findings imply that uncoupled metabolism stimulated by combined chemical uncoupler with the OSA process is a novel method to reduce excess sludge production. This method could reduce both capital and operating costs incurred by biologic wastewater treatment and, therefore, merits further research.

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