

Aim and Scope

The objective of the *Journal of Residuals Science & Technology* is to provide a forum for technical research on the management and disposal of residuals from pollution control activities. The Journal publishes papers that examine the characteristics, effects, and management principles of various residuals from such sources as wastewater treatment, water treatment, air pollution control, hazardous waste treatment, solid waste, industrial waste treatment, and other pollution control activities. Papers on health and the environmental effects of residuals production, management, and disposal are also welcome.

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Selected Papers Presented during the IWA-Sludge Conference Held in Harbin, China on August 8–10, 2009

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MANAGEMENT of residual sludge has become a significant issue in our global environment. Production of sludge from wastewater treatment plants has been continuously increasing worldwide for several years as well as stringent environmental regulations.

Due to rapid urbanization and population growth we have witnessed continued intensification of many serious environmental issues, including degradation of water quality. For example, China is making massive investments in water and wastewater treatment over the upcoming decades. New and upgraded treatment facilities will improve water quality there. They will also lead to large amounts of sludge generation, and this prospect is now being addressed with urgency.

We must work together toward sustainable approaches to solving environmental issues worldwide in order to stay ahead of the urbanization and population “exponential growth” curves.

This special issue of the *Journal of Residuals Science and Technology (JRS&T)* gathers some of the papers presented during the IWA-Sludge Conference held in

Harbin, China on August 8–10, 2009. Five categories of manuscripts provided from the IWA-sludge Conference include:

1. Sludge treatment technologies—thickening, dewatering, stabilization, and minimization
2. Innovative technologies in sludge treatment and management
3. Sludge disposal and reuse as a resource
4. Sludge characteristics and analysis methods
5. Land application controversies and solutions

Due to the number of articles provided from the IWA-Sludge Conference, this issue of the *JRS&T* will be quickly followed by the next issue which will contain a continuation of the IWA-Sludge Conference 2009 articles in addition to independently submitted articles. I would like to especially thank Dr. Steve Dentel from the University of Delaware and Dr. Goren Xu from the Harbin Institute of Technology for gathering the IWA-Sludge Conference articles for this special edition.

Can Capillary Suction Time be an Indicator for Sludge Disintegration?

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ABSTRACT: Sludge disintegration is a tool for sludge minimization goal. Sludge disintegration (or sludge pretreatment) principally converts the waste activated sludge into a more bioavailable form prior to anaerobic digestion and contributes to the enhancement of anaerobic digestion. The indicator of success for sludge disintegration is generally taken as the concentration of released organics to the liquid phase. There are different examples of expressing the success of disintegration; such as degree of disintegration, soluble COD release, ratio of soluble to total COD, the released sCOD per unit amount of solids present or constituents of released sCOD. Capillary suction time (CST) is a simple method used to measure dewaterability of sludge samples. Relationship between dewaterability measured by CST and a number of sludge properties such particle size, bound water content, etc. has been shown in the past. Therefore, this study investigates whether CST can be used to express the effectiveness of sludge disintegration. For this purpose a number of sludge pretreatment techniques were tested and correlations between CST and sCOD were analyzed. Results show that CST can act as a good indicator of disintegration for some pretreatment methods but is not useful for indicating the disintegration levels if any of the pretreatment method involves some sort of conditioning.

1. INTRODUCTION

SLUDGE minimization concept found wide application lately due to increasing quantities of sludge and absence of a reliable sludge disposal/reuse alternative. Sludge disintegration (or sludge pretreatment) acting as a tool for sludge minimization, aims to convert the waste activated sludge into a more bioavailable form prior to anaerobic digestion and contribute to the enhancement of anaerobic digestion (Müller, 2001). With this approach, hydrolysis step of anaerobic digestion is achieved externally using disintegration processes; therefore the rate-limiting step is eliminated up to a certain extent. In addition, biogas production and volatile solids reduction increase, amount of sludge to be disposed of and bulking and foaming decrease and in many cases sludge sterility improves (Müller *et al.*, 2004; Müller, 2001; Panter, 2002; Müller *et al.*, 1998).

There is significant number of studies reported and a number of ongoing research is still present in literature about sludge disintegration. Since the main idea behind

sludge disintegration is disturbing the formerly synthesized sludge flocs and bacterial cells, the indicator of success for sludge disintegration becomes the concentration of soluble organics released into the liquid phase. Commonly this release has been expressed either by degree of disintegration (Tiehm *et al.*, 2001), or measurement of soluble COD (Tiehm *et al.*, 1997), or solubilization factor (F_{sol}) i.e. taking the ratio of soluble to total COD (Baier and Schmidheiny, 1997), or normalizing the released sCOD with the solids concentration present in the system (Apul 2008).

Since there are practical disadvantages of other empirical parameters used in expressing the level of disintegration, this study investigates the usefulness of an alternative indicator for sludge disintegration during sludge pretreatment: Capillary Suction Time (CST). CST is not necessarily evaluated as a total substitute for sCOD but rather a supporting indicator for it. CST is an empirical test to analyze the dewaterability of sludge with many practical advantages. The test can simply indicate the dewaterability of the sample in short analysis periods. The CST test has been standardized and introduced into the “Standard Methods for the Examination of Water and Wastewater” as a sludge-related param-

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ter (APHA, AWWA, WEF, 2005). The advantages of CST test as a Standard Method such as less time consuming nature, reliability and ease of conduct made the test a desirable parameter for dewaterability (Vesilind, 1988).

Previously a correlation between dewaterability and particle size has been shown for sludge (Karr and Keinath, 1978; Vesilind, 1988). The smaller particle sizes, especially micron sized particles were shown to negatively affect the dewaterability. Since sludge disintegration methods disturb the sludge floc structure and cause cell break-up, there is an expected decrease in particle size distribution and a sharp increase in CST. King and Forster (1990) showed the relationship between disintegration by sonication and CST values; the decrease of mean particle size in pretreated sludge caused an increase in CST accordingly. On the other hand, sludge dewaterability is also known to be a function of extracellular polymeric materials (EPS) (Eriksson and Hardin, 1984) and the bound water content of sludge (Vesilind, 1994). Both the EPS and the bound water of sludge correlate negatively with sludge dewaterability; i.e. as their content in sludge increase, the dewaterability deteriorates. EPS constituting the 80% of cell mass, and largely being composed of organic matter such as polysaccharides, proteins and lipids have great potential for degradation during sludge pretreatment and further biodegradation during anaerobic digestion. The release of bound water from sludge is indicated to improve the dewaterability measured by CST (Panter, 2002). From these information, it seems that CST may be used to indicate the effectiveness of the pretreatment method pretty well. Therefore, this study aims to investigate the possibility of CST to evaluate the success of pretreatment applications acting as a good indicator of sludge disintegration. The study also aims to obtain the correlation of CST and sCOD.

Chemical, thermal, mechanical and various combinations of these pretreatment methods are examined. Methods working with different principles were selected on purpose for this investigation: acidic and alkaline pretreatment constituted chemical methods; ultrasonic pretreatment constituted mechanical method; and microwave pretreatment constituted the thermal method. Besides the application of separate methods, combinations of them were also investigated in order to observe the effects of different parameters such as temperature and pH acting together and help in explaining the mechanisms.

2. MATERIALS AND METHODS

2.1. Sludge Samples

Waste activated sludge (WAS) samples were supplied from the recycle line of activated sludge tank of Ankara Central Wastewater Treatment Plant. The MLSS and MLVSS concentrations are in the range of 5.5 and 4.0 g/L, respectively. Once in the lab, activated sludge samples were kept aerated until their use. The maximum storage time of samples was not allowed to be longer than 3 days; after which all samples were discarded.

2.2. Pretreatment

Chemical Pretreatment by Acid and Base

During chemical pretreatment 1N and 2N of HCl or NaOH solutions were used to adjust the pH of the samples to 1.5, 2.5, 4.5, 10, 11, 12 and 12.5. The reference was taken as the target pH value rather than the added acid or base quantity due to the fact that for each sampling, sludge may have slightly different buffering capacities. After the desired pH was adjusted, sludge samples were kept stirring for 30 minutes of treatment time.

Mechanical Pretreatment by Sonication

WAS was sonicated at different sonication times and intensities with a Labsonic P (Sartorius, Germany) sonicator operating at a maximum power of 400 W via two different ultrasonic probes with 14 mm and 22 mm diameters. In this study, 14 mm probe was nominated as mild and 22 mm probe was nominated as strong sonication. The details of sonication system are provided in Table 1.

Thermal Pretreatment by Microwave

Microwave pretreatment was conducted with Berghof, MWS-2 microwave system having a maxi-

Table 1. Specifications and Test Conditions of Mild and Strong Sonication.

	Mild Sonication	Strong Sonication
Sonication Frequency (kHz)	24	24
Sonication Density (W/mL)	0.2	0.51
Sonication Intensity (W/cm ²)	2.9	4.8
Temperature Control	No	No
Time of Sonication (min.)	1, 3 or 5	5, 10, 15, 20, 25 or 30

imum temperature of 220°C, maximum power of 1000 W, maximum pressure of 40 bars and the frequency of 2450 MHz. The applied average microwave treatment temperature was 160°C and the power applied was 600 W. In each batch 10 teflon vessels were loaded with 20 mL of sludge each and microwave irradiation was applied for 16 minutes.

Combined Pretreatment Methods

The combination of microwave with alkali and combination of ultrasound with acid treatments were investigated by applying simple combinations of the above-explained methods.

2.3. Analytical Methods

To evaluate the efficiency of disintegration sCOD, CST, MLSS and MLVSS concentration analysis were conducted.

MLSS and MLVSS analyses were conducted by following the Standard Method 2540D and 2540E procedures, respectively (APHA, AWWA, WEF, 2005).

CST analyses were conducted according to Standard Method 2710G (APHA, AWWA, WEF, 2005). *Triton Electronics Ltd. Type 304M Capillary Suction Timer* was used during the experiments.

HACH DR2000 spectrophotometer and *HACH low range (0–150 mg COD/L) and high range (0–1500 mg COD/L) digestion kits* were used for soluble COD determination. In COD analyses, United States Environmental Protection Agency (USEPA) approved dichromate COD method was followed (Jirka and Carter, 1975).

3. RESULTS AND DISCUSSIONS

Pretreatment methods applied in this work differ widely from each other, so they create different environmental conditions hence different impacts on sludge. The characteristics of sludge are strongly dependent on temperature, pressure and pH on the micro and the macro scale therefore each method has different bearings for cells and flocs. The results reflect these differences and influence the outcome of CST.

Figure 1 illustrates the effect of alkali and acidic pretreatment on both CST and sCOD and the plotted values are the % increase of the parameters compared to their untreated condition. As seen from the alkali pretreatment data, CST and sCOD show a nice correlation with increasing pH. As the pH is increased, sCOD in-

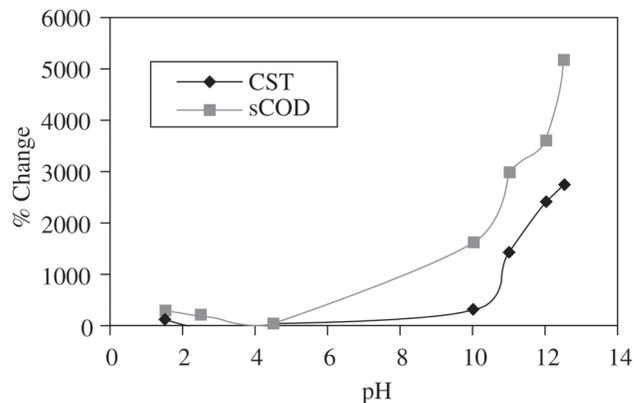


Figure 1. Percent change of CST and sCOD after acid and alkali pretreatment.

creases indicating the solubilization of cells and EPS. Parallel to this, the CST values also increase. Similarly when the pH is decreased by acidic pretreatment sCOD values increase indicating again the release of soluble materials from the flocs. However, the increase this time is not as remarkable as alkali application. The correlation between CST and sCOD is good as well, except for pH 2.5 which shows an irregular decrease of CST around 25% even with repeated trials. The reason of this decrease can be explained by the isoelectric point of sludge, since the isoelectric point of sludge lies between pH 1 and 3 (Forster, 1971), acid (i.e. hydrogen ions) act as a conditioner at this point and dewaterability of sludge increase. According to Neyens *et al.* (2003) at pH 1, a lower CST than the untreated sample was recorded similarly.

Figure 2 illustrates the % change of CST and sCOD for mechanically treated sludge by sonication. The graph on the top gives the sCOD release by low power sonication probe and the one on the bottom by high power probe. Here it is not possible to see a correlation, which is obvious in chemical pretreatment. Mild sonication influences the dewaterability of the sample significantly, however sCOD concentrations are not affected proportionally. These results show that mild sonication is able to disturb the sludge floc structure but is not able to release soluble COD into liquid phase. Mild sonication as in the case of acidic pretreatment cannot be counted as an effective disintegration method. In strong sonication, on the other hand, sCOD changes are remarkable but CST changes become insignificant. Strong sonication is known to be an effective method of sludge disintegration and the sCOD results clearly show that. The CST decrease however, is unexpected. This is possibly because of conditioning

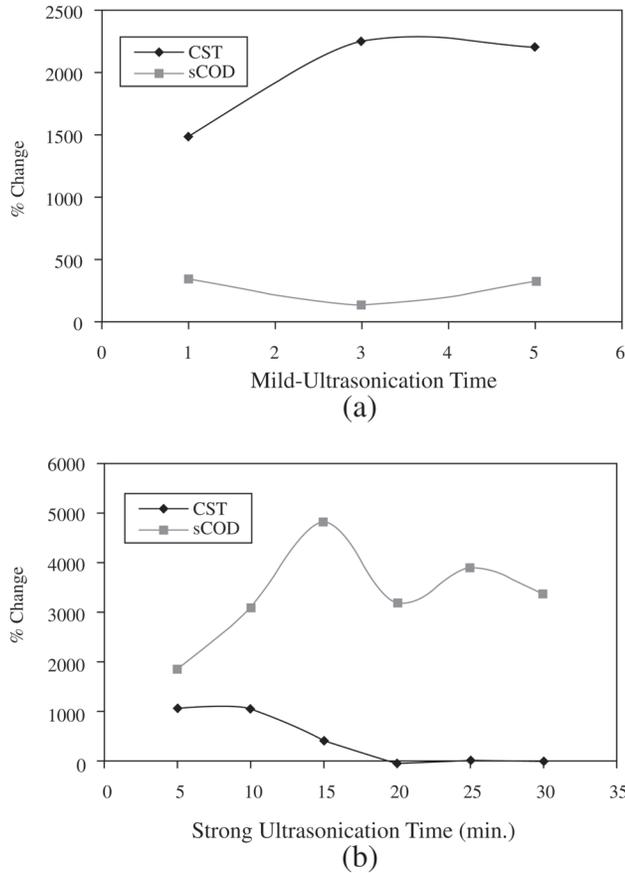


Figure 2. Percent change of CST and sCOD after mild (a) and strong (b) ultrasonic pretreatment.

effect of long sonication times due to increasing temperatures. King and Forster (1990) showed that sonication can be used as a conditioning treatment technique since thermal sludge treatment is used to enhance the sludge dewaterability (Weemaes and Verstraete, 1998). The temperature profile obtained in our work depending on sonication time can be seen in Figure 3. As temperature rises up to 80°C, CST increase becomes less and less significant compared to sCOD change for strong sonication.

As seen in Figure 4(a) combination of microwave and alkali pretreatments show a very nice synergy in constituting a very effective treatment. Combination of the two effective methods created an even more effective technique with the increase in sCOD indicating higher cell/floc breakup. This finding qualifies the combined method to be an effective treatment for subsequent anaerobic digestion due to the release of high sCOD (Dogan and Sanin, 2009). The CST values on the other hand, decrease with increasing pH in the combined method. The thermal effect of microwave does not allow for a sharp increase in CST. It acts as a condi-

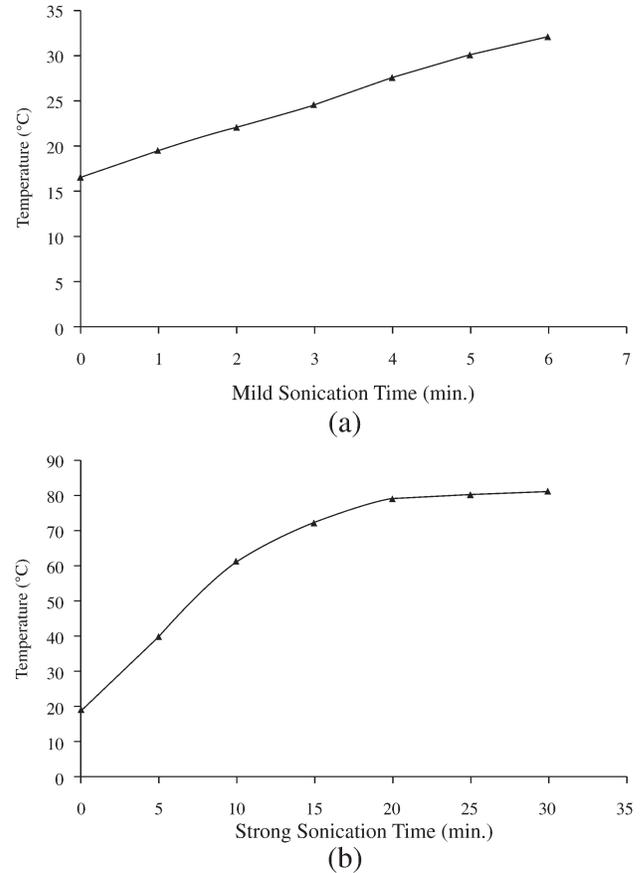


Figure 3. Temperature profile for waste activated sludge after mild (a) and strong (b) sonication.

tioning method for high pH imposed samples. On the other hand, the combination of acid and mild sonication [Figure 4(b)] was not able to release much sCOD especially when compared to MW and alkali combination. The sCOD increases are not to the synergistic levels. The acidic pretreatment had a very little effect in enhancing the dewaterability of mildly sonicated sludge but this was not significant. The observed sCOD release showed no parallelity to measured CST values.

3.1. The Correlations Between CST and sCOD

The correlations between CST and sCOD show great parallelity in pH related pretreatments with minor fluctuations (especially the one at pH 2.5); change in CST and change in sCOD show similar response in almost all pH values including the low and high pH ranges. The difference between the low and high pH ranges was the change induced in both CST and sCOD were minimal at values lower than pH 7 and sharply increasing with increasing pH above this level.

This correlation behavior was neither observed in

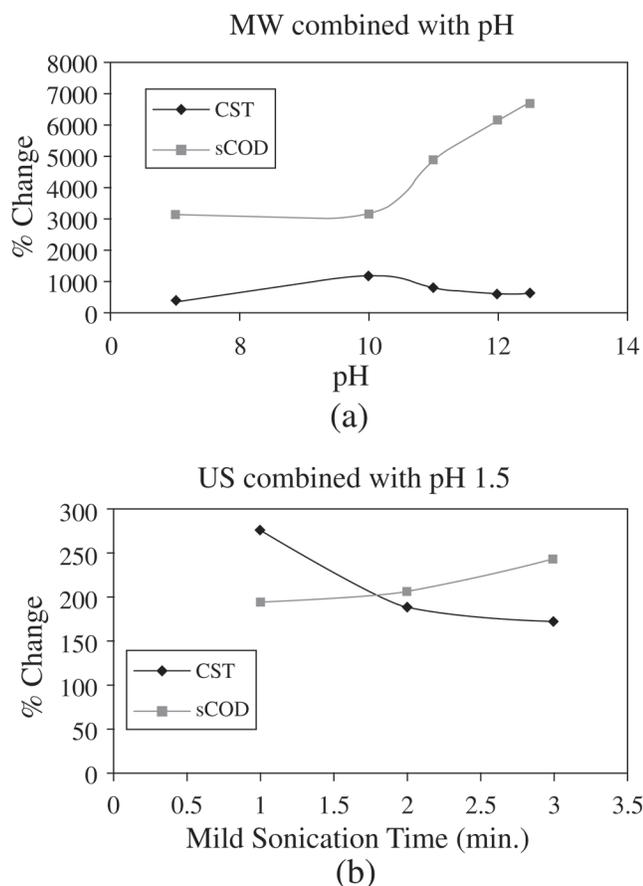


Figure 4. Percent change of CST and sCOD after combined pretreatment methods.

solely sonicated samples nor in combined applications. The reasons are discussed here one at a time. With the use of mild ultrasonication, the change induced on sCOD was very minimal at about the same level induced by the acidic pH range. The change in CST is sharper on the other hand, is at much higher levels and increasing with increasing sonication time.

These results indicate mild sonication, even though disturbs the floc structure and worsens the CST, it is unable to break the cells and solubilize the components. Therefore, there seems no correlation between the sCOD and CST.

In the case of strong sonication, there is a significant solubilization in COD indicated by a sharp percent change in sCOD. This qualifies the strong sonication as an effective method. However, CST data is not parallel to this. Rather than an increase, the percent change in CST decreases after 10 minutes of sonication. This trend can be explained by the heating effect of sonication especially in the strong sonication mode. As

shown in Figure 3, at 10 minutes the temperature is around 60°C, then at 15 and 20 minutes it increases to 70 and 80°C, respectively. This increase in temperature along with longer sonication times induces thermal conditioning effect and decreases the CST.

When the combined methods are examined, it can be seen that the parallelity of sCOD and CST is lost here too. The microwave application when combined with high pH values, increases the sCOD significantly. On the other hand the changes in CST remain rather low, and indicate first an increase and then a decrease after pH 10 (combined with microwave). This decrease is especially due to the sharp increase in CST with the high pH application (only alkali treatment) and significant improvement induced due to the addition of microwave treatment along with the alkali treatment.

When the mild sonication application is combined with pH 1.5 the change in sCOD and CST are insignificant compared to the ones achieved with combined microwave and alkali treatment. It seems that the trends in sCOD increase and CST decrease indicate that these two parameters do not behave parallel.

As the last step, relative changes of both sCOD and CST were calculated by measuring each parameter after pretreatment and calculating its % change compared to its untreated counterpart. Then the ratio of ‘% change’ of CST to ‘% change’ of sCOD was calculated. For each particular method, average, standard deviation and coefficient of variance (COV) of the calculated ratios were found. Table 2 summarizes the calculated COV for relative changes of CST and sCOD parameters for each pretreatment. When COV values are investigated alkali pretreatment seems to have the smallest value, which indicates that there is a relatively consistent correlation between CST and sCOD. Other methods show varying degrees of higher COV values. Different from other pretreatment methods, alkali pretreatment have no conditioning effects, therefore the correlation is relatively higher.

Table 2. Coefficient of Variance for Correlation Factors of Pretreatment Types.

Pretreatment Type	COV for Correlation Factors (%)
Acidic	119
Alkali	42
Mild Sonication	64
Strong Sonication	139
Microwave + Alkali	63
Sonication + Acid	130

4. CONCLUSIONS

Results point out that CST can be a good indicator of success of pretreatment for most pH based methods. Measured CST and sCOD values showed parallel trends for low pH (except for pH 2.5) and high pH treatments. On the other hand, CST does not seem to be as useful for indicating the disintegration levels in any of pretreatment method involving conditioning. Not only surface charge neutralization due to acid addition but also thermal conditioning during microwave treatment or ultrasonication enhances the dewaterability of sludge significantly. Therefore after pretreatment CST values and sCOD values do not show a consistent correlation. On the other hand when alkali pretreatment is investigated there is a promising correlation and the ratio between percent changes in CST to percent change in sCOD is 0.42 ± 0.20 . As a result, simply by using the CST parameter, level of disintegration can be found with certain amount of error after alkali pretreatment.

Another result of these data interpretations is that correlation factors are unique for different pretreatment methods and cannot be used without examining the characteristics of pretreatment methods.

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Characteristics of Different Extracellular Polymeric Substances (EPS) Fractions of Sludge Flocs from Brewery Wastewater Treatment Plant (WWTP)

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ABSTRACT: Distributions of extracellular enzymes, proteins (PN), and polysaccharides (PS) in the different extracellular polymeric substances (EPS) fractions of sludge flocs affect the wastewater treatment capability. The results showed that PN, PS, and extracellular enzyme had a distinct distribution pattern in the different EPS fractions. Both fourier transform infrared spectroscopy (FT-IR) and gel permeation chromatography (GPC) further suggested the difference of EPS fractions. These results suggest that by changing the distribution pattern of PN, PS, and extracellular enzyme, with some pretreatments before aerobic/anaerobic digestion, the performance of brewery wastewater treatment may be improved.

1. INTRODUCTION

IN China, there are several thousands of breweries (Fang *et al.*, 1990). For each cubic meter of beer produced, these plants generate about 20–30 m³ of effluent, which is much higher than those of the developed countries (Fang *et al.*, 1990; Wang *et al.*, 2007). Understanding the characteristics of brewery sludge will help to improve their treatment efficiency.

Extracellular polymeric substances (EPS) represent the major organic fraction and determine the structure, integrity and strength of sludge flocs (Park and Novak, 2007). Yu *et al.* (2008a) had showed that EPS had different fractions in sludge flocs and thus could be fractionated by the gravity settlement and centrifugation as well as ultrasound approaches. Specifically, EPS of sludge flocs possess a multi-fraction structure, i.e. supernatant, slime, loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS). The cells in the residue after EPS extraction form the pellet fraction (Yu *et al.*, 2007 and 2008a-d). Distributions of extracellular enzymes, proteins, and polysaccharides in the different EPS fractions of sludge flocs affect the wastewater treatment performance. Several investigators had shown that the disruption of EPS matrix can enhance

the rate and extent of the hydrolysis and acidification (Park and Novak, 2007; Yu *et al.*, 2008b).

In this study, sludge flocs were achieved from a brewery wastewater treatment plant (WWTP). The aim of this study was to understand the characteristics of brewery sludge from both macro- and micro-view levels in order to improve the process performance. Both fourier transform infrared spectroscopy (FT-IR) and gel permeation chromatography (GPC) were applied to explore the difference of organic composition from a micro-view level. Protease, α -amylase and α -glucosidase were reported to play essential roles in the hydrolysis of two major fractions of EPS: protein and polysaccharides (Goel *et al.*, 1998). Alkaline phosphatase hydrolyzes phosphomonoesters to provide an alternative source of phosphorus for the cells, whereas acid phosphatase was reported to be involved in internal cell metabolism (Kloeke and Geesey, 1999). Therefore, these five extracellular enzymes were selected for this study.

2. MATERIALS AND METHODS

2.1. Sludge Samples

Activated sludge sample was collected from the aerated basin of a full-scale brewery WWTP in Shanghai, China. The plant treats brewery wastewater using

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upflow anaerobic sludge blanket (UASB) process. The collected samples were transported to laboratory within 2 h after sampling. The sludge was first settled for 1.5 h at 4°C. The bulk solution was collected as supernatant, whereas the sludge sediments were collected and screened through a 1.2 mm screen with characteristics listed in Table 1. As shown in Table 1, soluble organic matter in sludge was lower than 1%. Meanwhile, PN and PS were the two major types of organic components in sludge, accounting for approximately 12.7% and 1.2% of volatile suspended solids (VSS), respectively.

2.2. Fractioning Protocol

Sludge fractioning protocol (Figure 1) was modified according to the method previously described by Yu *et al.* (2007 and 2008a). In brief, the bulk solution after settlement was collected as supernatant. Subsequently, one part of the settled sludge samples (Table 1) was re-suspended to their original volume using pH 7 buffer solution consisting of Na_3PO_4 , NaH_2PO_4 , NaCl and KCl at a molar ratio of 2:4:9:1 (Frølund *et al.*, 1995). The conductivities of the buffer were then adjusted through the distilled water to match those of the screened sludge samples (Table 1). Then, the re-suspended sludge samples were centrifuged at 2,000g for 15 min. The bulk solution was collected as the slime. The collected bottom sediments were re-suspended to

Table 1. Characteristics of Sludge Sediment Sample.

