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The objective of the *Journal of Residuals Science & Technology* (JRS&T) 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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# Approaches for Microbiological Characterization of a Landfill Leachate Treatment

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**ABSTRACT:** Landfill Leachates are a potential source of soil and water contamination. Biological remediation techniques are most often employed for treating landfill leachates. The nature of microorganisms dwelling in sludge flocs determines process performance. Samples were taken at different steps that constitute the nitrification-denitrification treatment of leachate in a municipal landfill located in Northern Spain. Process effectiveness was evaluated. Microbiological characterization of samples was performed by means of classical culturing. Also, molecular techniques (i.e., PCR-DGGE and DNA sequencing) were used to characterize the samples. An evolution of the microbial community composition was observed throughout the treatment process. Amplification, electrophoresis, and DNA sequencing steps were completed accurately and some  $\beta$ -proteobacteria related with waste treatment and composting processes could be identified.

## INTRODUCTION

**D**ISPOSAL of solid wastes in landfills is the most common method used for waste management [1]. After landfilling, degradation of wastes in combination with water infiltration generates a leachate with the potential to pollute soil and water.

Complexity of the landfill leachate was categorized on the basis of four major groups of pollutants: dissolved organic matter, inorganic macro-components, heavy metals, and xenobiotic organic compounds (e.g., aromatic hydrocarbons, phenols, pesticides, and more). Additionally, leachate chemical and microbiological composition varies depending on a complex set of interrelated factors (e.g., environmental conditions, manner of operation, and dynamics of decomposition process) [2].

Although, complexity of leachate composition makes it very difficult to set up general recommendations [3]. Biological techniques are commonly used due their reliability, simplicity, and high cost-effectiveness. Therefore, the most usual method for treating leachate is by on-site activated sludge [4]. Specifically,

municipal landfill leachates typically contain high ammonium concentrations ( $\geq 1,000$  mg/L) as well as high levels of organics. Thus, nitrification-denitrification processes are usually employed. Since microorganisms are critical in the biological process, many studies have investigated bacterial species. A great diversity of nitrifiers have been found in activated sludge of wastewater treatment plants. However, *Nitrosospira* and *Nitrospira* have been reported as dominant species among ammonia oxidizing and nitrite-oxidizing bacteria, respectively [5]. Likewise, *Pseudomonas* spp. has been frequently reported as the main denitrifying bacteria implied in the landfill leachate treatment process. Many other species such as *Azoarcus toluyliticus* or *Thiobacillus denitrificans* have also been described as nitrogen converters [6].

Until now most works regarding microbiological population of landfill leachates have been conducted in bench-scale systems. Additionally, few studies have evaluated microbial diversity in the context of biological treatments and almost none have assessed the treatment process *in situ*. Thus, the aim of this work was to investigate *in situ* the nitrification-denitrification treatment of leachate in a municipal landfill located in Northern Spain. Samples were taken from different points at the treatment plant. Performance of the bio-

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logical treatment was evaluated and a microbiological characterization of samples was attempted employing conventional culture and molecular based techniques.

## MATERIALS AND METHODS

### Description of Treatment Plant and Sample Collection

The treatment plant is sited next to the central landfill of Asturias in Northern Spain and treats mainly leachate from the non-hazardous waste landfill that has been in operation for approximately 30 years. During 2012 this landfill received approximately 600,000 t of wastes mainly municipal mixed wastes [e.g., organic matter (42%), papers and cardboard (15%), light packaging (11%), glass (5%), and others]. Other wastes landfilled here are engineering materials, vegetal wastes, non-hazardous industrial wastes, and waste water treatment plant (WWTP) wastes. Old and young leachates are mixed in a pool in order to homogenize wastewater before being treated. The leachate treatment plant also receives small contributions of effluents from the physical-chemical treatment of industrial wastes, treatment of Marpol wastes, and leachate from the hazardous waste landfill. The process begins with a pretreatment for removal of settleable solid followed by the nitrification-denitrification biological treatment in pressurized reactors that includes a subsequent ultrafiltration step separating treated water from the biological sludge (Biomembrat Technology). The plant counts with an active carbon treatment unit. Seen in Figure 1, five pressured tanks (2.5 bars) in the nitrification-denitrification process are involved: one tank of denitrification (DN), one mixed tank (MIX), and three tanks of nitrification (N0, N1, and N2). The volume of each reactor is 175 m<sup>3</sup> and the plant treats an average flow of 620 m<sup>3</sup>/day with a maximum hydraulic capacity of 700 m<sup>3</sup>/day. Hydraulic retention time for each reactor is around 1 h and operation temperature ranges from 37–40°C. Methanol and/or phosphoric acid are

supplied if necessary. Grab samples were taken in sterile plastic bottles at six points: leachate feed (S1), tanks of nitrification-denitrification (S2, S3, S4, and S5), and permeate (S6). See Figure 1.

### Analytical Methods

Samples were filtered through a 0.45 µm membrane before being analyzed. Analytical assays of COD (chemical oxygen demand), BOD (biochemical oxygen demand), SS (suspended solids), and pH were performed according to Standard Methods for the Examination of Water and Wastewater. BOD, pH, and SS measurements were developed within the following 24 hours after collection. COD and anions analyses were carried out using frozen samples.

Anions were determined by ion exchange chromatography (ICS-1000 DIONEX) employing an IonPac column (Dionex, AG23), an IonPac guard column (Dionex, AS23), and a suppressor column (Dionex, ASRS ULTRA II). Mobile phase was 4.5 mM CO<sub>3</sub><sup>2-</sup>/0.8 mM HCO<sub>3</sub><sup>-</sup>. Ammonium was assessed employing an IonPac column (Dionex, GC16), an IonPac guard column (Dionex, CS16), and a suppressor column (Dionex, CRS 300). Mobile phase was 30 mM of methanesulfonic acid. Flow was set at 1 mL/min and injection volume was 25 µL. Analyses were developed in duplicate.

### Microbiological Analyses by Classical Cultures

Total number of aerobic mesophilic microorganisms were counted employing a plate count agar (PCA) and Agar Endo was used to determine presence of total coliforms. Regarding both cases, Petri dishes were inoculated with 1 mL of sample or decimal dilutions thereof and were incubated at 30°C for 72 hr and 37°C for 24 hr, respectively.

To detect presence of sulphite reducing clostridia (SRC), dilution of samples (10<sup>-1</sup>) were heated to 80°C for 5 min and afterwards sulfite polymyxin sulfadiazine agar (SPS agar) tubes were inoculated in deep

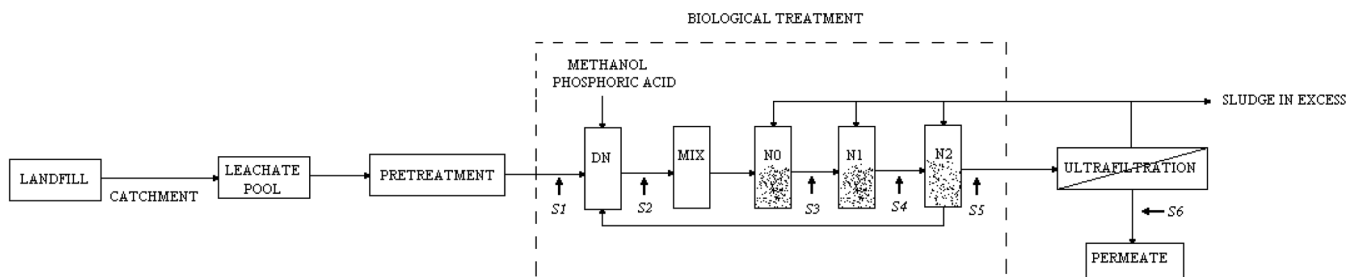


Figure 1. Schematic representation of the leachate treatment plant indicating points of sampling (S1, S2, S3, S4, S5, and S6).

with 1 mL of sample. Then, paraffin was added to create anoxic conditions. Tubes were incubated at 37°C for 48 hr and presence of SRC was identified as black colonies.

Since different genera of *Thiobacillus* are usually involved in nitrification-denitrification process, presence of *Thiobacillus* spp. was evaluated according to Fujimura and Kuraishi [7]. The specific culture medium employed was constituted by the following: 5 g/L bacteriological peptone, 5 g/L meat extract, 5 g/L yeast extract, 2.5 g/L NaCl, 0.1 g/L de  $K_2HPO_4$ , 0.05 g/L Mg  $SO_4 \cdot 7H_2O$ , and 20 g/L bacteriological agar. Petri dishes were inoculated with 1 mL of sample or decimal dilutions thereof and were incubated at 30 °C for 5 days. Microbiological cultures were carried out in duplicate.

### Further Characterization by PCR-DGGE Analysis of Leachates Samples

#### DNA Extraction

Leachate samples of 300 mL were centrifuged (20 min; 10,000 g). The pellet was suspended in 10 mL of sterile water, split into 1 mL aliquots, centrifuged again (10 min, 12,000 rpm), and stored frozen until use. Genomic DNA was purified from pellets using a Gene Matrix Soil DNA purification Kit (EURx).

#### PCR Amplification of 16S Sequences

16S rRNA genes were partially amplified from the extracted genomic DNA by PCR using a thermal cycler. The V3 region of the bacterial 16S rRNA gene was amplified employing the GC-F357 and R518 primers [8]. The final 40  $\mu$ L reaction mixture contained 23.4  $\mu$ L sterile water, 4  $\mu$ L PCR buffer 10 $\times$ , 4  $\mu$ L dNTP 50 mM, 2  $\mu$ L F357GC primer 80.6 nM, 2  $\mu$ L R518 primer 23.9 nM, 2  $\mu$ L  $MgCl_2$  50 mM, 0.6  $\mu$ L Taq-DNA polymerase, and 2  $\mu$ L of DNA sample. PCR conditions were one cycle at 94°C for 3 min, 34 cycles at 94°C for 50 s, 57°C for 60 s, and 72°C for 30 s. A final extension step was also used at 72°C for 10 min.

#### Electrophoresis and DNA Sequencing

DGGE was performed using a DCode apparatus (Bio-Rad) at 60°C employing 8% polyacrylamide gels with a denaturing range of 40–60%. Electrophoresis was performed at 75 V for 16 hr. Bands were visualized under UV light after staining with ethidium bromide (0.5 mg mL<sup>-1</sup>) and photographed. Representative

bands were excised from the acrylamide gels and their DNA eluted overnight in 25  $\mu$ L of sterile water at 4°C. DNA was then re-amplified using the same primer pair but without the GC-clamp. Amplicons were purified using a column to remove unincorporated primers and nucleotides and sequenced by cycle extension in an ABI PRISM® 3130xl Genetic Analyzer sequencer. Sequences were finally compared to those in the GenBank database using the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

## RESULTS AND DISCUSSION

### Nitrification-Denitrification Treatment Process

Concentration of suspended solids (SS) measured in the leachate coming from the pretreatment step was 16 mg/L and always below 65 mg/L. Concentration of SS inside the reactor ranges from 15,000 to 40,000 mg/L. Permeates are almost free of SS and the value measured in the permeate sample 6 mg/L. Regarding pH, all values were near neutrality (6.9–7.5) and consistent with the fact leachate pH is buffered throughout disposal of municipal solid waste during the operation period [9].

Within the DN tank  $NH_4^+$  concentration is reduced by dilution due to recirculation from the  $N_2$  reactor. This stream also contributes a high concentration of  $NO_3^-$  that is transformed into  $N_2$  in this first tank and explains the concentrations of  $NH_4^+$  and  $NO_3^-$  measured in *S1* and *S2*. During the following steps of the biological process, a decrease in  $NH_4^+$  concentration was observed as the cation was oxidized during the nitrification process and  $NO_3^-$  concentration increased (Table 1).  $NO_2^-$  was in very low concentrations for all steps because it is quickly oxidized to nitrate and was only detected in permeate (*S6*). Concentrations of  $NH_4^+$  were usually between 2,000 and 3,000 mg/L in the untreated leachate. This is below the 50 mg/L in the permeate. The nitrification-denitrification process was working efficiently with ammonium removal higher than 95%. This is a value much higher than that observed by Karthikeyan *et al.* [9] for a lysimeter-scale landfill bioreactor and similar to those reported by Liu *et al.* [10] for a membrane bioreactor fed with landfill leachate.

During the denitrification step, organic matter is consumed by heterotrophic bacteria responsible for the process. The COD value of the incoming sample was lower than values measured in samples taken from reactors. This is due to methanol added in the first reactor.

**Table 1. Physico-chemical Analyses (average values  $\pm$  SD).**

Sample	pH	COD (mg/L)	BOD <sub>5</sub> /COD	NH <sub>4</sub> <sup>+</sup> (mg/L)	NO <sub>2</sub> <sup>-</sup> (mg/L)	NO <sub>3</sub> <sup>-</sup> (mg/L)
S1	7.47 $\pm$ 0.01	2452 $\pm$ 2	0.26	1549 $\pm$ 8	< 1	20 $\pm$ 1
S2	7.42 $\pm$ 0.01	5364 $\pm$ 8	—	356 $\pm$ 7	< 1	78 $\pm$ 1
S3	7.38 $\pm$ 0.01	6660 $\pm$ 12	—	140 $\pm$ 1	< 1	1071 $\pm$ 16
S4	7.13 $\pm$ 0.01	4197 $\pm$ 5	—	47 $\pm$ 2	< 1	1418 $\pm$ 11
S5	6.98 $\pm$ 0.00	3844 $\pm$ 5	—	73 $\pm$ 2	< 1	897 $\pm$ 9
S6	6.89 $\pm$ 0.01	1600 $\pm$ 5	0.04	27 $\pm$ 2	1.6 $\pm$ 0.0	401 $\pm$ 15

This additional COD is consumed during the process as well as most of the biodegradable COD contained in the leachate. Besides, it is necessary to take in mind all samples were taken at the same time. The COD of the untreated leachate has important variability usually ranging between 2,500 and 7,000 mg/L. These values are within the range reported for different municipal landfill leachates located in Finland (642–8,037 mg/L) [11]. Effectiveness in reduction of organic matter can be observed in the BOD<sub>5</sub>/COD ratio. The BOD<sub>5</sub>/COD ratio of the untreated leachate (S1) was 0.26. This value for the ratio indicates it was a low biodegradable leachate resulting from decomposition of fresh and more stabilized waste. However, the outgoing sample (S6) was almost non-biodegradable with a ratio of 0.04. COD values of the permeate (S6) are generally 1,600 mg/L as a maximum with the percentage of COD usually removed ranging from 30–80%.

