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## REMOVAL OF CLOFIBRIC ACID AND DICLOFENAC DURING ANAEROBIC DIGESTION OF SEWAGE SLUDGE

Removal of two acidic pharmaceuticals, clofibric acid and diclofenac, present in sewage sludge using two kinds of anaerobic digestions was investigated. The effective isolation and purification pre-treatment to extract the target compounds from the sewage sludge samples was firstly established, followed by gas chromatography-mass spectrometry (GC-MS) analysis to identify and quantify them. Under mesophilic conditions, the removal efficiencies for clofibric acid and diclofenac with the initial concentrations of  $5 \mu\text{g}/\text{dm}^3$  were up to 95% and 97%. After thermophilic anaerobic digestion, the removal efficiency could be increased to 99.3% for clofibric acid, however, it did not increase for diclofenac. In general, anaerobic digestion could effectively remove the target compounds from the sewage sludge (all above 90%).

### 1. INTRODUCTION

With the spread of sewage treatment and the process efficiency's increase, the production of sludge is quite enormous, then the disposal of sewage sludge is becoming more and more emergent, while some micropollutants like pharmaceuticals existing in the sludge could exert direct environmental harms or potential risks to the safety of the environment [1]. The lipid regulating agent, clofibric acid (CA) and anti-inflammatory painkiller, diclofenac (DCF) as common acidic pharmaceuticals are pervasive in use for daily life, whereas a significant portion of the compounds without metabolism are excreted by the urine or faeces, subsequently discharged into the sew-

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age treatment plants (STPs). Therefore, sewage sludge is considered as one of the release pathways [2].

For the past few years, more and more investigations have been conducted on the presence and distribution of the compounds in the STPs of different countries and regions [3–7], including China where their existence in sewage has also been confirmed and described [8–10]. Besides, CA and DCF were found even in some natural water and drinking water [11, 12]. DCF and five of its intermediate products were detected in Malir River and Lyari River water as well as in effluent samples from Karachi, Pakistan, reported by Scheurell et al. [13], and the highest detected concentration of DCF reached  $4.4 \mu\text{g}/\text{dm}^3$ . In addition, CA and DCF were detected at the highest concentrations of  $17 \text{ ng}/\text{dm}^3$  and  $150 \text{ ng}/\text{dm}^3$  in Shijing River of China [14].

Considering the compounds CA and DCF with the property of low biodegradation rate, bioconcentration and bioaccumulation in aquatic organisms, inevitably they would pose unpredicted effects on the biology which are exposed to them for long term [15]. The toxic effects of the two pharmaceuticals at different concentrations were tested in a common carp *Cyprinus carpio* for a short-term period of 96 h under static bioassay method reported by Saravanan et al. [16]. At all concentrations, red blood cell, plasma sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), and glutamate oxaloacetate transaminase levels were decreased in the fish treated with CA and DCF. Bioassays of laboratory ecotoxicology of CA and DCF were performed on bacteria, algae, microcrustaceans and fishes [17]. The data could be used to calculate their predicted no-effect concentrations (PNEC) and to perform a preliminary risk characterization. Nassef et al. [18] also investigated the toxicity of three kinds of pharmaceuticals, carbamazepine, DCF, and triclosan which was examined by measuring their effects on feeding behavior and swimming speed of adult Japanese medaka fish (*Oryzias latipes*). Consequences of long-term exposure to DCF up to 3 months were evaluated using freshwater crustaceans (*Daphnia magna* and *Moina macrocopa*) and a fish (*Oryzias latipes*) by Lee et al. [19]. The presence of DCF at the concentration of  $1 \mu\text{g}/\text{dm}^3$  has been reported to damage the liver and kidney cell functions in fish [20].

So far, only a few researches concerned pharmaceuticals in sewage sludge, mostly focusing on their distribution and load analysis [21, 22]. A recent review published by the UK Environment Agency noted that no quantitative data for concentrations of priority control pharmaceuticals in sewage sludge were found, although this is a potential route to the terrestrial environment [23]. Moreover, little researches on the distribution and control of pharmaceuticals in sewage sludge of the mainland of China have been carried out nowadays, and the environmental risks in land use and utilization of sewage sludge caused by pharmaceuticals did not get enough attention. Thus, it is of great interest to investigate effective treatment technologies for pharmaceuticals of sewage sludge related to the sludge discharge and utilization. Anaerobic digestion (AD) has been extensively used and proven to be the most efficient technology to stabilize organic substrates in sewage sludge [22]. Mesophilic AD is most widely

adopted for the treatment of sewage sludge generated from STPs due to its relatively simple operation and control. In comparison with the mesophilic process, the thermophilic AD shows some advantages, such as acceleration of the biochemical reactions, a greater extent of pathogen reduction and an effective degradation of organic matter. The thermophilic treatment is also increasingly introduced because of the increasing demands on sewage sludge treatment.