Parameter	Content
pH	7.5 ± 0.1
TSS (mg L ⁻¹)	38.2 ± 0.7
VSS (mg L ⁻¹)	8.3 ± 0.2
COD (mg L ⁻¹)	13800 ± 400
SCOD (mg L ⁻¹)	124 ± 21
Proteins (mg g ⁻¹ VSS)	127 ± 27
Polysaccharides (mg g ⁻¹ VSS)	12 ± 2
Conductivity (μS cm ⁻¹)	285
CST (s)	12.5
Volume Weighted, D[4,3] (μm)	134

their original volume using the abovementioned buffer solution. Next, the suspensions were centrifuged again at 5,000g for 15 min with the bulk solution and solid phase collected separately. The organic matter in the bulk solution was the LB-EPS. The collected sediments were re-suspended again with the aforementioned buffer solution to the original volumes and then extracted using ultrasound at 20 kHz and 480 W for 10 min. The extracted solutions were centrifuged at 20,000g for 20 min. The organic matter in the bulk solution was the TB-EPS, while the residues (solid phase) re-suspended again with the aforementioned buffer solution to the original volumes were the pellets. After all the EPS fractions being extracted, membranes of 0.45 μm polytetrafluoroethylene (PTFE) (Shanghai Mosu Scientific Equipment Co., China) were used to filter out the particulates present in the supernatant, slime,

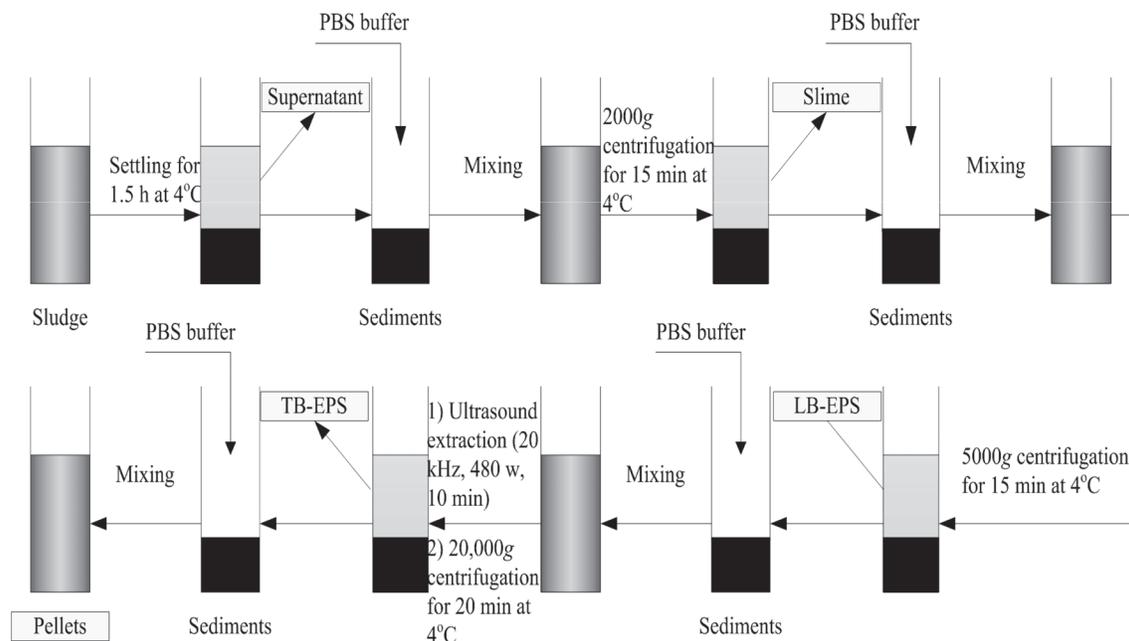


Figure 1. Fractioning protocol of sludge flocs.

LB-EPS and TB-EPS solutions. All the membranes before applied were ultrasonicated for 10 min in a water-bath type of ultrasonic equipment (KQ-300 DE, Kunshan ultrasonic equipment Co., Ltd, China), cleaned by Milli Q water, and dried in a 40°C constant temperature incubators (HHB11500-BS-II, Shanghai Yuejin Medical Instrument Factory, China).

2.3. Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

The extracted different EPS fractions from sludge flocs were lyophilized at -50°C for 48 h (Yu *et al.*, 2007). One milligram of freeze-dried EPS extraction fraction was mixed with approximately 60 mg of potassium bromide (KBr, IR grade). The IR spectra analysis of freeze-dried EPS fractions was then performed on a spectrometer (EQUINOXSS/HYPERION 2000, Bruker, Germany).

2.4. Gel permeation Chromatography (GPC) Analysis

The molecular weights (MW) of different EPS fractions were determined by a GPC (LC-10ADVP, Shimadzu, Japan) equipped with a differential detector (RID-10A) and a TSKgel column (G4000PWXL, TOSOH Co., Japan). The mobile phase was Milli-Q water. Polyethylene glycol/oxides (MW at 1169 kDa; 771 kDa; 128 kDa; 12 kDa; 4 kDa; 620 Da; 194 Da) were used as reference molecules for the calculation.

2.5. Analytical Techniques

Both the chemical analyses and enzymatic assays were carried out in triplicates. Protease activity was analyzed using the Lowry method (1951) with casein as the standard. The α -amylase activity was determined using the Bernfeld method (1955) and glucose was the standard. Alkaline phosphatase and acid phosphatase activities were performed according to Goel *et al.* (1998) with *p*-nitrophenylphosphate di-sodium salt (Sigma N 3254) as the standard.

All chemical analyses were carried out using chemicals of analytical grade. PN was determined by the modified Lowry method (Frølund *et al.*, 1995), using casein (Shanghai Sangon Biotechnology Co., Ltd, China) as the standard. PS was measured by the Anthrone method (Gaudy, 1962), with glucose as the standard. The COD of the filtrate was referred to as

SCOD. The SCOD analyses were done using HACH DR/2000 Spectrometer. The conductivity was determined by a conductivity meter (DDSJ-308A, Leici Co., Ltd, Shanghai, China). The CST of the sludge flocs was measured with a CST instrument (Model 319, Triton, UK) equipped with an 18-mm diameter funnel and Whatman no. 17 chromatography—grade paper. Other sludge parameters, including total suspended solids (TSS) and VSS, were analyzed following the standard methods (APHA *et al.*, 1998).

3. RESULTS AND DISCUSSION

3.1. PN and PS Distributions in the Different EPS Fractions and Sludge Flocs as a Whole

PN and PS are predominant in sludge flocs and represent most of organic matters (Yu *et al.*, 2007 and 2008a-d). Figure 2 shows the PN and PS distributions in the different EPS fractions and sludge flocs as a whole. It was noted that PN was the predominant component in sludge flocs when compared with PS. Among the different EPS fractions for brewery sludge, PN was occurred mainly (about 94.8%) in the TB-EPS and pellet fractions, with a minor percentage (about 5.2%) detected in the supernatant, slime and LB-EPS fractions. However, PS was dispersed nearly uniformly throughout the different EPS fractions (Figure 2).

The distribution pattern of PN was correlated with Sponza (2002) and Xavier *et al.* (2007) which concluded that PN constituted mostly at the core fractions of sludge flocs. In addition, the distributions of PN and PS in this study were consistent with the observa-

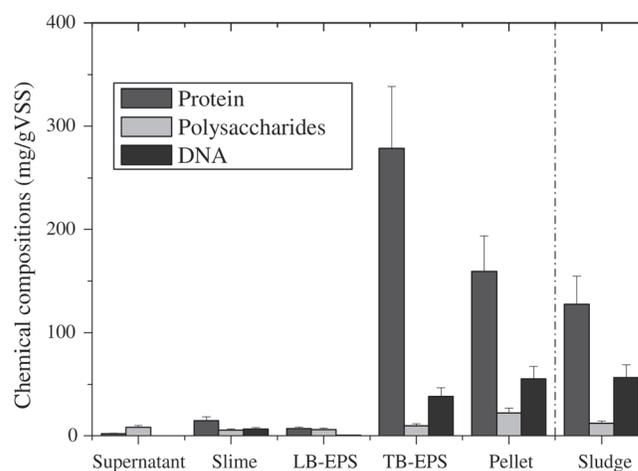


Figure 2. Distributions of proteins, polysaccharides and DNA in the different EPS fractions and sludge flocs as a whole. (Note: error bars represent standard deviation).

tions of Yu *et al.* (2008b) that they were predominant in the pellet and TB-EPS fractions, i.e. 84.6% for PN and 87.1% for PS, fewer in the LB-EPS and slime fractions, and virtually not in bulk solution.

DNA content in the different EPS fractions and sludge flocs as a whole ranged from 0 to 56.6 mg/gVSS (Figure 2), mainly presenting in the pellet and TB-EPS fractions. Hence, cellular lysis was minimal during most fractionation processing.

3.2. FT-IR Spectra of the Different EPS Fractions

FT-IR spectrum is a routine chemical technique used to study molecular structure (Naumann *et al.*, 1991). Figure 3 illustrates FT-IR spectra in the different EPS fractions of sludge flocs from brewery WWTP. Peaks at 3378–3446 cm^{-1} (OH), 1640–1671 cm^{-1} (CO, CN), 1540 cm^{-1} (CN, NH), 1110 cm^{-1} (OH), and 1047 cm^{-1} (OH) presented in the different EPS fractions, indicating that PN (1640 cm^{-1} , 1540 cm^{-1}) and PS (3378–3446 cm^{-1} , 1110 cm^{-1} , and 1047 cm^{-1}) (Schmitt and Flemming, 1998; Kim and Jang 2006; Comte *et al.*, 2006; Yu *et al.*, 2007) were distributed in every EPS fractions of sludge flocs. These peaks were also corre-

lated with the compositional analysis presented in Figure 4. It is noteworthy that the number of peaks presented in the slime and LB-EPS fractions was more than that of TB-EPS and pellet fractions. These peaks, including 1924 (C=O), 837 and 711 (aromatic groups), seemed to suggest that most of pollutants were adsorbed in the slime and LB-EPS fractions. Therefore, it could be inferred that the slime and LB-EPS fractions could act as a protective barrier for microorganisms embedded in the EPS matrix, and meanwhile the adsorption of pollutants by the slime and LB-EPS fractions may be a key first step in their biodegradation.

Ramesh *et al.* (2006 and 2007) investigated the soluble microbial products (SMP, i.e. the sum of supernatant, slime, and LB-EPS in this study), LB-EPS (i.e. partly TB-EPS in this study) and TB-EPS (i.e. partly TB-EPS in this study) of wastewater sludge by FT-IR spectra and found that they were markedly different. However, their studies could not present the FT-IR spectra of supernatant, slime and LB-EPS fractions owing to not further differentiate the supernatant, slime and LB-EPS. To the best knowledge of the authors, FT-IR spectra of different EPS fractions are the first to be reported in this study.

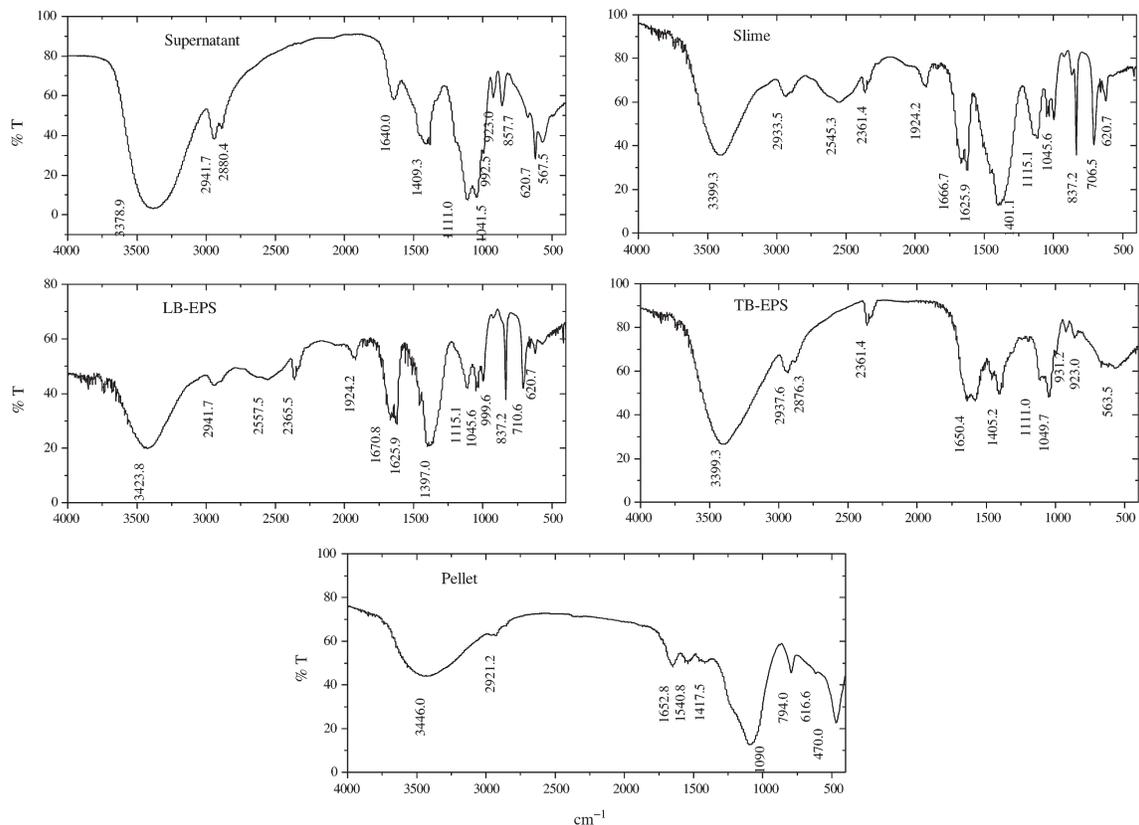


Figure 3. FT-IR spectra of different EPS fractions extracted from brewery sludge flocs.

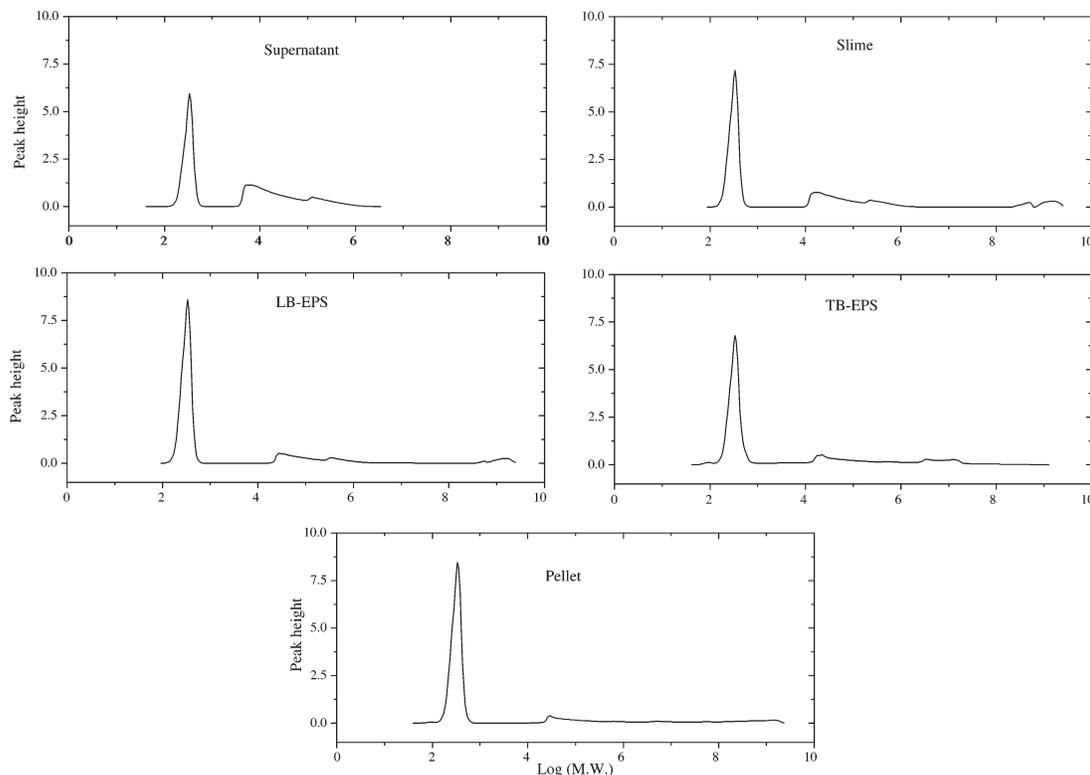


Figure 4. GPC chromatography of different EPS fractions extracted from brewery sludge flocs.

3.3. GPC Chromatography of the Different EPS Fractions

The study of the chromatographic fingerprints and molecular weight (MW) distribution of EPS has opened up a new approach to EPS characterization (Comte *et al.*, 2007). To provide a better understanding of the phenomena observed by FT-IR, the different EPS fractions from brewery WWTP were analyzed by measuring the GPC (see Figure 4). As shown in Figure 4, there was a group of strong peak [$2 < \log(\text{MW}) < 3$] presenting in all the EPS fractions of brewery sludge flocs. This group of peak represented carbohydrates (Shon *et al.*, 2006; Jarusutthirak and Amy, 2007). Two groups of weak peaks [$4 < \log(\text{MW}) < 6$] were detected in the slime and LB-EPS fractions, whereas only one group of weak peak [$4 < \log(\text{MW}) < 5$] was found in the TB-EPS and pellet fractions. This trend convincingly demonstrated that there are much more types of materials in the slime and LB-EPS fractions than those of the TB-EPS and pellet fractions. The phenomenon was validated by the observations of FT-IR spectra (see Figure 3). The two groups of weak peaks were generally attributed to PN, PS or extracellular enzymes (Shon *et al.*, 2006). As well, it is interesting to note that for the or-

ganic matters presented in the supernatant fraction, all the molecular weights were less than 10^7 Da, whereas they were as large as 10^9 Da in other EPS fractions. The results of Yu *et al.* (2009) were similar to those of this study by investigating the GPC chromatography of supernatant, slime, LB-EPS, and TB-EPS of excess sludge from the aerated basin of two municipal WWTPs and further verified the different of MW in the different EPS fractions, suggesting that the GPC chromatography of different EPS fractions is universal and not sludge-specific.

Both FT-IR spectra and GPC chromatography from a micro-view level showed that the EPS fractions had a distinct characteristic. By investigating the 3 dimensional excitation emission matrix (3D-EEM) spectra of SMP, LB-EPS, and TB-EPS, Tsai *et al.* (2008) also indicated that the chemical characteristics of SMP and LB-EPS were similar but not identical, whereas the TB-EPS and LB-EPS exhibited markedly different characteristics. Restate, the fractionation protocol of EPS fractions in Tsai *et al.* (2008) was different from that of this study. These findings strongly revealed that EPS should be differentiated into the different fractions despite of no standard procedures for EPS fractionation in sludge flocs.

3.4. Extracellular Enzyme Distributions in the Different EPS Fractions and Sludge Flocs as a Whole

Extracellular enzymes play a crucial role in the biological processes (Tcuber and Brodisch, 1977). Measurement of extracellular enzymes is an alternative method to assess microbial biomass and activity (Nybroe *et al.*, 1992). Figure 5 depicts the activities of protease, α -amylase, α -glucosidase, alkaline phosphatase and acid phosphatase in different EPS fractions and sludge flocs as a whole. It was found that in the brewery sludge flocs, protease, alkaline phosphatase and acid phosphatase were mainly distributed in the pellet and TB-EPS fractions, i.e. bound with cells, and almost none in the LB-EPS, slime and supernatant fractions. As for α -glucosidase, it was mainly distributed in the pellet fraction, few in the TB-EPS, LB-EPS, slime and supernatant fractions. However, α -amylase was almost uniformly distributed in the different fractions of sludge flocs. The results of enzyme distributions in brewery sludge flocs were consistent with the previous observation (Yu *et al.*, 2008b-d and 2009).

In addition, the gross activity (supernatant + slime + LB-EPS + TB-EPS + pellet) of the five extracellular enzymes was markedly higher than that of sludge flocs as a whole, with α -amylase predominating in all the EPS fractions, followed by alkaline phosphatase, acid phosphatase, protease and α -glucosidase. The results suggested that the enzymes which were originally embedded in the sludge flocs could be released with the extraction process. Therefore, the pretreatment method

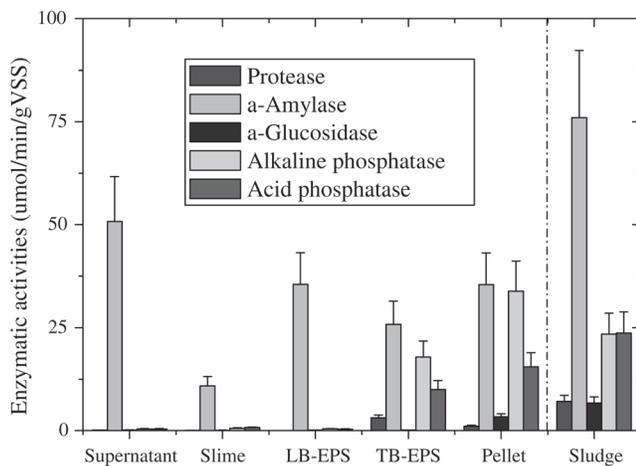


Figure 5. Distributions of extracellular enzymes of different EPS fractions and sludge flocs as a whole. (Note: error bars represent standard deviation).

could result in improved efficiency of aerobic/anaerobic digestion by increasing the contact and interaction among extracellular proteins, polysaccharides and enzymes (Yu *et al.*, 2008b).

The present results of enzyme distributions were similar to those of previous studies (Yu *et al.*, 2008a-d), revealing that the enzyme activity distributions of the studied five enzymes were found universal and not wastewater-specific. On the other hand, more than the 99% brewery sludge was found to be the particulate organic matters (Table 1). Yu *et al.* (2008b) had shown that the pretreatment could change the distribution pattern of enzymes and organic matters and released these enzymes and organic matters into solution. Therefore, the brewery wastewater treatment performance may be improved by adopting some pretreatment before aerobic/anaerobic digestion.

4. CONCLUSIONS

Our results provided a systematic distribution of PN, PS, and extracellular enzymes in brewery sludge flocs. PN was the predominant component in brewery sludge when compared with PS. Among the different EPS fractions for brewery sludge, PN was occurred mainly (about 94.8%) in the TB-EPS and pellet fractions, with a minor percentage (about 5.2%) detected in the supernatant, slime and LB-EPS fractions. However, PS was dispersed nearly uniformly throughout the different EPS fractions. Enzyme distribution showed that α -amylase had a distinct distribution pattern with alkaline phosphatase, acid phosphatase, protease, and α -glucosidase. Both FT-IR spectra and GPC chromatography suggested that there were much more types of materials in the slime and LB-EPS fractions than those in the TB-EPS and pellet fractions.

All the results of chemical composition, FT-IR spectra, GPC chromatography, and extracellular enzymes showed from the micro- and macro-view levels that the EPS fractions had a distinct property. These findings strongly reveal that EPS should be differentiated into the different fractions despite of no standard procedures for EPS fractionation in sludge flocs. In addition, a better understanding of the distribution of PN, PS, and, extracellular enzymes in the brewery sludge flocs should offer a more thorough understanding of biological processes, and possibly lead to even higher removal efficiencies or better control over the process.

ACKNOWLEDGEMENTS

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Characteristics of Ecological Stabilized Sewage Sludge (I)

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ABSTRACT: Ecological stabilization pilot scale experiment was conducted for wastewater sludge treatment. The study was performed over the period from June to November in 2005 and from May to November in 2006 on a bed with 80 m², which located in the third wastewater treatment plant, Changchun city, China. The system left spare in 2007 and 2008. The experiment investigated sludge stabilization degree from the aspects of sludge fluid state, organic matter degradation rate and microbial activity during four years ecological treatment. After two years running and two years resting, sludge moisture decreased from averaged 97% to 30.5%, getting 68.6% of removal; organic matter content decreased from 35–46% to average 10.24%, getting more than 70% of removal. Microbial activity indicated with triphenyltetrazolium chloride—dehydrogenase activity (TTC-DHA) obviously decreased with sludge stabilization time extension, which demonstrated sludge stabilization degree, i.e. longer sludge stabilization time responded to lower TTC-DHA and higher stabilization degree.

1. INTRODUCTION

SLUDGE stabilization is mainly aimed at the organic matter existing in the sludge, which is the process of organic matter turned into inorganic matter through physical, chemical or biological and chemical reactions under certain conditions (Wang and Jia, 2001). While the definition of “stabilized” sludge is not uniformly accepted. The United States use the value of 38% reduction of organic matter as the threshold for considering the sludge stabilization (Koottatep *et al.*, 2001). While sludge composting standards of China are more than 50% of organic matter degradation and less than 65% of moisture.

In China, most of existing wastewater treatment plant has no sludge treatment facilities, most of dewatering sludge are used to land-filling, all of these are mainly caused by the fact of the higher cost for sludge treatment and disposal (Yin and Tan, 2004). Sludge treatment technologies with low investment, operation and maintenance costs like constructed wetlands (Kadlec and Knight, 1995; Cooper *et al.*, 1996; Cui *et al.*, 2008) are promising treatment alternatives.

Constructed wetlands have been used in China since the mid-1990s, and there are now more than 100 plants

in operation for the purposes of municipal and industrial wastewater treatment, except for the sludge-drying reed bed. During last twenty to thirty years, interest has developed in using constructed wetlands (reed beds) to enhance conventional drying bed performance across some countries of Europe, North American and Asia (Cooper *et al.*, 1996; Burgoon *et al.*, 1997; Kengne *et al.*, 2006; Cui *et al.*, 2008).

Wastewater sludge ecological treatment technology was used for sludge stabilization. The core of ecological stabilization is constructed wetland, commonly reed bed. The principal of sludge ecological stabilization for drying the sludge more rapidly than old unplanted system are as follows (Cooper *et al.*, 2004): Stem, rhizomes and roots enhance water drainage by providing channels in depth; Wind-rock produces holes in the sludge surface at the base of stems; Evapotranspiration is enhanced by the presence of leaves; Mineralization.

The aim of this research was to investigate sludge stabilization degree in reed bed after four years operation based on the sludge characteristic parameters including sludge moisture, organic matter content and microbial activity.

2. METHODS

2.1. System Description

A pilot-scale vertical flow constructed wetland with a

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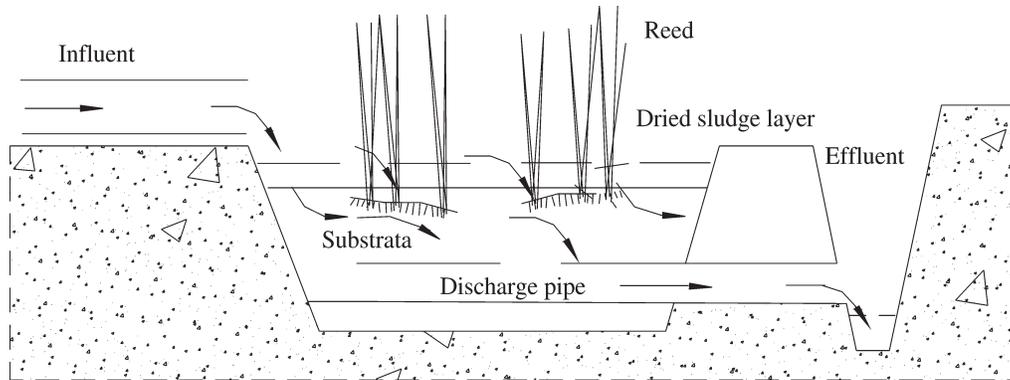


Figure 1. Constructed wetland sludge treatment system.

surface area of 80 m², having 60 cm sand-gravel matrix, supported by ventilated-drainage system and planting with *phragmites australi* (common reed) in 2005, was fed with thickened sludge from Cyclic Activated Sludge Technology (CAST) process, 3rd wastewater treatment plant located in Changchun, a city of the Northeast of China (Figure 1). The substrata in constructed wetland unit comprise a 20-cm layer of large gravel, a 25-cm layer of small gravel, a 10-cm layer of fine sand, and 10-cm layer of coarse sand from bottom to top, while a free board of 0.5 m was allowed for accumulation of dewatered sludge. The drainage system consists of perforated PVC pipes with a diameter of 20 cm on the bottom, ventilation pipes with 10 cm diameter were mounted on the drainage pipes, extending 0.5 m over the matrix surface.

2.2. Operation and Maintenance

Wetland was constructed before June 1, 2005. *Phragmites australi* harvested from nearby natural wetland were replanted in the unit on June 2, 2005 and watered with treated wastewater till plant length of 1.5 m, spending about forty days. Solids loadings were applied to the reed bed for two weeks from July 12 to 25, 2005 under the loading of 10 tons sludge per day (Run I), and then fourteen weeks from August to November 2005 under the loading of 10 tons per four days (Run II). Operated in a vertical flow mode, the sludge was uniformly distributed on the surface of the constructed wetland. The sludge loadings were stopped for the Winter from December and resumed in May 2006.

The second year experiment was conducted from May to November, and was divided into three stages:

Stage 1: May to July; loading rate: 10 tons sludge per four days.

Stage 2: August; loading rate: 15 tons sludge per four days.

Stage 3: September to November; loading rate: 7 tons sludge per four days.

The system left spare in the third year (2007) and the fourth year (2008).

2.3. Analyses

The sludge moisture and organic matter were measured according to Chinese Standard Methods for Examination of Urban Garbage Land Application (2002). Microbial TTC – DHA was measured according to Yin *et al.* (2005).

3. RESULT AND DISCUSSION

3.1. System Start-up in 2005

In Run I, the loaded sludge moisture ranged from 96% to 99.5%, stabilized sludge thickness raised to 6 cm within two weeks. Considering sludge dewatering and further increasing solid content, the experiment was adjusted to low loading rate (Run II).

According to the statistics of stabilized sludge moisture content within each period, the fastest sludge moisture decrease rate was generated at the first day, decreased from 97% to 75%, and to 65% at the fourth day. Loaded sludge did not have significant effect on the bottom layer sludge moisture during the length of the whole start-up experimentation.

3.2. Sludge Fluid State Stabilization

The system was operated from May to November in

2006. Moisture variation in surface and bottom sludge within the loading period were measured for the purpose of investigating moisture variation regularity within loading period and the variation trend with running time extension.

Moisture from different sludge layers had the similar variation trend, moisture decreased with running time within four days loading cycle, with 97% of surface sludge moisture in the first day to 85% in the fourth day, and 60.2% of bottom sludge moisture in the first day to 50.7% in the fourth day.