### Microbiological Characterization Classical Cultures

Mesophilic microorganisms include bacteria, yeast, and molds that grow best in moderate temperature (i.e., 20–45°C). The number of CFU per mL in the incoming sample (S1) was on the order of 10<sup>6</sup> CFU/mL, a value above the range (10<sup>3</sup>–10<sup>5</sup> CFU/mL) reported by Matejczyk *et al.* [12] for leachates from municipal solid waste landfill sites in Poland. In permeate sample (S6), the value was < 10 UFC/mL as microorganisms are retained during the ultrafiltration final step.

Sulphite Reducing Clostridia (SRC) were detected in samples taken from reactors (S2, S3, S4, and S5). Clostridia have been identified in landfills worldwide [13]. They are strictly anaerobic bacteria and can easily proliferate in denitrification and mixed tanks. Then, they are able to pass during the recirculation process to the nitrification tanks where they resist presence of oxygen due to their spore-forming ability. The system counts with a high recycle ratio so that hydraulic retention time in aerobic conditions is relatively short.

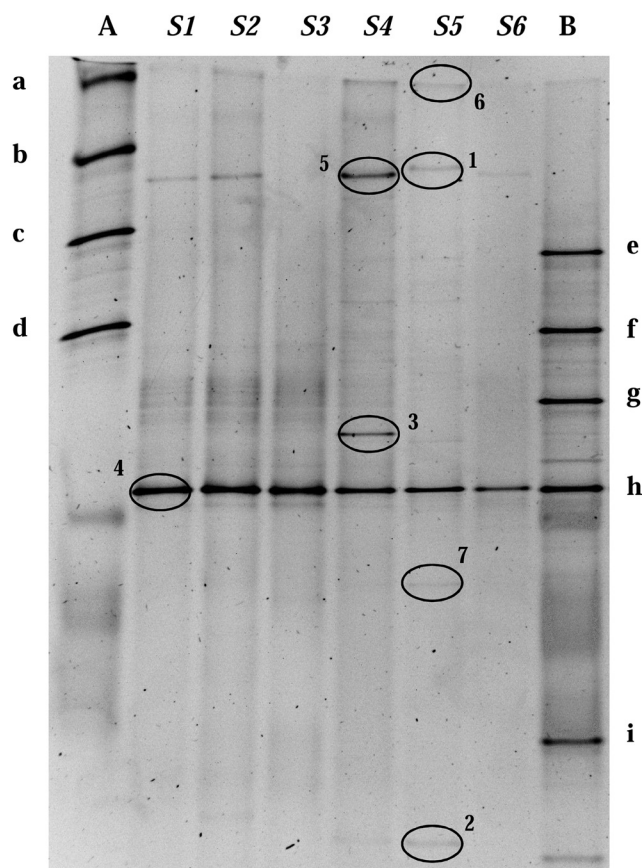
Coliform bacteria were recently described by Aziz *et al.* [14] as one of the most problematic pollutants in stabilized leachate. Whereas, Grisey *et al.* [15] reported very low levels of total coliforms in landfill leachates. Coliforms were not detected in any of the analyzed samples. Many factors such as presence of a high number of noncoliform bacteria may significantly affect coliform bacteria detection [16,17]. On the other hand, *Thiobacillus* spp. was detected in all samples as species of this genus are widely present in solid residues and lixiviates [18]. Specifically, *Thiobacillus nitrificans* has recently been described as one of the main nitrogen converters in a municipal landfill leachate bioreactor at lab scale [6].

### PCR-DGGE Analysis

PCR-DGGE has been extensively used in analysis of sediment, activated sludge, and manure slurry samples. However, few works have used this method to study microbial diversity in context of biological treatment of leachate and these studies most often have been developed at lab scale. Xie *et al.* [19] found that *Pseudomonas*, *Lysobacter*, and *Bacillus* were part of the main bacterial genus responsible for pollutant removal in landfill leachate reactors in experiments carried out at lab and pilot scale. Different factors such as nutrient availability or temperature are determinant on the landfill bacterial community structure.

Figure 2 shows DGGE profiles of microbiological populations contained in leachate samples. While number of bands on the gel reflects relative diversity of the bacterial community, band intensity indicates abundance of the bacterial group [8]. Sample S5 showed the highest biodiversity whereas in the S6 sample only a single band was clearly appreciated. Note that this sample was taken after a filtration step. A portion of the most intense bands were selected for sequencing and are marked in Figure 2. Although migration behavior of band 4 detected in all samples was the same as that of the *E. coli* strain used as control, the DNA sequenc-





**Figure 2.** DGGE profiles of microbial populations of leachate samples using universal primers to amplify the V3 hypervariable region of the bacterial 16S rRNA gene. Lanes A and B contain combined amplicons of identified strains used as controls: a. *Lactococcus garvieae*, b. *Lactobacillus plantarum*, c. *Leuconostoc mesenteroides*, d. *Streptococcus parauberis*, e. *Enterococcus faecium*, f. *Enterococcus faecalis*, g. *Lactococcus lactis*, h. *Escherichia coli*, and i. *Lactobacillus paracasei*. Bands marked and numbered were selected for sequencing.

ing process did not allow identification of this band. Absence of coliforms would be in accordance with results obtained by classical culturing as they were never recovered in the analyzed samples. Only the sequence of band 3 showed a percentage of nucleotide identity of 94% or higher to 16S rDNA sequences of *Delftia tsuruhatensis* (96% identity), *Comamonas composti* (95% identity), *Acidovorax defluvii* (95% identity), and *Diaphorobacter nitroreducens* (94% identity). These species are phylogenetically related and belong to the subgroup of  $\beta$ -proteobacteria [20] and all of them are related with waste treatment and composting processes. Actually, *D. tsuruhatensis*, *A. defluvii*, and *D. nitroreducens* have been respectively isolated from municipal wastewater activated sludge by different authors [21,22,23]. *C. composti* has been isolated from food waste compost by Young *et al.* [24]. The difficulty of identifying bands by sequencing is associated with co-

migration and doublebanding furthering the well recognized limiting factors of the DGGE technique [25].

## CONCLUSIONS

According to the results leachate nitrification-denitrification treatment is effective achieving important reductions in both organic matter and ammoniacal nitrogen concentrations higher than 30% and 95%, respectively. Regarding microbiological cultures, *Thiobacillus* spp. was identified in all samples. Whereas, sulphite reducing clostridia were found only in reactor samples. Coliforms were not detected. Additionally, DGGE profiles reflected a great variety or diversity in the microbial community composition of the samples. Sequencing techniques allowed for identification of one of the bands obtained from DGGE analysis showing a probable presence of *Delftia tsuruhatensis*, *Comamonas composti*, *Acidovorax defluvii*, and *Diaphorobacter nitroreducens*. Establishment of relations between microbial sludge composition and characteristics of treated leachate would contribute to shedding light on processes that occurs in reactors helping to control critical situations. Further development of purification steps of DNA bands is likely to enable a more complete identification of bacterial communities involved in this complex process.

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# Using *Ceratophyllum demersum* for Treatment of Saline Municipal Wastewater

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**ABSTRACT:** Salinity management is becoming a critical environmental issue for municipalities across nations. Wastewater effluent must remain a viable water resource for irrigation of crops and indirect potable use through groundwater recharge. The aim of this study was to investigate to what degree *Ceratophyllum demersum* (*C. demersum*) decreases salinity in aquatic environments. Treatments included raw municipal wastewater (RMW), treated municipal wastewater (TMW), and diluted fresh latex (DFL). It was concluded that *C. demersum* could be used to remove salinity from wastewater and merits further study as a saline reduction method for wastewater.

## INTRODUCTION

An ideal way to assess water quality should be via use of physical, chemical, and biological parameters providing a complete spectrum of information for water quality management. However, such study requires much more time and resources than study of biological parameters alone. It is widely accepted and may reliably give all the information about water quality assessment depending on the case at hand [4,5]. Water quality assessment is an important activity in agricultural water management. Ionic composition of water has significant influence on plant growth. Irrigation with water of insufficient quality might retard plant growth and may contaminate soil rendering it less suitable for agriculture because of salinity. The concentration of positively charged ions, especially sodium, is more important than others. High concentrations of sodium both in absolute and in relative terms make water unsuitable for irrigation [1] since sodium adversely affects soil structure and soil permeability by replacing calcium and magnesium [6].

Application of organic wastes like sewage sludge or municipal solid wastes to degraded soils may produce both beneficial and harmful effects [10,11]. Reclamation of soils with organic wastes may be subjected to

restrictive regulations. In the case of quarries, it may be possible to add higher waste doses without causing environmental problems or food pollution. This type of application must be compatible with environmental protection preventing translocation of dissolved pollutants into drainage waters [13]. On the other hand, the amount of dehydrated sewage sludge that is subsequently composted or thermally dried is more and more important with only a small proportion of it is used in agriculture [9]. Furthermore, electrical conductivity regulations could make use of this biosolid difficult for agricultural purposes [8,12].

Electrical conductivity (EC) is an important parameter in groundwater quality assessments for drinking and irrigation since it is related to concentration of charged particles in water. EC is measured by an electronic probe which applies an electric voltage between two electrodes. The resistance of water is measured by a drop in voltage. EC, which is inversely proportional to the resistance, is then conductance per unit distance. Pure liquid water has a very low EC. Presence of charged particles in water increases conductivity. Important water quality classification systems such as the Wilcox (1948) diagram and the USA Salinity Laboratory (1954) classification system use EC as input. A Wilcox diagram classifies water quality based on the EC and sodium concentration of water. For this diagram, an EC level between 0 and 750  $\mu\text{S}/\text{cm}$  is determined to be excellent-good and an EC level between

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750 and 2,000 mS/cm is good or permissible for irrigation. Water with an EC level greater than 2,000 mS/cm is considered not good for irrigation.

Using aquatic organisms for surface water quality assessment started more than a century ago (Liebmann, 1962) based on observation that organisms occurring in polluted water are different from those in clean water. Biological quality can be assessed by different kinds of organisms: diatoms, riparian and aquatic vegetation, invertebrates, and fishes. Here the interest is in how *Ceratophyllum demersum* may affect electrical conductivity (EC) of wastewater and how this aquatic plant may refine EC from wastewater for reuse in agricultural activities.

## MATERIALS AND METHODS

*Ceratophyllum demersum* plants which are aquatic plants collected from the Zayanderood river in the spring of 2009 (Isfahan, Iran, 32° 38' 30" N, 51° 39' 40" E). Samples were thoroughly washed with distilled water to remove any soil /sediment particles attached to plant surfaces. Then, it was placed in 12 transparent buckets which established three treatments of raw municipal wastewater (RMW), treated municipal wastewater (TMW), and diluted fresh latex (DFL) in four replications. Each bucket was filled with 6 L of each treatment. Each microcosm was planted with 100 g (fresh weight) of *C. demersum*. This study was done at a temperature between 28 and 32°C. However, temperature of treatments plants were located in was between 24 and 26°C. Experiments were performed in an outdoor area of Khorasgan University under natural daylight for 18 days without aeration and 18 days with aeration afterwards.

Samples were collected for three times during the 18

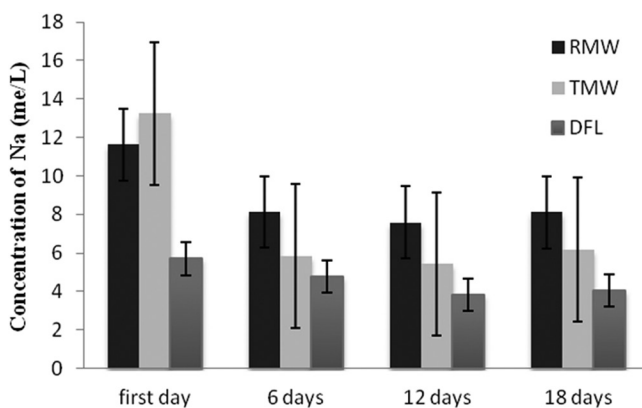


Figure 1. Variation of Na concentration during without aeration in three different treatments along with standard deviation.

days of the experiment at noon and compared against the primary sample (before using *C. demersum*). After each collection of samples, volume of TMW, RMW, and DFL were evaporated and replaced with an equal amount of distilled water.

EC was measured according to comparison and extracts with saturation extracts [3]. Na and K concentrations were measured by a flame photometer [14].

All data collected during this experiment was analyzed using SPSS statistical software package (version 16.0) based on a randomized block design. This was compared to Duncan's multiple range tests.

## RESULTS AND DISCUSSIONS

Data obtained during the investigation revealed that the experimental set containing the aquatic plant (*Ceratophyllum demersum*) removed substantial amounts of Sodium (Na) (Figure 1) and Potassium (K) during the without aeration phase (Figure 2).

The greatest decrease in Na occurred in TMW treatments during this phase. Less than 2–5% of potassium (K) was removed from any solution.

Analysis of plant tissue (stem and leaf) of *E. crassipes*, *L. minor*, and *S. Polyrhiza* indicated a large amount of crude protein before and after the removal experiment and mineral nutrients (N, P, K) were present in plant tissues. Results revealed crude proteins and other mineral nutrients increased in plant tissues after 21 days of incubation. Chemical composition of roots and leaves of aquatic plants showed a higher concentration of nutrients than in another study [7].

Pollutant-removal efficiency of certain macrophytes such as *Eichhornia crassipes*, *Microcystis aeruginosa*, *Scenedesmus falcatus*, and *Chlamydomonas mirabilis* has been performed in laboratory conditions to evalu-

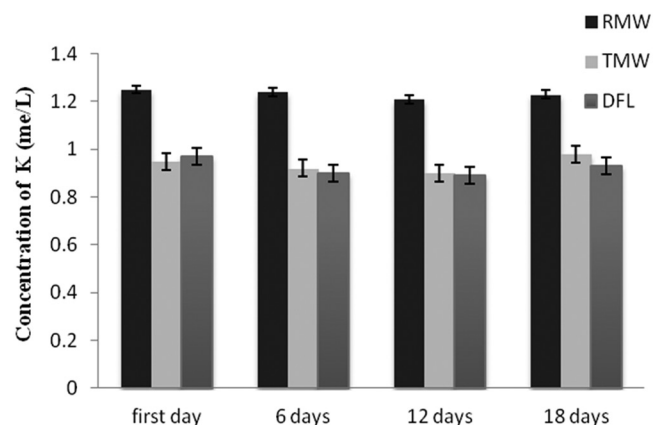


Figure 2. Variation of K concentration during without aeration in three different treatments along with standard deviation.

ate their potential role in the wastewater treatment process. These aquatic plants could reduce total alkalinity up to 74.6% in wastewater [15,16].