In this study, the mesophilic and thermophilic anaerobic digestion processes were adopted to remove the two target compounds, anti-inflammatory painkiller DCF and lipid regulating agent CA, which are common in use for daily life. Effective isolation and purification to extract the compounds from the sewage sludge samples were also established before the analysis and quantification of analytes by GC-MS. The results of this work might be combined with previous research achievements to perfect the basic data of sewage sludge as well as toxic pollutants management of the sludge discharge and utilization.

## 2. MATERIALS AND METHODS

*Chemicals and materials.* The reference compounds, CA)and DCF, were purchased from Shanghai ANPEL Scientific Instrument Co., Ltd., China. Their purities were all above 99%. Standard stock solutions of 1 g/dm<sup>3</sup> were prepared in methanol, stored at -20 °C in the dark, and successively diluted with methanol to prepare working solutions. Acetone, methanol, and dichloromethane were obtained from Fisher, USA. Methyl tert-butyl ether (MTBE), N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) (containing 1% of TMCS, derivatization reagent) were obtained from Sigma-Aldrich, USA. All the solvents used were HPLC grade or higher. Other reagents used were of analytical grade or better. Ultrapure water was obtained with Aq-uapro ultrapure water system (China).

Glass fiber filters GF/B (1 µm) were purchased from Whatman, USA. The filters were prewashed 3 times with acetone before use. Cartridges (C8 opposition, 6 cc/200 mg) for solid-phase extraction (SPE) were bought from Supelco, USA.

*Sewage sludge.* Most of STPs employing anaerobic digestion processes in China usually use common tanks for the digestion of mixtures of primary and biological (secondary) sewage sludge. Raw sewage sludge used in this work was collected from a STP located in east district of Shanghai, China. The STP was built in 1920s, and is currently the only one on stable operation in China. It serves the population of about 800 000, and is capable of treating sewage at 28 000 m<sup>3</sup>/d, 80% of which is from domestic waste water and the rest is from industry. The sludge was obtained from the sludge circulation lines of the biological treatment units. Table 1 shows some of the main indicators of the sludge.

Table 1

Main indicators of the sewage sludge used in this work

Indicator	pH	COD	SS	VSS	NH <sub>3</sub> -N	TN	TP	VFA
		[mg/dm <sup>3</sup> ]						
Value	7.3	5000	7000	3200	6.8	11.9	0.72	50

*Anaerobic digestion.* Two lab-scale continuously stirred anaerobic digesters (total volume of 25 dm<sup>3</sup> for each) were installed in parallel and started up (Fig. 1). One was operated in the mesophilic range (37 °C), and the other in the thermophilic range (55 °C). The temperature was maintained by the external jacket of the digesters with auto temperature controller. There were one inlet and two sampling mouths in each digester. The outlet for sludge discharge was set at the bottom of the digester. After condensing, raw sewage sludge which was collected from the sludge circulation lines mentioned above was pumped into the each digester using peristaltic pump. Wet gas flow meter was linked to each digester to count the gas production directly, together with auto-control temperature system guaranteeing stable temperature condition and automatic stirrer keeping mixing uniformity of the digester.