Figure 2 indicated stabilized sludge moisture variation from May to October in 2007. The system was stopped loading, thus moisture for plant growth mainly came from accumulated sludge in the system.

The research did not get the balance between evapotranspiration and rain, thus used stabilized sludge moisture variation indicating the effect of sludge dewatering. Stabilized sludge moisture in the system had the trend of decrease with time extension, moistures in surface, middle and bottom sludge layers decreased from 61.6% to 34%, from 75% to 35% and from 50% to 34% during May to October 2007. The sludge moisture in different layers in October tended to the same lower value of about 34%, meets the stabilization standard of sludge composting in China, which require less than 65% of sludge moisture.

Based on the analytical results in September, 2008, the stabilized sludge moisture decreased further to average 30.5%, getting more than 68.6% of removal.

3.3. Organic Matter Stabilization

Organic matter contents variation in surface and bot-

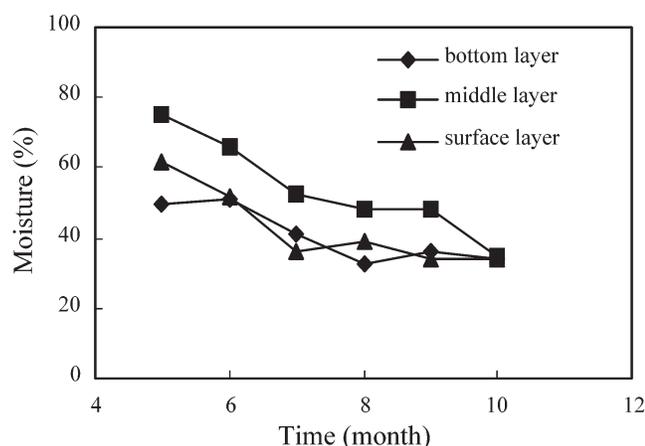


Figure 2. Stabilized sludge moisture variation with time in 2007.

tom sludge within the loading cycle were measured in 2006 for the purpose of investigating organic matter content variation within loading cycle and the variation trend with running time.

Organic matter contents from different sludge layers had the similar variation trend, organic matter contents decreased with running time within four days loading cycle, with 35–46% of organic matter in surface sludge and 16.6–22% of organic matter in bottom sludge. The bottom sludge was loaded in the first year, thus the organic matter degradation efficiencies in the second year were more than 50%.

Figure 3 indicated stabilized sludge organic matter content variation in 2007. It is obviously to found that sludge organic matter contents have the trend of decreasing with time extension. From May to November 2007, organic matter contents in bottom, middle and surface sludge layers decreased from 25.3% to 16.1%, from 30.7% to 17.8%, and from 34.5% to 16.5%, individually. Middle sludge layer possessing higher organic matter content was possible caused by sludge dried time and the degree of sludge contacting air.

Considering the fresh sludge organic matter content ranged from 35% to 46%, while stabilized sludge organic matter content was averaged 16.8% in November 2007, then organic matter degradation efficiency was more than 52%, meeting the stabilization standard of sludge composting in China, which require more than 50% of organic matter degradation efficiency.

Based on the analytical results in September, 2008, the stabilized sludge organic matter content decreased further to average 10.24%, getting more than 70% of degradation efficiency.

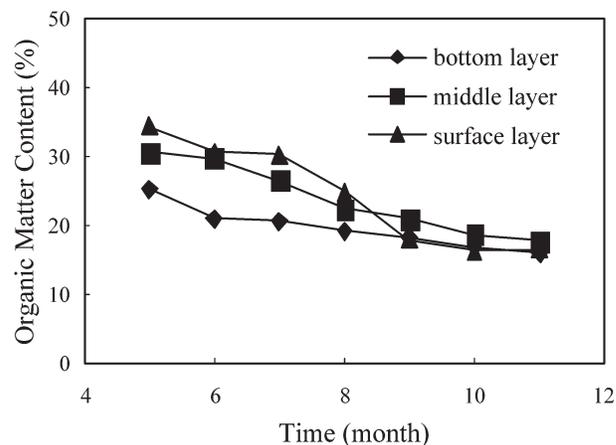


Figure 3. Stabilized sludge organic matter content variation with time in 2007.

3.4. Microbial Stabilization

The sludge samples were taken from surface layer, 10 cm, 5 cm and 0 cm layers from the top to the bottom of stabilized sludge in 2006. The thickness of stabilized sludge during the first year was below 10 cm, so the sludge from 0 cm and 5 cm was the first year's stabilized sludge, while sludge from 10 cm and above layers was produced in the second year, surface layer sludge represents fresh sludge. TTC-DHA of olden sludge accumulated in the first year was obviously lower than that of second year sludge, sludge activity in the bottom layer ranged from 0.25 to 1.54 $\mu\text{g TF/mg VS}\cdot\text{h}$ and sludge activity in 5 cm layer ranged from 0.37 to 1.31 $\mu\text{g TF/mg VS}\cdot\text{h}$ (Figure 4), which demonstrated the degree of sludge stabilization indirectly.

There was big sludge activity difference between 10 cm sludge layer and fresh sludge, TTC-DHA of latter was higher. Sludge TTC-DHA decreased with drying time extension in 10 cm layer, which indicated that sludge stabilization degree was improved with sludge retention time. Based on the mechanism of TTC-DHA analysis, VS represents the amount of microbial, if VS contain more olden and dead microbial cells, TTC-DHA should be lower, corresponding to higher degree of sludge stabilization.

The system was spare in 2007 and 2008. Under the condition of no sludge loading, except of May in which plant began to grow, stabilized sludge TTC-DHA decreased and tended to stabilization with drying time extension. In comparison with the year of 2006, stabilized sludge TTC-DHA decreased further and ranged from 0.05 to 0.18 $\mu\text{g TF/mg VS}\cdot\text{h}$ after June 2007, and kept similar lower level in September, 2008.

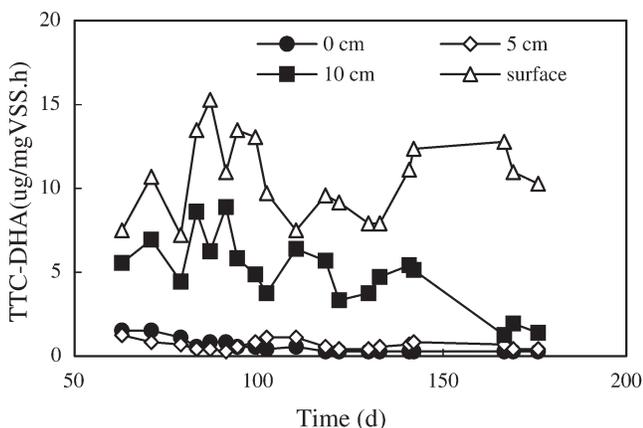


Figure 4. Stabilized sludge TTC-DHA variation with time in 2006.

4. CONCLUSIONS

Wastewater sludge ecological stabilization is a feasible technology for sludge treatment. Sludge moisture and organic matter degradation efficiency met the stabilization standard of sludge composting in China after two years' system batch running and two years resting. Sludge TTC-DHA could indicate the degree of sludge stabilization, longer sludge drying time corresponded to lower TTC-DHA and higher degree of sludge stabilization.

ACKNOWLEDGEMENTS

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Characteristics of Organic Matters During Anaerobic Digestion of Ultrasonically Pretreated Sludge and the Effects on Sludge Dewaterability

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ABSTRACT: The present study evaluated the sludge dewaterability and further explored the relationship between the sludge dewaterability and the molecule weights (MW) of organic matters in the different fractions of sludge flocs. The sewage sludge was sampled and fractionated into four fractions: (1) slime, (2) loosely bound extracellular polymeric substances (EPS) (LB-EPS), (3) tightly bound EPS (TB-EPS), and (4) pellet. The results show that proteins (PN) and polysaccharides (PS) were mainly distributed in the TB-EPS and pellet fractions (75.1%), fewer in the slime and LB-EPS fractions. The sludge dewaterability was improved during anaerobic digestion, and the normalized CST correlated with PN, PS, and MW in the LB-EPS fraction. The results suggest that the size exclusion chromatography (SEC) technique could be an effective method to be potentially applied as an indicator to sludge dewaterability.

1. INTRODUCTION

MORE and more excess sludge is produced in biological wastewater treatment (BWT) systems in recent years. Normally, the excess sludge handling and disposal account for 50–60% of the total construction and operation costs of whole wastewater treatment plants (WWTPs) [1]. Anaerobic digestion and dewatering of sludge are two widely used methods to reduce the volume of excess sludge [2,3]. Whereas, the digestion efficiency was limited and the sludge dewaterability would become poor because of the increase in fine particle part during anaerobic digestion [4]. In order to improve the anaerobic digestion efficiency, ultrasound as the pretreatment for digestion had been studied previously [5]. However, information regarding the sludge dewaterability of anaerobic digestion followed by the ultrasound pretreatment is still largely lacking.

Size exclusion chromatography (SEC) technique was previously applied to investigate the compositions and characteristics of dissolved organic matters (DOM) from nature water or landfill leachate or aquatic and ter-

restrial systems [6,7]. To the best of our knowledge, no previous work has yet been done to evaluate the sludge dewaterability by this technique. The purposes of this work were to investigate the variations of organic matters and sludge dewaterability during anaerobic digestion and further explored the relationship between the sludge dewaterability and molecular weights (MW) in the different fractions of sludge flocs.

2. MATERIALS AND METHODS

2.1. Sludge Sample

The activated sludge sample was collected from the aerated basin of a municipal WWTP in Shanghai, China. This plant treats 75,000 m³/d of wastewater (93% from domestic and 7% from industrial origin) using anaerobic-anoxic-oxic process. They were transported to the laboratory within 30 min after sampling and settled for 1.5 h at 4°C with supernatant decant, then the sludge sediments were collected and screened through a 1.2-mm screen and subsequently centrifuged at 1500 r/min for 10 min with supernatant decant again. The sediments were pretreated using an ultrasonic reactor (FS-600, Shanghai Sonxi Co., Ltd., China) operated at the condition of 20 kHz, 480 W and 10 min [8,9]. The

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transducer was immersed 10 mm into 50 ml sludge samples, and the temperature was maintained at about 25°C in an ice water bath to prevent possible temperature effects. At last, the sonicated sludge was inoculated by anaerobic sludge sampled from our laboratory, the ratio of inoculated sludge to sonicated sludge was about 1:20. The TSS and VSS of mixed sludge were 19.14 ± 0.08 mg/L and 6.87 ± 0.52 mg/L, respectively.

2.2. Anaerobic Digestion

Anaerobic digestion was carried out in an airtight vessel with a working volume of 4.0 L. 3.0 L of sludge were encased and pH was adjusted automatically between 6.8 and 7.5 by 6 M HCl and NaOH solutions during anaerobic digestion. Oxygen in the vessels was removed by nitrogen gas (N₂) sparging for 2 min. The vessels were then sealed up with rubber stoppers, keeping the environment temperature at 37 ± 1°C. About 100 ml of sludge samples were collected at regular intervals and used for the stratification extraction and other analysis.

2.3. Sludge Structure and Stratification Protocol

Sludge stratification protocol was modified according to our previously described method [8,9]. Extracellular polymeric substances (EPS) in sludge flocs are composed of soluble EPS (i.e., slime) and bound EPS. The latter exhibits a dynamic double-fractioned structure and can be classified as loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) based on the extraction methodology. After the EPS is extracted, the cells in the residue form a pellet. Hence, from the outer surfaces to the cores, the sludge flocs possess a multifractioned structure consisting of slime, LB-EPS, TB-EPS, and pellet.

2.4. Analytical Methods

The chemical analyses were carried out in duplicate using chemicals of analytical grade. PN was determined by the modified Lowry method [10], using casein (Shanghai Sangon Biotechnology Co., Ltd, China) as the standard. PS was measured by the Anthrone method [11], with glucose as the standard. CST has been widely accepted for the evaluation of sludge dewaterability due to the simple equipment required, and a high value of CST usually implies a poor dewaterability [12]. In order to measure the

dewaterability potential of sludge flocs, the CST values were normalized by dividing them by the initial TSS concentration and then expressed in units of seconds per liter per gram TSS. The MW of organic matters in the different fractions were determined by a SEC (LC-10ADVP, Shimadzu, Japan) equipped with a differential detector (RID-10A) and a TSK gel column (G4000PWXL, TOSOH Co., Japan), the mobile phase was Milli-Q water. Polyethylene glycol/oxides (MW at 1,169 kDa; 771 kDa; 128 kDa; 12 kDa; 4 kDa; 620 Da; 194 Da) were used as reference molecules for the calculation. Other sludge parameters, including total suspended solids (TSS) and volatile suspended solids (VSS), were analyzed following the standard methods [13]. The statistical analysis was carried out using the software SPSS version 11.0 for Windows (SPSS, Chicago, IL, USA). Pearson's coefficient is always between -1 and +1, where -1 denotes a perfect negative correlation, +1 denotes a perfect positive correlation, and 0 denotes the absence of a relationship. The correlation was considered statistically significant at a 95% confidence interval ($p < 0.05$).

3. RESULTS

3.1 The Variations of PN and PS during Anaerobic Digestion

Figure 1 depicts the variations of PN and PS with time during the anaerobic digestion. The process could be separated into three phases according to the anaerobic digestion time: phase 1 (digestion time < 5 d), phase 2 (5–27 d) and phase 3 (> 27 d). The total PN and PS concentration followed the order: phase 2 > phase 1 > phase 3. It is well known that the anaerobic digestion consists of three stages: hydrolysis, acidification and methanation. Hydrolysis of the sludge flocs can be described as the breakdown of organic matters, resulting in the fragment of cell wall and the release of intracellular compounds [14], and it happened mainly at phase 1 and partly at phase 2, so the phase 2 had the highest concentration of PN and PS. Chen *et al.* [15] also reported that the concentration of organic matters increased significantly under not only acidic but also alkaline conditions. The organic matters at phase 3 would be the sludge remaining mainly consisting of non-biodegradable fractions and inorganic matters [2].

The initial PN and PS concentrations in the loosely bound fractions (slime and LB-EPS) were 159.5 ± 3.0 mg/g-VSS and 37.2 ± 8.9 mg/g-VSS, respectively.

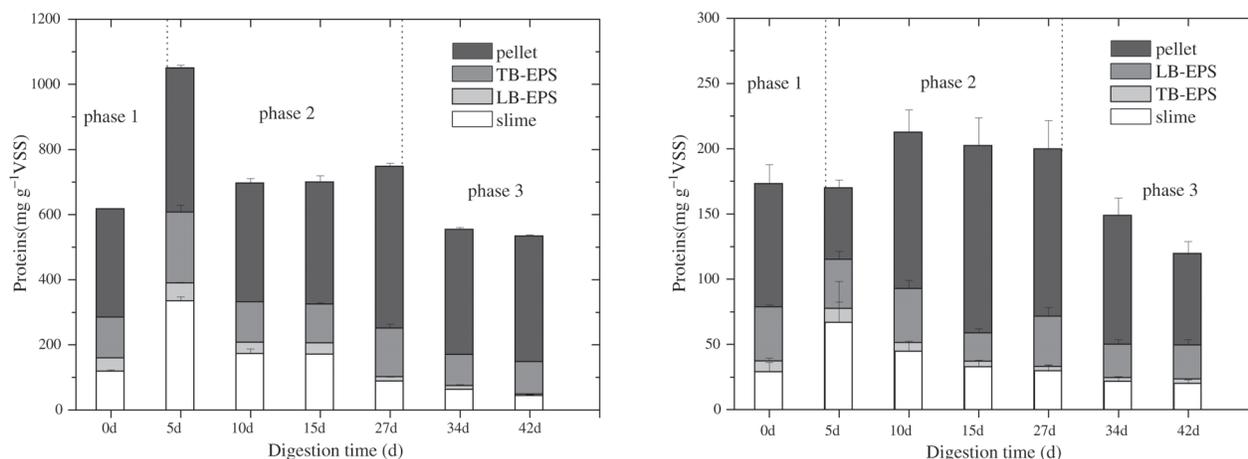


Figure 1. The variations of PN and PS during anaerobic digestion (Note: error bars represent standard deviation).

Subsequently, they decreased to 46.3 ± 1.4 mg/g-VSS and 23.0 ± 3.3 mg/g-VSS at 42nd day. While in the tightly bound fractions (TB-EPS and pellet), the PN concentration increased from the initial 458.4 ± 1.4 mg/g-VSS to 487.9 ± 3.0 mg/g-VSS at 42nd day, and PS concentration decreased from 135.3 ± 16.0 mg/g-VSS to 95.9 ± 13.4 mg/g-VSS. In other words, the organic matters in the loosely bound fractions were easily degraded when compared with those in the tightly bound fractions, suggesting that the organic matters in the tightly bound fractions were mainly large molecule matters or colloids that were not easily decomposed.

3.2 The Sludge Dewaterability during Anaerobic Digestion

Figure 2 presents the variation of normalized CST

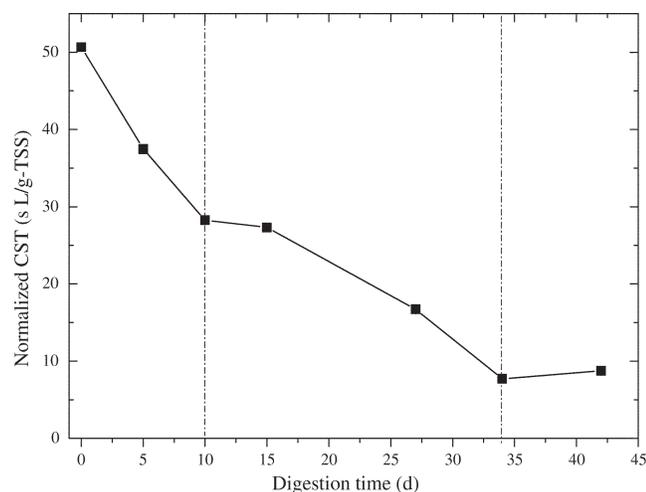


Figure 2. The variation of normalized CST during anaerobic digestion.

during anaerobic digestion. The initial normalized CST was 50.6 s L/g-TSS, it rapidly decreased to 28.3 s L/g-TSS at 10th day and slowly fell to 7.73 s L/g-TSS at 34th day, then approached the plateau value until the end of test period. Some researchers had reported that the anaerobic digestion would deteriorate the sludge dewaterability [4,16]. However, we interestingly found that the sludge dewaterability was improved during the anaerobic digestion followed by ultrasound pretreatment.

3.3 The Transformation of Molecular Weights during Anaerobic Digestion

SEC reveals a wide range of molecular weights (MW). For the sake of further discussion, the MW distributions of organic matters in different EPS fractions could be categorised into two groups according to the retention time: $MW > 210$ kDa (retention time < 13 min) and $MW < 210$ kDa (retention time > 13 min). The anaerobic digestion could convert the complex organic matters into low molecular weight organic matters [4], we took the LB-EPS fraction for example [Figure 3(b)], the large molecule matters ($MW > 210$ kDa) occupied 100% at 0th day, they decreased to 36.0%, 25.4%, and 22.3% at 10th day, 27th day, and 34th day, respectively. While the small molecule matters ($MW < 210$ kDa) increased from none to 64.0%, 74.6%, and 77.7% at 10th day, 27th day, and 34th day, respectively. Hence, complex organic matters in the LB-EPS fraction were mainly degraded (78.7%) into less complex organic matters during anaerobic digestion. The organic matters in the TB-EPS and pellet fractions [Figures 3(c) and 3(d)] also had the similar degradation tendency as

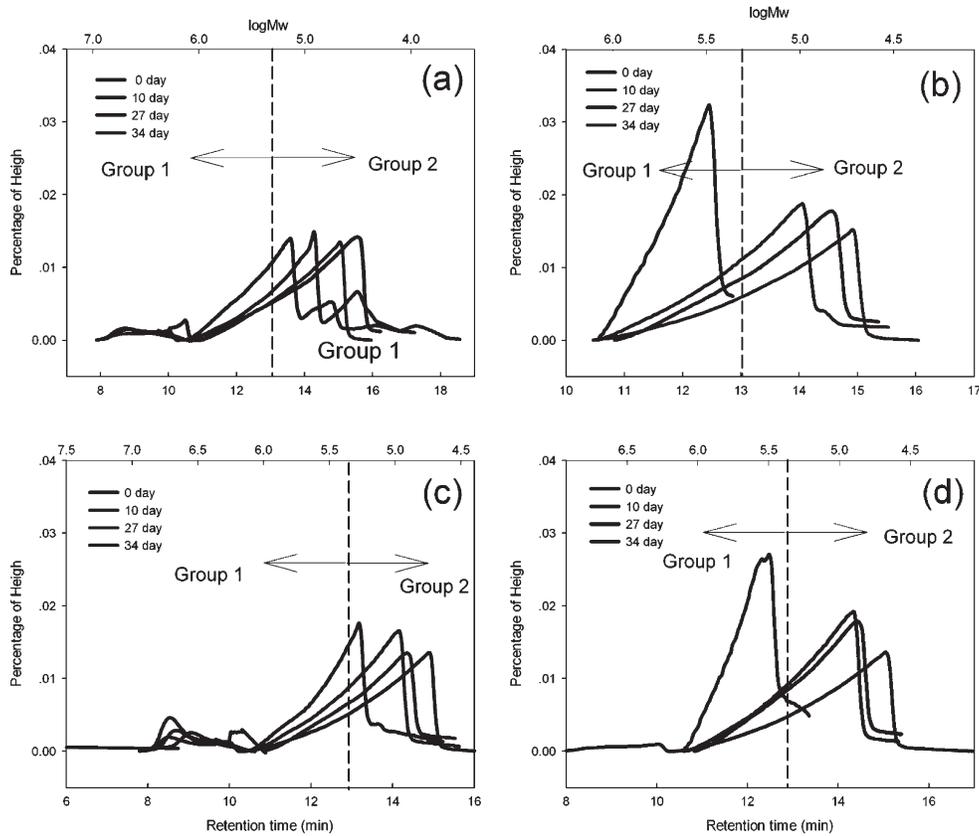


Figure 3. The SEC profiles in the different fractions of sludge flocs. (a) slime; (b) LB-EPS; (c) TB-EPS; (d) pellet.

in the LB-EPS fraction. However, the organic matters in the slime fraction mainly consisted of less complex matters ($52.1\% < 210$ kDa) and the MW of organic matters varied fluctuately during anaerobic digestion [Figure 3(a)].

4. DISCUSSION

4.1 Correlation Analyses between the Normalized CST and PN and PS

Table 1 shows the Pearson correlations between the normalized CST and the PN and PS in the different fractions of sludge flocs. The normalized CST only correlated with PN ($R^2 = 0.92$, $p = 0.005$) and PS ($R^2 = 0.95$, $p = 0.011$) in the LB-EPS fraction, there was no correlation with PN ($R^2 < 0.98$, $p > 0.09$), or PS ($R^2 < 0.97$, $p > 0.14$) in other fractions.

Several studies have been aimed at investigating the effects of EPS and their compositions on sludge dewaterability. Novak *et al.* [17] reported that the digested sludge dewaterability was directly affected by the amount of biopolymers released into the solution during the anaerobic process. Higgins and Novak [18]

found that PN was of more significance than PS to sludge dewaterability, the degradation of PN by protease resulted in poorer dewaterability, while the degradation of PS had no noticeable effect on sludge dewaterability. Li and Yang [19] reported that the LB-EPS (corresponding to slime and partly LB-EPS in this study) played a greater role than the TB-EPS on sludge dewaterability. Owing to the complicated matrix of sludge structure, those results above did not identify which parts or which compositions of EPS were the strongest determinants to the sludge dewaterability. The present study demonstrated that the organic matters in the LB-EPS fraction had more important influence on the sludge dewaterability.

Table 1. The Pearson Correlation between the Normalized CST and PN and PS ($n = 7$).

Fractions (Layers)	Normalized CST			
	PN		PS	
Slime	0.89	0.091	0.89	0.148
LB-EPS	0.92	0.005	0.95	0.011
TB-EPS	0.95	0.224	0.97	0.137
Pellet	0.98	0.470	0.88	0.743

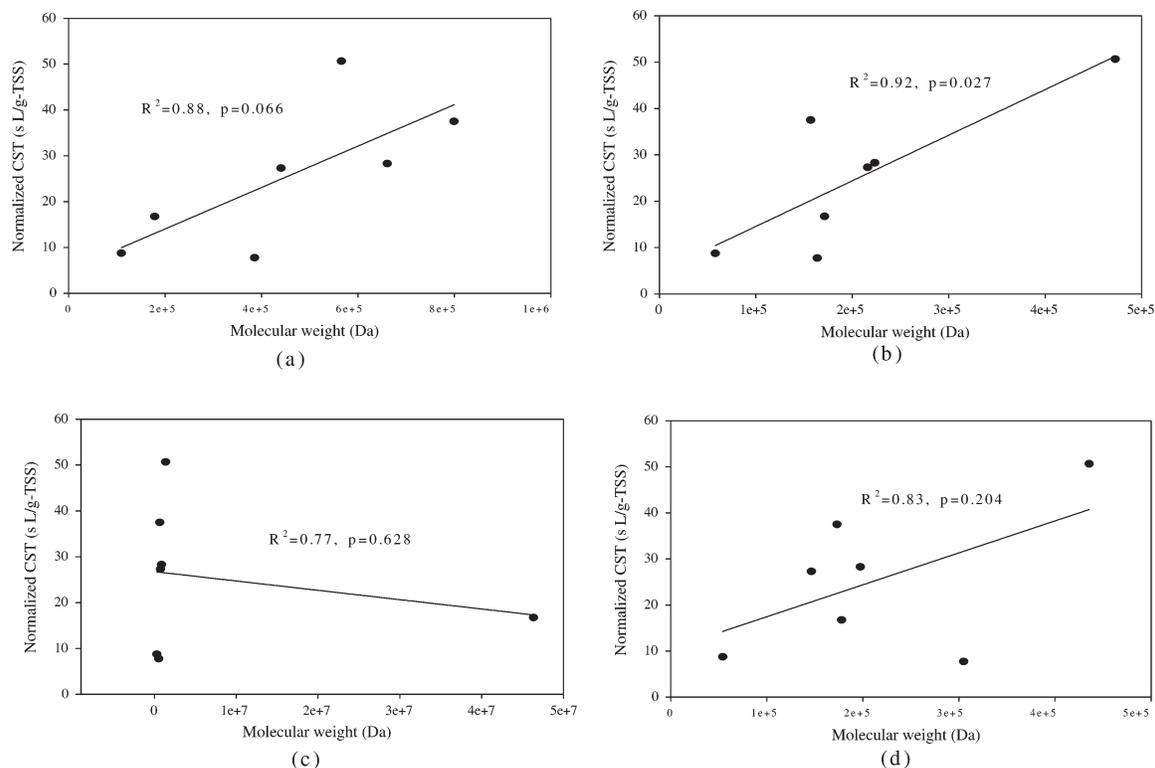


Figure 4. The correlations between the normalized CST and the MW of organic matters in the different fractions of sludge flocs. (a) slime; (b) LB-EPS; (c) TB-EPS; (d) pellet.

4.2 Correlation Analyses between Sludge Dewaterability and Molecular Weights

As mentioned above, the SEC technique was widely applied in many domains [6,7]. However, they were mostly qualitative analysis, and no previous work has yet been done to evaluate the sludge dewaterability by this technique. In this study, we for the first time found that there was a strong positive correlation between the sludge dewaterability and the MW of the organic matters in the LB-EPS fraction ($p = 0.027$), suggesting that the organic matter in the LB-EPS fraction was a significant contributor to the sludge dewaterability. So we could detect the MW of organic matters in the LB-EPS fraction to predict the sludge dewaterability. This would be a simple and effective technique for the environmental researchers to better understand the organic substance properties and the sludge dewaterability and could help to design and optimize the sludge/wastewater treatment facilities.

5. CONCLUSIONS

The sludge dewaterability during anaerobic digestion followed by ultrasound pretreatment was evalu-

ated and the correlation between the sludge dewaterability and the organic matters in the different fractions of sludge flocs was explored. The organic matters in the sludge flocs were mainly distributed in the TB-EPS and pellet fractions, fewer in the slime and LB-EPS fractions. The sludge dewaterability was improved during the anaerobic digestion, and the normalized CST significantly correlated with organic matters in the LB-EPS fraction. In this study, we for the first time reported that there was a strong positive correlation ($p = 0.027$) between the sludge dewaterability and the MW of organic matters in the LB-EPS fraction. The results suggest that the SEC technique could be an effective method to be potentially applied as an indicator to the sludge dewaterability.

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Experimental Research on Treatment of Housing Estate Wastewater by Non-Residual Sludge Hydrolytic Acidification Process

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ABSTRACT: With the increasing of the housing estate sewage quantity, the quantity of residual sludge was increasing at the same time. The proportion of investment and operation cost used for treatment of residual sludge was higher in total investment and operation cost. That brought some difficulties in treatment of housing estate sewage. In this paper, the non-residual sludge hydrolytic acidification process was taken to treat housing estate sewage. This cycle system was composed of medium-temperature anaerobic acidification reactor and aerobic biosystem. Aerobic and anaerobic microorganism died and could be degraded continuously through a variable anaerobic-aerobic process. Tests showed that operation of acidification reactor would have better effect under the condition of temperature 34°C, HRT 1.5 day, PH 5.5–6.0 and residual sludge could be decreased or zero emission. In this paper design and effective control of acidification reactor were also discussed.