Measurements of EC in three solutions indicated that the EC reduced from 2.68 to 2.12 (Ds/L) in RMW, from 1.34 to 0.95(ds/m) in TMW, and from 0.93 to 0.75 Ds/L over the initial 18 days without aeration (Figure 3).

Submerged aquatic vascular plants are known to absorb nutrients [17,18]. Thus, considerable amounts of nutrients like Na and K can be stored in plant dominated littoral areas of aquatic ecosystems [7].

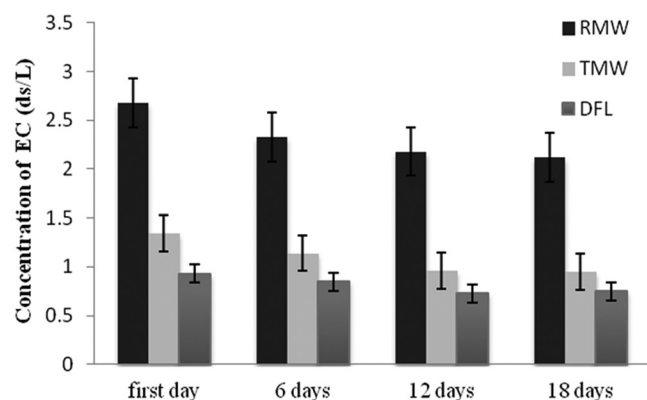
Tripathi *et al.* (1991) has tested aquatic plants in laboratory conditions to evaluate their potential role in wastewater treatment. Their results showed these aquatic plants could reduce the alkalinity of wastewater.

After the initial 18 days without aeration, all of the samples were subjected to aeration for an additional 18 days. One of the reasons for this experiment was to determine if *C. Demersum* is more capable of removing elements such as K and Na that contribute to EC.

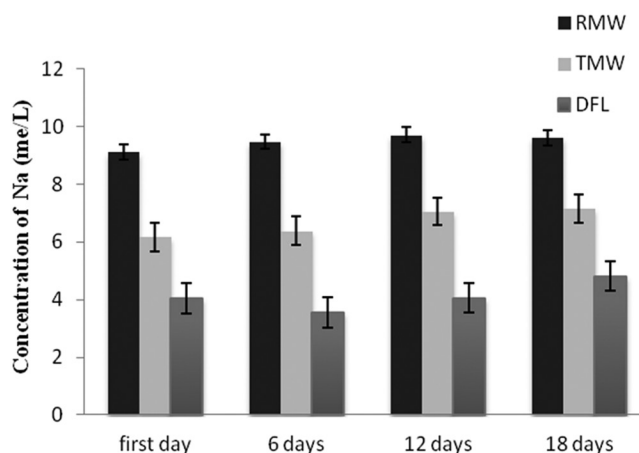
Figure 4 shows the concentration of Na in each of the samples aerated. Analysis of Na of all solutions subject to aeration indicated that there was no significant change in sodium concentration after 18 days of aeration.

The variation of K in each of the aerated solutions is shown in Figure 5. Analysis of K after 18 days of aeration indicates a statistically significant decrease in K from the RMW. Plotting the other samples shows that the K concentration in both TMW and DFL increased after aeration. However, the increase was not statistically significant.

Analysis of EC on samples of three sample types



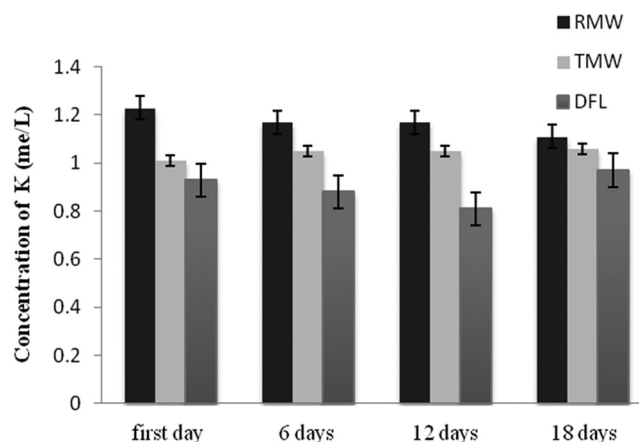
**Figure 3.** Variation of EC concentration during without aeration in three different treatments along with standard deviation.



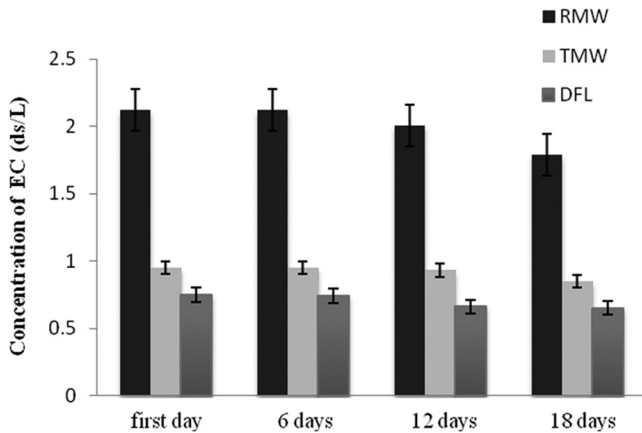
**Figure 4.** Variation of Na concentration during aeration in three different treatments along with standard deviation.

that were aerated shows that they decreased from 2.68 to 2.12 ds/L in RMW, from 2.12 to 1.79 ds/L in TMW, and from 0.75 to 0.65 ds/L in DFL samples (Figure 6).

Salts and other contaminants in the water can dissociate into components called ions. In most cases ions in water are considered as impurities, especially when referring to pure water. Yet, in other aqueous solutions such as hydrochloric acid or sodium hydroxide the ions define the actual chemical composition. As discussed, in water the hydrogen ion ( $H^+$ ) and the hydroxyl ion ( $OH^-$ ) are extremely mobile due to their geometry and size of ions relative to each other in comparison with the  $Na^+$  and  $Cl^-$  ions in NaCl aqueous solutions. Resulting, NaCl shows a much higher conductance compared to that of liquid water. Electrolytes also increase the electrical conductivity of water. There is a relationship between conductivity and concentration of electrolytes. Solutions do not always have a linear relationship between increases in conductivity and



**Figure 5.** Variation of K concentration during aeration in three different treatments along with standard deviation.



**Figure 6.** Variation of EC concentration during aeration in three different treatments along with standard deviation.

increases in total solids and salts. In dilute solutions an increase in concentration causes a linear increase in conductivity provided that there are no interactions between the solution and dissolved electrolyte [2]. The authors conclude that use of *C. demersum* in aqueous solutions with high EC such as wastewater and diluted fresh latex could be effective in decreasing the EC of those aqueous solutions.

## CONCLUSION

EC decreased in polluted water treated with *C. demersum* both with and without aeration. The aquatic plant *C. demersum* might play a major role in the environmental conditions of stagnant and flowing waters. This plant could adsorb elements and decrease pollutants in sewage and wastewater. *C. demersum* can be used for refining wastewater.

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# Regulatory Framework in Sludge Management: Examples from Around the World

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**ABSTRACT:** Treatment and disposal/beneficial use are the most important aspects of municipal sludge management. Particularly, the application guidelines and limit values for treatment systems and the major disposal routes including landfilling, land application and incineration are covered in the legislations. This study aims to review the legislations about municipal sludge treatment and disposal from different countries such as Turkey, USA, EU, Canada and South Africa. Evaluations show that the current legislations place a greater emphasis on the beneficial use of sludge, rather than the mere disposal. The specifics of regulations related to combustion changes between different countries such that in some countries separate regulation for sludge combustion is implemented, whereas in others sludge is not specifically mentioned but included among the big group of wastes to be combusted. Similarly, some countries have particular regulations for landfilling of sludge, whereas the others consider sludge within the greater category of biodegradable wastes together with the organic fraction of solid wastes. This study compares and contrasts these issues and current legislations of the aforementioned countries.

## INTRODUCTION

LARGE quantities of sludge produced each day create one of the greatest burdens on environmental sector worldwide. How to process and dispose of this mountainous material are the two important questions to be addressed daily during sludge management. The framework of applications is established by the legislations worldwide, which may be in the form of laws, rules, regulations, guidelines, etc. Legislations include some general issues that are addressed by most countries, as well as many country specific issues. This study examines the legislations of different countries about sludge management by comparing their important elements.

Table 1 gives a list of the regulations that touch upon the issue of sludge management. As can be seen from this table, in great majority of countries examined,

sludge management is included in more than one regulation or law. EU legislations concerning sludge management (landfilling, land application and incineration) are included in different directives (Table 1). On the other hand in USA, EPA 40 CFR Part 503 includes all aspects such as land application, surface disposal and incineration of sludge in one rule. Rule 503 has 6 chapters and 3 appendices. Chapters 1 to 5 are about use or disposal of sewage sludge/biosolids, land application of biosolids, surface disposal of biosolids, incineration of biosolids, pathogen and vector attraction reduction requirements and sampling and analysis, respectively. Similarly there is a main guideline in South Africa, which has five different parts. Volume 1 to volume 5 of this guideline include selection of options, requirement for the agricultural use of sludge, requirement for the on-site and off-site disposal of sludge, requirement for the beneficial use of sludge and requirements for the incineration of wastewater sludge, respectively. Canada does not have a national regulation on the management of sludge that is applicable in all states. Ca-

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**Table 1. International Legislations Related to Sludge Examined within the Context of Study.**

Country/State	Name of the Guideline Document/Regulation/ Directive	Enforcement Date/Number
Turkey	Regulation on the General Principles of Waste Management	05.07.2008/26927
Turkey	Regulation Regarding the use of Domestic and Urban Sludge on Land	03.08.2010/27661
Turkey	Water Pollution Control Regulation	31.12.2004/25687
Turkey	Solid Wastes Control Regulation	14.03.1991/20814
Turkey	Hazardous Wastes Management Regulation	14.03.2005/25755
Turkey	Regulation Regarding Soil Pollution Control and Sites Polluted by Point Point Sources	08.06.2010/27605
Turkey	Regulation Regarding the Incineration of Wastes	06.10.2010/27721
Turkey	Regulation Regarding the Landfilling of Wastes	26.03.2010/27533
Turkey	Urban Wastewater Treatment Regulation	08.01.2006/26047
Turkey	Wastewater Treatment Plants Technical Aspects Bulletin	20.03.2010/25527
EU	The European Waste Catalogue	2000/532/EC
EU	Legislation on Waste Shipment	1013/2006/EC
EU	Waste Incineration Directive	2000/76/EC
EU	Criteria and Procedures for the Acceptance of Waste at Landfills	2003/33/EC
EU	The Landfill Directive	99/31/EC
EU	Integrated Pollution Prevention and Control Directive	2008/1/EC
EU	Urban Wastewater Treatment Directive	91/271/EEC
EU	Packaging and Packaging Waste Directive	94/62/EC
EU	Protection of the Environment, and of the Soil in Particular, when Sewage Sludge is used in Agriculture	86/278/EEC
EU	Waste Framework Directive	2008/98/EC
EU	Working Document on Sludge—3rd Draft	27.04.2000
EU	Mining Waste Directive	2006/21/EC
EU	Waste Electrical and Electronic Equipment Directive	2002/96/EC-2002/95/EC
EU	Used Batteries and Accumulators Directive	91/157/EEC& 98/101/EC
EU	End of Life Vehicles Directive	2000/53/EC
EU	Directive on the Disposal of polychlorinated biphenyls (PCBs) and polychlorinated terphenyls (PCTs)	96/59/EC
EU	Directive on the Promotion of the Use of Energy from Renewable Sources	2009/28/EC
USA	U.S. EPA 40 CFR Part 503	1993
Canada/BC	Organic Matter Recycling Regulation	B.C. Reg. 18/2002
Canada/Alberta	Guidelines for the Application of Municipal Wastewater Sludges to Agricultural Lands	March 2001
Canada/Saskatchewan	Land Application of Sewage Sludge Guidelines	June 2004
Canada/Ontario	Fertilizers Act	1985
Canada/Ontario	Environmental Protection Act	2009
Canada/Quebec	Document on Improvement of Soil by Organic Fertilizer (Bureau de Normalisation du Quebec)	2003
S. Africa	South African Sludge Guidelines Volume 1: Selection of Options	2009
S. Africa	South African Sludge Guidelines Volume 2: Requirement for the Agricultural use of Sludge	2009
S. Africa	South African Sludge Guidelines Volume 3: Requirement for the on-site and off-site Disposal of Sludge	2009
S. Africa	South African Sludge Guidelines Volume 4: Requirement for the Beneficial Use of Sludge	2009
S. Africa	South African Sludge Guidelines Volume 5: Requirements for the Incineration of Wastewater Sludge	2009

nadian Council of Environmental Ministers plans the management strategies for the wastes originating from the municipal wastewater treatment plants. In accordance with these, the state and regional governments and local environmental agencies implement the regu-

lar controls on sludge management. Examining different legislations from different countries one can see that almost all regulations give priority to the beneficial use of sludge. In most countries, the regulatory limits have evolved as a result of risk based studies.



## COMPARISON OF LEGISLATION ON BENEFICIAL USE OF SLUDGE

Concept of beneficial use of wastes became an integral part of the sustainable waste management. Beneficial use of sludge is also addressed in regulations in EU, USA and other countries examined. In EU and some other countries in Europe, beneficial use as it is considered in legislations puts the main emphasis on land application in agriculture and mono or co-incineration of sludge. On the other hand, in USA in addition to these two common uses another alternative, surface disposal, or in South Africa, application on land at unusually high rates for rehabilitation and use in the manufacturing of commercial products such as fertilizer products (compost, pellets, etc.) and construction materials (bricks, cement, pumice and artificial aggregates) are defined with their specific requirements under beneficial use. The suggested beneficial uses for sludge in Canada constitute agricultural use, incineration and soil rehabilitation.