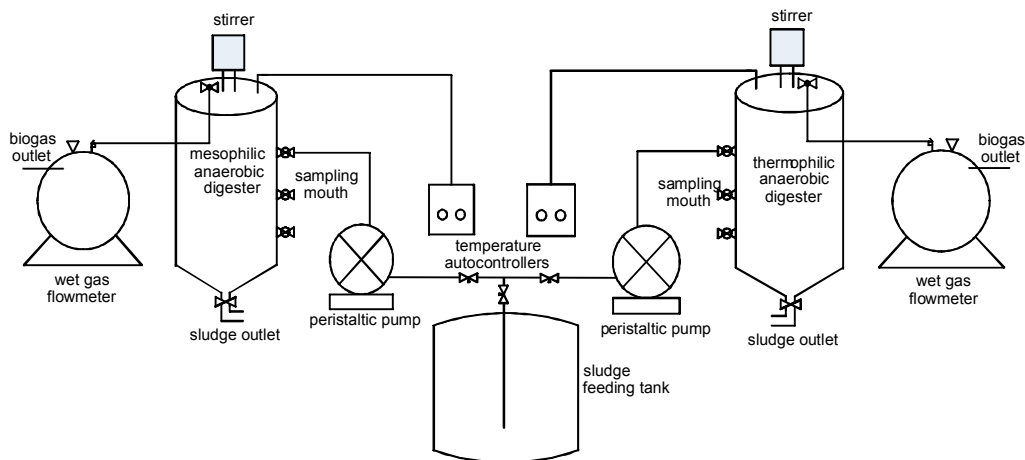


Fig. 1. Schematic diagram of the laboratory scale anaerobic digestion system

The start-up of the two digesters consisted of two periods. During the first period (ca. 25 d), the raw sewage sludge was firstly pumped into the reactors, and the final filled volume was kept to 20 dm<sup>3</sup>. At the first week, there was no sludge discharge or feeding. Then, the digesters were operated on batch mode with the lower initial feeding load, i.e. 0.5 dm<sup>3</sup> sewage sludge per digester per day. Three operating factors were measured online: temperature, pH and biogas production. Apart from the online measurements, the operation was monitored in terms of solids, COD<sub>cr</sub>, volatile fatty acids

(VFA),  $\text{NH}_3\text{-N}$  and TP twice or 3 times per week. The period lasted till the variations of the factors became basically stable. During the next period, a continuous feeding at a relatively low organic load rate (OLR) where solid retention time (SRT) was kept at 20 d was applied for about one month. Then, a gradual increase of the OLR was applied in order to obtain the settled operational conditions, i.e. SRTs of 10 d and 7 d for the mesophilic and thermophilic anaerobic digesters.

The whole start-up period lasted about two and a half months. Table 2 shows some of main indicators of mesophilic and thermophilic anaerobic digesters after the start-up. From then on, the digesters were fed with the sewage sludge previously spiked with CA and DCF at certain levels. The system was used to the following tests through a short-term stabilizing operation.

Table 2

Main indicators of the two anaerobic digesters after the start-up

Indicator	Mesophilic anaerobic digestion	Thermophilic anaerobic digestion
Temperature, °C	37–39	48–52
pH	6.6–7.2	6.6–7.1
COD <sub>cr</sub> , g/dm <sup>3</sup>	10.6–15.8	14.3–18.5
MLSS, g/dm <sup>3</sup>	12.4–17.4	14.6–17.2
MLVSS, g/dm <sup>3</sup>	5.8–7.1	5.3–8.2
VFA, mg/dm <sup>3</sup>	248–425	270–480
$\text{NH}_3\text{-N}$ , mg/dm <sup>3</sup>	5.5–9.2	6.2–9
TP, mg/dm <sup>3</sup>	0.8–1.2	0.8–1.04
Gas production, dm <sup>3</sup> /d	0.5	0.8

After the start-up of the anaerobic digestion system, the mesophilic anaerobic digester was operated at a SRT of 10 d, and the thermophilic reactor operated at a SRT of 7 d. The tests were launched after the two digesters ran for two or three SRT periods. Each test was done repeatedly. Samples of digested sludge were collected everyday for seven days. Each sample was immediately per-treated so as to accurately represent the variations of the target compounds by the anaerobic digestion processes.

*Sample pretreatment.* The sewage sludge sample of 500 cm<sup>3</sup> was pre-filtered using a vacuum pump with a GF/B glass fiber filter. The filtrate was introduced to a C8 cartridges (6 cc/200 mg) on a vacuum 12-position extraction manifold (Supelco, USA) by means of a PTFE tube for the solid-phase extraction (SPE) to extract the analytes. Figure 2 shows the procedure of SPE. The biosolid/sludge fraction of the sample retained on the fiber was dried at 60–70 °C in a drying oven. Then the biosolid/sludge was ground in a mortar, mixed thoroughly and stored in amber bottles. For analysis, the biosolid/sludge fraction was firstly pretreated using ultrasonic solvent extraction.

An aliquot (0.02 g) of the stored sludge was extracted with 8 cm<sup>3</sup> of methanol/H<sub>2</sub>O (5:3) and then three times with 5 cm<sup>3</sup> of methanol. In each extraction step, the sample slurry was ultrasonicated for 30 min at 50 °C. The supernatant was collected after centrifugation at 10 000 rpm for 15 min, combined, and diluted to a final volume of 500 cm<sup>3</sup> using ultra-pure water. Afterwards, it was extracted using the same SPE procedure for the aqueous samples (Fig. 2).