1. INTRODUCTION

WITH the rapid development of housing estate, the size expanded and the sewage quantity was increasing at the same time. There were COD, BOD, suspend solid, ammonia nitrogen, phosphorus and other pollutants in the sewage. The average content showed as follows: COD 220–400 mg/L, BOD 140–300 mg/L, SS 200–400 mg/L and PH 6–9. The sewage belonged to low concentration wastewater and its biodegradability was better.

At present, biochemical techniques were widely used in housing estate sewage treatment at home. Although these techniques had better efficiency, too much residual sludge was produced and the proportion of investment and operation cost used for treatment of residual sludge was higher in total investment and operation cost. That brought some difficulties to practical application. In this condition, people paid more attention to searching for a new technique with low disposal cost, high efficiency, easy controlling and less residual sludge. In this aspect, we had made some bolder tries and hoped that these could bring consultable treatment process for the project of housing estate sewage.

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2. THE SOURCE, QUALITY AND QUANTITY OF WASTEWATER

The water sample was taken from domestic sewage of housing estate in Harbin. The domestic sewage passed through septic tank and flowed into urban drainage pipeline. The wastewater quality was shown as Table 1.

Treated water quality executed primary standard of national “Standard for Discharge of Wastewater” (GB8978-1996). COD = 60 mg/L, BOD = 20 mg/L, SS = 20 mg/L.

3. TEST OF TECHNOLOGICAL PROCESS

Non-residual sludge hydrolytic acidification process was shown as Figure 1. Domestic sewage was reserved in collecting tank and then pumped to hydrolytic acidification tank to hydrolyze and acidulate. Macromolecule organic matter was hydrolyzed and acidulated to micromolecule organic matter. Most of undissolved organic matter was resolved into dissolved organic matter that brought opportunities for the disposal of contacting oxidation [1]. Effluent from hydrolytic acidification tank flowed into contacting oxidation tank. Organic matter was adsorbed and degraded by aerobic microorganism. Separated from sludge in secondary sedimentation tank, effluent dis-

Table 1. Water Quality Index of Housing District Sewage.

Water Quality Index	COD (mg/L)	BOD (mg/L)	SS (mg/L)	PH	NH ₃ -N (mg/L)	TP (mg/L)
Variation range	250–400	170–300	210–380	6–8	25–35	1–4
Average	320	195	280	7.2	28	2.1

charged. Residual sludge produced in contacting oxidation tank flowed into sludge hydrolytic acidification tank to medium-temperature anaerobic acidulate. In order to achieve the goals of internal recycle degradation without discharge in the whole system, it flowed back into hydrolytic acidification and contacting oxidation system to be degraded continually.

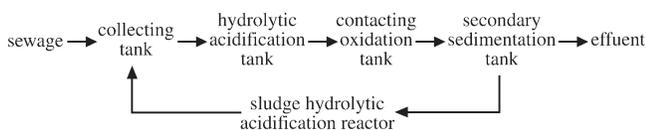
4. TEST AND ANALYSIS

4.1. Test of Hydrolytic Acidification Tank

The sludge of hydrolytic acidification tank was taken from the digestion sludge of Dong-Jiao Sewage Disposal Plant of Tianjin. Apparent sludge was black and the rate of water content was 75–80%. One day after inoculum sludge was plunged into hydrolytic acidification tank, a small quantity of sewage was added intermittently and inflow load increased step by step. PH value of inflow was controlled between 6.0–6.5 and DO was controlled between 0.1–0.3mg/L. Inoculum sludge degraded organic matter under the anoxic-aerobic conditions. 25 days later, SV of sludge in hydrolytic acidification tank got to 35–40%. Microorganism was in logarithmic phase and its growth curve was shown as Figure 2. The sewage of whole hydrolytic acidification tank appeared dust-black and had a special kind of hydrolytic acid taste. This showed that hydrolytic acidification phase had begun. By microscopy, zoogloea appeared irregular type and there were a few colpidia. This showed that the phase of cultivation and domestication of strains had completed.

4.2. Test of Contacting Oxidation Tank

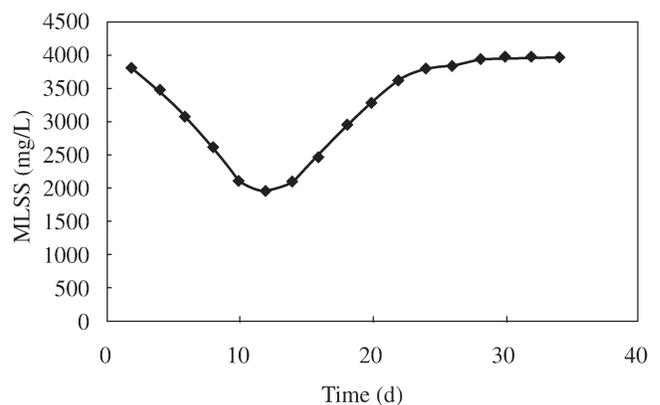
With the increasing of sludge in hydrolytic acidification tank, a part of sludge flowed into contacting oxida-

**Figure 1. Technological process sketch map.**

tion tank with effluent. Nitrogen and phosphorus nutriment was plunged into contacting oxidation tank at the ratio of BOD:N:P = 100:5:1 and adjusted SRT according to the variety of SV. 28 days later, SV value achieved to 15–20% in contacting oxidation tank, which was shown as Figure 3. By microscopy, there was plenty of microorganism in filling in contacting oxidation tank. A better biosystem was composed of abundant activated zoogloea, vorticella and rotatoria. Deciduous biofilm had a good compact structure and high velocity of sedimentation. Sludge separated from water rapidly. The supernatant fluid was limpid and had good water quality. It showed that biota had adapted to the sewage of housing estate and the phase of cultivation and domestication of strains had completed in contacting oxidation tank.

4.3. Test of Sludge Hydrolytic Acidification Reactor

Sludge hydrolytic acidification reactor was actually an acidogenic-phase reactor in two-phase anaerobic digestion [2]. Residual sludge was translated into monose, amino acid, acetic acid, etc. and back flowed into collecting tank to be disposed after a series of hydrolytic acidification reaction. The method of mixing in hydrolytic acidification reactor was water pump mixing. Sludge was pumped from the upper part of sludge hydrolytic acidification reactor and brought to

**Figure 2. Grown curve of microorganism in hydrolytic acidification tank.**

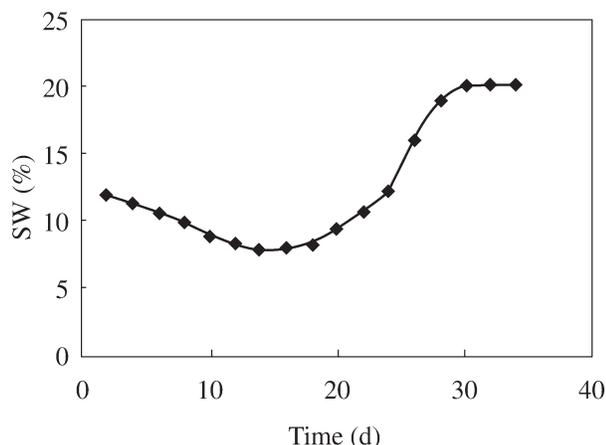


Figure 3. SV curve in contacting oxidation tank.

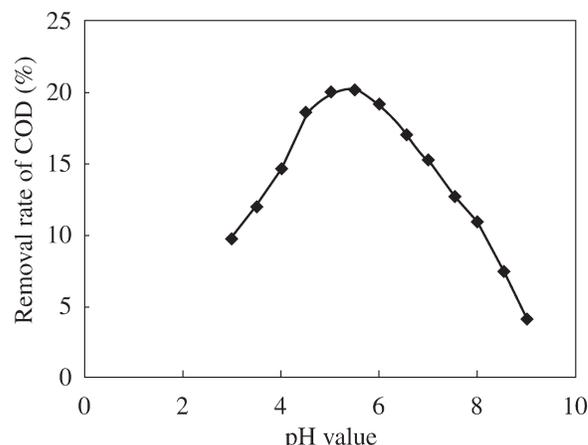


Figure 4. PH curve of hydrolytic acidification liquid.

the bottom to make the acid liquid be mixed completely. In test the temperature was controlled at 34°C in hydrolytic acidification reactor to medium acidulate. The sludge retention time had direct relation to the degree of acidification. The retention time was often taken between 0.8–2 d. In test, 1.5d was taken and remained adjustable space.

The operation effect of hydrolytic acidification reactor had direct relation to the PH value. In test sludge quantity, sludge retention time, temperature and other factors were fixed. By changing the PH of hydrolytic acidification liquid, the relations curve between the degree of hydrolytic acidification and PH was shown as Figure 4. Based on Figure 4, when the PH of hydrolytic acidification liquid was between 5.5–6.0, it was advantageous to acidification. In operation we could adjust dosing quantity and retention time to change PH value of hydrolytic acidification liquid.

4.4 Treatment Results

The test of housing estate wastewater treatment by non-residual sludge hydrolytic acidification process showed that the effluent met the demands of “Standard for Discharge of Wastewater”. The removal rate of COD was 90%, BOD was 95% and SS was 96%, which was shown as Table 2. Residual sludge produced by contacting oxidation tank flowed into sludge hydrolytic

acidification reactor to be disposed and then back flowed to internal recycle system to hydrolytic acidification degrade. Basically there was no residual sludge to be discharged.

4.5 Analysis

In general biochemical treatment system, the sludge produced always needed to be disposed separately and the cost of sludge treatment was higher. In non-residual sludge hydrolytic acidification process, sludge hydrolytic acidification reactor, hydrolytic acidification and contacting oxidation treatment system composed an internal recycle system. Hydrolytic acidification sludge in hydrolytic acidification reactor back flowed to collecting tank and was disposed again in biosystem. Recycle sludge complemented the sludge of hydrolytic acidification reactor and avoided the phenomena of slow increasing or negatively growing of sludge. It made enough activated sludge for hydrolytic acidification tank.

Sludge hydrolytic acidification reactor had important effect on biochemical system operation. Microorganism experienced a variable anaerobic-aerobic process in system. The inner of hydrolytic acidification reactor was anaerobic. Aerobic microorganism died in anaerobic process and degraded by anaerobic microorganism. Anaerobic microorganism flowed into hydrolytic tank

Table 2. Treatment Results.

Water Quality Index	COD (mg/L)	BOD (mg/L)	SS (mg/L)	PH	NH ₃ -N (mg/L)	TP (mg/L)
Variation range	28–49	7.8–12	6.5–12.5	6.8–7.4	12–21	0.3–0.7
Average	38	9.9	11	7.1	13	0.5

and contacting oxidation tank. Anaerobic microorganism died in aerobic process and degraded by aerobic microorganism in contacting oxidation tank.

Poor degradable organic matter adsorbed by residual sludge could be acidulated to degradable micromolecule matter in the sludge hydrolytic acidification reactor. This method avoided the accumulation of poor degradable organic matter and enhanced the efficiency of the whole treatment system.

5. CONCLUSIONS

The test of housing estate wastewater treatment by non-residual sludge hydrolytic acidification process showed that reasonable design of sludge hydrolytic acidification reactor and proper HRT were very important. Sludge hydrolytic acidification reactor actually utilized the acidogenic phase in two-phase anaerobic digestion. Medium temperature acidification was taken in test. It had a better effect on acidification under con-

ditions of temperature 34°C, HRT 1.5 day, and PH 5.5–6.0. In operation, we could adjust dosing quantity and retention time to change PH value of hydrolytic acidification liquid. Sludge hydrolytic acidification reactor could be realized as a part of biochemical treatment. Residual sludge produced by contacting oxidation tank flowed into hydrolytic acidification reactor to preacidification-degrade and back flowed to aerobic biochemical system. Microorganism of residual sludge died and was degraded in variable anaerobic and aerobic process. Through this method, it would achieve the goals of non-residual sludge discharge in aerobic biochemical system.

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Investigation into the Fate of a Metabolic Uncoupler, 2,6-dichlorophenol in the Sequence Batch Reactor System

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ABSTRACT: The fate of a metabolic uncoupler, 2,6-dichlorophenol (2,6-DCP) was investigated in the sequence batch reactor systems for 3 months by determination of 2,6-DCP in the dissolved and particulate phases. Two sequence batch reactors were used: one system was used as a control, while the other received 2,6-DCP concentrations equal to 20 mgL⁻¹. The results revealed that 70 ± 5% of 2,6-DCP was still in the final effluent during the day of 1–40. During the next days (days 40–70), 10 ± 4% was biodegraded with the adsorption fraction increasing from 25 ± 5% of day 40 to 50 ± 2% of day 70, while the effluent part decreasing from 65 ± 4% to 40 ± 3%. Up to the end of the experiment (days 70–90), 20 ± 2% of 2,6-DCP was biodegraded, 35 ± 2% was removed via the effluent, while 45 ± 3% was removed by the activated sludge. The addition of 2,6-DCP initially deteriorated the removal of biological nutrient (days 1–40). After acclimatization of biomass, the biological nutrient removal was recovered and 2,6-DCP could reduce sludge generation by about 5% (days 70–90).

INTRODUCTION

ACTIVATED sludge process plays an important role in treating a wide variety of wastewater, in which organics are converted to carbon dioxide, water and bacterial cells. The produced cells are separated from the purified water and concentrated in the form of excess sludge. However, with the expansion of population and industry the increased excess sludge production generates a real challenge in biological wastewater treatment, e.g. treatment and disposal of excess sludge may account for 40–60% of the total capital and operation cost [1]. Therefore, intensive attention has been received during the past years about the technologies for minimization of excess sludge production applied in the wastewater purification processes.

Addition of metabolic uncoupler to the aeration tank can reduce excess sludge production without process changing. Therefore, chemical uncoupler has been given increased attention during the past years. Recent research focused on the feasibility of using metabolic uncouplers to reduce excess sludge production from the

wastewater biological treatment process [1–8]. Significant sludge reduction was observed in all the studies, e.g. Strand *et al.* [9] compared 12 chemicals and 2,4,5-trichlorophenol (TCP) was screened as the most effective uncoupler which could reduce the sludge yield by about 50% after TCP addition. Low *et al.* [2] investigated the effectiveness of pNP on reducing biomass production in a bench-scale activated sludge process. The results revealed that the biomass reduction was 49% but the total substrate removal efficiency was also decreased by 25%.

Chemical uncoupler may provide a promising way for sludge reduction because it only needs to add a set of chemical uncoupler dosing, however, most of chemical uncouplers tested are xenobiotic and potentially harmful to environment in practical application. If some fraction of the chemicals were released into the final effluent discharge, this would produce serious ecological problems, which may be the key factor in determining the application of chemical uncoupler for excess sludge reduction. Furthermore, previous works only concentrated on the evaluation of their effects on excess sludge reduction while little information is available in literature about the fate of chemical uncoupler in the activated sludge culture. Therefore detailed investigations

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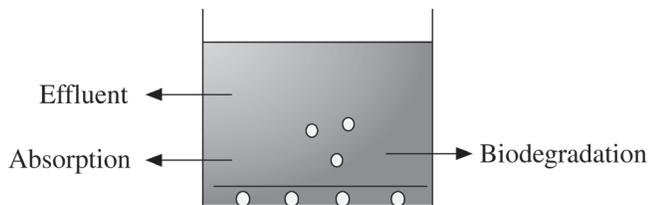


Figure 1. The fate of 2,6-DCP in SBR system.

concerning the fate of chemical uncoupler in the activated sludge process are required.

In an early study, Langford *et al.* [10] pointed out that pure adsorption to the sludge, biological degradation and transformation, volatilization and chemical degradation have to be distinguished as major removal pathways for organic compounds in the activated sludge. In our earlier works, batch test systems were utilized to examine the influence of volatilization and biological degradation on the removal of chemical, which revealed that abiotic and biodegradation loss of 2,6-DCP accounted for a small part of removal efficiency. The relative amounts of chemical uncoupler that is likely to be adsorbed onto sludge and discharge in the effluent water was determined in this study. Therefore, the specific objective of this work is to investigate the fate of a metabolic uncoupler, 2,6-dichlorophenol between the dissolved phase of wastewater and the adsorbed phases of sludge in the sequence batch reactor systems during three months (Figure 1). Moreover, the effects of 2,6-DCP on the substrate removal efficiency, excess sludge reduction and activated sludge characteristics were studied.

MATERIALS AND METHODS

Seed Sludge

The activated sludge used in this work was taken from Taiping Municipal Wastewater Treatment Plant in Harbin, China. It was cultivated in a 80 L fill-and-draw reactor at room temperatures of $25 \pm 2^\circ\text{C}$ and a mean solids retention time of 16 days. The dissolved oxygen (DO) level was kept above 3 mgL^{-1} . 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. A synthetic wastewater was used for cultivation, which composed of glucose (200 mgL^{-1}), dissolved starch (200 mgL^{-1}) (final concentration equal to 400 mg CODL^{-1}), ammonium chloride (95.5 mgL^{-1}), carbamide (32.1 mgL^{-1}),

K_2HPO_4 (29.4 mgL^{-1}), KH_2PO_4 (17.6 mgL^{-1}), MgSO_4 (40 mgL^{-1}), CaCl_2 (5 mgL^{-1}). In addition, other minerals were added, which contained: $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (1.25 mgL^{-1}), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (1.25 mgL^{-1}), CoCl_2 (0.3 mgL^{-1}), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (1.25 mgL^{-1}), CuSO_4 (0.25 mgL^{-1}). Sodium bicarbonate of 300 mgL^{-1} was introduced to reactors to maintain the pH around 7.0. The same synthetic substrate was used in the following batch and continuous operation experiments. The cultivation continued for two weeks without the addition of 2,6-DCP.

Batch Experiments

Batch experiments were conducted in 7 L reactors to investigate the effects of pH and temperature on the activated sludge yield and system performance under 2,6-DCP dosing and the optimization of pH and temperature were chosen for the following long-term operation tests. For all the batch tests, the influent 2,6-DCP concentration, the biomass and influent substrate concentrations were fixed at 20 mgL^{-1} , $2000 \text{ mg MLSSL}^{-1}$ and 400 mg CODL^{-1} , respectively. The pH of each reactor was adjusted to the required value with Sodium bicarbonate and temperature was maintained in a water bath. Experiments conducted to study pH effects were performed at a constant temperature of 25°C . Reactors were operated in a batch mode which were conducted sequentially in 8 h cycle with 10 min for the fill phase, 6 h for the aeration phase, 1.5 h for the settling phase and 10 min for the drawing phase. Batch tests were carried out for 10 days and the reactors were aerated with air pumps to maintain a dissolved oxygen concentration of at least 3 mgL^{-1} . Meanwhile, control tests without the addition of 2,6-DCP were also conducted in parallel.

Sludge Growth in Activated Sludge Culture Operated with the Presence of 2,6-DCP

In order to identify the fate of 2,6-DCP in the activated sludge culture for a long-term operation, two 13 L batch reactors (Figure 2) filled with the activated sludge described previously were operated in a batch mode in parallel. The first reactor received a dose of 2,6-DCP at a 20 mgL^{-1} level three times per day, while another (A) served as the control system without the addition of 2,6-DCP. Both reactors were operated sequentially in 8 h cycle with 10 min for the fill phase, 6 h for the aeration phase, 1.5 h for the settling phase and 10 min for the drawing phase. These experiments were conducted for three months.

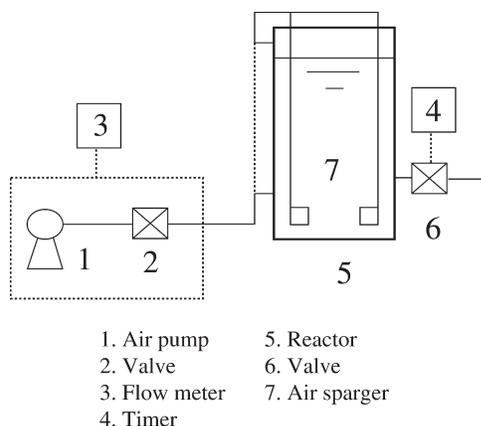


Figure 2. Schematic diagram of the experiment installation.

Prior to the 2,6-DCP dosing, both reactors were first run for 14 days, in which COD and $\text{NH}_4\text{-N}$ removal efficiencies were greater than 90% and stable sludge settleability was obtained with the SVI values ranging between 100 and 110 mlg^{-1} indicating the system reached a steady state, then 20 mgL^{-1} of 2,6-DCP in influent wastewater was fed into reactor B.

To start the experiments with an initial MLSS level at 2000 mgL^{-1} in each reactor, sufficient amount of sludge was transferred from the sludge cultivation reactor. The same synthetic wastewater as that used in the batch experiments was fed to all the reactors with a COD strength at about 400 mg CODL^{-1} . The MLSS concentration in each reactor was maintained at 2000 mgL^{-1} during the experiments which can be determined from the MLSS and COD concentrations measured before sludge withdrawing in each cycle.

Analytical Methods

Before analysis, samples were centrifuged at 5000 g for 10 min to separate the aqueous phase from the sludge, the supernatant fluid filtered immediately through 0.45 μm membranes. Analytical parameters, including dissolved COD (S-COD), MLSS, mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), $\text{NH}_4\text{-N}$ were measured according to the Standard Methods [11]. Measurement of specific oxygen utilization rate (SOUR) was according to the method [1]. The activated sludge yield (Y) was determined from the increase in MLSS divided by the decrease in COD during the batch tests.

The 2,6-DCP concentration in the effluent was determined with a high-performance liquid chromatograph (HPLC; Shimadzu, LC-10AT) equipped with a Waters

C-18 column (4.6 \times 150 mm) and UV detector set at 203 nm. A mixture of acetonitrile (70%) and water (30%) was used as solvent at a flow rate of 1 mLmin^{-1} at room temperature. The sample injection volume was 20 μl .

A mixture of acetone-hexane (80 ml, 1:1 vv^{-1}) was used to extract 2,6-DCP from sludge solids because acetone is an extremely polar solvent assisting the penetration into the matrix. The weighed sample and anhydrous sodium sulfate (1 g, dehydrated, extra pure) were placed into the thimble. The sample was extracted by Soxhlet for 20 h (124 cycles). The extracts were further dried with anhydrous sodium sulfate and concentrated to 10 mL for the GC analysis under nitrogen flow.

The 2,6-DCP concentration of activated sludge were determined with Gas Chromatography (GC) operated on a Agilent 7890 chromatograph. The optimal condition was set for 15°C, injector temperature; 270°C, FID temperature; 30 kPa, inlet pressure; 35, split ratio and the temperature program followed as 150°C (5 min), then to 280°C at 55°C min^{-1} .

RESULTS AND DISCUSSION

Effects of Temperature and pH on Wastewater Treatment Performance

Temperature and pH are important factors in determining the activated sludge system performances, which should also be crucial for the metabolic uncoupler dosed activated sludge process. Previous studies focused on the sludge reduction performed only under specific operation conditions while little is known about the effects of operating parameters on the system performances after metabolic uncoupler dosing. Thus, batch experiments were conducted to screen the optimization of temperature and pH for the 2,6-DCP dosed activated sludge cultures. The effects of temperature on the COD removal efficiency and activated sludge yield (Y) during the 10-day batch experiments were investigated in the temperature range of 15–25°C at 2,6-DCP concentration of 20 mgL^{-1} .

The results are presented in Figure 3. As shown in Figure 3, the COD removal efficiency enhanced with raising the temperature up to 25°C for both the blank and 2,6-DCP dosed reactors indicating that a high temperature favored the substrate removal. At 25°C, the effluent COD removal efficiency in the control was 92% on average, while it was 85% for 2,6-DCP dosed reactor which revealed that 2,6-DCP dosing slightly influenced the COD removal.

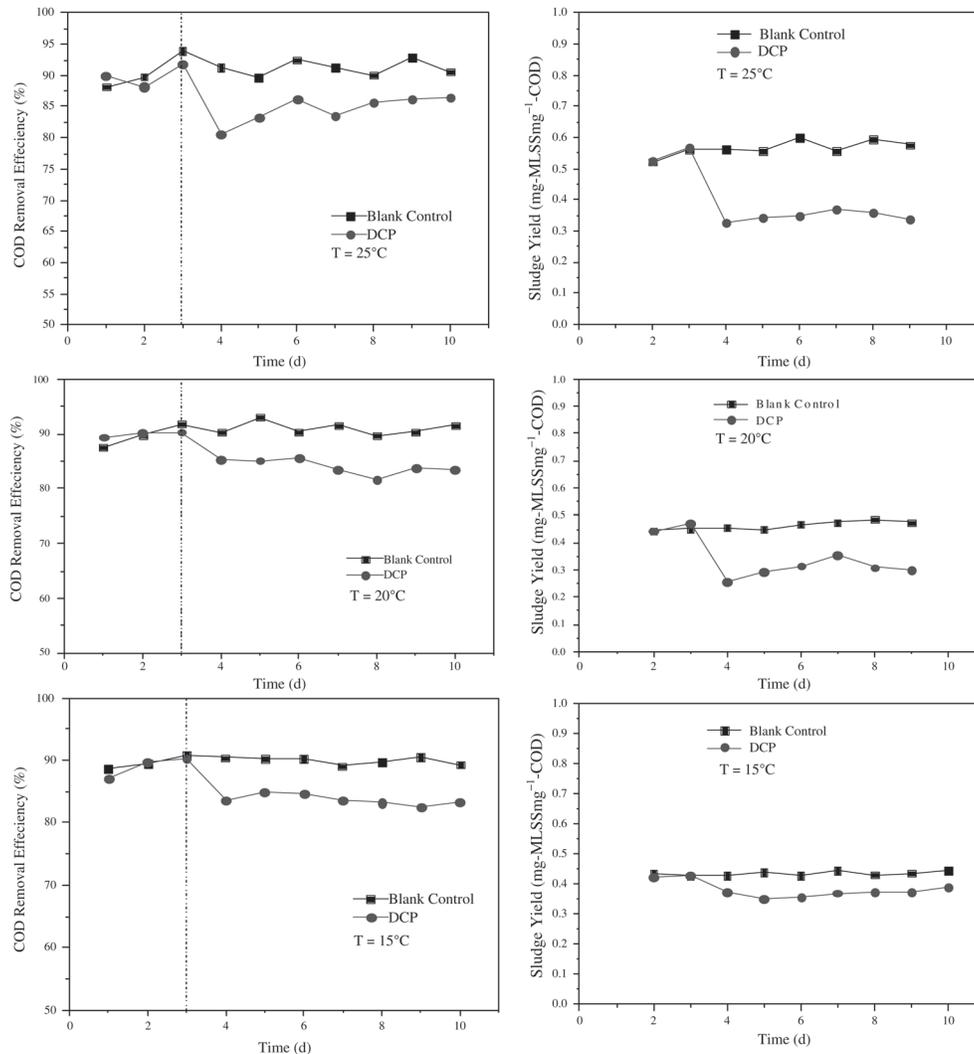


Figure 3. Effect of temperature on the sludge yield (Y) and COD removal efficiency with 2,6-DCP dosed.

The yield of activated sludge is a direct index describing the amount of excess sludge produced in biological wastewater treatment systems. As shown in Fig. 3, the sludge yield significantly decreased with an increase in temperature from 15°C to 25°C. At temperature of 25°C, the sludge yield was reduced by 45.5% compared with 33.3% of 20°C and 22.2% of 15°C. Comparison of effects of different temperatures on the COD removal efficiency and sludge reduction rate suggests that 25°C is the optimal temperature of the investigated.

Figure 4 shows the effects of pH on the COD removal efficiency and activated sludge yield at different pH values ranging from 5.5 to 7.5 for the blank and 2,6-DCP dosed reactors. For each pH value, the temperature (25°C) was kept constant. As seen from Figure 4, at pH = 7.5 ± 0.3, COD removal efficiency was decreased by 7%

as compared to the control test without addition of 2,6-DCP, while it was 9% at pH of 6.5 ± 0.3 and 11% at pH of 5.5 ± 0.3. It demonstrated that COD removal efficiency increased with the increase in pH. In the case of sludge yield, with raising the pH, the sludge reduction did not change significantly up to pH 7.5 ± 0.3. Thus, pH 7.5 ± 0.3 was chosen as the optimal parameter for the following long-term operation experiment.