### Beneficial Use of Sludge on Land

Beneficial use of municipal sludge requires a number of limitations on several different components of sludge as well as on the application practices. Even though there are some differences about the parameters included in the regulations between different countries, no country examined allows for unstabilized sludge to be applied on land. Heavy metal limit values in sludge are one common aspect of legislations in many countries examined. The second component included most commonly is the pathogens and indicator organisms and the associated limit values. Trace organic contami-

nants are addressed by only few countries. Subsections below compare the approaches of selected countries within this context.

### Limits on Heavy Metals

A comparison of heavy metals included in the regulations of selected countries as well as their limit values is given in Table 2. As can be seen in Table 2, both the heavy metals included and their limit values change among the countries examined. Table 2 has two rows showing USA limit values as pollutant concentrations and ceiling concentrations. If biosolids are below the pollutant concentrations, a permit is not needed if other regulatory requirements are met. On the other hand, the ceiling concentrations are the values that biosolids to be land applied cannot exceed at any time. Plausibly, the pollutant concentrations are lower than the ceiling concentrations. Table 2 lists two different limit values for EU, too. The first one is from the current sewage sludge directive (Protection of the Environment, and of the Soil in Particular, when Sewage Sludge is used in Agriculture—86/278/EEC) and the second one is from the Working Document on Sludge, 3rd Draft (2000), which is a proposed regulation and has not been accepted yet. Comparing these two regulations, the limits in the proposed regulation can be seen to be considerably reduced. The main factor for this is the growing concern in Europe about the risks of land application posed to the human and animal health as well as the environment due to the presence of heavy metals. In addition to the limits of heavy metals enforced in sludge, the heavy metals in soil that the sludge would be applied are also listed in many regulations.

Table 2 includes limit values from Turkey, Neth-

**Table 2. Heavy Metal Limit Values for Land Application in Different Countries (mg/kg).**

Heavy Metals	U.S.A.*		EU		Turkey	France	Denmark	Netherlands
	Pollutant Concentrations	Ceiling Concentrations	86/278/EEC	Working Document on Sludge, 3rd Draft				
As	41	75	—	—	—	—	25	15
Cd	39	85	20–40	10	10	20	0.8	1.25
Cr	—	—	—	1000	1000	1000	100	75
Cu	1500	4300	1000–1750	1000	1000	1000	1000	75
Hg	17	57	16–25	10	10	10	0.8	0.75
Mo	—	75	—	—	—	—	—	—
Ni	420	420	300–400	300	300	200	30	30
Pb	300	840	750–1200	750	750	800	120	100
Se	100	100	—	—	—	—	—	—
Zn	2800	7500	2500–4000	2500	2500	3000	4000	300

\*Limit values for USA on the left column is for Pollutant Concentrations and the ones on the right column is the Ceiling Concentrations.

erlands, France and Denmark. Comparing these with each other and with the current EU directive, it is seen that these countries selected to apply more stringent levels and tended to adopt the proposed directive rather than continuing to apply the current directive. A comparison of limit values in USA and EU reveals that the EU limits are more stringent than the US limits except for that of lead. The heavy metal limit values in Canada, both in the type of metal covered and the limit values, differ from state to state. On the other hand, in South Africa 8 heavy metals (arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc) are considered in three different classes of concentrations. The guidelines specify which class is permitted to be used in which type of beneficial application.

#### *Limits on Microorganism*

While, the current EU directive on sludge does not contain specific limits on pathogenic or indicator microorganisms, the proposed directive (working document) suggests two different quality levels for *Salmonella* and *E Coli*. In Rule 503 by USEPA, sludges are evaluated in two classes: Class A and Class B. The microorganisms included are *Salmonella*, Fecal Coliforms, Enteric Viruses and Helminth Ova. Class A sludge should either have the density of fecal coliforms in sewage sludge be less than 1000 MPN/ g dry sludge or the density of *Salmonella* sp. be less than 3 MPN/ 4 g dry sludge. Class A sludge can be applied on land without any site (entrance) restrictions. Class B requires the density of fecal coliforms to be less than 2,000,000 MPN (or CFU)/g dry sludge. Class B, since it identifies lower quality sludge, necessitates some site restrictions. In Turkey, the effectiveness of sludge stabilization is judged based on the reduction of *E Coli*. During stabilization a reduction in *E Coli* concentration by 2 logs is required by the current regulation related to the land application of sludge. In Canada the issues about the pathogens and indicators are mostly similar to the ones in USA, with the organisms to be monitored being either *Salmonella* or Fecal Coliforms/*E Coli*. South African guidelines separate the sludges in three classes based on the levels of Fecal Coliforms and Helminth Ova. Different limitations are enforced on these three classes of sludge for their use on land.

#### *Vector Attraction Reduction Requirements*

Even though vector attraction reduction requirements are not required in European countries, USA and

South Africa have a number of these requirements listed. In both countries regardless of the class of sludge at least one of the vector attraction reduction requirements should be met to be able to land apply the sludge.

#### *Trace Organic Contaminants*

There are wide differences on whether limit values exist for trace organic contaminants in sludge in regulations of different countries. Table 3 lists this situation for some selected countries. Neither the US rule nor the current EU directive has any limit values issued for trace organic contaminants. On the other hand, the proposed regulation in EU specifies 7 different contaminants and limit values for them. Turkey, France and Denmark are among the countries applying limit values for a number of organic micro-pollutants. USA and Canada state that the levels and the potential risks for these contaminants are extremely low; therefore they don't include these pollutants in their regulations. On the other hand, concerns about the health effects of these chemicals motivated some European countries to put regulatory limits for these chemicals. In South Africa the limit value for the sum of 8 PAH compounds in sludge is enforced.

#### **Beneficial Use of Energy in Sludge by Combustion Processes**

In USA and South Africa, there are dedicated regulations or guidelines for sludge mono or co-combustion. Table 1 lists the relevant regulations. EPA requires that in an incineration facility combusting municipal sludge, the stack gases have to be continuously monitored for carbon monoxide (CO), beryllium and mercury and shown that the emissions are not exceeding the levels in National Emission Standards. In addition, arsenic, cadmium, chromium, nickel and hydrocarbon levels should not exceed the allowed daily average values listed in the regulation. In South Africa, when the sludge is used as auxiliary fuel, or combusted together with other wastes a significant number of organic pollutants are regulated together with the usual air pollutants. For other non-combustion processes including pyrolysis, NO<sub>x</sub>, CO, total dust, TOC, HCl, HF, SO<sub>2</sub>, temperature, oxygen, pressure and water vapor have to be monitored. In EU as well as in Turkey, there is not a single regulation dedicated to the combustion or co-combustion of sludge. Sludge is included in the broader regulation regarding the combustion of wastes and it is defined as 'residue'. These regulations do not have

**Table 3. Trace Organic Pollutant Limit Values (mg pollutant/kg biosolids DW) for Land Application of Sludge in Legislations of Selected Countries.**

Organic Pollutants	U.S.	EU		Turkey	France	Denmark
		86/278/EEC	Working Document on Sludge, 3rd Draft			
AOX <sup>1</sup>	–	–	500	500	–	–
DEHP <sup>2</sup>	–	–	2600	2600	–	50
LAS <sup>3</sup>	–	–	100	100	–	1300
NP/NPE <sup>4</sup>	–	–	50	50	–	10
PAH <sup>5</sup>	–	–	6	6	Fluoranthene: 4 Benzo(b)fluoranthene: 2.5 Benzo(a)pyrene: 1.5	3
PCB <sup>6</sup>	–	–	0.8	0.8	0.8	–
PCDD/F <sup>7</sup>	–	–	100 (ng toxic equivalent (TE)/ kg DW)	100 (ng toxic equivalent (TE)/ kg DW)		–

–Not included.

<sup>1</sup>Sum of halogenated organic compounds.

<sup>2</sup>Di(2-ethylhexyl)phthalate.

<sup>3</sup>Linearalkylbenzenesulphonates.

<sup>4</sup>Nonylphenol and nonylphenolethoxylates with 1 or 2 ethoxy groups.

<sup>5</sup>Sum of the following polycyclic aromatic hydrocarbons: acenaphthene, phenanthrene, fluorene, fluoranthene, pyrene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno(1, 2, 3 c,d)pyrene.

<sup>6</sup>Sum of congeners 28, 52, 101, 118, 138, 153 and 180.

<sup>7</sup>Polychlorinated dibenzodioxins/ dibenzofuranes.

specific items or limit values for sludge. There are general air emission limit values for mono or co-combustion of wastes. In addition, all regulations specify the combustion conditions, trial burn plans, air pollution control devices and how to treat the wastewater originating from air pollution control systems. General air emission parameters followed includes CO, SO<sub>2</sub>, NO<sub>x</sub>, TOC, acid gases (HCl and HF), dust, some metals and dioxins and furans. Regulations also describe the monitoring frequency and record keeping requirements.

## COMPARISON OF LEGISLATIONS ON LANDFILL DISPOSAL OF SLUDGE

The examinations of legislations in different countries regarding the disposal of sludge by landfilling yielded that there are not specific regulations in majority of the countries. There are items related to sludge in some regulations regarding the disposal of solid or hazardous wastes into landfills. The Landfill Directive in EU required all the solid waste landfills in member countries either to be brought up to the standards required by the Directive or to be closed by July 2009. The directive separates the wastes into categories of municipal waste, hazardous waste, non-hazardous waste and inert waste. The Directive does not allow for the co-disposal of these different categories of wastes. The landfills described in this Directive are of three types: landfills for hazardous wastes, landfills for non-

hazardous wastes, and landfills for inert wastes. Municipal sludge, if obeys the required limit values, can be landfilled in non-hazardous wastes landfill. There are reduction targets for the landfilling of biodegradable wastes based on data for 1995 such as to 75% by 2006, 50% by 2009 and 35% by 2016. Turkey adopted the Landfill Directive in 2009, therefore should meet similar reduction targets in the upcoming years.

State of British Columbia in Canada has a landfill regulation that covers in details the responsibilities of the operators, landfilling methods and environmental impact assessment reports to be prepared. Sludge has to be dewatered if it is to be disposed in landfills. In Quebec and Ottawa, Canada, landfilling is allowed only if no beneficial use of sludge is possible and requires this to be demonstrated. In South Africa, the relevant guideline includes items related to soil quality requirements, landfill liner requirements, management of methane and monitoring frequency. If there is no leachate collection system, the maximum allowable limits for metals in soil have to be obeyed. In USEPA Rule 503, there is no item related to the landfilling of sludge. The landfilling of wastes is covered in EPA 40 CFR Part 258 rule that is related to the landfilling of solid wastes in USA.

## CONCLUSIONS

When the different regulations in different countries

about sludge management are evaluated, it is seen that the legislation related to the use of sludge on land is more extensively developed. None of the examined regulations allow for the raw sludge to be applied on land. These regulations share a number of common approaches, even though the parameters covered, limits values, etc. are specific to the country. All the examined regulations had limit values of heavy metals in sludge, some in soil that the sludge is applied; some countries had limits for pathogens or indicator microorganisms; only few had limit values for trace organic contaminants. Sludge or waste combustion (mono or co) and energy recovery is addressed in many countries by regulations examined. The main emphasis is to control the emissions that have the potential to cause air pollution problems. Landfilling on the other hand, is only permitted when no beneficial use of sludge is possible in some countries. In others, organic matter disposal into landfills is limited in a gradual manner over a short time scale. Landfilling does not seem to be a sustainable method to be applied widely in the near future in sludge management.

## ACKNOWLEDGEMENTS

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# Enhancement of Odor Removal and Dewaterability of Anaerobically Digested Sludge by Protease Addition

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**ABSTRACT:** Breaking the gel-like structure of sludge by disrupting the extracellular polymeric substances (EPS) with specific enzyme -protease- application was investigated. After 8 hours of incubation, the final cake solid content of sludge increased more than 20% with protease pretreatment. FESEM images taken from protease treated-unconditioned samples revealed the significance of proteins in sludge structure. Protease pre-treatment improved the removal of major sulfur odors. Decrease in total amount of MT, DMS, DMDS, CS<sub>2</sub> and H<sub>2</sub>S production in the headspace was found 32%, 41%, 36%, 34% (5 mg/l protease) and 58% (15 mg/l protease) respectively in the unconditioned samples. Addition of protease led to an increased rate of hydrolysis of the matrix by breaking the peptide bonds maintaining the integrity of sludge floc, resulting to an improvement in both odor removal and dewaterability.

## INTRODUCTION

**C**ONDITIONING and dewatering of digested sludge is a major economical factor in wastewater treatment plant operation due to high water content of sludge. Recently, conventional dewatering methods such as belt filters and centrifuges have been mechanically improved by higher torque centrifuges or heat/vacuum application during belt filtration. Although these methods increased the dewatering efficiency, they were found to be more expensive because of high energy requirement. They also led serious drawbacks such as microbial regrowth and increase in odor production. Instead of applying mechanically improved methods, changing the structure of sludge may be more beneficial for the enhancement in both odor reduction and dewatering. The complexity of sludge structure which includes particles, bacteria, and polymeric substances that are excreted from bacteria, may lead sludge to be recalcitrant in sludge management [1]. The major organic fraction of sludge is extracellular polymeric substances, called EPS, form a network structure between particles [2]. Lately, a new insight into sludge structure suggested it to be as a gel-like material because of its

network structure [3,4]. In mid-80's, Goodwin and co-workers [5] reported that EPS are mix of proteins and polysaccharides. Afterwards, Wilén *et al.* [6] showed that EPS mainly consist of proteins, and carbohydrates, also humic compounds, uronic acids, and DNA. Novak *et al.* [7] demonstrated the effect of EPS constituent amounts on determining filterability and polymer dose relationships. Their results showed a fairly linear relationship of concentrations to both dewaterability and polymer demand. Proteins are major constituents in EPS and digested sludge. Therefore, selective degradation of EPS constituents may enhance dewaterability and filterability of sludge. Yu *et al.* [8] reported increasing soluble organics in enzyme-treated sludge due to improved degradation of EPS constituents. Same study also showed reduction in mean particle size in the enzyme-treated sludge samples, which yields better filtration and dewaterability. Enzyme treatment was also found to increase methane-rich biogas production rate by increasing biodegradation efficiency of sludge during the digestion process [9].