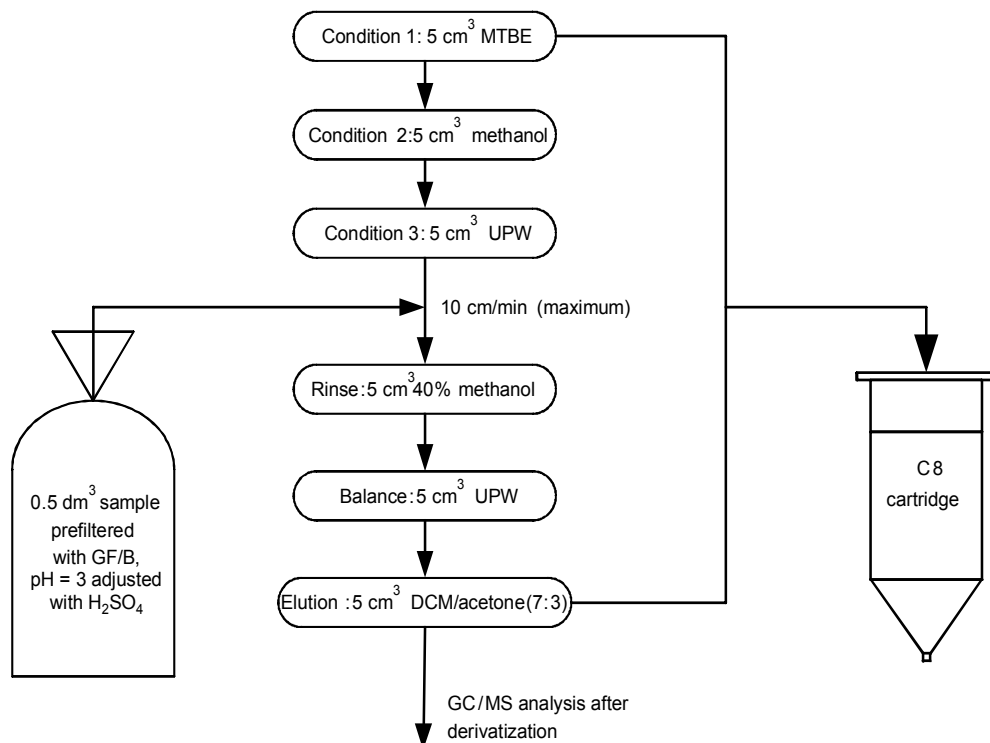


Fig. 2. The procedure of solid-phase extraction (SPE) for the aqueous samples (MTBE – methyl tert-butyl ether, UPW – ultrapure water, DCM – dichloromethane)

*GC-MS analysis.* The elute of 5 cm<sup>3</sup> as shown in Fig. 2 was evaporated till dryness under a gentle nitrogen stream, and then 0.1 cm<sup>3</sup> of derivatization reagent, BSTFA (containing 1% of TMCS) as well as 0.05 cm<sup>3</sup> of pyridine were added. The derivatization was performed at 70 °C for 1 h. The derivative was further dried, and the residue was redissolved in 0.1 cm<sup>3</sup> of *n*-hexane for GC/MS analysis.

GC/MS system, Agilent 7890/5975C-GC/MSD, USA, was used for the analysis of target compounds. The parameters for GC/MS described by Zhou et al. [24] were adopted with minor modification. For the identification and qualitative analysis of the analytes, the full scan mode ( $m/z = 50\text{--}650$ ) was firstly applied (Fig. 3).

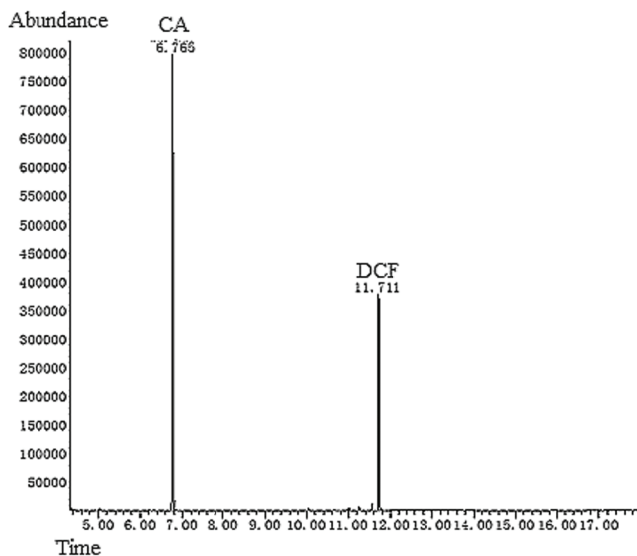


Fig. 3. Chromatograms of clofibric acid and diclofenac in the full scan mode using GC/MS (the concentration of each reference compound in aqueous solution is  $50 \text{ ng/dm}^3$ )

The HP-5MS capillary chromatographic column was used for separation of the compounds. Their identification and verification was achieved via the comparison of retention times and mass spectrum in samples with those of standard compounds.