Fate of 2,6-DCP in Activated Sludge Process

To investigate the mechanisms of 2,6-DCP removal in the sequence batch reactor system, 2,6-DCP concentration on the activated sludge was also determined. Using the measured 2,6-DCP concentration in the influent, effluent and activated sludge samples, the total 2,6-DCP mass flux in both systems was deter-

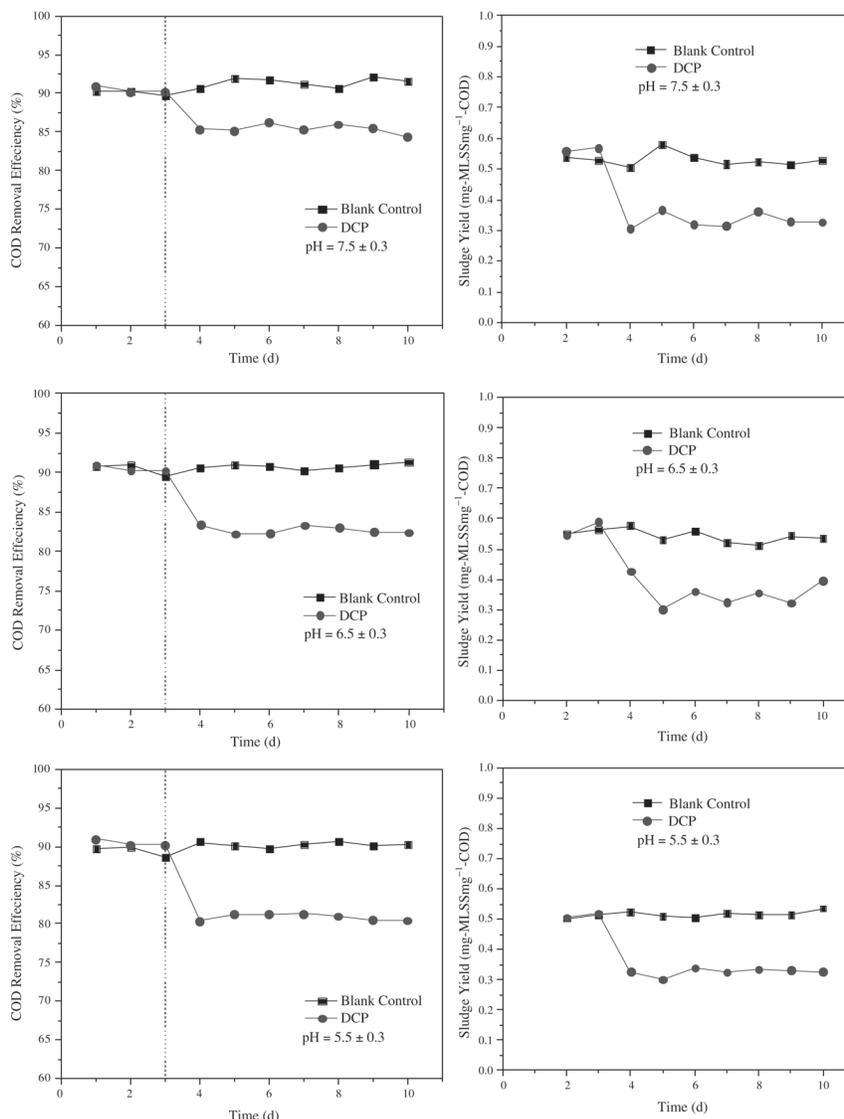


Figure 4. Effect of pH on the sludge yield (Y) and COD removal efficiency with 2,6-DCP dosed.

mined. In our earlier works, batch test systems were utilized to examine the influence of volatilization on the removal of chemical, which revealed that abiotic loss of 2,6-DCP accounted for a small part of removal efficiency, thus biodegradation was the major process governing the rate and extent of 2,6-DCP loss observed in this study. Using Equation (1), the amount of biodegraded 2,6-DCP was calculated as the closure of the mass balance around the systems for each sampling day[12]:

$$M_b = M_{in} - (M_{out} + M_{exc.sludge}) \quad (1)$$

where M_b is the biodegraded 2,6-DCP mass (mg d^{-1}) and M_{in} , M_{out} and $M_{exc.sludge}$ are 2,6-DCP mass (mg d^{-1})

in influent wastewater, effluent wastewater and excess sludge, respectively.

The results are presented in Figure 5. During the 90 days of operation, three different periods, namely 1, 2 and 3, were clearly differentiated based on the variation of 2,6-DCP concentration in the effluent and activated sludge.

During phase 1 (days 1–40), 2,6-DCP concentrations in the effluent and activated sludge kept stable relatively. A significant part of 2,6-DCP (70%) was persistent in the final effluent, 25% was adsorbed by the activated sludge and a little part (around 5%) was biodegraded in the sludge. During phase 2 (days 40–70), biomass acclimatization to 2,6-DCP resulted to a gradual increase of its biodegradation potential, dur-

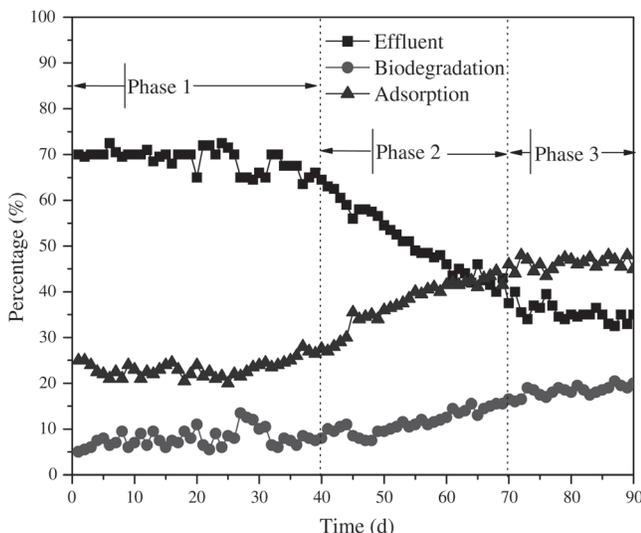


Figure 5. 2,6-DCP mass balance in SBR system.

ing which 10% of 2,6-DCP was biodegraded with the effluent part decreased from 65% to 40% and the adsorption fraction increased from 25% to 50%. During phase 3 (days 70–90), 2,6-DCP concentrations in the effluent and activated sludge seemed to get stable again. 35% of 2,6-DCP was persistent in the final effluent, 45% was removed by the activated sludge and 20% of 2,6-DCP was biodegraded.

Compared phase 3 to phase 1, a significant part of 2,6-DCP was adsorbed by activated sludge and biodegraded, both accounted to 65% of the total mass. 2,6-DCP concentration persistent in the final effluent had a fast drop decreasing from 70% of phase 1 to 35% of phase 3.

To summarize the above experimental data, the distribution profiles of 2,6-DCP in the activated sludge process are clearly shown in Figure 6. At the first days of 2,6-DCP dosed in system B, persistent in the liquid phase was the major mechanism affecting its fate during activated sludge process resulting from the xenobiotic property of 2,6-DCP. During the next days of the experiment, biomass acclimatization to 2,6-DCP resulted to a great increase of its adsorption potential. The data from the experiment indicated that, in an activated sludge system, 2,6-DCP was removed from the dissolved phase and accumulated on the suspended solids. Afterwards, a direct biodegradation of adsorbed 2,6-DCP seemed to be performed.

Effect of 2,6-DCP on Sludge Yield

After 14 days operation, the sludge yield of both re-

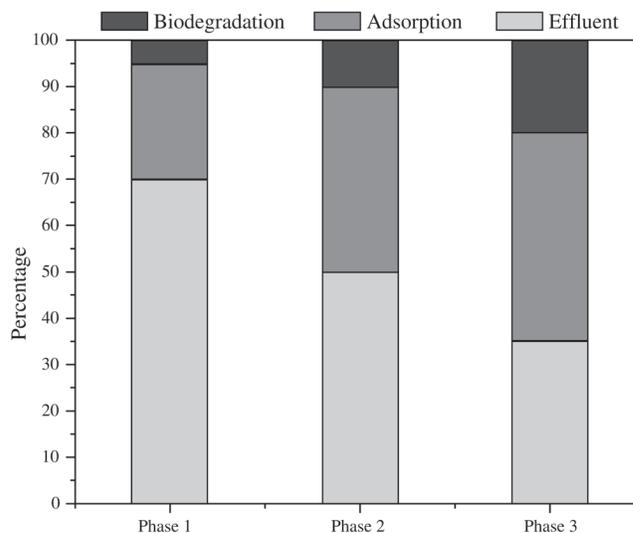


Figure 6. Fate of 2,6-DCP in SBR system.

actors was maintained at 0.55 ± 0.05 indicating both systems reached the steady state. The effect of 2,6-DCP on the accumulative excess sludge (withdrawn sludge) production in the two SBRs is shown in Figure 7 over 90 days. It can be seen that the sludge yield and excess sludge accumulative rate in the two reactors exhibited obvious differences after feeding 2,6-DCP. In reactor A, the Y and sludge generation rate remained at $0.55 \pm 0.05 \text{ mg MLSSmg}^{-1}\text{COD}$ and $0.90 \pm 0.05 \text{ gd}^{-1}$, respectively. The total accumulated sludge after 90 days was 81 g. In the reactor B, during the day of 1–40, the Y and sludge generation rate of system B dropped to $0.34 \pm 0.02 \text{ mg MLSSmg}^{-1}\text{COD}$ and $0.54 \pm 0.05 \text{ gd}^{-1}$ (a reduc-

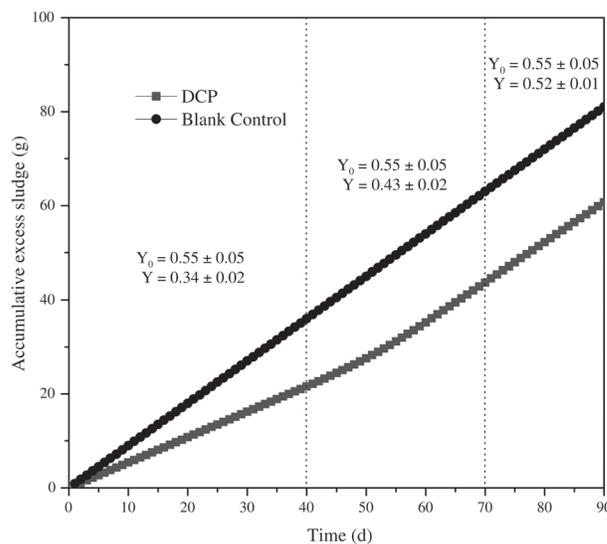


Figure 7. Accumulative excess sludge in the two reactors during the operation period.

tion of approximately 40%) compared with the control reactor. Then in the next days (days 40–70), the Y increased from $0.34 \pm 0.02 \text{ mg MLSSmg}^{-1}\text{COD}$ to $0.50 \pm 0.02 \text{ mg MLSSmg}^{-1}\text{COD}$ and sludge generation rate got to $0.81 \pm 0.05 \text{ gd}^{-1}$ indicating the sludge production was decreased by about 10% compared with the control reactor. At the end of the experiment (days 70–90), the Y and sludge accumulative rate were recovered to $0.52 \pm 0.01 \text{ mg MLSSmg}^{-1}\text{COD}$ and $0.85 \pm 0.05 \text{ gd}^{-1}$ and the total accumulated sludge after 90 days was 60 g which demonstrated the uncoupling metabolism effect of 2,6-DCP weakened with the biomass adaptation to the metabolic uncoupler.

Figure 8 shows the effect of 2,6-DCP on the reduction of sludge growth yield. It can be seen that three periods were also shown in this figure. At first, approximately 40% reduction of the sludge yield was observed (days 1–40), while the reduction potential of the growth yield decreased gradually from 40% of day 40 to 9% of day 70. At last, the growth yield reduction seemed to get stable again, which maintained at 5% (days 70–90) indicating that after acclimatization of biomass, the activated sludge yield was recovered. In conclusion, the most effective sludge reduction period of 2,6-DCP lasted for 40 days. In the next 30 days, the sludge reduction decreased, but it was still effective in limiting the sludge growth. Therefore, 70 days could be considered as the effective uncoupling periods for the metabolic uncoupler 2,6-DCP. After that, acclimatization of biomass resulted to the gradual recovery of activated sludge yield, which may be the reason why 2,6-DCP concentration decreased in the effluent while the adsorption part had a fast increase in the sludge.

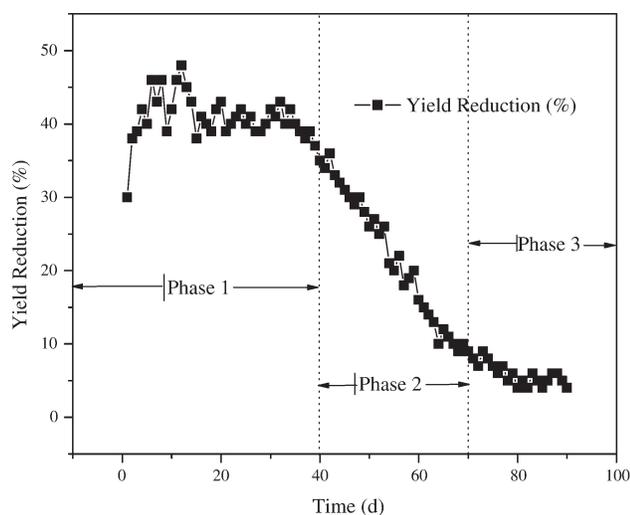


Figure 8. Reduction of the growth yield.

Compared Figure 8 with Figure 5, some potential correlations may be revealed. As shown in both pictures, three periods showed the same changing trends, in which day 40 and 70 are the critical boundary point of both figures. This phenomenon deserves the next further study.

Effect of 2,6-DCP on Biological Nutrient Removal

The COD removal efficiency of the two SBRs during the 90 days is shown in Figure 9. It is apparent that the initial addition of 2,6-DCP resulted in a fast drop of COD removal efficiency. This could be due to the death of some microbes supersensitive to 2,6-DCP in the activated sludge. However, in several days, the sludge had adapted to the presence of uncoupler, and the COD removal efficiency in the uncoupling metabolic reactor recovered to a higher level again. During 90 days, the average COD removal efficiency of the reactor with 20 mgL^{-1} 2,6-DCP reached about 85%, which only reduced by 8% compared to the control reactor demonstrating that addition of 2,6-DCP has little impact on the carbon removal efficiency and it can get to recovery soon.

The effect of 2,6-DCP on the $\text{NH}_4\text{-N}$ removal efficiency of the two SBRs during the 90 days is shown in Figure 10. As is shown, during the 90 days of operation, three different periods were also clearly differentiated.

During phase 1 (days 1–20), the initial addition of 2,6-DCP resulted in a fast drop of $\text{NH}_4\text{-N}$ removal efficiency, during which about 65% decline was observed.

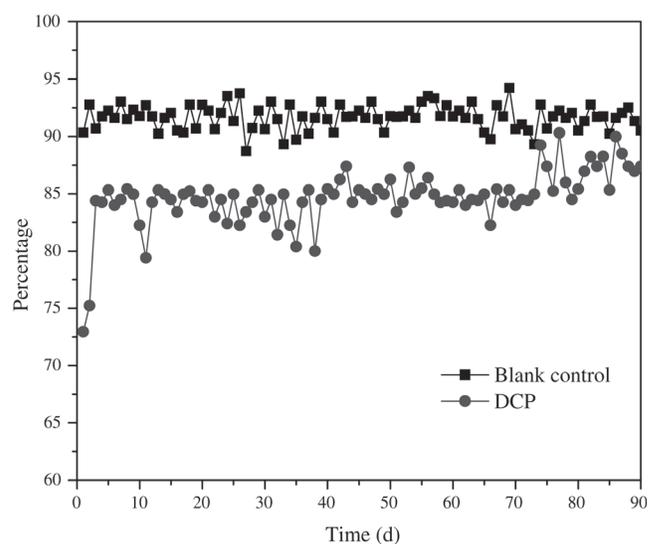


Figure 9. COD removal efficiency of the two reactors during the operation period.

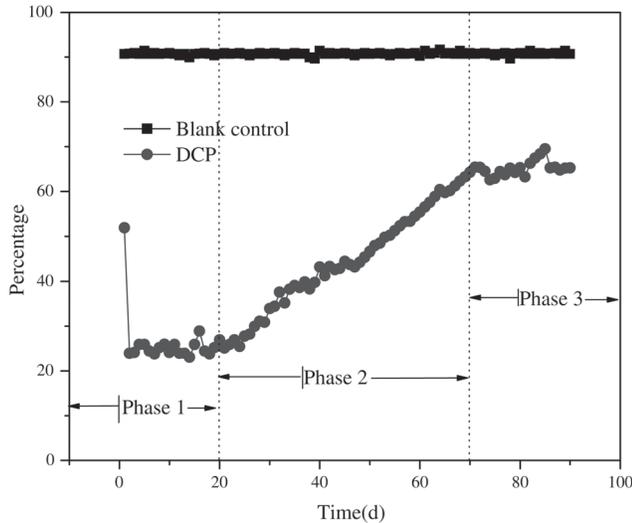


Figure 10. $\text{NH}_4\text{-N}$ removal efficiency of the two reactors during the operation period.

The variation in ammonia removal rates can be attributed to the toxicity effect of 2,6-DCP on the ammonia oxidizing bacteria (AOB), suggesting that this species may be quite sensitive to the xenobiotic substances in the activated sludge system. During phase 2 (days 20–70), biomass acclimatization to 2,6-DCP resulted to a gradual increase of $\text{NH}_4\text{-N}$ removal potential, which increased from 25% of day 20 to 65% of day 70. During phase 3 (days 70–90), $\text{NH}_4\text{-N}$ removal efficiency in the effluent seemed to get stable again, which kept 65% of $\text{NH}_4\text{-N}$ removal efficiency or reduced by 25% compared to the control reactor, indicating that $\text{NH}_4\text{-N}$ removal efficiency in reactor B was still influenced by 2,6-DCP.

Compared with Figure 5, it can be seen that the fate of 2,6-DCP in the activated sludge system has direct impact on the $\text{NH}_4\text{-N}$ removal efficiency, which may influence the ammonia oxidizing bacteria (AOB) activity of activated sludge. While compared with COD removal effect, it can be drawn that 2,6-DCP effect on the nitrogen removal is larger than the carbon removal. The former has a longer recovery period than the latter.

Effect of 2,6-DCP on the Characteristics of Activated Sludge

The effect of 2,6-DCP on the sludge settleability was also studied, and the results are shown in Figure 11. It is clear that after feeding 2,6-DCP, the SVI value of the sludge showed a significant increase, but after 50 days, the SVI value generally recovered to normal levels and remained at about 90 during the later stages.

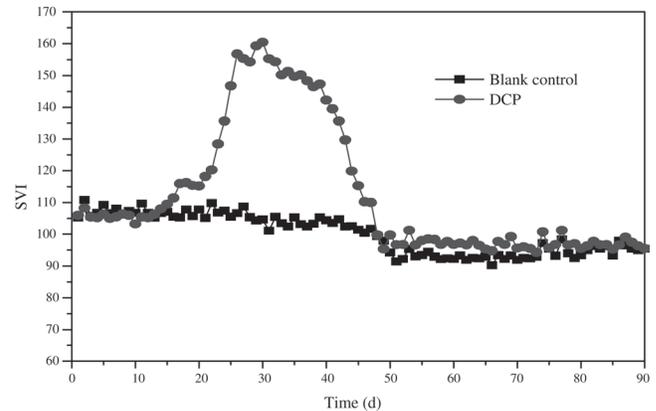


Figure 11. SVI values of sludge in the two reactors during the operating period.

Figure 12 shows the SOUR rates of the two reactors during the operating period. It indicates that the 2,6-DCP-containing culture had a 50% higher SOUR than the control at first days of 2,6-DCP dosing, which demonstrated that the microorganisms became more active in the presence of 2,6-DCP. Similar results were also reported in the cases of TCS and TCP (Chen *et al.*, 2002; Strand *et al.*, 1999). The TCS-containing activated sludge had a 50% higher SOUR than the control (Chen *et al.*, 2002), and the TCP-dosed activated sludge also had a higher SOUR (Strand *et al.*, 1999). After that, the SOUR of the Reactor B gradually decreased and reached a steady state at last, in which the 2,6-DCP-containing activated sludge had a 20% higher SOUR than the control. Considering the reduced sludge yield, the increase in SOUR caused by the 2,6-DCP dosing should be associated with the lost of substrate to

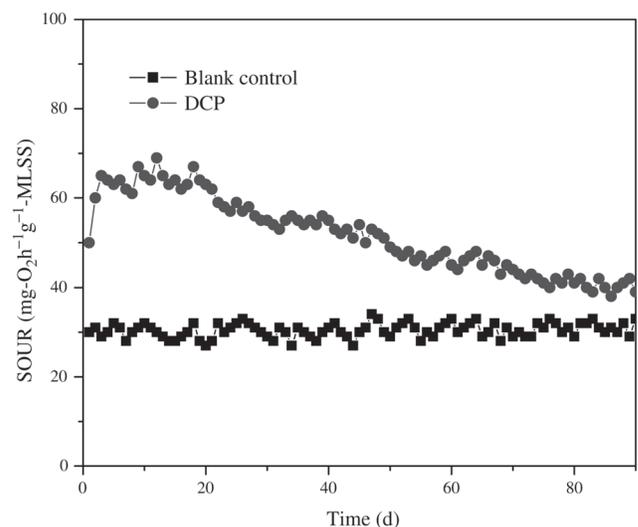


Figure 12. Sludge SOUR of sludge in the two reactors during the operating period.

the non-growth consumption. Thus, the extra-consumption of oxygen implies a high level dissipation of energy for metabolic regulation, which might be the reason responsible for the reduction in sludge yield [1].

CONCLUSIONS

The objective of this work was to study 2,6-DCP behavior in activated sludge system, and to investigate the role of biodegradation on the 2,6-DCP mass balance. The results have revealed that a significant part of 2,6-DCP ($70 \pm 5\%$) was persistent in the final effluent in Phase 1 (days 1–40), in which biodegradation removal of 2,6-DCP accounted for a small part of removal efficiency ($5 \pm 1\%$) and $25 \pm 4\%$ of 2,6-DCP was removed by the excess sludge. During the next days of the experiment (days 40–70), biomass acclimatization to 2,6-DCP resulted to a gradual increase of its biodegradation potential. As a result, over experiment phase 2, $10 \pm 4\%$ of 2,6-DCP was biodegraded with the adsorption fraction increasing from $25 \pm 5\%$ of day 40 to $50 \pm 2\%$ of day 70, while the effluent part decreased from $65 \pm 4\%$ to $40 \pm 3\%$. Up to the end of the experiment (days 70–90), $20 \pm 2\%$ of 2,6-DCP was biodegraded, $35 \pm 2\%$ was removed via the effluent, while $45 \pm 3\%$ was removed by the activated sludge. The decrease of COD and $\text{NH}_4\text{-N}$ removal efficiencies and sludge growth yield were 17%, 65% and 40% compared with the control reactor in the first phase, then the parameters recovered to 8%, 25% and 5%, respectively, which demonstrated that 2,6-DCP effect on the nitrogen removal is larger than the carbon removal. The potential correlations among the fate of 2,6-DCP in the activated sludge process and its effects on the COD, $\text{NH}_4\text{-N}$ removal efficiencies and activated sludge growth yield need to be further investigated. The current concern over the metabolic uncoupler entering the aquatic environment would indicate the need for further careful investigation the bio-safety of the remaining uncoupler in the effluent.

ACKNOWLEDGEMENTS

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Mechanochemical and Biological Degradation of PCB in Contaminated Marine Sediments

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ABSTRACT: Large amounts (megatons) of marine sediments, partly contaminated by toxic recalcitrant organics like polychlorobiphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH) and as well as by heavy metals and other inorganic pollutants, result from dredging operations at industrial harbors worldwide. In order to avoid the unsustainable burden of sanitary landfilling of this huge amount of special/hazardous waste and to find out technical and cost effective methods for its detoxification and eventual reuse as building material for new marine embankment, an R&D project has been undertaken. The project is based on two complementary advanced technologies: short mechanochemical (MC) pretreatment, wherein chemical reactions are activated at nano-particle level by collisions with milling bodies in special high energy milling devices, followed by biological treatment (B) with purposely isolated aerobic bacteria like *Burkholderia xenovorans*. The experimental results aimed at determining the kinetics and the overall technical efficiency of the MC+B treatment of artificially contaminated marine sediments from the harbor of Taranto (S. Italy) indicated that, in the best operating conditions, PCB degradation may be achieved in very effective (~50 %) and fast (<8 d) manner.

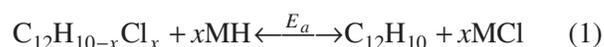
1. INTRODUCTION

CONTAMINATED marine sediments represent a critical health threaten for coastal ecosystems, where hydrophobic contaminants like PCB, in particular, arriving via rain flood, river discharge etc., tend to accumulate by partitioning between dissolved and particulate phases (Neff, 1979; Khan *et al.*, 2004).

In spite of PCB biorecalcitrance, their biological degradation in contaminated sediments is considered the main (and cheapest) way on their natural attenuation, although poorly efficient and extremely slow, with years required to reach completion (Sinkkonen and Paasivirta, 2000; Field and Sierra-Alvarez, 2008). Many bacteria have been isolated to that aim, either Gram negative, e.g., some species of genera *Burkholderia*, *Acinetobacter*, *Ralstonia* e *Pseudomonas* (Furukawa *et al.*, 1979; Hofer *et al.*, 1984; Bedard *et al.*, 1986; Gibson *et al.*, 1993; Novakova *et al.*, 2002) or Gram positive like many species of *Rhodococcus* (Asturias and Timmis, 1993; Chung *et al.*, 1994; Seto *et al.*, 1995). It has been ob-

served, however, that only low-chlorinated (with ≤ 4 Cl atoms) PCB congeners are biodegraded (Ahmed and Focht, 1973; Clark *et al.*, 1979; Yagi and Sudo, 1980; Furukawa and Chakrabarty, 1980; Massé *et al.*, 1984; Focht and Brunner, 1985; Ahmad *et al.*, 1991; Fava *et al.*, 1994; Borja *et al.*, 1995) while higher congeners are biorefractory, with the biodegradation of penta- and hexa-chlorobiphenyls occasionally reported (Bedard *et al.*, 1987 a and b).

On the other hand, chemical degradation of PCB by reduction with nucleophilic reagents may occur according to the reaction:



where $C_{12}H_{10-x}Cl_x$ is a generic PCB congener ($1 < x < 10$), MH is the hydrogen donor metal hydride, MCl is the resulting salt and E_a is the activation energy; it however requires high temperature ($\leq 350^\circ\text{C}$) and organic solvents and this makes it very expensive (Pittman and Young, 2001).

Mechanochemistry (MC) is a well-known nano-technology, where chemical reactions are activated at room temperature by collisions with milling

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bodies (usually steel balls) in special high energy milling devices (shaker, vibratory, centrifugal, jet, nutational or planetary mills) (Thissen *et al.*, 1967; Boldyrev and Meyer, 1973). Although largely used in several fields (e.g., for producing catalysts, functional ceramics, special-purpose materials, hydrogen-storing materials etc.), in the last decade MC has also been proposed for environmental application (Hall *et al.*, 1996; Saeki *et al.*, 2001; Welham, 2001; Cavalieri and Padella, 2002; Korolev *et al.*, 2003; Plescia *et al.*, 2003; Saeki *et al.*, 2004; Ryou, 2004; Miyoshi *et al.*, 2004; Drebushchak *et al.*, 2007; Stellacci *et al.*, 2008a and b). When applied in slurry phase, in particular, MC showed that the degradation of PCB according to reaction (1) may occur at appreciable reaction rate even at room temperature (Rowlands *et al.*, 1994; Mio *et al.*, 2002; Nomura and Hosomi, 2002; Korolev *et al.*, 2003; Aresta *et al.*, 2003; Birke *et al.*, 2004; Tanaka *et al.*, 2004; Pizzigalli *et al.*, 2004).

PCB, PAH (Polycyclic Aromatic Hydrocarbons), heavy metals and other inorganic pollutants largely occur in marine sediments of industrial harbors all over the world (Kuo *et al.*, 1999; Wiegel and Wu, 2000; Frignani *et al.*, 2001; Fava *et al.*, 2003; Sprovieri *et al.*, 2007). This is the case, in particular, of the industrial harbor of Taranto (Apulia, S. Italy), listed among Italy's most polluted sites, deserving national priority and public funds for reclamation (Italian Law No. 426/1998). A huge amount of contaminated marine sediments (approx. 20 Mtons) should be dredged from Taranto harbor and this causes an unaffordable economic burden in terms of safe disposal of such partially hazardous waste. In order to reduce reclamation costs, an experimental investigation has been carried out on MC dry treatment of Taranto marine sediments largely contaminated by PCB and PAH (Intini *et al.*, 2005; Cangialosi *et al.*, 2007a and b). Despite the encouraging results achieved, the treatment resulted quite expensive due to the consumption of energy (≥ 30 min ultra-milling) and the chemicals (NaBH_4) required, according to the reaction:



A two-stage process has then been considered where a short (≤ 1 min) MC pre-treatment in slurry condition of the PCB-contaminated marine sediments is followed by their aerobic biodegradation by selected microorganisms. Preliminary laboratory experiments have been carried out and the results obtained are presented.