Since biodegradation of proteins may lead to an increased odor emanation due to the nitrogen and sulfur content in the structure of aminoacids, using enzymatic degradation of the proteins in EPS may help to reduce odor production and to improve dewaterability by altering the structure of sludge. Using enzymes for sludge

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pre-treatment is a fairly new area of interest. Initially, Forster [10] investigated enzyme additions to sludge in terms of viscosity effects. Then, Thomas *et al.* [11] reported enzyme application for sludge pretreatment, using a mixed enzyme product to improve dewaterability in laboratory and with a full-scale belt press. Recently, it was found that a commercial enzyme mixture (Envirozyme 216) significantly increased the final solids content of samples [12]. However, the possible effect of specific enzyme treatment may be more informative on the structure of sludge compared to the mixed enzyme composition.

The objective of this study was to determine whether the favorable effects reported for enzyme mixtures could be correlated with the activity of protease, a specific enzyme. We investigated the effect of proteins on sludge filterability, dewaterability, solid content, and odor production by selectively degrading the proteins present in the EPS by protease addition. This would also give us an insight into chemical reasons of poor dewaterability and high odor production.

## MATERIALS AND METHODS

### Sludge Type and Collection

Anaerobically digested sludge was sampled from the municipal wastewater treatment plant in Wilmington, (DE, USA) which is a conventional activated sludge plant treating domestic and industrial wastewaters. Primary and activated sludges are combined for mesophilic anaerobic digestion with a typical sludge retention time of 15 to 20 days. The sludge was collected directly from the digester and transported to the laboratory, where all experiments were completed within three days of collection.

### Enzyme Pretreatment

A protease from *Aspergillus oryzae* (Sigma P 6110), was used as pretreatments. A stock solution was prepared and then added to digested sludge samples in three different concentrations. All samples were incubated at 35°C under anaerobic conditions, for total 16 hours. A sample was taken every 4 hours and each sample was separated into two aliquots. The first was analyzed without polymer addition, and the second was analyzed after conditioning with a cationic polymer. All analyses were conducted as soon as possible following incubation (generally within two hours) since the enzymes were still active at room temperature.

### Conditioning

A flocculant polymer Zetag 7557 (Ciba Specialty Chemicals, Suffolk, VA) was used to condition the digested sludge. Zetag 7557 is a high molecular weight, cationic copolymer of 65% (mole percent) acrylamide monomer and 35% acryloyloxyethyl trimethylammonium chloride. A 0.5% (w/v) stock solution was prepared using the procedure of Dentel and Abu-Orf [13]. A polymer dose of 300 mg/L, found to be optimal in preliminary tests, was applied to the enzyme-conditioned samples. Using a Phipps & Bird paddle stirrer, the samples were mixed at 330 rpm for 2 min and followed by mixing at 30 rpm for 5 min.

### Filterability and Dewaterability

The filterability and dewaterability of samples were evaluated using a capillary suction time (CST) device (Venture Innovations, Lafayette, LA) according to Section 2710G of Standard Methods [14]. A bench-scale simulation of belt press dewatering was also employed to investigate sludge behavior under pressure and shear conditions. For simulating the gravity drainage step of a belt filter press, a 300 ml sludge sample was poured onto a gravity drainage simulator assembly, developed by Severin and colleagues [15] as modified by Ayol and Dentel [16]. The cake formed in gravity drainage step was transferred to a Crown Press (Neogen Corporation, Lansing, MI) that simulates belt press. Real belt filter cloths were used in these experiments. The sludge cake that was formed in gravity drainage was squeezed between filter cloths for controlled periods of time and pressure. Details about use of the gravity drainage kit and Crown Press can be found in references [17,18]. The solid content of the final cake was measured according to method 2540C of Standard Methods. The filtrate was also collected after applying the Crown Press and analyzed for turbidity, using methods 2130 of Standard Methods [14].

### Cryo-FESEM

The freeze-dried method was selected based on the results of Poxon [4], who reported that this process conserved structural characteristics of sludge. The structural differences of treated sludge were observed by using a Hitachi 4700 field emission scanning electron microscope (FESEM). Samples were initially filtered and mounted dropwise on an aluminum stub and plunged into liquid nitrogen slush at -130°C. The sam-

ple was then warmed up to  $-95^{\circ}\text{C}$  in a Gatan specimen preparation chamber and kept there for 15 min. The prepared sample was then transferred to the cryostage and viewed at  $-120^{\circ}\text{C}$  under 0.7 and 1.0 kV.

### Protein Analysis

Samples were analyzed for protein content by using protein assay kits [19].

### Odor Analysis

Digested sludge samples were anaerobically incubated to investigate possible effects of protease on odor formation. For consistency, samples for odor analysis were prepared from the same batches that were used in filterability and dewaterability tests. After sludge was incubated with protease for 4 hours at  $35^{\circ}\text{C}$ , samples for odor analysis were prepared with and without polymer addition with enzyme doses of 0, 5, 15 and 25 mg/l. Conditioning was performed with the addition of 300 mg/l cationic polymer. Conditioned and unconditioned digested sludge samples were prepared in a bench-top glove bag under Nitrogen gas flow, and incubated for 16 days in amber glass bottles under anaerobic conditions. The bottles, each containing 150 ml of digested biosolids, were sealed with Mininert™ valves and kept in a water bath at  $30\text{--}130^{\circ}\text{C}$ . Gas production, as an indicator of microbial activity, was measured on a daily basis by using a Mariotte flask. This also equilibrated the headspace pressure to atmospheric pressure prior to sampling.

### Analytical Methods

Odor compounds including dimethyl sulfide (DMS), dimethyl disulfide (DMDS), methane thiol (MT), hydrogen sulfide, and carbon disulfide were sampled by solid phase micro extraction (SPME) prior to the injection to Hewlett-Packard 5890 gas chromatograph coupled to an HP 5970 mass spectrometer. A 75 m Carboxen-polydimethylsiloxane (CAR-PDMS) coated fiber was selected for sampling due to its relatively high efficiency in extracting vapors and low-molecular weight analytes, especially sulfur compounds [20,21]. Extraction (sorption) time and GC injection (desorption) time of 5 minutes with SPME were applied for the analysis of odor samples as recommended by Supelco [22]. A 30-m DB-5MS column (0.25 mm ID, 0.5 m film thickness) was used with the following temperature program: isothermal at  $32^{\circ}\text{C}$  for 4.5 min,

**Table 1. Analytes, GC Retention Times, MS Target Ions, and Odor Thresholds for Human Detection.**

Analyte	Molecular Mass (g/mol)	Qualification Ions (g/mol)	GC Retention Time (min)	Odor Threshold* (ppm)
H <sub>2</sub> S	64	34	1.77	0.00047
MT	48	47, 48	2.105	0.0011
DMS	62	47, 62	2.80	0.001
CS <sub>2</sub>	76	76	3.10	0.02
DMDS	94	79, 94	8.00	0.0003

[23], Adapted from [24].

ramped at  $15^{\circ}\text{C}/\text{min}$  to  $110^{\circ}\text{C}$  and then at  $30^{\circ}\text{C}/\text{min}$  to  $250^{\circ}\text{C}$ , and held at  $250^{\circ}\text{C}$  for 1 min. Injector and detector temperatures were  $240^{\circ}\text{C}$  and  $280^{\circ}\text{C}$ , respectively. The duration of each sample run was 15 minutes. A selective ion monitoring based GC-MS method was developed by performing series of test injections with odor standards under full-scan mode to obtain the optimal GC conditions, retention times, targets (quantification), and confirmation ions (qualification) for each odor compound. Before each sampling, blank injections were performed to obtain a clean baseline. These methods are currently used to quantify very low levels of odorants including dimethyl sulfide (DMS), dimethyl disulfide (DMDS), methane thiol (MT), hydrogen sulfide, and carbon disulfide. The odor compounds of interest, their retention times, quantification ions, and human threshold limits are shown in Table 1.

## RESULTS AND DISCUSSION

### Filterability

Figure 1. summarizes the CST results for 4 different protease doses (0, 5, 15 and 25 mg/l) and 5 different incubation times (0, 4, 8, 12, 16 hours). Not only the effect of enzyme addition but also conditioning was analyzed and the results are shown with the dashed lines. CST is widely used as an indicative of filterability and the lower the CST results are, the better the filterability is.

A significant decrease (%40) in CST results was observed for all enzyme treated samples after 8 hours of incubation, pointing out an improvement in filterability. The minimum CST values were obtained by increasing enzyme dose for both conditioned and unconditioned samples. However after 8 hours of incubation, the CST stayed relatively constant—especially for conditioned samples— even with increasing enzyme dose. This suggests that lower enzyme doses could be effective if the incubation period is eight hours or more.

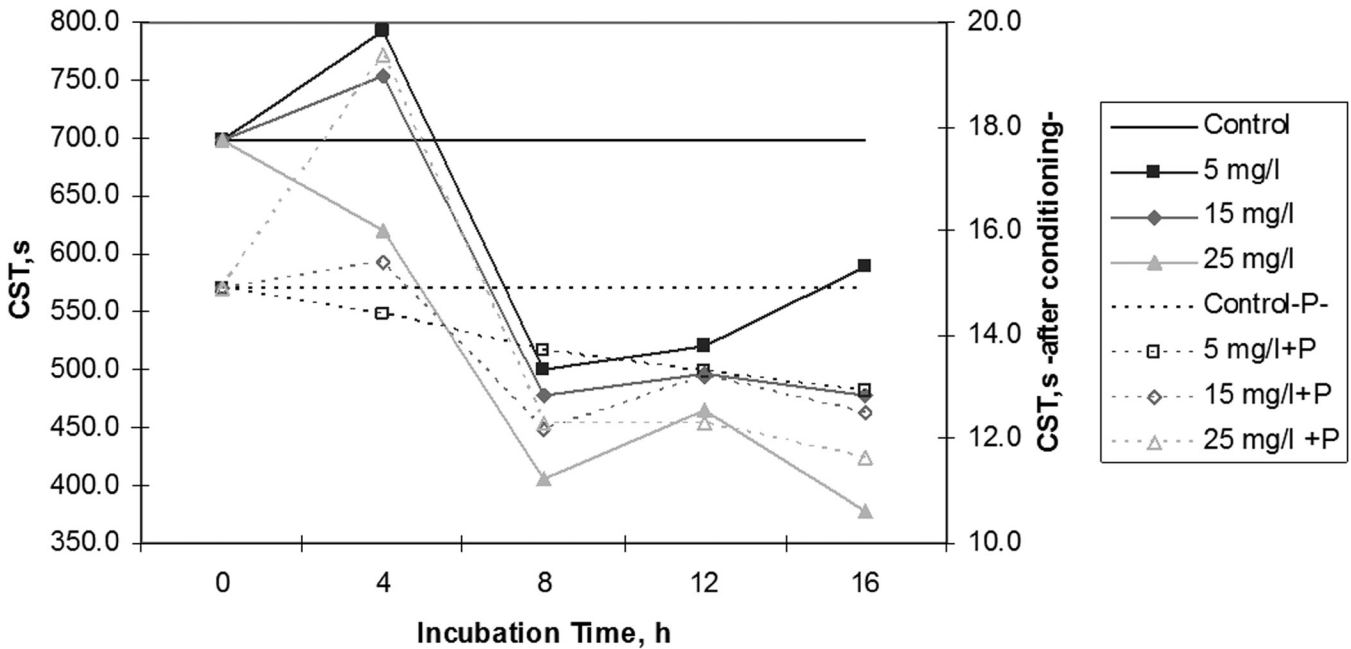


Figure 1. CST results for protease treated digested sludge.

**Dewaterability**

Figure 2 depicts the cake solid concentrations of the sludge samples after gravity drainage and crown press application. A general increasing trend can be seen for all samples with increasing incubation time and protease dose. Similar to CST results, solid content results also stabilized around 8 hours of incubation. However, the results changed drastically after 8 hours of prote-

ase treatment. Without conditioning, the solid content reached up to 9% from original content of 7% and final solid concentration of conditioned samples was found higher than 22% whereas the initial value was lower than 18%. These differences might look like slight increases, however they corresponds to at least 20% of reduction in sludge mass. The importance of protease addition could be more favorable if the economics of sludge treatment and disposal is taken into account.

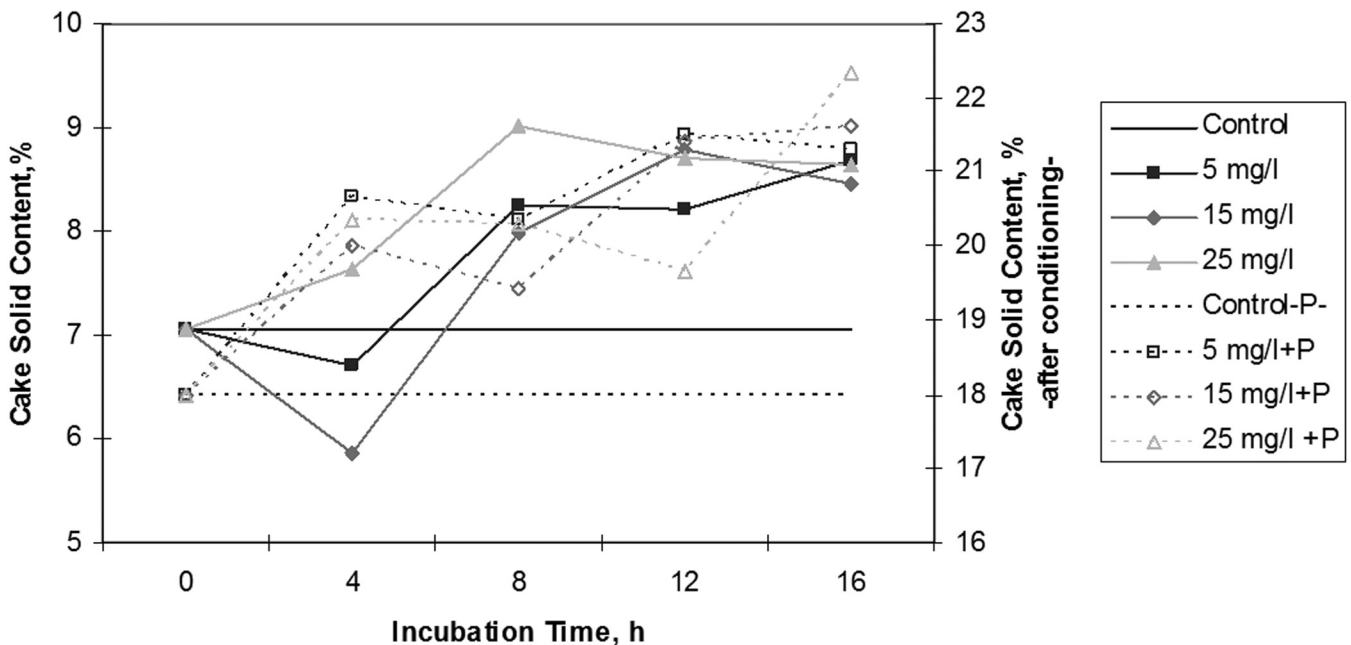


Figure 2. Effect of protease addition on cake solid content.