Table 3

The recoveries of the target compounds  
in the sewage sludge samples

Compound	Spiked standard concentration [ $\text{mg/dm}^3$ ]	Recovery [%]	Relative standard deviation (RSD) [%]
CA	0.05	104	15.2
	0.1	105	5.4
	0.2	101.8	2.2
DCF	0.05	98	5.8
	0.1	104.3	10.6
	0.2	99.8	3.6

The concentrations of the target compounds were the sums of both the aqueous part and the bio-solid/sludge fraction of the sewage sludge samples.

Quantification was performed by comparing the integration of the selected ion chromatograms of each compound with that of the instrumental internal standard in

the mode of selected ion monitoring (SIM). Calibration was made from 2 to 2000  $\mu\text{g}/\text{dm}^3$  (10 points) using standard solution containing the two selected compounds. The calculated concentrations of analytes were corrected by the recoveries of the analytes in the samples (Table 3). The mean recoveries were 101.8–105% for CA, and 98–104.3% for DCF. In addition, procedure blanks and solvent blanks were also treated and analyzed alongside with the measurements. The concentrations of the target compounds were calculated with both the aqueous part and the biosolid/sludge fraction of the sewage sludge samples.

### 3. RESULTS AND DISCUSSION

#### 3.1. CA AND DCF IN THE SEWAGE SLUDGE

The two target compounds, CA and DCF, were detected in the raw sewage sludge at the mean concentrations of 27.8  $\text{ng}/\text{dm}^3$  and 114.2  $\text{ng}/\text{dm}^3$ . In order to ensure the detection of the two compounds and represent their variations more effectively and clearly during the anaerobic digestion processes, reference compounds were spiked to the raw sewage sludge and the initial concentrations of the compounds were up to around 5  $\mu\text{g}/\text{dm}^3$ , higher than their environmentally relevant concentrations.

Regulation and control of SRTs of anaerobic digestion processes was realized by the daily load of the spiked sewage sludge volume, considering the biosolid contents in the sewage sludge nearly consistent. In this study, the mesophilic anaerobic digester was operated at an SRT of 10 d and for the thermophilic reactor, SRT was 7 d. The variation and removal of CA and DCF under various operating conditions were examined. Each sewage sludge sample was pre-treated into two sub-samples, i.e. an aqueous phase sample and a biosolid/sludge phase sample. Therefore, the detection and analysis for the target compounds was conducted on the two phase subsamples, and the variation and removal of the compounds in the two phases were investigated.

#### 3.2. MESOPHILIC ANAEROBIC DIGESTION

The SRT of the mesophilic anaerobic digestion were adjusted at 10 d. CA was dramatically removed during the early sampling period of mesophilic anaerobic digestion and the removal rate reached 92% at the fourth day of reaction, and then the elimination tendency became smooth (Fig. 4). The removal efficiency of DCF was quite low at the early sampling period, being only 43% on the first day. An immense increase on removal efficiency did not appear until the fourth day, up to about 95%. The final removal efficiencies of CA and DCF in the sewage sludge were about 95% and 97% in the mesophilic anaerobic digestion (SRT 10 d).



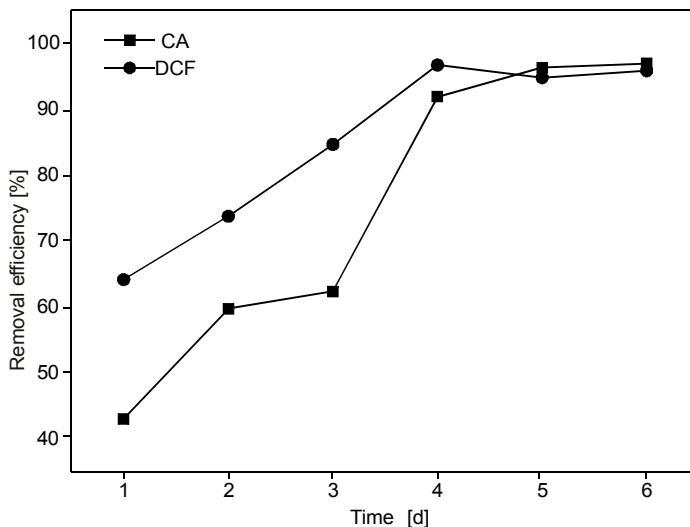


Fig. 4. Removal efficiencies of CA and DCF in the sewage sludge in the mesophilic anaerobic digestion