2. MATERIALS AND METHODS

Sample Preparation and PCB Analysis

Due to bureaucratic difficulties for withdrawing real contaminated marine sediments from Taranto harbor, uncontaminated samples with similar geo-physical characteristics taken from the near-by Mar Grande Gulf as described elsewhere (Cardellicchio *et al.*, 2007) were artificially contaminated with PCB. To that aim Aroclor 1260 (Supelco, PA, USA), a mixture of 209 PCB congeners containing approx. 12% $\text{C}_{12}\text{H}_5\text{C}_5$, 38% $\text{C}_{12}\text{H}_4\text{C}_6$, 41% $\text{C}_{12}\text{H}_3\text{C}_7$ and 8% w/w $\text{C}_{12}\text{H}_2\text{C}_8$, was used. The average PCB formula in Aroclor 1260 is $\text{C}_{12}\text{H}_{3.58}\text{Cl}_{6.42}$ (MW 375 g/mol) hence, based on his stoichiometry, an average of 6.42 chloride ions should be released into solution by the complete degradation of one PCB molecule adsorbed in the sediment. According to USEPA (2001), the sediment was artificially contaminated with PCB by dissolving Aroclor in hexane and pouring the solution on it to yield $\approx 2,500 \text{ mg kg}^{-1}$ (6.5 mmol kg^{-1}) PCB concentration. The sample was then added with hexane until complete wetting and, after homogenization by gentle stirring for 1 h in a glass bottle, the hexane was allowed to fully evaporate in a rotavapour. According to USEPA (1995), the analysis of PCB in the contaminated samples was carried out by Soxhlet[®] extraction for 30 min with hexane (40 ml g^{-1} of sediment), cleaning-up the extract from organic interference by means of sulfuric acid (ca. 1 ml per 4 ml of hexane) and final PCB analysis by gaschromatography (Mod. CP-3800 with ECD, Varian Inc., Palo Alto, USA) under the following operating conditions: capillary column 30m long and 0.25mm thick; initial temperature 75°C for 2 min, first ramp to 170°C ($20^\circ\text{C min}^{-1}$), second ramp to 290°C ($2.5^\circ\text{C min}^{-1}$), maintained for 10.25 min; gas carrier Helium.

Bacterium *Burkholderia xenovorans* st. LB400 (DSMZ, Braunschweig, Germany), isolated from a PCB-contaminated soil and capable to use biphenyl as unique source of carbon and energy, was employed for the biodegradation experiments. Two culture broths were used (all chemicals from Fluka Italia, Milan, I):

- Luria Bertani (LB) medium, prepared from a lyophilized commercial mixture (LB Broth High salt) with the bacterium stored on Petri plates at 4°C
- Phosphate Ammonium Salts (PAS) medium, composed by 75.5 ml of PA stock solution (56.77 g l^{-1})

K_2HPO_4 , 21.94 g l⁻¹ KH_2PO_4 , 27.61 g l⁻¹ NH_4Cl), 10 ml of PAS100x stock solution (19.5 g l⁻¹ $MgSO_4$, 5 g l⁻¹ $MnSO_4 \cdot H_2O$, 1 g l⁻¹ $FeSO_4 \cdot 7H_2O$, 0.3 g l⁻¹ $CaCl_2 \cdot 2H_2O$), 50 mg of yeast extract and distilled water until 1 litre final volume. PAS100x solution was sterilized by filtration and added after the other components had been sterilized in autoclave (121°C for 20 min).

Biphenyl and PCB were used as the only carbon and energy source for bacterial growth.

MC Experiments

The Hicom 15 High Energy Mill (Hicom Technologies, Pinkenba, Australia) was employed adopting the best operating conditions ascertained in previous experiments (Cangialosi *et al.*, 2007b) (see Table 1). The sediment was ultramilled in slurry condition (90% v/v water content) to achieve better MC performance.

Na_2S , NaOH and CaO nucleophilic chemicals (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) were tested as reducing reagents according to the following redox dehalogenation reaction:



Humic acid (HA) (Fluka Italia, Milan, I) was also added for its well-known surfactant capability. Small sediment aliquots (≤ 1 g) were withdrawn after given milling time and analyzed for residual PCB concentration while the chlorides released in the aqueous phase were analyzed by ion chromatography (Mod. 761 Compact IC, Metrohm AG, Herisau, CH, with 1 mM $NaHCO_3$ and 3.2 mM Na_2CO_3 as mobile phase).

Table 1. Technical Characteristics and Operating Conditions during the Slurry MC Pre-treatment.

Technical Characteristics of Hicom 15 High Energy Mill	
Vial net volume and material	5.3 l AISI 304 stainless steel
Milling balls diameter and material	Ø 10 mm AISI 420C stainless steel
Operating Conditions	
Sediment	49.4 g
Reducing reagent	19.8 g (1:2.5 w/w of sediment)
Distilled water	578 ml (12:1 w/w of sediment)
Total milled mass	647.2 g
Milling mass (steel balls)	6472 g
Milling-to-milled mass ratio	10:1
Vial filling ratio	30% v/v
Milling rotation speed	975 rpm

MC+Biological Experiments

Samples of the artificially contaminated sediment were submitted to a very short slurry pre-ultramilling in the operating conditions reported in Table 1 using CaO as reducing reagent and then subjected to the biological treatment in different experimental conditions as indicated below.

Erlenmeyer Tests

Two Erlenmeyer flasks (500 ml each) were filled with pre-ultramilled sediment (20g) and its own milling aqueous solution (180 ml) and added with the required volume of PA and PAS100x stock solutions to yield the PAS medium. H_2SO_4 98% was added to adjust pH at 6.8, i.e., the optimum value for *B. xenovorans* growth. According to Fava and Piccolo (2002), 0.3 g of HA (1.5% w/w of the sediment) were added to the second flask. Both flasks were inoculated with 3% v/v of a *B. xenovorans* culture (grown with 1 g l⁻¹ of biphenyl only into PAS medium) and incubated at 30°C for 28 days on a rotary shaker at 120 rpm to ensure an oxygen concentration into water near half the saturation level. A small slurry aliquot (10 ml) was withdrawn and centrifuged from each flask every 7 days and the centrate and the solid phases were analyzed for chlorides and PCB concentration respectively.

Bioreactor Tests

Further experiments were carried out using a 3.5 l bioreactor (Esedra plus 3.5, Solaris Biotechnologies, Porto Mantovano, MN, I) agitated with a Rushton paddled impeller at 500 rpm to avoid sediment settling and aerated with sterile air at 1Nm³ min⁻¹ flow rate. Temperature was maintained at 30°C, pH at 6.8 and oxygen saturation near 100%. Like in the Erlenmeyer tests, the bioreactor was filled with pre-ultramilled sediment (250g) and its own milling aqueous solution (2300 ml), supplemented with 3.75 g of HA (1.5% w/w of sediment) and added with PAS until a 2,750 ml slurry final volume. The slurry was then inoculated with 3% v/v of a *B. xenovorans* culture (grown with 1 g l⁻¹ of biphenyl in a PAS medium). Two tests were carried out using pre-ultramilled and unmilled sediment respectively. Small slurried aliquots (10 ml) were withdrawn and analyzed for chlorides and PCB every second days for a total of 8 days. Table 2 summarizes the overall experimental plan.

Table 2. Summary of the Experimental Plan.

Mechanochemical Treatment Tests				
	Sediment (g)	PCB (mg kg ⁻¹)	Reagent	Reagent Concentration and Solution Volume
1	84	2,500	Na ₂ S	27.1 g l ⁻¹ (1330 ml)
2	84	2,500	NaOH	27.1 g l ⁻¹ (1330 ml)
3	84	2,500	CaO	27.1 g l ⁻¹ (1330 ml)
Mechanochemical + Biological Treatment Tests				
Erlenmeyer Flasks (30 C, pH 6.8 P _{O2} 50%)				
	Sediment (g)	[PCB] (mg/kg)	HA (g)	PAS Medium Volume (ml)
1	20 p.u.	2,500	0	180
2	20 p.u.	2,500	0.3	180
Bioreactor (30 C, pH 6.8 P _{O2} 100%)				
	Sediment (g)	[PCB] (mg/kg)	HA (g)	PAS Medium Volume (ml)
1	250 p.u.	2,500	0	2,500
2	250 p.u.	2,500	3.75	2,500

p.u. = pre-ultramilled

3. RESULTS AND DISCUSSION

As shown in Figure 1, in the experimental conditions investigated a fast and effective PCB degradation occurs in the very early stages of the MC treatment (20 to 50% PCB is degraded in the first 30 seconds depending on the reducing chemical used). PCB degradation then proceeds much more slowly until a steady $\approx 60\%$ value is achieved irrespective of the reducer employed. This confirms the enormous potential of MC treatment to ease solid state reactions by extended comminution of the solid particles (Korolev *et al.*, 2003; Birke *et al.*, 2004).

In the subsequent MC + Biological treatment tests,

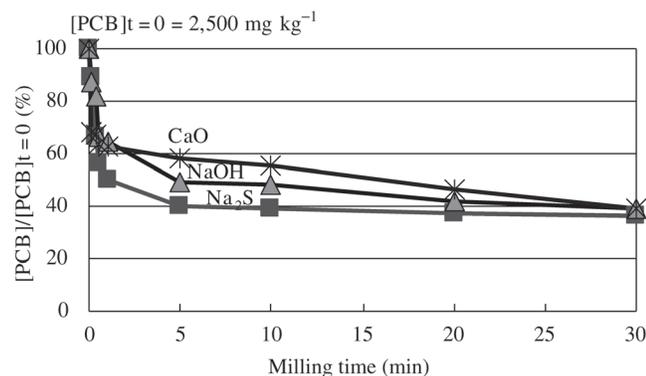


Figure 1. PCB degradation in artificially contaminated marine sediments by MC treatment.

accordingly, the sediment underwent a very short (30 seconds) ultramilling pretreatment with the cheaper CaO reducing agent with the double aim of achieving an initial $\geq 20\%$ PCB degradation and a larger reactivity of the comminuted solid phase. Special attention was paid to the addition of HA, whose surfactant behaviour was expected to improve the biological degradation process at the solid phase.

As shown by the results of the MC + Biological degradation in Erlenmeyer flasks (Figure 2), PCB biodegradation in the pre-milled sediments continued at a very small rate in the absence of HA (approx. $0.25 \text{ mmol kg}^{-1}$ in 28 days, i.e., $0.89 \times 10^{-2} \text{ mmol kg}^{-1} \text{ d}^{-1}$, see Figure 2(a)). By addition of 1.5% HA, however, the reaction rate showed a 4-fold increase (approx. 1 mmol kg^{-1} in 28 days, i.e., $3.57 \times 10^{-2} \text{ mmol kg}^{-1} \text{ d}^{-1}$, see Figure 2(b)), thus supporting the role of the surfactant to improve PCB bioavailability into the slurry water phase (Fava and Piccolo, 2002).

In both cases, the experimental molar concentration ratio between PCB decrease in the sediment and chlorides increase in solution was ≈ 4 (see Figure 2). Compared with the 6.42 ratio expected for the complete degradation of the average PCB congener ($\text{C}_{12}\text{H}_{3.58}\text{Cl}_{6.42}$)

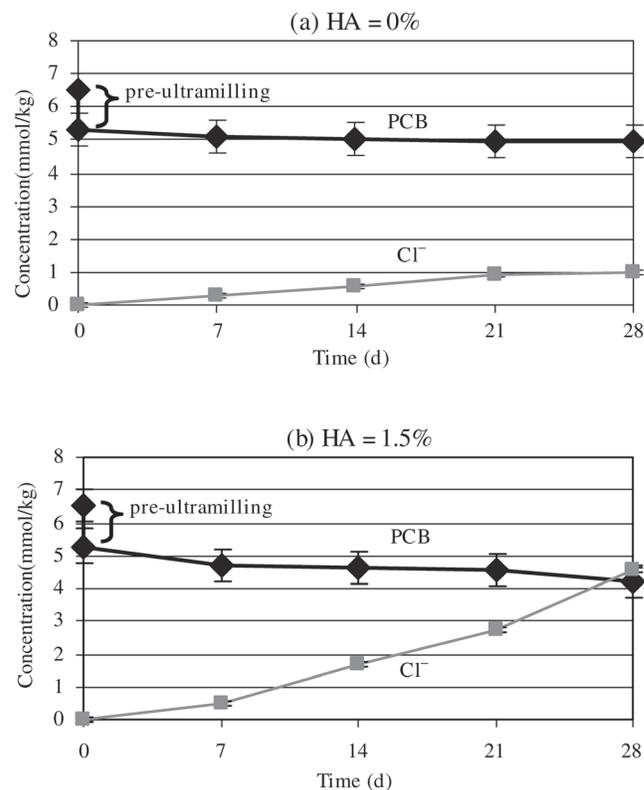


Figure 2. PCB biodegradation in pre-milled artificially contaminated sediment (Erlenmeyer tests).

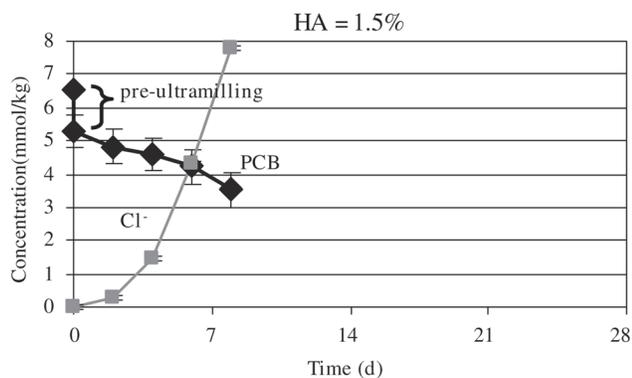


Figure 3. PCB biodegradation in pre-milled artificially contaminated sediment (Bioreactor tests).

used in the present experiments, this result confirms that the aerobic biodegradation is effective mainly towards low-chlorinated PCB congeners (with ≤ 4 Cl atoms).

Much better results were obtained with the Bioreactor tests. More efficient mixing and higher oxygenation, besides better control of all cultivation parameters, clearly allowed a quicker bacterial growth and a faster biodegradation rate. As indicated by the results in Figure 3, indeed, both the reaction rate and the biodegradation amount of PCB increased consistently in presence of 1.5% HA (approx. $1.75 \text{ mmol kg}^{-1}$ in 8 days, i.e., $21.9 \times 10^{-2} \text{ mmol kg}^{-1} \text{ d}^{-1}$).

After only 8 days of treatment (MC + Biodegradation) the overall PCB degradation amounted to approx. 47% (20% due to the very short MC pretreatment plus 27% due to the following biological treatment). Approx. $1,200 \text{ mg kg}^{-1}$ PCB were degraded from its initial $2,500 \text{ mg kg}^{-1}$ concentration in the sediments, as summarized in Table 3.

4. CONCLUSIONS

A two-stage treatment of marine sediments artificially contaminated with PCB, based on a very short ultra-milling pretreatment by MC technology followed

Table 3. Summary of the Experimental Results in MC+B Treatment of Marine Sediments Artificially Contaminated by PCB.

Set-up	HA (%ww)	Biodegradation Rate Constant ($\text{mmol kg}^{-1} \text{ d}^{-1}$)	Overall PCB Degradation (%)
Erlenmayer	0	0.89×10^{-2}	20 + 5
Erlenmayer	1.5	3.57×10^{-2}	20 + 18
Bioreactor	1.5	21.9×10^{-2}	20 + 27

by aerobic biological degradation with a commercial bacterium (*Burkholderia xenovorans* st. LB400), has been investigated. The preliminary results were very promising. Compared with the poorly effective and lengthy biological aerobic process and with the expensive MC treatment previously investigated, a very short (30 seconds) MC pretreatment allowed an overall $\leq 47\%$ biodegradation in only 8 days in laboratory tests on synthetic sediments highly contaminated by PCB.

Overall experimental process stoichiometry confirmed that only low-chlorinated PCB congeners (with ≤ 4 Cl atoms) are biodegraded.

Further experiments are planned to investigate the possibility of increasing the extent of overall PCB degradation by weakening the biorecalcitrance of higher PCB congeners through longer MC pre-treatment.

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Moisture Removal Mechanism of Sludge Conditioning by Freeze-Thaw Process

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ABSTRACT: The purpose of this study is to investigate the effects of sludge floc size and solids content on freeze-thaw conditioning and to enhance our understanding of the freeze-thaw conditioning mechanism. Digested activated sludge and an unidirectional freezing apparatus were used to perform the experiment. The results indicated that if sludges of small floc size was frozen at low speed, i.e., 10 mm/h, floc migration and aggregation occurred with the formation of ice front causing dehydration. This resulted in increasing floc compactness and narrowing the particle size distribution (PSD). For sludges composed of large floc, the floc were easily destroyed by the migrating ice front resulting in a smaller mean floc diameter and PSD than sludges composed of small floc. At low freezing speed, development of ice front for sludge with high solid concentration is slower than that of sludge with low solid concentration. When high freezing speed was employed to freeze sludge, floc destruction by ice front was more obvious, and the compactness of particle layer was lower than that caused by low freezing speed. Furthermore, under high freezing speed sludge with high solid concentration was easily entrapped by the ice crystal, and floc diameter was increased. However, gross migration of sludge was not found under this condition. Based on the experimental results, a modified model for the sludge freeze conditioning is proposed with the consideration of the effect of floc size and solids concentration. In addition, pretreatment to increase the solid concentration and decrease floc size before freeze-thaw conditioning are recommended to enhance the efficiency of this technique.

1. INTRODUCTION

FREEZE-THAW conditioning of sewage sludge has been demonstrated to enhance the dewatering characteristics of sludge (Martel, 1989; Vesilind and Martel, 1990; Vesilind, 1990; Vesilind and Chen, 1994). The mechanisms for improving the dewatering characteristics include reducing bound-water content of the sludge and restructuring the sludge floc into a more compacted and denser form (Vesilind and Martel, 1990; Lee and Hsu, 1995; Lee, 1994, Parker and Collins, 1999 a, b). The main advantages for using freeze-thaw conditioning is that no chemicals is used, which can significantly reduce the operating cost of sludge treatment. This technique is especially attractive in locations where natural freeze-thaw is available (Hellstrom and Kvarnstrom, 1997).

The performance of freeze-thaw conditioning depends on several factors including initial solid concen-

tration, time of frozen period, freezing rate, and dissolved solid concentration (Logsdon and Edgerley, 1971; Jean and Lee, 1999; Martel, 2000; Ormeci and Vesilind, 2001; Tao, *et al.*, 2006). Several conceptual models have been developed to describe the interaction between the ice front and sludge particles during freezing (Vesilind and Martel, 1990; Parker and Collins, 2000). When the ice front approaches sludge flocs, it also pushes the flocs at a certain speed. Depending on the freezing rate and other factors, the flocs either got entrapped in ice or pushed ahead of the ice front. Once the flocs is trapped in the ice, dewatering process initiates, resulting in releasing of bound water (dehydration) and more compacted sludge particles (Parker and Collins, 2000; Chen *et al.*, 2001). On the other hand, advancing flocs ahead of the ice front results in the thickening of flocs (Vesilind and Martel, 1990). Tao *et al.* (2006) concluded that the difference between the advancing speed of the ice front and the speed of the flocs is the main reason determining whether the flocs are entrapped in the ice or pushed ahead of the ice front.

Although solid concentration and freezing rate have

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been demonstrated to be significant factors affecting the performance efficiency of freeze-thaw process, the relationships between floc particle size and solid concentration and the operating conditions of freeze-thaw, i.e., freezing rate, has not been well characterized. As the entrapment of sludge flocs by ice front significantly affects the results of freeze-thaw conditioning, initial as well as final particle size of flocs could be an important indicator for the performance efficiency of this process. Thus, the objective of this study is to characterize the effects of freezing rate and initial solid concentration on particle sizes of sludges before and after freeze-thaw conditioning.

2. EXPERIMENTAL SETUP

Freezing of sludge was performed by advancing test tubes containing sludge samples into a water bath (Firstek Scientific, B403-L) maintained at -10°C with ethanol. The desired freezing rate was achieved by placing the test tubes rack in a step motor, of which advancing speed was controlled by a computer equipped with a signal input/out software (Smart Vision LS-4000). Two motor advancing speeds, 10 mm/h and 25 mm/h, were used in this study. Under such condition, the direction of the freezing was vertical. After complete freezing the samples were kept at -5°C for another 2 h. This was followed by the thawing step, which was conducted by placing the frozen sludge under room temperature for 12 h.

The sludge sample used in this study was obtained from the anaerobic digester of wastewater treatment facility of the Taichung Distillery, Taichung, Taiwan. The wastewater was comprised of the refuse and liquid, of which the chemical oxygen demand (COD) is high, from the fermentation processes. Pure kaolinite clay was used to prepare samples with high solid content and subjected to the same freeze-thaw conditioning of the sludge.

To achieve a smaller particle size distribution, the sludge was pretreated with ultra sound (Ikasonic, model UP-50H). The output of the ultra sound machine was set at 40 W and the pretreatment period was 30 s. On the other hand, to achieve a larger particle size distribution, cationic polymer was added to the sludge before the freeze-thaw conditioning process.

To prepare samples with different solid concentrations, the sludge was centrifuged at 5000 rotation-per-minute (rpm) for different periods of time.

3. ANALYTICAL METHODS

The dewaterability of the sludge was evaluated with a capillary suction time (CST) device (Triton-200). Solid concentration, COD, and turbidity of the sludge were analyzed according to standard methods.

The settling velocity of sludge particles was analyzed with the aim of a device consisting of settling cylinders, a camcorder (SONY DCR-TRV110) linked to a computer equipped with a still image capture board (SONY DVBK-2000) and a monitor (Figure 1). The settling cylinders has two chambers: chamber A is where the settling of particles took place, whereas chamber B is hydraulically connected to chamber A at the bottom to offset the upward flow and disturbance caused by the settling of particles. The camcorder focused on a marked section in chamber A to record the time and distance travelled by a particle. Each settling experiment was conducted with at least 200 sludge particles. The settling velocities recorded were used to calculate their densities by using Stokes law.

Particle sizes of sludge in each settling experiment were also analyzed. The captured still images of settling sludge particles were analyzed with a Matrox Inspector image analysis software. After the individual particle sizes were analyzed, the mean particle size of sludge was evaluated with formula (1).

$$\bar{x}_{NL} = \frac{\int_0^1 x dF_n}{\int_0^1 dF_n} \quad (1)$$

\bar{x}_{NL} = number-length mean size of the sludge

x = individual particle size

F_n = fraction of particles of size x

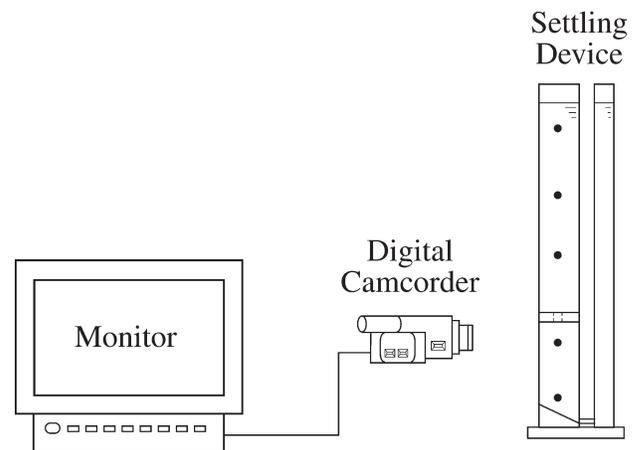


Figure 1. Schematic display of the settling and recording devices.

After the density and mean particle size were determined, the log-log relationship between the two variables was regressed according to the following formula to estimate the value of b .

$$y = ax^b \quad (2)$$

y = density

x = mean particle size

b = constant

Once the value of b is determined, the fractal dimension (FD) of the sludge particles is evaluated according to formula (3) (Wu *et al.*, 1997).

$$FD = b + 3 \quad (3)$$

4. RESULTS AND DISCUSSION

4.1. Effects of Floc Size

Ultrasonic and polymer addition were used as the pre-treatment units for producing different floc size. After ultrasound treatment, the mean floc size decreased from approximately 2000 to 1500 μm , whereas it increased from 2000 to 2400 μm after polymer treatment (Table 1). Freeze-thaw caused the mean floc size to decrease regardless of the treatments and freezing speeds (Table 1). However, the degree of change depends on method of treatments. Freeze-thaw caused minor change of floc size of sludge treated with ultrasound, whereas it significantly decreased the floc size of sludge pretreated with polymer. Previous researches reported that after the freeze-thaw treatment, slow freezing speed usually causes the floc size to increase and high freezing speed causes floc size to decrease.

Consequently, the speeds tested in this study are regarded as the so-called fast freezing based on the observed change in floc size.

In addition to the mean floc diameter, the size distribution factor (FSD) of sludge flocs was measured (Table 1). Just like the mean floc diameter, ultrasound caused FSD of sludge to decrease whereas polymer addition caused FSD of sludge to increase. Also, freeze-thaw caused FSD to decrease regardless of the treatments. The decrease of the FSD indicates the size distribution of sludge flocs were shifted from a wider into a more narrow range by freeze-thaw, which means the floc size became more uniform.

The fractal dimension value, FD, of sludge floc increased after freeze-thaw treatment at both freezing speeds indicating the floc structure of sludge became more compact after treatment. Furthermore, the increase on FD value of samples under 10 mm/h freezing speed are greater than that of 25 mm/h (Table 1). In general, under the freeze-thaw with either 10 or 25 mm/h freezing speed, the sludge flocs became smaller but had more compact structure and more uniform size distributions.

Figure 2 illustrates the CST change of samples with the freeze-thaw conditioning under two different freezing speeds. The CSTs for all samples after freeze-thaw are much less than that before freezing indicating a markedly improvement on dewaterability. It is noted that all CSTs of freeze-thaw treated sludges were about 220–250 sec and the effects of freezing speed on CST were minor. Hung *et al.* (1996) found that if the sludge has been frozen completely, the sludge filterability is improved regardless of the freezing speed and further reduction in freezing speed only slightly reduce the CST.

Table 1. Changes of Mean Floc Diameter (d μm), FSD and FD for the Sludge with Pre-treatments and Freeze-thaw Under Different Freezing Speeds.

Freezing Speed	10 mm/h		25 mm/h	
	Before	After	Before	After
	d / FSD / FD			
U	1510 / 0.48 / 1.61	1500 / 0.42 / 1.82	1533 / 0.62 / 1.74	1524 / 0.39 / 1.82
O	1994 / 0.61 / 1.62	1553 / 0.45 / 1.81	2066 / 0.70 / 1.67	1487 / 0.52 / 1.75
P	2429 / 0.76 / 1.66	1419 / 0.63 / 1.81	2449 / 0.69 / 1.68	1425 / 0.52 / 1.83

O = Original sludge

U = Ultrasonic treated

P = Polymer added

d = mean diameter of floc

FD = fractal dimension value

FSD = $(L_{25} - L_{75})/L_{50}$

L_x is defined as a floc size that $x\%$ of floc size are larger than this size

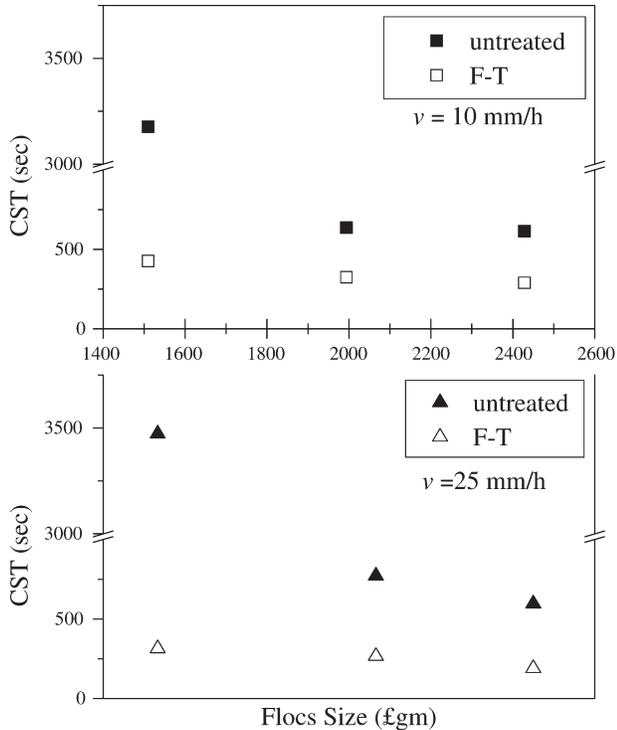


Figure 2. CST of samples with freeze-thaw conditioning.

As the sludge is freezing, the ice-front is forming and floc migration also happen at once. In this study, we followed the index RM used by Hung *et al.* (1996) to present the degree of particle migration in the sludge after freeze-thaw. In Hung's study, the frozen sample was cut along its radial direction into two sections with top 1/4 and bottom 3/4 of total length, respectively and the ratio of the solid content in the top section to that of the original is defined as RM. In this study the frozen sample was cut into four sections along the sample length and labeled as RM1–RM4, respectively, to illustrate the floc migration.

As shown in Table 2, there were insignificant RM differences between sludges treated with different freezing speed, indicating negligible solid migration during freezing and most solids were trapped by the ice front.

Table 2. The Floc Migration Degree for Test Samples After Freeze Thaw.

Freezing Speed	10 mm/h			25 mm/h		
	Floc Size (m)	1510	1994	2429	1533	2066
RM1	1.08	1.06	1.05	1.05	1.02	1.03
RM2	1.00	–	1.03	–	1.14	1.06
RM3	0.99	–	1.09	–	0.97	1.02
RM4	0.93	–	0.83	–	0.87	0.89

– = Test Failed.

4.2. Effects of Solid Content

At low freezing speed, freeze-thaw caused the floc size of all except sludge with solid content (SC) of 1.77% to increase (Table 3). On the other hand, high freezing speed caused the floc size of all the sludges to decrease. After freeze-thaw, FSD of samples decreased from 0.64–0.69 to 0.39–0.48. In addition, FSD values of freeze-thaw treated sludges decreased with SC values (Table 3).

Previous research found that the floc size of sludge with low SC is smaller than that of sludge with high SC after freeze-thaw treatment (Martel *et al.*, 1998). Based on the finding of this study, floc size change is affected not only by the solid content but also by the freezing speed used.