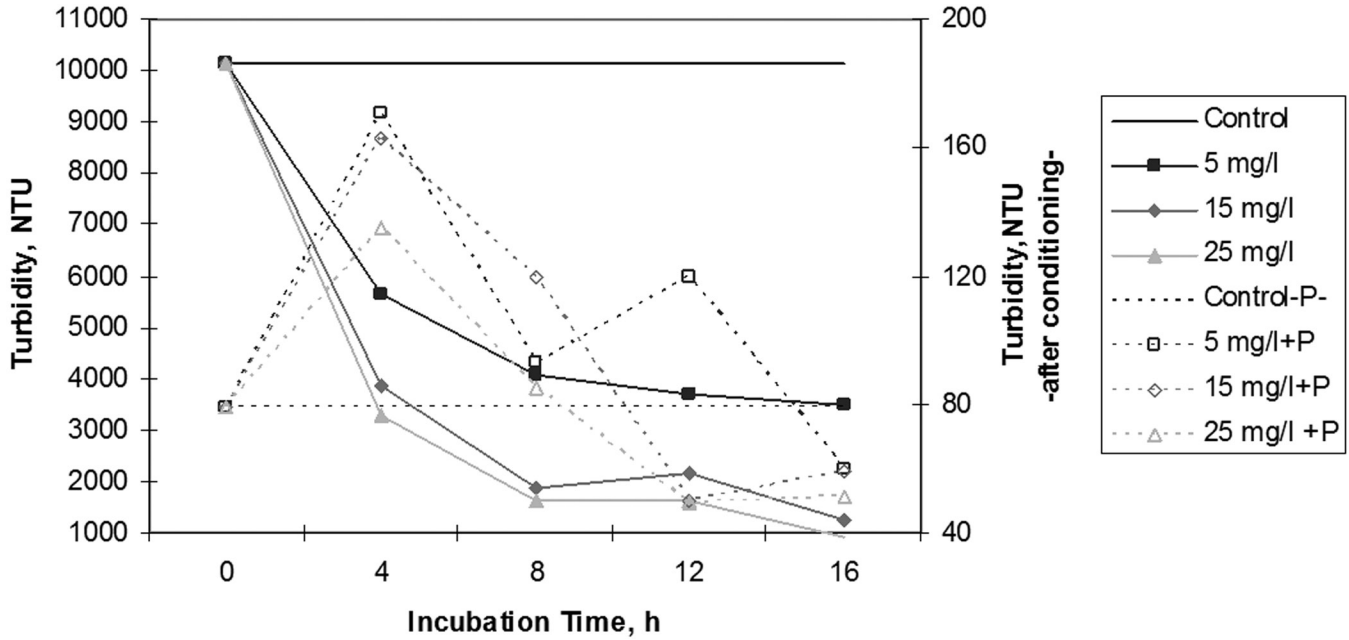


Figure 3. Turbidity results for protease treated digested sludge.

Optimal enzymatic conditions need to be identified. Although low doses of protease provoked the hydrolysis of sludge constituents, higher doses created further hydrophilic end groups which counteracted dewatering.

**Turbidity**

The filtrate turbidity results are given in Figure 3. The values decreased with increasing protease dose and incubation time. However shorter periods of incubation increased the turbidity for the conditioned samples. This might be the result of decreasing particle size, as hypothesized by Watson and colleagues [25].

Poor capture of the smaller particles would then be anticipated. The suspended solid results of filtrate are not shown here, but they exhibited similar trends with the filtrate turbidity results.

**Protein Content**

Protein content of sludge, measured after 16 hours of incubation (Figure 4), showed a decrease in protein concentrations in both conditioned and unconditioned sludge. For unconditioned samples, the protein degradation somewhat stopped at 15 mg/l. Interestingly, the protein content of polymer conditioned sludge found to have significantly lower than raw sludge and increasing

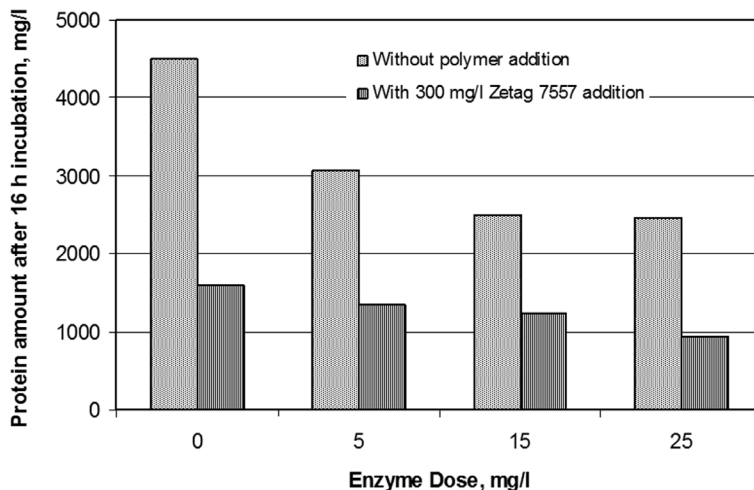


Figure 4. Protein content of digested sludge after enzyme addition.

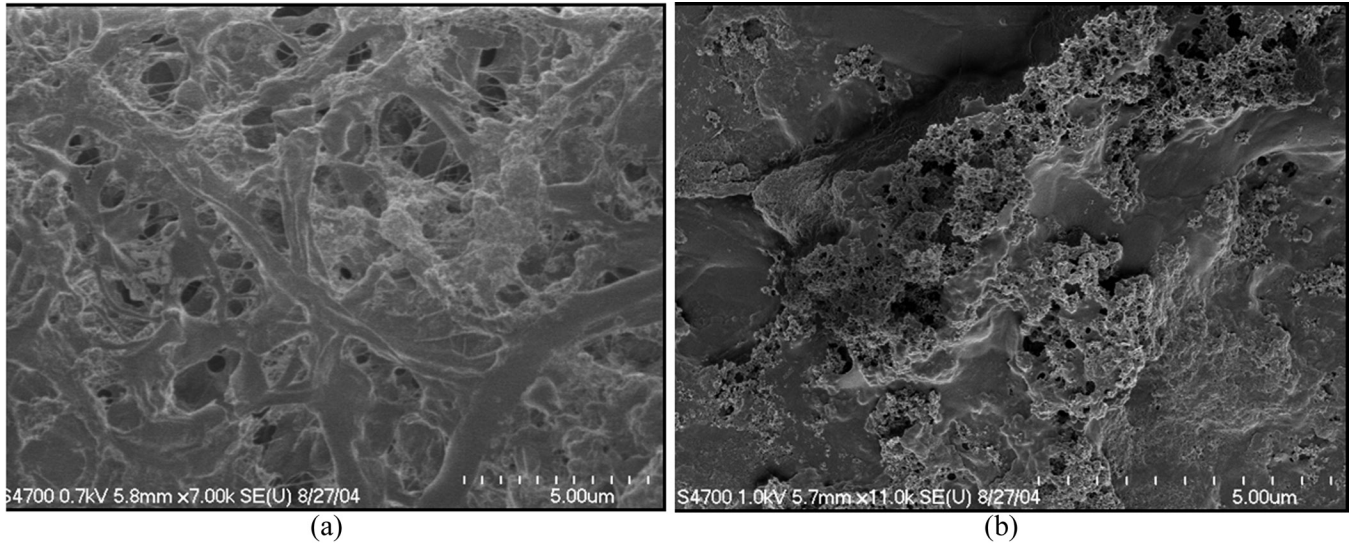


Figure 5. FESEM images: Raw sludge (a) and 25 mg/l Protease treated sludge incubated for 16 hours (b).

protease addition resulted higher protein degradation. It was shown that specific enzyme addition increased the degradation of protein around 40%. The efficiency of this treatment might be higher under different conditions such as longer incubation times and different incubation temperatures.

**FESEM Images**

The structural changes of protease treated sludge are given in Figure 5, confirming the gel like behavior of digested sludge as Poxon [4] showed earlier. Figure 5(b) shows the structure after 25 mg/l of protease was added to the sample and then incubated for 16 h. Prote-

ase addition led severe changes in the sludge structure by resulting a porous surface and compact structure due to the degradation.

**Gas Production**

Gas production is one of the most important parameters in monitoring microbial activity for systems such as activated or digested sludge. Knowing the gas production from each sample is also essential to be able to calculate the total odor amount produced during entire incubation period. Releasing excess pressure that builds up during the incubation period is also crucial for SPME sampling since the amount of

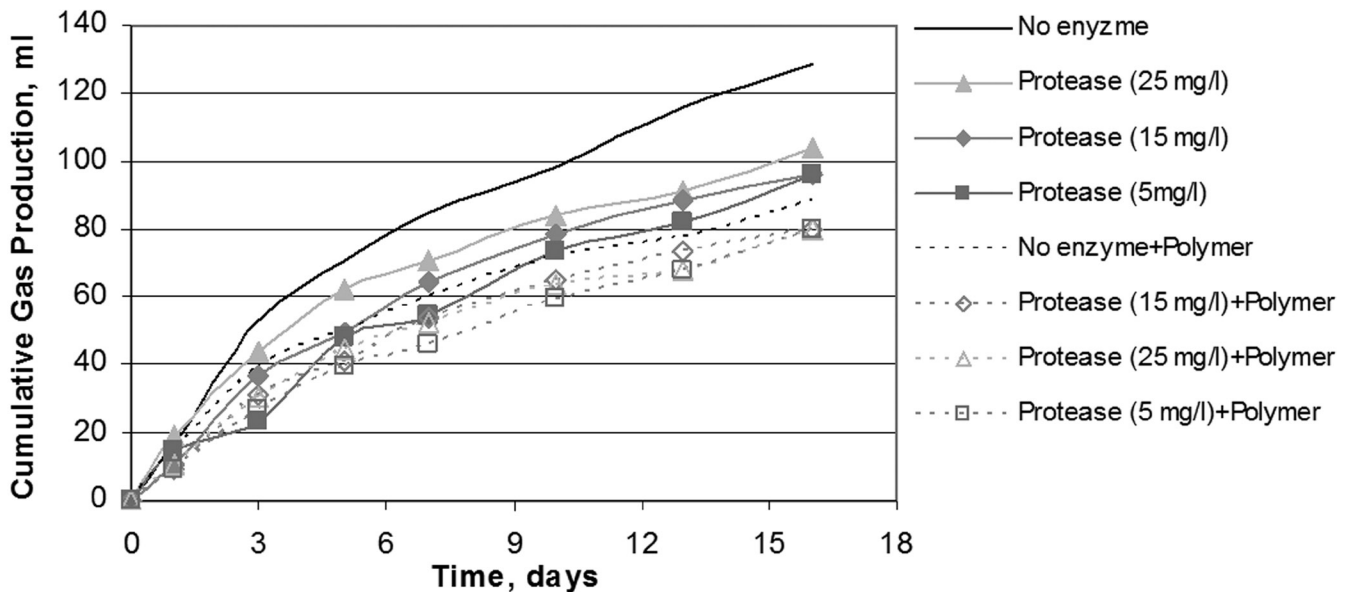


Figure 6. Cumulative gas production from protease pre-treated digested sludge samples, with/without polymer addition.

analyte adsorbed by the fiber is affected by the pressure. Cumulative gas production from the samples during 16-day incubation period is shown in Figure 6. Results exhibited that protease addition slightly decreased total gas production in both conditioned and unconditioned samples. However a higher decrease in total gas production was monitored in the sam-

ples with polymer. During conditioning, Zetag 7557, builds a strong network structure between particles in the sludge and the polymer chain. This strong network structure limits the availability of biodegradable molecules for the microorganisms in the sludge and leads to a lower methane and carbon dioxide production in the conditioned samples.

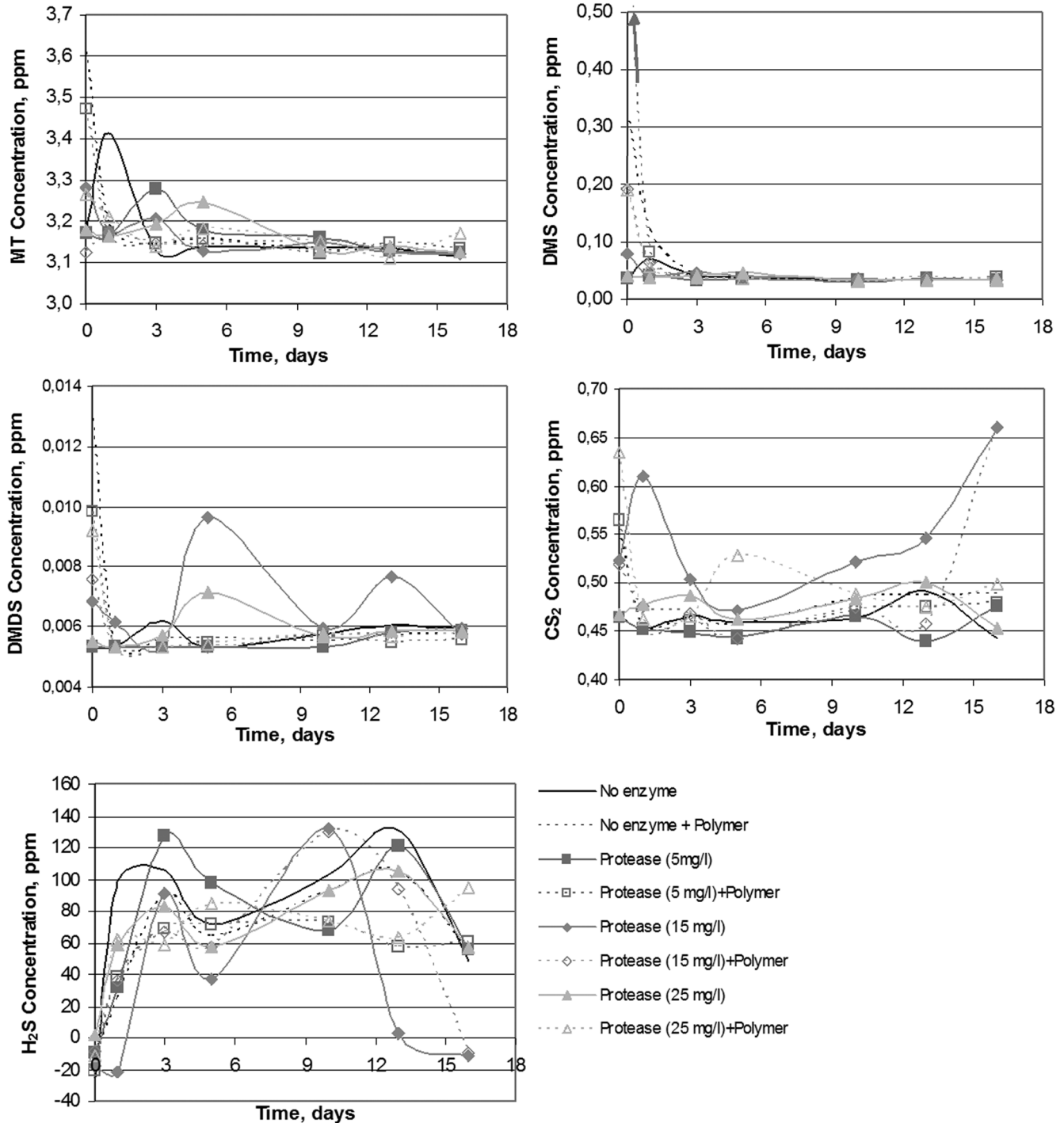


Figure 7. Headspace concentrations of MT, DMS, DMDS, CS<sub>2</sub> and H<sub>2</sub>S during the incubation of protease pre-treated digested sludge with/without polymer at 30°C.