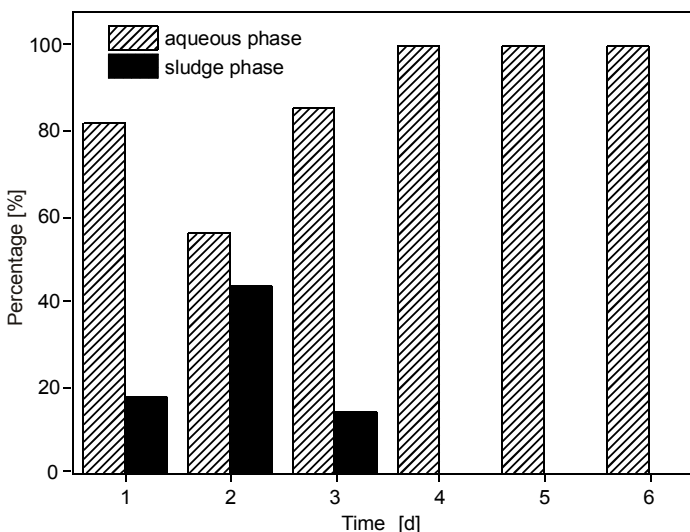


Fig. 5. Distribution of CA in the aqueous and biosolid/sludge phases of the sewage sludge in the mesophilic anaerobic digestion (all the data obtained from the means of the repeated samples)

The distribution of the target compound CA in the aqueous phase and bio-solid/sludge phase is shown in Fig. 5. The data given in the figure as well as in the following figures are normalized using the related concentrations of the compounds in the sewage sludge samples. CA was found in both phases, and the maximum percent-

age for the sludge phase reached 43.8% in the early sampling stage. While with the proceeding, the contents in the sludge phase were decreased sharply, there was no CA found from the fourth day, and the two compounds were detected in the aqueous phase.

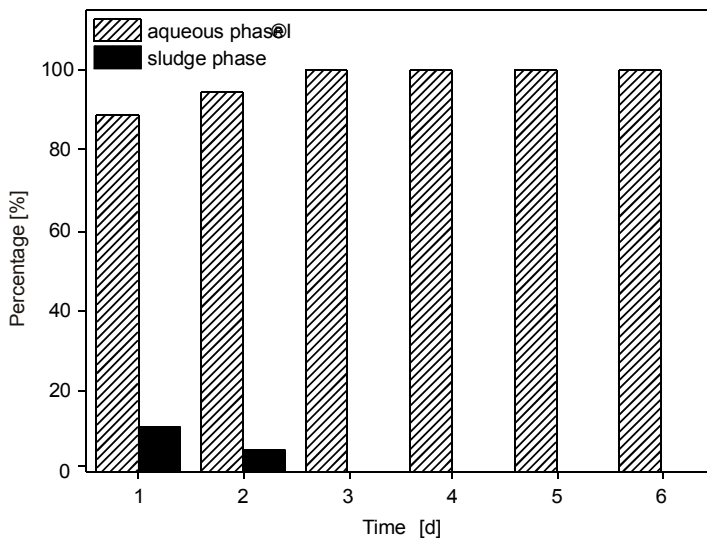


Fig. 6. Distribution of DCF in the aqueous and biosolid/sludge phases of the sewage sludge in the mesophilic anaerobic digestion (all the data obtained from the means of the repeated samples)

The distributions in the aqueous and biosolid/sludge phase of DCF are shown in Fig. 6. Unlike CA, the percentage of DCF in the sludge phase was quite low even at the early sampling period, and the highest was only 11.2%. Subsequently, no DCF was detected in the sludge phase. The variations of the two compounds mentioned above suggested that both adsorption and biodegradation contributed to their removal in the early reaction stage of the mesophilic anaerobic digestion, and then biodegradation performed inside and around the digested sludge predominated in the following reaction stage. The adsorption of CA was more perceptible than that of DCF possibly due to larger hydrophobic nature of CA, leading to the bigger contents in the sludge phase of the compound in the early reaction stage. The two selected compounds could be degraded to below the detection limits in the sludge phase with the further reaction, showing the risks of the two compounds caused by the digested sludge discharge or reuse might be reduced.