Table 4 shows the degree of floc migration for sludge samples after freeze-thaw. From the RM1 to RM3, no significant gross migration were occurred. For RM4, there are four data less than 1.0 and are different from RM3 at the samples with SC value at 5%. However, no gross migration found on the top section, although some particles were really rejected by the ice front and pushed forward to top-sections especially on the low freezing speed operation. The effect of solid content of sludge to floc migration can be therefore concluded that only slightly local floc migration could happened on the bottom section when the solid content of sludge less than 5 w/w (%) and the gross floc migration did not appear even a low, 10 mm/h, freezing speed applied.

Table 3. The Change of Mean Floc Diameter and FSD of Sludge with Different Solid Contents by Freeze-thaw Conditioning.

Freezing Speed	10 mm/h			25 mm/h		
	w/w (%)	1.77	3.36	5.51	1.85	3.57
	d / FSD					
Before	2013/0.65	1975/0.65	1992/0.64	2042/0.68	1902/0.69	1956/0.66
After	1580/0.46	2118/0.43	2233/0.39	1520/0.48	1823/0.44	1732/0.44

Table 4. The Floc Migration of Activated Sludge After Freeze-thaw.

Freezing Speed	A (10 mm/h)			B (25 mm/h)		
	w/w (%)	1.77	3.36	5.51	1.85	3.57
RM1	1.15	1.18	1.02	1.12	1.12	0.94
RM2	1.08	1.16	0.96	1.10	1.09	0.94
RM3	1.09	1.07	0.97	0.97	1.03	0.92
RM4	0.68	0.59	1.05	0.81	0.76	1.20

4.3. Solid Migration Mechanisms

Based on the findings of this study, a conceptual model for freeze-thaw conditioning of sludge is proposed as described in the following.

1. High Freezing Speed; High Solid Content

When the ice front contacts small floc, it will reject the flocs and push the flocs forward. However, when the floc size is large, the ice front either entraps the floc or pierces the floc. As the ice front grows forward, small floc migrated ahead of the ice front whereas the pierced large floc imploded into smaller pieces (fragments) as a result of ice formation. When the migrating flocs contact other flocs, a larger floc formed, a process called aggregation, which eventually creates flocs that are too large to be pushed by the ice front. Consequently, the aggregated flocs are entrapped by the ice front. The entrapped fragments and aggregates undergo dehydration as a result of ice formation within the floc. This results in a more compacted floc structure. High SC increases the amount of floc aggregates and high freezing speed prevent the floc to migrate as a front. The conceptual model described above is shown schematically in Figure 3.

2. Low Freezing Speed; Low Solid Content

When the ice front contacts the flocs, it pushes small flocs while entrapping large flocs. As the ice front continues to grow, small flocs migrate and large flocs are completely entrapped within the ice formation. The migration of small flocs eventually stops and entrapment occurs, causing dehydration of flocs as the water is drawn by the ice formation. Dehydration of flocs causes the floc structure to be denser and more compact. When the freezing process is complete, most flocs are entrapped in the ice as individual particles since the solid content is low. The conceptual model described above is schematically shown in Figure 4.

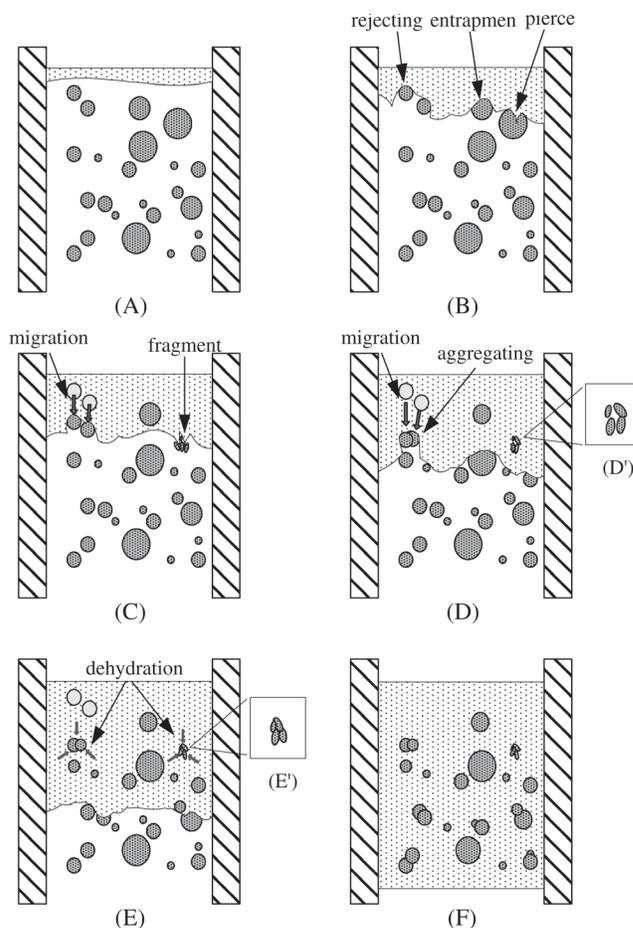


Figure 3. Conceptual Model for High Speed Freezing of High Solid Content Sludge. Freezing direction: top to bottom. (A) Formation of ice front; (B) Rejection of small particles and entrapment of large particles; (C) Migration of small particles and fragmentation of large particles; (D) Aggregation of small particles and (D') Dehydration; (E) More compacted particles and aggregates; (F) Completion of freezing.

The conceptual models of freeze-thaw conditioning of sludge can be used to describe the results of this study. Both freezing speed and particle size affects the degree of migration of flocs. Degree of migration then affects the floc diameter after freeze-thaw process. Entrapment of flocs causes dehydration and subsequent fragmentation of flocs, leading to more uniform and compacted flocs sizes. Therefore, large size flocs are usually pierced and fragmented by the ice front, resulting in the decrease in floc size. On the other hand, small size flocs are pushed forward by the ice front at slow freezing speed and large aggregates of flocs formed as a result.

In addition, sludges with high solid content tend to form aggregates during freezing as the flocs pushed by the ice front has more chance to contact other flocs.

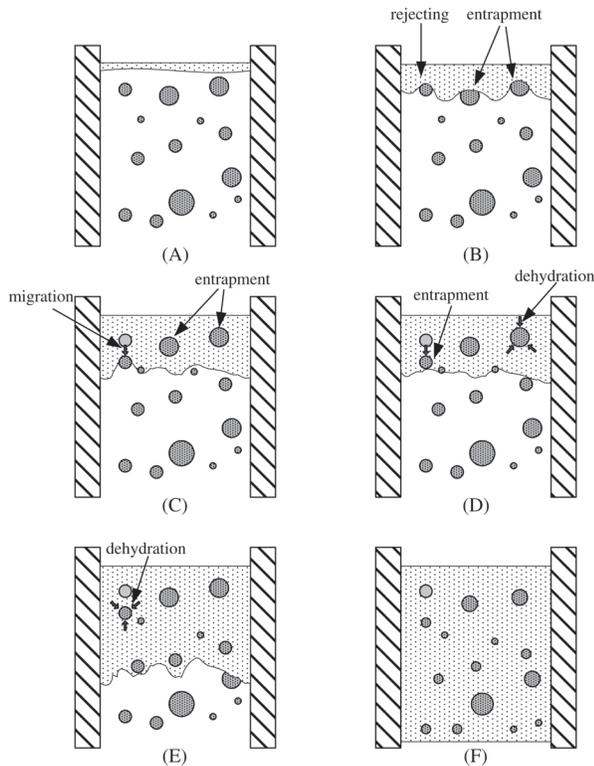


Figure 4. Conceptual Model for Low Speed Freezing of Low Solid Content Sludge. Freezing direction: top to bottom. (A) Formation of ice front; (B) Rejection of small particles and entrapment of large particles; (C) Migration of small particles; (D) Dehydration of small and large flocs; (E) More compacted individual particles; (F) Completion of freezing.

5. CONCLUSION

This study characterized the change in particle size and distribution of sludge floc treated with freeze-thaw conditioning. According to the results of this study, freezing speed, original floc size, and solid content of sludges affect the floc size distribution and dewaterability of sludges after freeze-thaw conditioning. Two conceptual models describing the interaction between the ice front and sludge floc during freezing stage are proposed and used to explain the results of this.

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On the Necessity of Sludge Conditioning with Non-organic Polymer: AOP Approach

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ABSTRACT: Organic polymers have long been used as sludge conditioners to improve its dewaterability in sludge management practice. Although polymers can bring about a great dewatering performance of the sludge, their potential health related risk remains unknown regarding their residual in dewatered sludge cakes in the environment when the sludge is finally disposed as landfill especially in long term point of view. For this regard, as an initiative action, Fenton ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$) and its related reagents were tested in this study as a potential alternative an alum sludge conditioners for the purpose of eliminating the perceived long term risk associated with polymer residual in the environment.

1. INTRODUCTION

THE global use of chemicals in wide range of types plays important role in water purification and wastewater treatment engineering. The use of synthetic organic polymers or polyelectrolytes (with molecular weights varied normally from 10^4 to 10^6 Daltons) in drinking water treatment as flocculent aid and mainly in conditioning of water treatment sludge has been a worldwide practice for a long time. Great performance of sludge dewatering devices was obtained when various types of polymers were adopted as sludge conditioners. In recent year, however, such use of polymers has raised increased concerns of their residuals in the surrounding environment, which was associated with the cycling/reuse and even the landfilling of the treated or dewatered sludge. Although organic polymers are specifically manufactured for water industry, the monomers used in the manufacture of many polyelectrolytes are toxic (Bolto and Gregory, 2007). Long-term effects of polymer residual on aquatic organisms, human health and the biota related to the disposal of such sludge are still remaining unknown. Therefore, as an initiative action, research work to seek the alternative chemicals in water treatment processes

especially in sludge conditioning is desirable in terms of sustainable development.

In this paper, the necessity of the sludge conditioning to avoid using organic polymers was discussed based on the updated information from the literature. Thereafter, a new approach of using Fenton ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$) and its related reagents as one of the advanced oxidation processes (AOPs) (Neyens and Baeyens, 2002) for conditioning of an aluminum salt-flocculated drinking water treatment sludge was summarily presented.

2. THE NECESSITY OF NON-POLYMER APPROACH: MINI REVIEW

2.1 Concerns of Organic Polymers

A recently critical review of the polymers used in water industry was given by Bolto and Gregory (2007). As is known the polymers are mainly made of acryl amide and acrylate, they can be one of possible toxic chemicals to aquatic animals and human bodies at certain concentration even though they are sometimes biodegradable. Unfortunately, techniques of appropriate monitoring of the polymer residual are shortage (Zhao *et al.*, 2008), this makes it unknown of the polymer residual in water treatment and sludge conditioning process although the amount of the polymer addition is ac-

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curately known. Furthermore, information on the fate of the added polymers in water and sludge treatment is extremely lack. As a result, it is unclear regarding the risk of the polymers used from the long-term point of view. It has been noted that the use of polyelectrolytes in Japan and Switzerland are not permitted in the drinking water treatment, while Germany and France located a strict limit for such use (Bolto and Gregory, 2007). Accordingly, a stringent limit for the polymer use to prevent the environmental damage has been proposed (Majam and Thompson, 2006). Therefore, new concepts and principles for sludge conditioning/treatment in line with sustainable development remain a great challenge to engineers and scientists who are called upon to develop solutions that are technically, economically, and socially sound.

Due to a lack of research and information as to the toxicological effects of the polymer residual that could possibly leach out from landfills when the dewatered sludge cake is deposited, much of the information on the toxicity of polymers is in relation to the effects on aquatic organisms. The presence of residual polymer is a cause of concern around the world but no research has been undertaken to determine if it is actually in attendance. It is generally found that the cationic polymers are more toxic especially to aquatic organisms. Anionic and non-ionic polymers are usually of low toxicity. Some countries have taken drastic measures with regard to the use of polymers in water treatment due to concerns about contaminants. Examples of these include Japan and Switzerland disallowing their use in water treatment and France and Germany setting severe limits on their usage. Criddle (1990) indicated that the monomers are more toxic than the polymers. Where limits have been placed on the level of monomer these are strictly controlled. In particular the level of acrylamide is scrutinised where the maximum allowable content of free acrylamide is 0.025%, and the residue for drinking water is limited to 0.05ug/l. In Europe the level of monomer for PDADMAC is 0.5% and 2% in USA (BSI, 1998; NSF International, 2001). Recommended limits have also been set for regularly used commercial polymers in drinking water production by the National Sanitation Foundation. They are as follows: < 20 mg/l for ECH/DMA polymers, < 1 mg/l for PAM's, and < 50 mg/l for PADMAC.

Hamilton *et al.*, (1994) maintained that aquatic organisms are not inclined to readily absorb the synthetic polymer and alterations to their toxicity may take place by key aquatic components. They also found that

cationic polymers are noticeably more toxic to aquatic organisms than anionic and non-ionic polymers. Cationic polymers are also regarded as moderate to high toxicity. Cationic polymers are detrimental to fish because of mechanical gill blockage that causes suffocation (Biesinger and Stokes, 1986; Cary *et al.*, 1987). If however solids such as clays are added to the receiving water this problem is greatly reduced as the sediments in the water absorb the cationic polyelectrolytes. Humic acids also affect the toxicity (Goodrick *et al.*, 1991).

Timofeeva *et al.*, (1994) found during their study that the toxicity of polymers is greatest for polymers of high charge density. This was observed during the study of the toxicity of CPAM's of various molecular weight and charge density towards daphnia and minnows. A greater toxicity was found in daphnia that were exposed to higher molecular weight polymers at low charge density, however concentrations of 0.2 mg/l, which greatly exceeded the likely polymer concentration in the final product water, were found. Toxic level for minnows was found to be ten times higher. Narita *et al.*, (2001) found in another study that cationic polymers drastically disturbed yeast cells at a dose of ca.70mg/l while non-ionic and anionic were not found to disturb them.

In a study undertaken by Takigami and Taniguchi (1998) looking into toxicity assays and their evaluation on organic flocculants, two different toxicity assays were identified *Closterium ehrenbergii* algal toxicity test and the *Bacillus subtilis* rec-assay. Investigations were made with and without the addition of a flocculant into the algal toxicity of the effluents from a pilot scale sewage treatment plant. Lethal effects in this study were mainly contributed by a polymer fraction of molecular weight greater than 100,000. However it is thought that the genotoxicity of the flocculants could be perhaps caused by a combination of effects of various components such as monomer, oligomers, and additives.

2.2 Non-polymer Approaches

In recent years, considerable efforts have been made to explore the non-polymer approaches for sludge conditioning/treatment. These approaches may be studied for thriving the sludge conditioning research, rather than dealing with the concern of the potential polymer threat. From the literature, the non-polymer approaches include: (1) non-reagent approaches of freeze-thaw

conditioning (Vesilind and Martel, 1990; Parker *et al.*, 1998; Ormeçi and Vesilind, 2001); microwave heating (Jones *et al.*, 2002; Wojciechowska, 2005); ultra-sound conditioning (Yin *et al.*, 2004), and (2) non-organic polymer approaches of sludge ozonation (Park *et al.*, 2003) and Fenton's reagent conditioning ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$) of wastewater treatment sludge (Neyens *et al.*, 2003; Buyukkamaci, 2004; Dewil *et al.*, 2005).

Fenton's reaction (using reagent of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$) as one of the advanced oxidation processes (AOPs) (Neyens and Baeyens, 2002) has long been employed in many areas. However, little has been found in conditioning of aluminum salt-flocculated drinking water treatment sludge (termed as alum sludge thereafter). In our previous study, the effectiveness and optimization of Fenton's reagent for an alum sludge conditioning was preliminarily investigated (Tony *et al.*, 2008). The addition of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ led to a considerable improvement in the alum sludge dewaterability as evaluated by the CST (capillary suction time).

3. ALUM SLUDGE CONDITIONING USING FENTON'S AND ITS RELATED REAGENTS

3.1. Alum Sludge and Fenton and Fenton-like Reagents

The alum sludge samples used in this study were taken from a water treatment plant in Southwest Dublin, Ireland where raw water from a local reservoir was coagulated with aluminum sulphate. The sludge had a suspended solids concentration of 2,850 mg/L. The capillary suction time (CST), specific resistance to filtration (SRF), pH, and Al content of the alum sludge were 67.5 s, 6.3×10^{11} m/kg, 5.7–6.0 and 194 mgAl/g sludge, respectively. Six dibasic salts ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, ZnCl_2 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) and two ferric salts ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{Fe}_2(\text{SO}_4)_3$) were used individually to make different metal solution in Fenton and Fenton-like reagents. Hydrogen peroxide in liquid form (30% by wt.) was obtained from a commercial supplier.

3.2. Experimental Methods

The experimental investigation of the alum sludge conditioning was conducted in a standard jar-test apparatus. Initially metal solution was added to a series of 250 ml sludge samples, Fenton or Fenton-like reagent

reaction was then initiated after adding hydrogen peroxide. Thereafter, the sludge was subjected to rapid mixing (for 30 second) and slow mixing (for 30 second) to generate reaction. This conditioning procedure, especially the reaction time, has been investigated previously for the time of reaction from 1 min to 4 hrs (Tony *et al.*, 2008). The optimum doses of hydrogen peroxide and metal salt were also optimized previously on the response surface methodology (RSM), which is a collection of mathematical and statistical techniques for optimising purpose. According the previous study (Tony *et al.*, 2008), the dosage of metal ions in Fenton and Fenton-like reagents of 20 mg/g DS (dry solids) and the dosage of H_2O_2 of 125 mg/g DS are applied in this study.

The CST of the sludge samples before and after various conditioning was measured by CST apparatus (Triton-WPRL, Type 130 CST). Dewaterability of the sludge under Fenton and Fenton-like conditioning is evaluated by the percentage reduction (E%) of CST via the following equation:

$$E(\%) = \frac{C_0 - C}{C_0} \times 100$$

where C_0 and C are, respectively, the CST of alum sludge before and after conditioning.

3.3. Results and Discussion

Table 1 presents the results (in a manner of average data) of the alum sludge conditioned with Fenton and Fenton-like reagents. As can be seen in Table 1, a clear advantage of conditioning efficiency with Fenton reagent ($\text{FeCl}_2/\text{H}_2\text{O}_2$) was identified among all the Fenton and Fenton related reagents tested. FeCl_3 and FeSO_4 used with H_2O_2 also showed considerable im-

Table 1. Results of an Alum Sludge Conditioning with Fenton and Fenton Related Reagents.

Reagent	Mean CST Reduction (%)
FeCl_2	47
FeCl_3	38
FeSO_4	37
CuSO_4	11
CoCl_2	7
MnCl_2	7
$\text{Fe}_2(\text{SO}_4)_3$	3
ZnCl_2	1

Note: Doses of Fe^{2+} , Co^{2+} , Cu^{2+} , Mn^{2+} , Zn^{2+} , Fe^{3+} are all at 20 mg/g-DS (dry solids). Dose of H_2O_2 is 125 mg/g-DS. Experiments were conducted at pH = 6.0.

provement of sludge dewaterability. This may be due to the fact that they are useful coagulants in water purification. Testing of FeCl_3 and FeSO_4 alone without H_2O_2 for alum sludge conditioning showed that FeCl_3 do have the same function of combined use of it with H_2O_2 for CST reduction, but FeSO_4 did not.

Also seen from table 1, the Cu, Co, and Mn salts can only bring about a quite similar CST reduction rate in a range of 7% to 11% when they are used as Fenton-like reagents, reflecting a marginal effect regarding sludge dewaterability improvement. However, the CST reduction rate obtained using Zn salt was 1%, indicating its ineffectiveness.

The reason of the difference in the abilities of the various transition metal salts in Fenton and Fenton-like reagents conditioning of alum sludge may be related to the different abilities of transition metals to react with oxygen in a variety of ways, due to the unpaired oxygen electrons (Mustranta and Viikari, 1993). The hydroxyl radicals formed is not enough to attack the sludge particles to form the new intermediates which is able to treat the sludge in order to enhance its filtration properties. However, for the iron ions, the produced hydroxyl radicals are much more sufficient than for the other transition metals. More significantly, our previous investigation (Tony *et al.*, 2008) has demonstrated (by the molecular size distribution measurement of sludge samples before and after Fenton reagent conditioning) that the Fenton reaction degraded/broke the organics from large molecular sizes into smaller ones via highly reactive hydroxyl radicals. Thus improving sludge dewaterability through the release of both interstitial waters, which were trapped among organics, and adsorbed and chemically bound water by the degradation of organics.

It should be pointed out that the Fenton's reagent ($\text{FeCl}_2/\text{H}_2\text{O}_2$) cannot achieve the same level of CST reduction as that achieved by the polymers (data not shown), Thus, the application of the Fenton reagent for alum sludge conditioning as alternative conditioner lies in its advantage over polymer on environmental safety, rather than the efficiency regarding the CTS reduction. The study of using Fenton reagent/reaction for alum sludge conditioning as an alternative options to overcome unknown potential health related risk of polymer conditioning may provide a showcase of proactive treatment engineering. However, further investigation is obviously desirable, which includes the cost-effective analysis of the cost increase of equipment installation, operation and control process.

4. CONCLUSIONS

Fenton reagent ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$) can be considered as an alternative option for alum sludge conditioning for the purpose of eliminating the perceived long term risk associated with polymer residual in the environment. From the results of this study, CST reduction of 47% can be achieved when Fenton reagent was used for an alum sludge conditioning. Obviously, it is not the same level regarding the CST reduction of using polymers. In addition, it should be noted that there may be some increase of cost associated with the Fenton's reagent due to its dual-reagent addition. Such costs may be related to cost increase of equipment installation, operation and control process. Full consideration and review should be conducted before any large application of such conditioning approach.

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Optimized Coagulation on a Municipal Wastewater Treatment: Competitive and Synergetic Effect Among Different Pollutants

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ABSTRACT: Iron(II) shows an increasingly attractive option among the main metal salts available for phosphorous removal by chemical precipitation. But other anions in the changing wastewater may compete with phosphorous for iron(II) also. Meanwhile, in the absence of oxygen, the iron(II) will not be oxidized and produce a very fine precipitate with phosphorous which is difficult to settle down easily. A case study towards an optimized coagulation system in a municipal WWT plant was therefore carried out to illustrate the competitive and synergetic effect during iron(II) coagulation. The results showed that FeSO₄ can effectively reduce the dissolved total phosphorus (DTP) and sulfide (S²⁻), but total phosphorus (TP) is not reduced. Alum and polyaluminum chloride (PACl) could act as the coagulant aid to develop an effective coagulation process with the emphasis of phosphorus removal from wastewater.

1. INTRODUCTION

It is well-known, phosphate discharged into the surface water accelerates eutrophication (Inamori *et al.*, 1993; Kiely, 1997). This can in turn disturb the balance of organisms present in the water and affect water quality, mainly through the depletion of the oxygen level as the algae decay. Reduced oxygen level can have harmful effects in fish and other aquatic life, causing reduction in biodiversity.

In order to minimise eutrophication, treatment facilities must reduce phosphorus levels to less than 1 mg P/L. Phosphorus removal techniques fall into three main categories: physical, chemical and biological. Chemical removal techniques, using metal salts, are reliable and well-established processes (Jenkins *et al.*, 1971; Cooper *et al.*, 1993). Among the main metal salts available for phosphorous removal by chemical precipitation, the cost benefits and optimum pH requirements 7.2–8.0 of using iron(II) make it an increasingly more attractive option (Fytianos *et al.*, 1998). But in the absence of oxygen, the iron(II) will not be oxidized and produce a very fine precipitate with phosphorous which

is difficult to settle down spontaneously. The large quantity of iron(II) dose, however, increase largely the sludge load. Moreover, other ions in the wastewater body may compete with phosphorous for iron(II) (Xie *et al.*, 2005; Zhou *et al.*, 2008). Therefore the wastewater exhibits quite complicated nature including various factors affecting the phosphorus removal. A case study is then carried out to develop an optimized process for phosphorous removal and sludge control at a local wastewater treatment plant. The synergetic removal of co-existing competitive sulfide ion is discussed based on the source water characteristics.

1.1. Instructions for Authors

Prof. Wang is a Professor in Environmental Engineering, Aquatic Chemistry and Colloid and Interface Sciences at Research Centre of Eco-Environmental Sciences, Chinese Academy of Sciences, and an Adjunct Professor at University of South Australia. He is an international leader in developing new inorganic coagulants for water treatment, such as High Performance Poly-Aluminium Chloride (HPAC) and Inorganic Polymeric Flocculant (IPF). For the last 5 years, he has published over 60 refereed journal papers in the area of coagulation mechanism, high efficient coagulant de-

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velopment, and fractal floc structure characterisation and control. He works closely with water treatment industry on in-situ process control to optimise coagulation process.

2. METHODS

All chemicals used were analytical grade except those being pointed out specifically. Distilled water was used in all experiments. Phosphate solutions were prepared from $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (their concentration was 100 mgP/L). Sulfide solutions were prepared from Na_2S (their concentration was 100 mgS/L). FeSO_4 solutions (were prepared from $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 70 mg FeSO_4/g), AlCl_3 solutions (were prepared from $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, 100 mg $\text{Al}_2\text{O}_3/\text{g}$) and PACl (100 mg $\text{Al}_2\text{O}_3/\text{g}$, Wanshui company, Beijing) were used as coagulant. Treated wastewater was collected from Dongying municipal wastewater plant, Shangdong Province, China. The precipitation experiments were carried out by a conventional jar test procedure in a six-unit multiple stirrer system. Phosphate analysis, sulfide analysis and COD analysis are all in conformity with Chinese Water and Wastewater Measurement Method (4th Edition).

3. RESULT AND DISCUSSION

3.1. Experimental Results of Real Wastewater

The water sample was from Dongying municipal wastewater plant with the main character of high sulfide concentration at 5.77 ppm, much higher than normal municipal wastewater. The respective concentration of TP and DTP were 3.99 and 2.81 ppm, and pH was 7.8. The precipitation experiments were carried out by a conventional jar test procedure in a six-unit multiple stirrer system.

As increasing FeSO_4 dosage, the effluent DTP and S level were brought down effectively. When the molar Fe/DTP ratios were 1.0, the respective DTP and sulfide maximum removal were 56% and 44% for the effluent. The FeSO_4 dosage actually used was much lower than stoichiometric requirement. But the TP concentration almost didn't change and this was related to the phenomenon that the flocs failed to settle down (J. Rajesh Banu *et al.*, 2008). These results are presented in Figure 1.

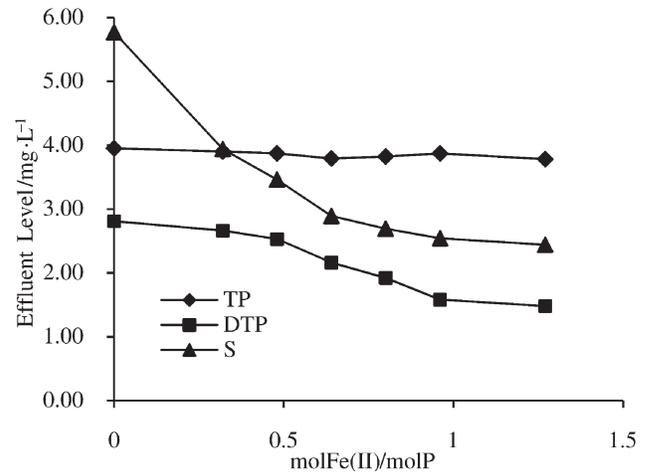


Figure 1. Changes of pollutants removal in effluent with increasing Fe(II) dosage.

3.2. Effect of Particles on Phosphate Removal

To explain the results above, artificial water samples were used to study the phosphorous removal using iron(II) in the presence of sulfide. First, without other competitive ions, the corresponding molar ratio to achieve a removal of 80% phosphorous lied in the region of 1.5(Fe/P), which was consistent with the stoichiometric requirement. Second, with both phosphorous and sulfide existing, the sulfide got removed effectively as increasing the iron(II) dosage. To achieve 80% removal, the Fe/S ratio was about 2.22, which far exceeded the stoichiometric requirement. At this point, both TP and DTP were not removed at all (Figure 2).

The solubility product constant of FeS is much lower than $\text{Fe}_3(\text{PO}_4)_2$, it is indicative that iron(II) preferred to

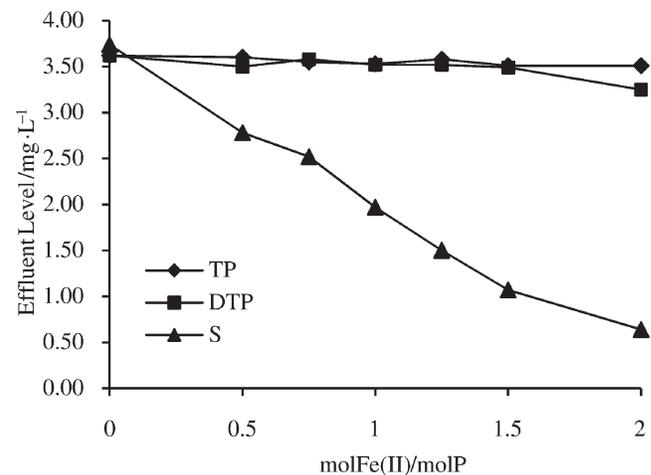


Figure 2. Changes in effluent level with increasing Fe(II) dosage: without kaolin.