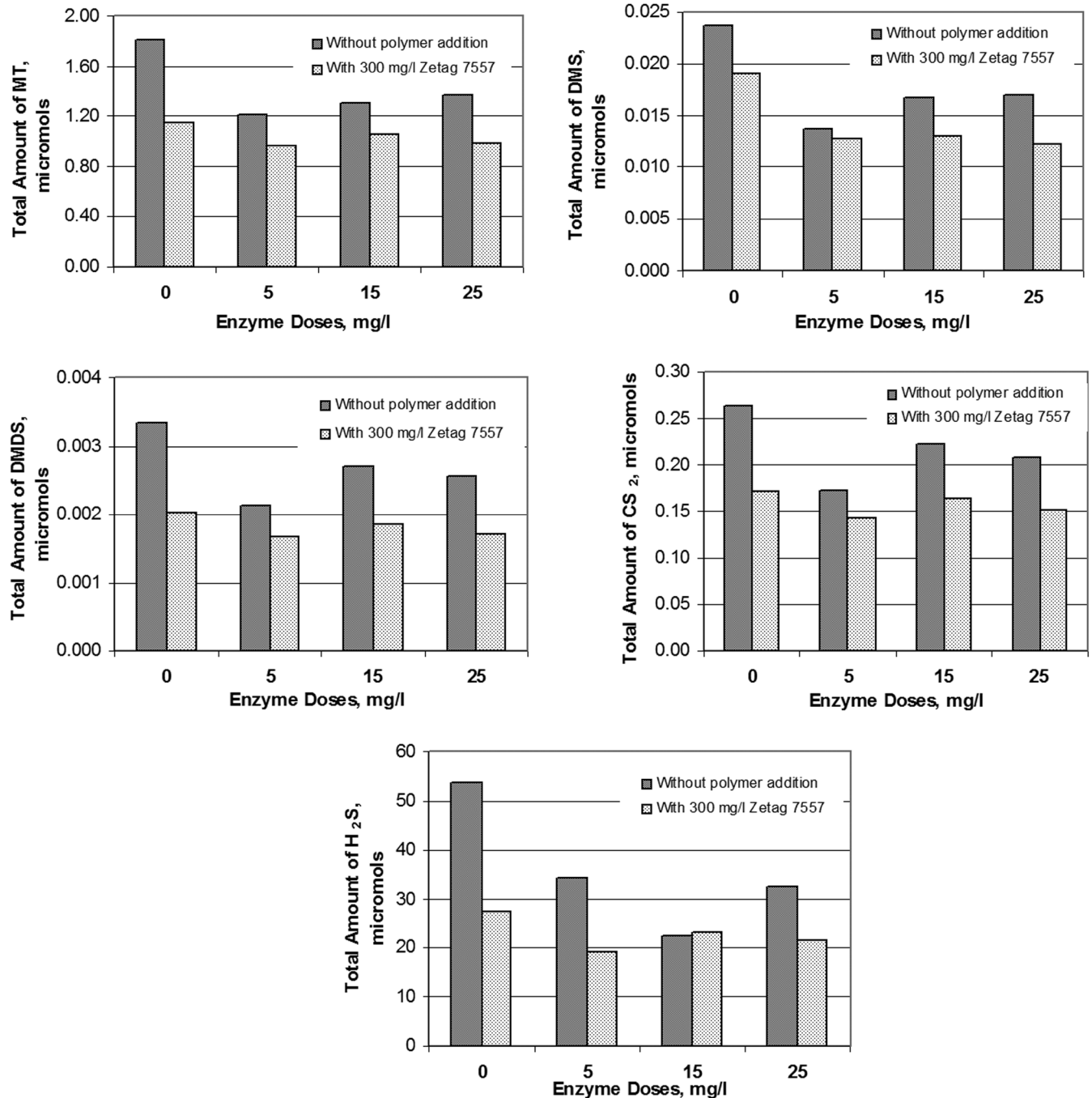


Figure 8. Total amount of MT, DMS, DMDS, CS<sub>2</sub> and H<sub>2</sub>S produced during the incubation of protease pre-treated digested sludge with/without polymer at 30°C.

### Headspace Odor Concentrations

Change in odor concentrations is given in Figure 7. Results demonstrated that higher initial headspace MT, DMS and DMDS concentrations were measured in the conditioned samples compare to those were measured in unconditioned samples. Concentrations of all the odorants were found above human threshold con-

centrations. Different doses of protease did not make a significant difference in headspace odor concentration in absence of polymer. However, H<sub>2</sub>S concentration decreased with the increasing protease concentration until the protease dose of 25 mg/l in the unconditioned samples. Lowest H<sub>2</sub>S concentration was measured in the unconditioned digested sludge sample with 15 mg/l, above which protease itself results an increase

in organic load which can also be observed as a slight increase in total gas production. Concentration of MT was found higher than both DMS and DMDS.

### Total Amount of Odor Production in the Headspace

Total amount of each odorant was calculated by using gas production increments of a sample and headspace odorant concentration. It is important to know the potential effect of a known sample by using not only the headspace odor concentrations, but also gas production by which the same concentration of odorant in the headspace can be transported to the target. Total amount of odor in the headspace of protease pre-treated digested sludge samples (conditioned and unconditioned) are given in Figure 8. Results illustrated that ultimately lower amount of odor was produced from both the unconditioned and conditioned samples. However, odor amount didn't significantly change with the change in protease dose in the conditioned sludge samples. On the other hand, effect of protease dose was considerably higher on the amount of total odor production in the headspace of unconditioned digested sludge. For instance, decrease in the amount of H<sub>2</sub>S was found 15% in the conditioned samples, whereas it was found 58% in the unconditioned samples.

Likewise, percent decrease in the amount of DMS production after the treatment with 5 mg/l protease was found 32% and 41% in the conditioned and unconditioned samples respectively. All of the odorants except H<sub>2</sub>S reached their minimum in the samples with 5 mg/l protease by following the same pattern. However a slight increase in the amount of odor production was observed with the further increase in protease. As mentioned earlier, protease itself, as an organic molecule is higher than a certain dose, can also be biodegraded leading an increase in the production of volatile organic compounds. It's been known that biodegradation of proteins is one of the major sources of odors due to nitrogen and sulfur in their structure [26,27].

### CONCLUSION

Breaking the gel-like structure of sludge by disrupting EPS with specific enzyme (protease) application (Figure 5) led to 40% of enhancement on filterability.

At least 8 hours of incubation was required to improve the filterability and dewaterability, whereas 4 hours of incubation gave a negative effect. After 8 hours of incubation, the final cake solid content of

sludge increased more than 20% with Protease pre-treatment.

The practical application of protease addition prior to conditioning and dewatering is favorable since the conditioned samples had higher cake solid contents and lower filtrate turbidity.

The results without conditioning suggest the importance of proteins in gel structure and filterability of sludge.

Protease pre-treatment contributed on removal of major reduced sulfur odors produced from the anaerobically digested sludge.

Headspace odor concentrations did not exhibit a significant difference with the increase in protease amount. However, when total gas production is taken into account, decrease in total amount of MT, DMS, DMDS and CS<sub>2</sub> production in the headspace was found 32%, 41%, 36% and 34% respectively in the unconditioned digested sludge samples with the protease dose of 5 mg/L.

Decrease in the total amount of H<sub>2</sub>S in the headspace was found 58% with the addition of 15 mg/l protease in unconditioned sludge samples.

Study also demonstrated that polymer addition decreases the gas production by limiting the availability of biodegradable organics for the existing microbial population. Despite the lower gas production, higher initial headspace odor concentrations were measured in the conditioned samples.

Some odors and sludge mass can be successfully reduced by enzyme pretreatment depending on the enzyme type, dose and operational conditions; however the properties of sludge might also play a crucial role.

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# Polycyclic Aromatic Hydrocarbons (PAHs) Removal Applications in the Industrial Treatment Sludge using UV and TiO<sub>2</sub>

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**ABSTRACT:** In the present study, the removal of PAHs from industrial treatment sludge by using ultraviolet (UV) irradiation and titanium dioxide (TiO<sub>2</sub>) was investigated. Twelve PAHs ( $\Sigma_{12}$  PAHs) were targeted and their levels in the sludge were studied. TiO<sub>2</sub> was added at the ratio of 5% and 20% of the dry matter (DM) of the sludge. At 45°C, 35% of the  $\Sigma_{12}$  PAHs were removed with only UV light. Higher PAH removal ratios were obtained employing TiO<sub>2</sub> as a photocatalyst. At 45°C,  $\Sigma_{12}$  PAH removal ratio was reached to 83% with using TiO<sub>2</sub> at the ratio of 20%.

## INTRODUCTION

**P**OLYCYCLIC AROMATIC HYDROCARBONS (PAHs) are semi-volatile organic compounds (SVOCs) formed as a result of two or more benzene rings being arranged and combined in a linear, angular or clustering manner [1]. It is known that PAHs emerge due to incomplete combustion of organic matters and are introduced to the environment from natural or anthropogenic sources. These pollutants, which tend to remain and accumulate in the environment for a long time, may have a reverse effect on biological balances and can damage ecosystems. PAHs have potential mutagenic and cancerogenic effects on human body [2]. For this reason, it has become necessary to remove these pollutants from all matrices such as air, soil and sludge.

Various SVOCs, such as PAHs, exist in treatment sludge along with other pollutants [3–6]. PAHs reach wastewater treatment plants and treatment sludge through the water in sewer systems after they are washed from the soil and also through wet and dry deposition via the atmosphere [6–7]. Several studies on the amount of PAHs in wastewater treatment sludge have been carried out [6–9]. Furthermore, Cai *et al.* [9] studied the removal of PAHs from sludge by applying composting technique. Flotron *et al.* [10] investigated PAH removal through chemical methods such as the Fenton process. However, research on the removal of these pollutants with UV applications is limited. It is

known that SVOCs can be degraded in different matrices with UV technology. It is also known that UV light is absorbed by SVOCs, including PAHs, polychlorinated biphenyls (PCBs) and pesticides in liquid matrices, soil and synthetic form [11–14]. Specifically the short wavelength UV-C provides high energy for the degradation of PAHs [15–16].

Several additives are used in UV applications to enhance PAH removal. TiO<sub>2</sub> is one of the most frequently used photocatalysts. TiO<sub>2</sub> is a semiconductor and it can be chemically activated by UV light [17]. Heterogeneous photocatalysis of organic pollutants using TiO<sub>2</sub> under UV-irradiation and/or solar light has demonstrated successful performance in PAH removal applications [18]. It is expected that these photocatalyst will accelerate the photodegradation process of PAHs in industrial treatment sludge and increase PAH removal efficiencies.

In the present study, a special UV apparatus was designed and removal of PAH compounds from organized industrial district (OID) treatment sludge was investigated by this apparatus. In this content, beginning and residual PAH concentrations in the sludge were investigated. The effect of temperature, UV radiation, TiO<sub>2</sub> dose on the PAH removal were also explained.

## MATERIALS AND METHODS

### Sludge Sampling

Sludge samples were taken from a decanter of an OID wastewater treatment plant. The samples were brought to the laboratory in glass containers with alu-

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minimum lids and the preliminary processes (dry matter analysis and extraction) were completed within 24 hours. Initial PAH amounts in the sludge were measured by employing a GC-MS. Twelve PAH compounds identified by the United States Environmental Protection Agency (USEPA) were targeted: phenanthrene (Phe), anthracene (Ant), fluoranthene, (Fl), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene, (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene, (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-c,d] pyrene, (InP), dibenzo[a,h]anthracene (DahA), and benzo[g,h,i]-perylene (BghiP). The initial concentration of the  $\Sigma_{12}$  PAH compounds were measured as 3268 ng/g dry matter (DM). Characterization of the sludge is provided in Table 1.