### 3.3. THERMOPHILIC ANAEROBIC DIGESTION

The thermophilic anaerobic digestion was operated at SRT 7 d. The proportions of the two compounds in the sludge phase (Figs. 7 and 8) showed a similar variation tendency in this thermophilic mode to the mesophilic mode. However, there were still

some differences between the two modes considering the concrete data. The highest ratio of the CA in the sludge phase was only 10.8% which appeared in the second sampling day under the thermophilic mode, and the compound was not detected in the fourth sampling day. The ratio of DCF in the sludge phase under the thermophilic mode was very low and the maximum was only 5.3%.

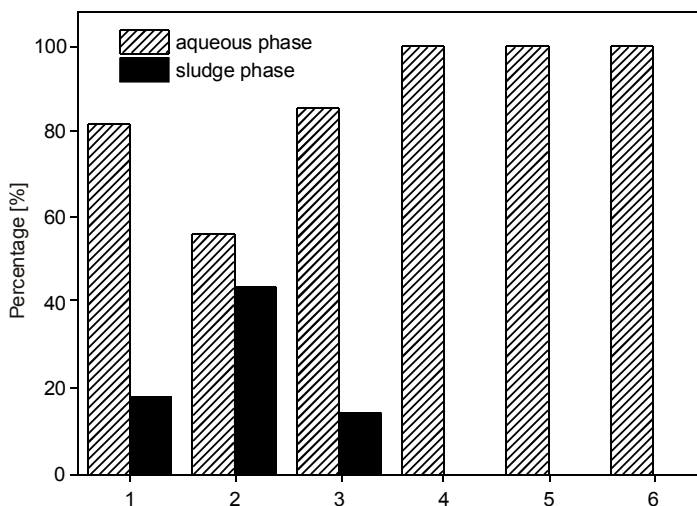


Fig. 7. Distribution of CA in the aqueous and biosolid/sludge phases of the sewage sludge in the thermophilic anaerobic digestion (all the data obtained from the means of the repeated samples)

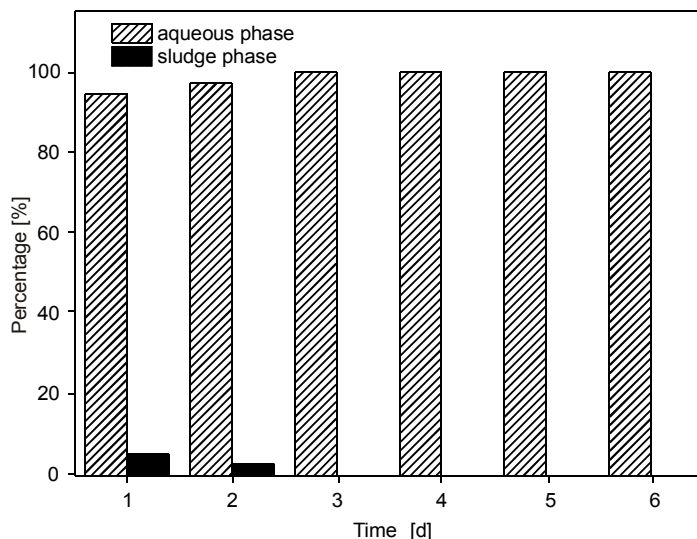


Fig. 8. Distribution of DCF in the aqueous and biosolid/sludge phases of the sewage sludge in the thermophilic anaerobic digestion (all the data obtained from the means of the repeated samples)

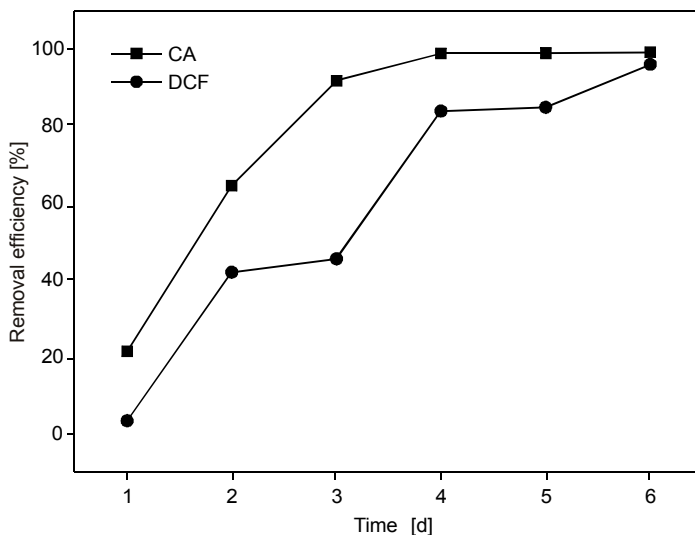


Fig. 9. Removal efficiencies of CA and DCF in the sewage sludge in the thermophilic anaerobic digestion

As seen in Fig. 9, the removal efficiencies of the two compounds were quite low in the early sampling stage under the thermophilic mode, which were about 21.4% and 3.0% for CA and DCF. However, the removal efficiency of CA increased significantly in the third sampling day, up to 91.9%. As for DCF, the removal efficiency was raised to about 85% in the fourth sampling day. The final removal efficiencies of CA and DCF in the sewage sludge amounted to 99.3%, 96.2% in the thermophilic anaerobic digestion.