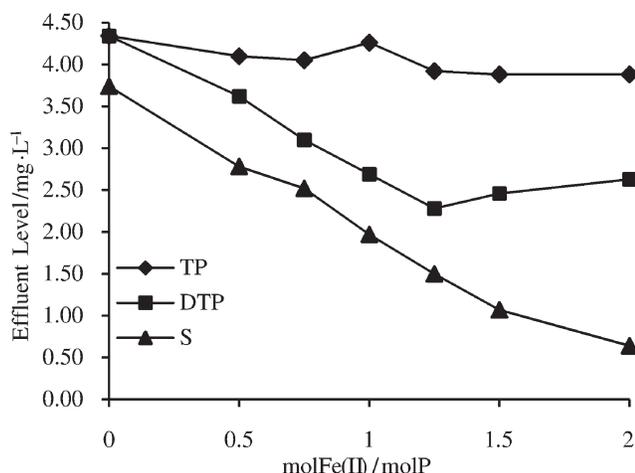


Figure 3. Changes in effluent level with increasing Fe(II) dosage: with kaolin.

precipitate sulfide. Comparing with the results of actual water sample, it is speculated the outgoing of the extra iron(II) were the hydrolysis of iron(II) and the precipitates of iron(II), phosphorous and maybe sulphide also were tiny enough to pass through the 0.45 μm membrane. So the third experiment was carried out by adding kaolin to the artificial water sample. With the same sulfide removal trend, the DTP got removed also this time. At the maximum DTP removal ratio of 47%, the molar Fe/P ratio was 1.15 (Figure 3).

The result was in consistent with the actual wastewater. Kaolin couldn't adsorb dissolved ion salts by themselves, but it could accelerate the aggregation and aggregate the precipitates to grow up large enough to be prevented by the 0.45 μm membrane (Shogren, 2009). This result confirmed the previous hypothesis. In both actual and artificial water samples, the adopted iron(II) dosage for phosphorous and sulfide removal was much lower than the stoichiometric requirement. It illustrated that the removal of phosphorous and sulfide were not strictly according to the stoichiometric proportion, there was synergetic removal mechanism between phosphorous and sulfide, both the hydrolysis product of iron(II) and solid particles present in the bulk water affected the removal rate.

3.3. Aiding Effect of Organic Flocculants on Phosphate Removal

The results above show clearly that the settlement performance is very important in phosphate removal. Therefore flocculants such as polyacrylamid (PAM) and polymerized dially dimethyl ammonium chloride

(HCA) was tested to improve the settlement performance of FeSO_4 . The molar Fe/DTP ratio was kept at 1.0. As PAM and HCA can not be combined with phosphate to produce insoluble phosphate, DTP can not be removed more efficiently (Figure 4). It seemed that PAM and HCA did not achieve the desired results on TP and COD removal. Further evidence of the ferrous ion itself does not form an effective floc, and flocculant PAM and HCA can not play its role of bridging bond.

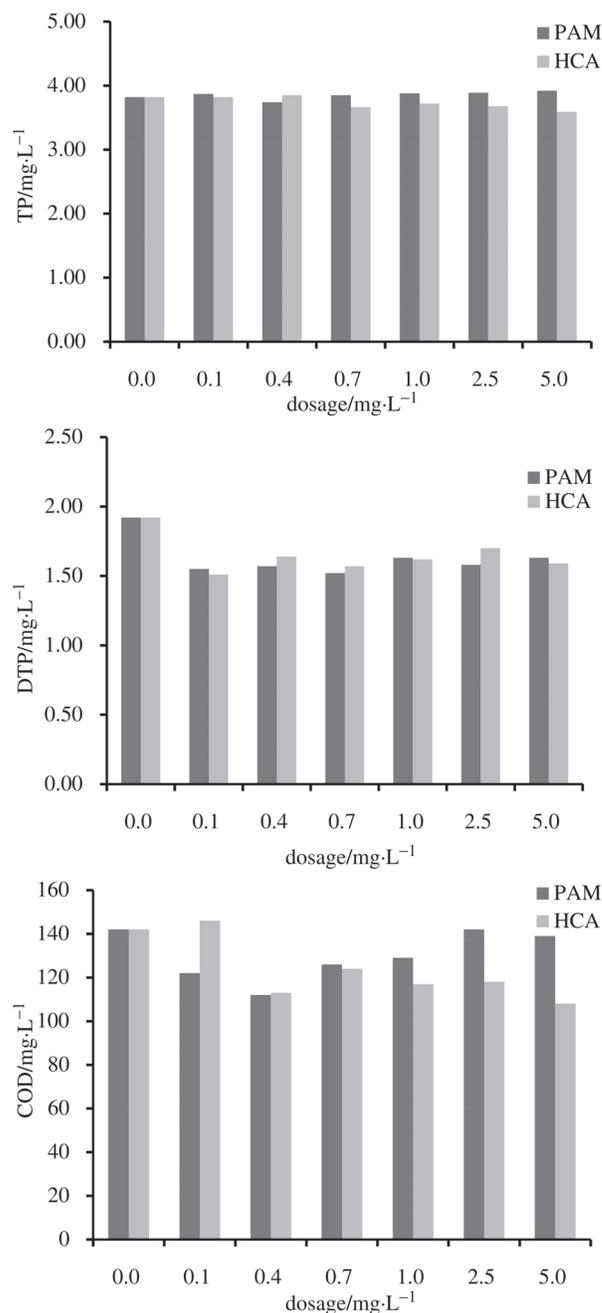


Figure 4. Changes in effluent level with increasing organic flocculant dose.

3.4. Aiding Effect of Inorganic Coagulants on Phosphate Removal

AlCl_3 and PACl were then chosen to associate with FeSO_4 to improve the settlement performance in the actual wastewater treatment. The molar Fe/DTP ratio was

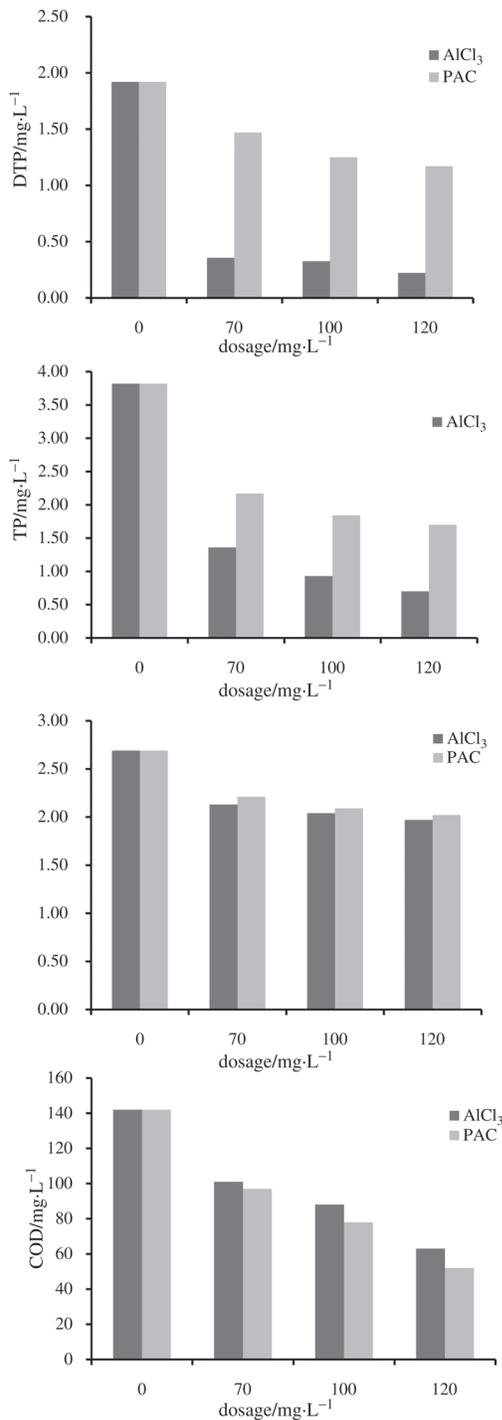


Figure 5. Changes in effluent level with increasing inorganic coagulant dosage.

also kept at 1.0. It seemed that AlCl_3 was much more efficient in phosphorous removal than PACl. Both TP and DTP became further removed effectively (Figure 5). When the dosage of AlCl_3 was increased to 100 mg/L, the effluent TP level was below 1mg/L, meeting the phosphorous discharge requirement. PAC brought down the effluent TP level but failed to DTP. It indicated when Al monomers prevail, it could further react with dissolved phosphate and form insoluble salts (Georgantas *et al.*, 2007). But the components of PAC were aluminium hydroxy polymers, being OH-complexed the capability to complex with phosphate becomes decreased (Bashan *et al.*, 2004). It also illustrated that direct adsorption of dissolved phosphate ions on the aluminum hydrolysis products was weak. Both AlCl_3 and PAC exhibited favorable flocculation performance, so they improved the precipitate performance and TP removal effect. At the same time, COD got removed also about 60% at coagulant dose of 120 mg/L. Sulfide removal was almost out of relation to coagulant and mainly depended on iron(II).

4. CONCLUSIONS

In this work, we find that chemical phosphorus removal mainly includes two stages, chemical precipitation and flocculation. After adding coagulant into the sewage, the metal ion formed instantly the non-soluble phosphate salts or hydrogen oxides (depending on the pH). The settlability of precipitate formed depends largely on raw water quality, especially the DOM and inorganic anions. Although the solubility product constant of FeS is much lower than $\text{Fe}_3(\text{PO}_4)_2$, phosphorous and sulfide got removed simultaneously in both actual and artificial water samples. The iron(II) dosage actually used was much lower than the stoichiometric requirement, it indicated that there was co-operating removal effect between the two pollutants. The precipitate formed only by iron(II), sulfide and phosphorous was conformed tiny enough to pass through the 0.45 μm membrane. Aquatic particulates were of great importance to precipitate phosphorus and sulfide, which could adsorb the formed fine precipitates, making them aggregate large enough to settle down efficiently from the bulk water. Both AlCl_3 and PACl exhibited favorable flocculation performance, they improved the settlement performance by complexation, neutralization and adsorption, at the same time, COD got removed also. But AlCl_3 was much more efficient in phosphorous removal than PACl.

ACKNOWLEDGEMENTS

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Ultrasonic lysis of Biological Sludge: A Three-stage Process

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ABSTRACT: Ultrasounds have been known very effective for biological sludge disintegration, which can be used as a pretreatment method prior anaerobic digestion or sludge minimization. This paper is a preliminary investigation of the involution of sludge matrix during sonication. The sludge was treated with 0.8 W/ml ultrasounds at 20 kHz and the changes of the sludge size, supernatant chemical oxygen demand (SCOD), supernatant protein, nitrogen, phosphate, protein, and polysaccharides during sonication were recorded. The results showed that ultrasonic sludge disintegration could be treated as a three-stages process. The first stage lasted around 5 minutes; during which the macro-flocs were destroyed, and the entrapped matters and free-water were released. The second stage lasted 10–15 minutes; during which the micro-flocs were destroyed, the extracellular polymer substances (EPS) were dissolved, the bacteria were disinfected, and the capillary water was released. The primary particulates dominated the last stage; and their small size prevented complete disintegration. As a result, the increase of supernatant organics showed a saturation effect.

1. INTRODUCTION

ACTIVATED sludge processes are the dominating wastewater treatment method. Large quantities of hazardous excess sludge are generated in the processes, and elimination of the waste sludge has been a major problem for the last decade worldwide. The disposal of waste sludge is subject to various environmental, social and economic problems; and the cost consists about 50% of the operational cost of WWTP [Friedler, 2006]. Thus, various techniques allowing sludge reduction have been developed recently. Among which, ultrasonic treatment is one of the most promising technologies [degaard, 2004].

High-energy ultrasound has been shown to disrupt the flocs and lyse the bacterial cells, leading to a reduction in particle size in the solution and improving its biodegradability [Gonze, 2003; Bougrier, 2005; Dewil 2006; Nickel, 2007]. Sludge is treated by ultrasounds to provide organic materials for further biological treatment prior to anaerobic digestion [Tiehm, 2001; Mao, 2006; Onyeche, 2002; Neis, 2001] or recycling into aeration tank [Zhang 2007b; Yoon, 2003]. Numerous studies showed that such a pre-treatment could reduce

the sludge digestion time from 20 days to 8 days and increase the biogas production [Tiehm, 2001; Mao, 2006]; or to reduce the mass of excess sludge by 50%-100% [Zhang 2007b; Yoon, 2003].

The effects of sonication on physico-chemical characteristics of sludge including the sludge size, viscosity, capillary suction times, organic contents, soluble organic contents, and supernatant protein and DNAs were reported [Bien 1997; Chiu 1997; Chu 2001; Bougrier 2006; Zhang 2007a; Na 2007]. Impacts of important parameters such as the ultrasonic frequency, power intensity, and duration were investigated [Chiu 1997; Tiehm 2001; Gonze 2003]. The process was optimized in terms of time, power density and sludge concentration [Show 2007]. Kinetic models were developed for the ultrasonic sludge disintegration [Wang 2005; Li 2007]. A recent review summarizes the mechanisms, applications and effects of sludge sonication [Khanal 2007]. A threshold was found to exist but the accurate value was inconsistent in different studies [Tiehm 2001; Gonze 2003; Bourgrier 2005]. Mechanical shear forces resulting from acoustic cavitation were believed to be the main mechanism for sludge disintegration while reactive oxidants such as hydroxyl free radical had little influences. Hence low sound frequency (20–40 kHz) and high energy intensity were preferred for the operation.

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Table 1. Characteristics of the Biological Sludge.

Moisture (%)	pH	VS/TS	COD _{NaOH} (mg/L)	protein (mg/L)	polysaccharid (mg/L)
99.01	6.90	0.79	6884	5746	1211

However, the evolution of the sludge under ultrasonic irradiation is still unclear. Therefore, there is still need for a detailed study of the development of sludge and supernatant characteristics in order to understand the progress of sludge sonication.

2. EXPERIMENTAL

2.1. Sludge

The sludge used in this study was obtained from a subsequent biological reactor (SBR) in the lab using sewage as the feed. The sludge was collected in the settling period of the SBR and was stored at 4°C before use. No adjustment was adopted prior sonication. Table 1 reported the characteristics of the sludge and all data were the average values of samples.

2.2. Chemicals

The standards, DNA sodium salt from calf thymus, bovine serum albumin (BSA), and glucose were purchased from Altrich. All other chemicals were purchased from Beijing Huating Chemicals. All chemicals were used as received without further purification.

2.3. Sonication

A probe system, KS-250 from Kesheng Inc., was used for the sludge lysis. The electric power was between 0 to 250 W, the sound frequency was 20 kHz, and the probe tip diameter was 9 mm. For each experimental run, 100 ml sludge was sonicated in an open 150 ml glass beak. The depth of the sludge was 4.5 cm and the surface area of the sludge was 32.15 cm². The ultrasonic probe was placed in the center of the beaker, and the tip was dipped 1 cm into the sludge. During sonication, the sludge pH and temperature were not controlled.

2.4. Analytic methods

In order to measure the sludge COD_{NaOH}, the sludge sample was digested at 70°C for 24 h with the aid of 0.5 mol/L NaOH using a Lianhua 5b-2 digester. The di-

gested solution was then used for COD measurement. The sludge pH and temperature were monitored using a Hach pH meter. The sludge activity was evaluated using the OUR (oxygen utilization rate) test. The sludge OUR value was determined at 25°C after 5 min aeration of the sludge sample and addition of 1 g/L glucose as food. An YSI-DO200 meter was used to get the dissolved oxygen level in the solution. The particle size was measured using a Malvern Mastersizer granulometer.

The supernatant was separated from the sludge by centrifuging at 4000 rpm for 30 min. The supernatant was filtered through a 0.45 µm filter before measurement of dissolved nitrogen (DN), dissolved phosphorous (DP), and organic matters. The supernatant nitrogen and phosphorous were analyzed with alkaline potassium persulphate digestion-UV spectrophotometric method and ammonium molybdate spectrophotometric method. The protein, polysaccharide, and deoxyribonucleic acid (DNA) were measured using modified Lowry method, diphenylamine method, and anthrone method respectively [Wang, 2006; Zhang, 2007a]. A Pharmacia Ultra-Spec 2000 spectrophotometer was used for above analyses.

Other parameters, COD, volatile solid (VS), and total solid (TS), were quantified according to the standard water and wastewater analytic methods [APPA, 2000].

2.6. Indexes

Ultrasonic power density was calculated as: $D = \text{ultrasonic power} / \text{volume of sludge sample}$, and the unit was W/ml. The power density evaluates the energy efficiency of the sludge lysis process.

The sludge disintegration ratio (DD_{COD}) was defined as: $DD_{\text{COD}} = (\text{SCOD} - \text{SCOD}_0) / (\text{COD}_{\text{NaOH}} - \text{SCOD}_0)$, in which the SCOD was the supernatant COD and SCOD_0 was the SCOD of the untreated sludge samples.

The protein release ratio was similarly defined as: $D_{\text{protein}} = (S_{\text{protein}} - S_{\text{protein0}}) / (T_{\text{protein}} - S_{\text{protein0}})$, in which S_{protein} was the supernatant protein concentration and T_{protein} was the protein content of the untreated sludge sample.

The polysaccharide release ratio was defined as:

$D_{\text{polysaccharide}} = (S_{\text{polysaccharide}} - S_{\text{polysaccharide0}}) / (T_{\text{polysaccharide}} - T_{\text{polysaccharide0}})$, in which $S_{\text{polysaccharide}}$ was the supernatant protein concentration and $T_{\text{polysaccharide}}$ was the protein content of the untreated sludge sample.

Disinfection ratio was calculated as following: $(1 - \text{OUR} / \text{OUR}_0) * 100\%$, in which OUR was used to represent the bacteria activity and OUR_0 was the initial OUR of the sludge.

3. RESULT

Figures 1–4 report the changes of the sludge during sonication. From Figure 1, we suspected that three stages existed in the process of sludge sonication: (1) 0–5 min in which the particle size dropped dramatically to ~10 μm; (2) 5–15 min in which the particle size further decreased to ~5 μm; and (3) after 20 min the particle size virtually stabilized.

Figure 2 showed that the bacteria disinfection also followed a three-stage pattern. But unlike the size reduction, very low disinfection occurred in the 1st five minutes, the most significant change occurred in the 2nd stage, and most bacteria were deactivated after 20 minutes.

Figure 3 reports the increases of supernatant COD, nitrogen, and phosphorus during sonication. The patterns of supernatant changes (SCOD, DN and DP) were quite different with those of sludge changes (size and biological activity). The SCOD increased almost linearly with the sonication time during the whole period (0–30 min) and showed virtually no difference for the three stages. The DN increased according to a pseudo-first-order reaction for 15 minutes and then smoothered; the turning-point was within the 2nd stage. Similarly, the TP increase was quicker in the first 15 minutes and then slowed down.

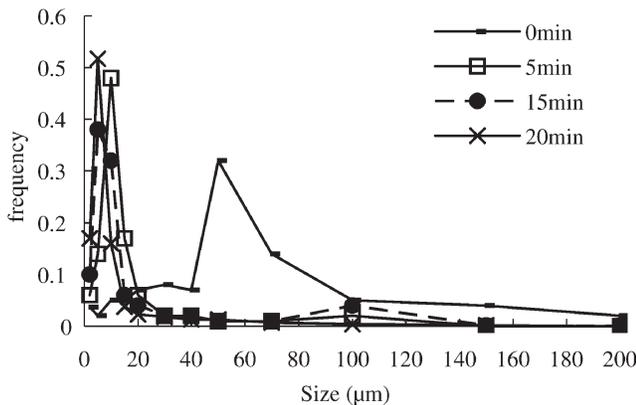


Figure 1. Sludge size change during sonication, 0.8 W/ml.

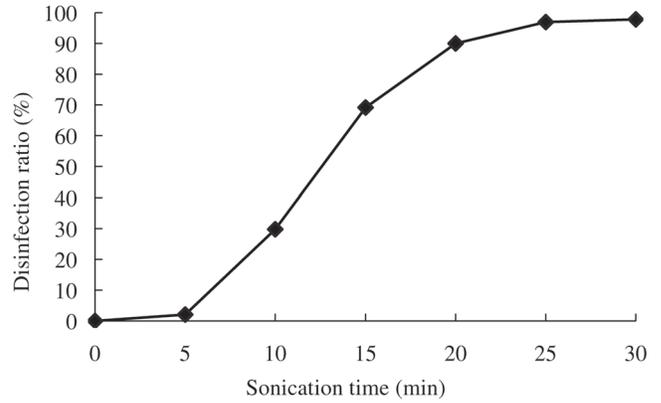


Figure 2. Sludge disinfection during sonication, 0.8 W/ml.

Figure 4 compared the disintegration efficiency of COD, protein, and polysaccharide during sonication. Protein and polysaccharide are the most important organic components of biological sludge. Protein counts for more than 50% of the dry mass while polysaccharide is the major component of EPS and plays a crucial role in ‘gluing’ together the sludge flocs. DD_{COD} increase was almost linear with the sonication time, just as SCOD did. The D_{protein} showed a pseudo-first order increase with the sonication time, just as DN did, but with a much sharper slope and slowed down after 10 minutes (in the 2nd stage). The $D_{\text{polysaccharide}}$, on the other hand, was quickest during the 1st stage and slowed down after 5 minutes.

4. DISCUSSION

The biological sludge can be viewed as a multi-level object that includes primary particles (~ 2 μm), microflocs (~ 13 μm), and highly porous flocs [Chu, 2001]. Extracellular polymer substances (EPS) and

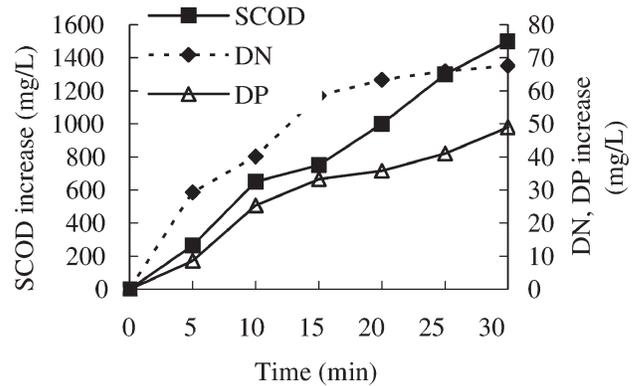


Figure 3. Increase of supernatant COD, DN, and DP during sonication, 0.8 W/ml.

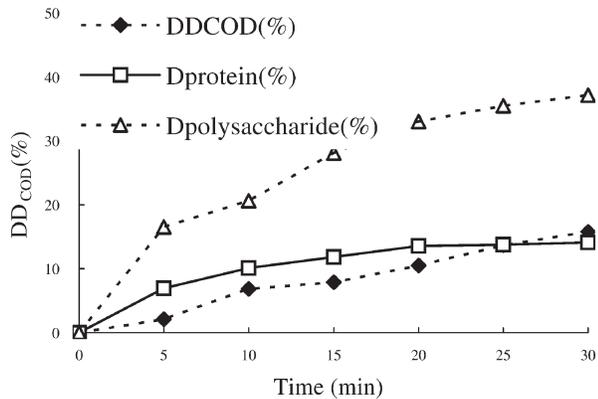


Figure 4. COD, protein, and polysaccharide release during sonication, 0.8 W/ml.

bridging ions were believed to play an important role in the formation of sludge flocs [Jorand 1995].

We understand that the primary particles are most of inert inorganic particulate and bacterium cell. The primary particles are linked together to form microflocs with the aid of EPS, which is mainly composed of proteins and polysaccharides; some organics matters are also adsorbed onto the surface of bacteria cells. The macro-flocs are formed by microflocs connected by bridging ions such as Ca^{2+} and Mg^{2+} . Some large molecules are trapped into the macro-flocs. Water exists inside the macro-flocs as free water, in the microflocs as capillary water, and adsorbed on the primary particles. Furthermore, the bacteria cells have a high content of water. Organic matters, evaluated by COD, exist in all these types of water and will be transferred into SCOD during sludge disintegration.

4.1. Stage I

In the initial period of sonication (0–5 min), the porous macro-flocs, the weakest structure of biological sludge, were rapidly broken. As a result, the sludge size decreased immediately to around $10\ \mu\text{m}$, indicating that microflocs were the main component of the sludge at that stage. Literature also showed that macro-flocs disappeared shortly and high concentration of Ca^{2+} and Mg^{2+} were released after 5 min of sonication [Wang, 2006; Chu, 2001, Gonze, 2003]. Note that the sludge size decrease was quicker in this study compared to the report of Gonze, which could be explained by the fact that higher power density (0.8 W/ml) was used in this study compared to that (0.55 W/ml) in Gonze's study.

The free water (together with dissolved organic mat-

ters) and trapped organic molecules are released into the supernatant, which led to the increase of SCOD, DN, and DP. After this initial period, the sludge DDCOD reached 4%, indicating that roughly 4% of organic matters existed inside the pores of the macro-flocs. The values were not available SCOD increase of 2581 mg/L after 5 min. Given the fact that the sludge concentration was 3% and VS/TS ratio was 0.6 in their work, we could reasonably estimate a DDCOD of ~9% for their work, which was slightly higher than our data. The authors reported a power density of 0.96 W/ml in the graph and 0.768 W/ml in the text for the same experimental run. Therefore, we could not tell which power density was the correct one. Both the values were close and supported our hypothesis.

The high $D_{\text{polysaccharide}}$ and DN values at the 5th minute (Figures 3 and 4) indicated that EPS might also dissolve in the first stage. However, the low DDCOD (Figure 4) did not support the suspicion. The contradiction cannot be explained yet.

4.2. Stage II

The 2nd stage seemed to last 10–15 minutes. After the initial rapid drop, the sludge size further decreased from $\sim 10\ \mu\text{m}$ to $\sim 5\ \mu\text{m}$ in the second stage, indicating that microflocs were destroyed and primary particles were freed. The capillary water (together with dissolved solutes) and molecules entrapped inside the microflocs were released into the supernatant. We suspected that most EPS were also released during this period. On the other hand, since the particle decreased dramatically, the corresponding surface area of the particles increased significantly. As a result, more water was adsorbed by the particles, which was supported by the extended CST values [Bougrier 2006]. The additional water-adsorption might trap more solutes onto the surface of the small particles ($\sim 2\ \mu\text{m}$). Simultaneously, the destruction of microflocs exposed bacteria cells to the sound waves. Shear forces could damage the cell membranes and release some intracellular materials such as DNA. The comprehensive result was a relatively stable increase of SCOD, DN, DP; a rapid increase of supernatant proteins and polysaccharides, which are the main components of EPS; and a slight increase of DNA, which was mainly inside the cells. Deactivation of bacteria also started in this period. Other researchers reported a 5–20 min delay in bacteria disinfection [Chu 2001].

4.3. Stage III

In the last stage (after 20 minutes), all sludge flocs, macro or micro, disappeared and primary particles were directly attached by the ultrasound waves. It has been reported that the role of chemical oxidation could be ignored [Wang 2005; Gronroos, 2005] and mechanical forces were the dominating factor during sludge sonication. The mechanical forces result from collapsing of transient bubbles, and the theoretical resonance or maximum radius of the bubbles can be estimated by [Show, 2007]:

$$R = 3.28/f$$

where R has a unit of mm and f is the sound frequency. According to above equation, the theoretic resonance bubble size in water is around 8 μm when the sound frequency is 20 kHz, which is larger than most bacteria cells. The sludge is very different from pure water with high concentration of particles and the resonance size should be different.

However, it is still reasonable to conclude the resonant cavitation bubbles in the ultrasonic field are larger than bacteria cells when the sound frequency is 20 kHz. Therefore, the bacteria cells are unlikely to behave as the cavitation bubble and collapse. It is more likely that cells are damaged by the collisions of gaseous bubbles; such an indirect damage tends to have low efficiency in cell lysis. The longer the sonication time is, the smaller the particles remain, and the harder for the particles to be lysed. Therefore, the particle size reduction slowed down and almost stabilized. Correspondingly, the increase rate of SCOD, DN, DP, protein, and polysaccharides was lower than that of the 2nd stage.

5. CONCLUSIONS

The paper studied the changes of sludge and supernatant during ultrasonic lysis, and proposed a three-stage evolution for sludge sonication basically according to the sludge size change. In the first stage, which lasted for a few minutes, the macro-flocs were destroyed and the sludge size was reduced to around 10 μm . In the second stage, which lasted for 10–15 minutes when the ultrasound power density was 0.8 W/ml, the micro-flocs were further destroyed and the sludge size was reduced to around 2–5 μm . Bacteria disinfection and release of most EPS, protein, and polysaccha-

rides also occurred in the second stage. In the third and last stage, further size reduction was limited by the relative large resonance size of cavitation bubble in a low-frequency sound field. Accordingly, the increase of supernatant organics slowed down.

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Table 5. Comparison of state-of-the-art matrix resins with VPSP/BMI copolymers.

Resin System	Core Temp. (DSC peak)	T _E	Char Yield, %
Epoxy (MY720)	235	250	30
Bismaleimide (H795)	282	>400	48
VPSP/Bismaleimide copolymer			
C379: H795 = 1.9	245	>400	50
C379: H795 = 1.4	285	>400	53

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