### The UV Apparatus

The apparatus, which is specifically designed by our group, used in our studies is provided in Figure 1. The apparatus, manufactured from stainless steel, is designed with the purpose of identifying the impacts of UV-C rays and temperature on PAH removal in the sludge samples in a controlled system. Materials without organic content were used in the apparatus to prevent interference with PAH. Three lamps (254 nm UV-C, Philips TUV G8T5) with a total power output of 24 W were installed on the top of the apparatus (Figure 1). All experiments were repeated 3 times and details such as measuring the luminous intensity and positioning of

**Table 1. Sludge Characteristics.**

Parameter	Value
Dry matter	20%
Inorganic Matter (CaCO <sub>3</sub> ve NaCl)	13%
Mineral Oil	0.3%
Iron	1.5%
Zinc	1.7%
$\Sigma_{12}$ PAH	3268 ng/g DM

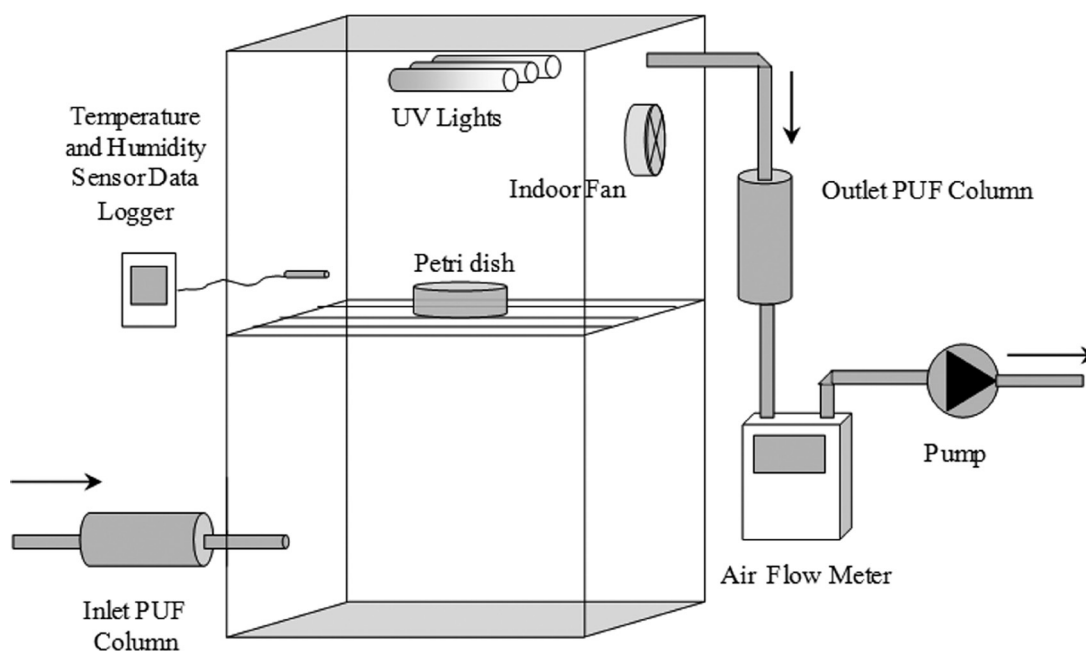
sludge samples in the apparatus etc. were explained in our previous publications [19–20].

### PAH Removal Applications

Thirty g of wet sludge was laid on each petri dish in all PAH removal experiments. UV experiments were carried out at 15°C and 45°C (Group 1–2). During UV-TiO<sub>2</sub> experiments, TiO<sub>2</sub> were added to the sludge as a photocatalyst (Group 3–6). These samples were exposed to UV-C light at two different temperatures (15°C and 45°C) for 24 hours. TiO<sub>2</sub> were dosed to the sludge at the ratio of 5% and 20% of dry sludge weight, respectively.

### PAH Extraction, Cleanup and Determination of Sludge Samples

The PAH content in the sludge prior to and after performing PAH removal applications were measured in



**Figure 1.** UV apparatus [4].



this study. Targeted PAHs were chosen from the priority pollutants list issued by the USEPA. Ten grams of the homogen sludge samples were weighted and placed in amber colored glass vials. Thirty mL Aceton/Hexane (ACE/HEX), (1/1) by volume was added to the sample. Also, 1 mL surrogate standard was added to each vial including sludge and ACE/HEX mixture. Initial  $\Sigma_{12}$  PAH concentrations were determined after extraction and cleanup procedures. Sludge samples that were treated in the apparatus were also placed in amber colored glass vials and subjected to extraction and cleanup procedures after the addition of 30 mL of ACE/HEX, (1/1). All sludge samples were ultrasonically extracted for 30 min and filtered. Thirty mL mixture of ACE/HEX (1:1, v/v) were added to the sludge and extracted again for 30 min. Thirty mL ACE/HEX was added to the sample for a third time and it was waited in the freezer for 24 hours. Then, the sample was extracted for 30 min again. Extracts were filtered through a sodium sulfate column in order to remove any existing water and impurities. After this, sample was concentrated to 5 mL in a rotary evaporator. The sample volume was reduced to 2 mL under a gentle nitrogen stream. Then it was cleaned up with a cleaning column as described. Gentle nitrogen stream were again used to decrease volume of the sample to 1 mL. A detailed explanation about PAH extraction and other procedures can be found in Karaca [20].

### Quality Assurance/Quality Control

Field blanks at the rate of 10% of the number of sludge samples were taken to determine any contamination during the experimental process [20]. The PAH tests that were applied to the sludge samples were also applied to the field blanks containing 20 g of Na<sub>2</sub>SO<sub>4</sub>. Details of the blank sample handling can be found elsewhere in detail [3]. PAH surrogate standard was added to each sample [21–22]. Phenanthrene-d10 was used as a surrogate for Phe, Ant, Fl, Pyr and BaA. Chrysene-d12, which served as a surrogate for Chr, BbF, BkF and BaP. Perylene-d12 served as a surrogate for InP, DahA and BghiP. The PAH concentrations measured at the GC-MS were corrected according to the surrogate standard. PAH concentrations were measured using an Agilent 7890 Model Gas Chromatography (GC) equipped with an Agilent 5975C inert XL mass selective with triple axis detector (MSD). Limit of detections (LODs) were defined for each species of PAH. The LODs are calculated as the mean blank plus three standard deviations. All reported values were blank corrected and

they were above the LOD values. Prior to analysis of the samples, GC-mass spectrometry (MS) was calibrated for seven concentration levels (0.01, 0.1, 0.5, 1.25, 2.5, 5 and 10  $\mu\text{g/mL}$ ) to determine the linearity of the responses. The  $r^2$  value of the calibration curve was  $\geq 0.99$ . System performance was verified by analysis of the midpoint calibration standard every 24 h during the analysis period. The quantifiable PAH amount for a 1- $\mu\text{L}$  injection was 0.1 pg.

## RESULTS AND DISCUSSION

### UV Applications

While UV lamps were open and the thermostat was closed the average temperature was 15°C. The temperature while the UV lamps and thermostat were open was 45°C. It was observed that UV light was not effective on the removal of  $\Sigma_{12}$  PAHs at 15°C. In other words, no PAH removal was ensured at this temperature. However,  $\Sigma_{12}$  PAH removal ratio was calculated as 36% during UV application at 45°C. Removal ratios of 3-, 4-, 5, 6-ring PAH compounds were 34%, 41%, 43%, %40, respectively. It was thought that increasing of evaporation and acceleration of photo-degradation reactions contributed to the removal of PAHs, at 45°C. Similarly, studies in the literature showed that increasing the temperature from 10–30°C, increased the photodegradation of PAHs [23–24]. PAH concentrations in the sludge before and after PAH removal applications are shown in Figure 2.

### UV-TiO<sub>2</sub> Applications

PAH concentrations in the sludge samples during UV-TiO<sub>2</sub> applications are given in Figure 3. While, at 15°C, the decrease of  $\Sigma_{12}$  PAH amount in the samples containing 5% and 20% TiO<sub>2</sub> were 65% and 77%, respectively yet at 45°C, these values were 60% and 83%, respectively.  $\Sigma_{12}$  PAH removal ratios increased with increasing TiO<sub>2</sub> amounts at both 15°C and 45°C. The PAH removal results in the present study consistent with the literature results in a several sludge experiments [19,25]. Karaca and Tasdemir [19] reported that higher PAH removal efficiencies were obtained in automotive treatment sludge with increasing TiO<sub>2</sub> dose at 40°C. Furthermore, Karaca and Tasdemir [25] showed that after UV applications PAH removal efficiencies in food industry treatment sludge containing 20% TiO<sub>2</sub> were higher than the efficiencies in the sludge containing 5% TiO<sub>2</sub>.

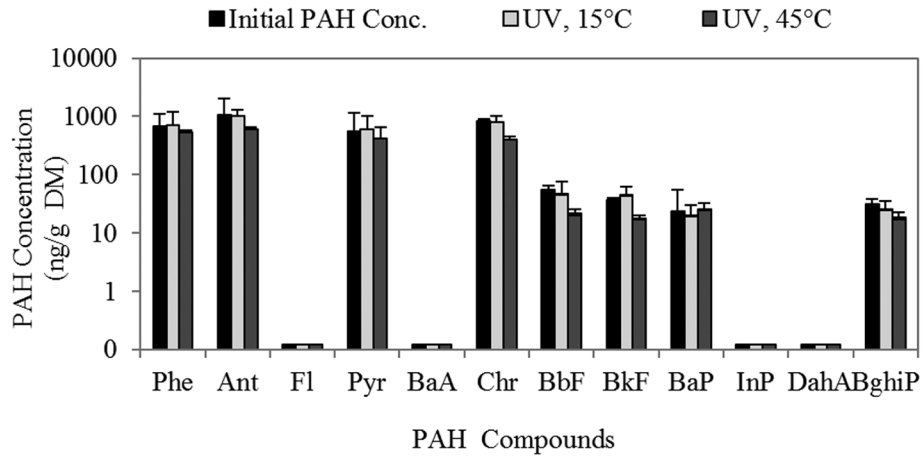


Figure 2. The PAH concentrations in the sludge during UV applications.

When TiO<sub>2</sub> is exposed to UV light, electron hole pairs are created. The photogenerated holes in the valance band diffuse to the surface and react with adsorbed water in order to produce hydroxyl radical (OH\*) [26–27]. OH\* degrade organic compounds [17]. On the other hand, electrons in the conduction band react with molecular oxygen in the air to produce the

superoxide radical anion (O<sub>2</sub><sup>-\*</sup>). In the present study, the humidity of the sludge was 80%. It was believed that the H<sub>2</sub>O molecules adsorbed by the TiO<sub>2</sub> on the surface and produced OH\* radicals [17,28]. Therefore, it is possible for this radical to break the benzene ring in the PAHs.

At 15°C, the removal efficiencies for 3-, 4-, 5- and

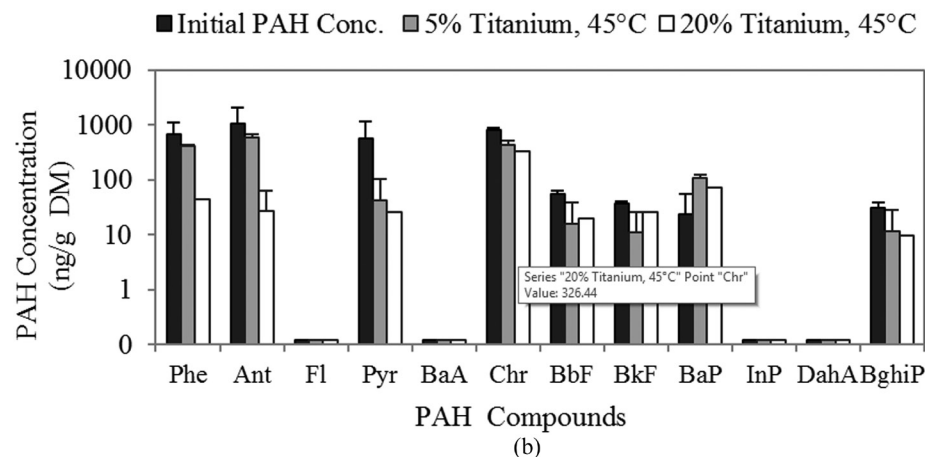
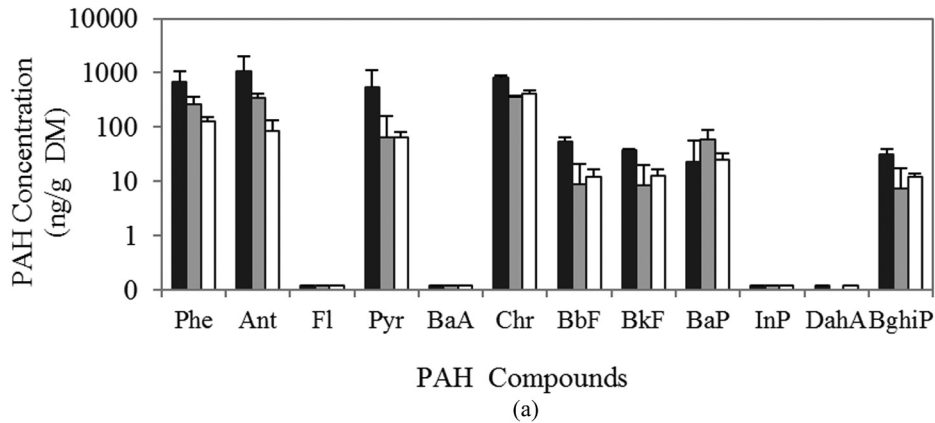


Figure 3. The PAH concentrations in the sludge during UV-TiO<sub>2</sub> applications: (a) 15°C and (b) 45°C.

6-ring compounds of the samples containing 5% and 20% TiO<sub>2</sub> were calculated as %65, %69, %20, %69 and %87, %65, %45, %47, respectively. These values were %43, %65, %0, %52 and %96, %74, %0 and %61 at 45°C. It was determined that the 3-, 4-ring PAHs removal efficiencies were higher than removal efficiencies of 5-, 6- ring PAH compounds. The lighter compounds (3-, 4-ring) tended to evaporate more than the heavier ones (5-, 6- ring) based on high vapor pressures which were promoted by increasing temperature [29–30].

## CONCLUSION

The aim of the present study was to contribute to the relevant existing literature by determining the removal of PAHs in OID treatment sludge during UV applications. UV technology was used here for the first time for this type sludge. The results obtained in this study are summarized as follows:

- Only 36% of  $\Sigma_{12}$  PAH from the sludge were removed with UV application (without photocatalysts).
- TiO<sub>2</sub> addition contributed to PAH removal process and higher PAH removal efficiencies were obtained with using TiO<sub>2</sub> during UV applications.
- Higher PAH removal ratios were obtained at 45°C than at 15°C. It was concluded that the rate of photodegradation increases with the synergistic effect of UV radiation and temperature rise.
- $\Sigma_{12}$  PAH removal ratios increased with increasing TiO<sub>2</sub> amounts at both 15°C and 45°C.
- Maximum PAH removal ratio (83%) was obtained with 20% TiO<sub>2</sub> addition at 45°C. It was concluded that PAHs can successfully be removed from OID treatment sludge with the applications UV-TiO<sub>2</sub>.

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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 <sub>E</sub>	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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