#### 3.4. COMPARISONS OF CA AND DCF IN THE MESOPHILIC AND THERMOPHILIC ANAEROBIC DIGESTION

The distribution and removal efficiencies of the target compounds, CA and DCF, were examined in the anaerobic digestion with different SRTs via changing load rate of sludge. The proportions of the target compounds in the sludge phase under the mesophilic mode far exceeded those in the thermophilic mode at the early sampling period, whereas the compounds showed no presence in the sludge phase at the later sampling period under the mesophilic and thermophilic modes.

Anaerobic digestion could effectively remove the two selected compounds and the final removal efficiencies all exceeded 90% in the both mesophilic and thermophilic modes. CA could be eliminated from the sewage sludge nearly completely under the thermophilic condition with the final removal efficiency of 99.3%. Although the removal efficiency of 96.9% for CA under the mesophilic condition was slightly lower, the compound was also greatly removed from the sewage sludge. Based on the final

removal efficiencies, a relatively higher removal of DCF from the sewage sludge could be obtained in the mesophilic anaerobic digestion with SRT 10 d and CA could be nearly completely removed from the sewage sludge in the thermophilic anaerobic digestion with SRT 7 d.

As can be seen from Figs. 4 and 7, there are some differences between removal of the two compounds under anaerobic conditions. Mesophilic anaerobic digestion demonstrated better removal of the compounds in the early sampling stage while the thermophilic anaerobic digestion removed the compounds significantly in the middle sampling period. Especially for the DCF, mesophilic anaerobic digestion showed a gentle increase in the removal efficiency which was considerably high in the early sampling period. In contrast, the removal efficiency of the compound was only around 3% in the first sampling under the thermophilic condition, and subsequently it sharply increased.

The final removal efficiencies of the target compounds were all above 90%, which were much higher compared with the previous study reported by Carballa et al. [22] in which the mesophilic and thermophilic anaerobic digestion of selected pharmaceuticals including CA and DCF at various SRTs were investigated. The maximum removal of DCF from the sewage sludge was about 75% under mesophilic conditions with SRT of 10 d and 72% under thermophilic conditions with SRT of 6 d. The differences in the removal efficiencies between the two studies maybe partly contributed to the source of the raw sewage sludge. In this study, the raw sewage sludge was from the circulation lines of the biological treatment units. The sludge possessed the property of excellent bioactivity and facultative anaerobic bacteria, which could be cultivated into anaerobic activated sludge with no great difficulty, maintaining stable content of bacteria for anaerobic digestion. Besides, the operating factors of the experimental processes were also different which might lead to some influences on the performances of the removal of the compounds in the anaerobic digestion. It is still uncertain that the eco-environmental risks caused by the two compounds can be greatly reduced through sludge anaerobic digestion although high removal efficiencies of them were achieved. The two compounds could be biodegraded into intermediate products [13, 25], whose presence was not detected in this study, and may still cause, even enhance the eco-environmental risks. Further studies are being conducted in our lab to analyze the intermediate products and their final removal paths of the two compounds and assess the capacities of eco-environmental risk control reasonably during the anaerobic digestion of sewage sludge.

#### 4. CONCLUSIONS

Anaerobic digestion processes, widely used for sludge stabilization, has been applied here to investigate the variations and the performances of the removal of the two

selected acidic pharmaceuticals, diclofenac and clofibrac acid, frequently detected in the sewage sludge. The distributions between the aqueous and biosolid/sludge phases and the removal efficiencies of the two compounds were explored under the mesophilic and thermophilic modes. On the whole, the anaerobic digestion was effective in removal for diclofenac and clofibrac acid. Diclofenac could be highly removed by means of the mesophilic anaerobic digestion with SRT of 10 d and clofibrac acid was almost eliminated in the thermophilic anaerobic digestion with SRT of 7 d. The two compounds could be partly adsorbed on the sludge phase in the early period of the anaerobic digestion, and both adsorption and biodegradation contributed to their removal. Little amounts of the compounds were detected in the sludge phase in the later period of the anaerobic digestion, suggesting that the biodegradation inside and around the digested sludge predominated in the period.

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