

Aim and Scope

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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C O N T E N T S

Research

- Optimization of a Rapid DNA Extraction and Purification Protocol for Wastewater Biosolids**1
HONGPING CHEN and YEN-CHIH CHEN, Ph.D.
- Influence of Raw Sludge Quality on the Efficiency of Microaerobic Sulfide Removal during Anaerobic Digestion of Sewage Sludge**11
P. JENICEK, C. CELIS, A. PICHA and D. POKORNA
- A Solar Sludge Drying Model: Study of Drying Phenomena—Validation of an Industrial Solar Dryer**17
D. JUNG, S. COUTURIER, J. PANNEJON, J. JOSSENT and D. CHENU
- Effects of Biosolids Treatment Processes on Nitrogen Cycling and Carbon Accumulation under Various Tillage Practices**29
JINLING LI, GREGORY K. EVANYLO, XUNZHONG ZHANG and ERIK H. ERVIN
- Combination of Hydrogen Sulphide Removal from Biogas and Nitrogen Removal from Wastewater**41
D. POKORNA, J. MACA and J. ZABRANSKA
- Degradation of Hydroxytyrosol in Olive Oil Mill Wastewaters using Thermosensitive Zinc Phthalocyanine—Modified Titanium Dioxide**47
HOUDA BEN YAHIA SMIDA, MOEZ BEICHEICKH and BASSEM JAMOSSI
- Developments of European Standardisation on Sludge: Guidelines for Good Practice**55
L. SPINOSA and C. VIGNOLES

Optimization of a Rapid DNA Extraction and Purification Protocol for Wastewater Biosolids

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ABSTRACT: The aim is to optimize a rapid DNA extraction protocol for biosolids that provides high recovery and purity, and to evaluate potential biases on both quantitative and qualitative microbial analyses. A protocol with 3-step sequential DNA extractions using a modified lysis buffer and purification by a commercial DNA kit was developed and compared to a previously known high recovery solvent-based extraction method, a commercial soil DNA kit with single extraction, and the same commercial kit protocol except extracted with the modified lysis buffer. The developed protocol showed more than 7 times DNA recovery compared to the commercial kit, and comparable *E. coli* concentrations to the solvent-based method. Direct adoption of the commercial soil kit showed significantly lower recovery and underestimation of *E. coli* compared to the high recovery protocols. A simple switch of the lysis buffer also improved the DNA recovery by 5 times. Species diversity indexes from pyrosequencing analysis on the other hand showed scatter results from all protocols and only as much as 76% similarity was observed among any paired protocols. Phylogenetic analysis showed a shift of dominance as DNA recovery increases. Overall, this work shows that the developed protocol allows extraction of high quality and quantity DNA within 3 hr. However, qualitative comparisons of species structure may still vary.

INTRODUCTION

MOLECULAR DNA technologies have been widely adopted for both quantitative and qualitative analyses of microbial populations in both natural environments and in engineered systems. These techniques are culture-independent, require relatively short processing time, and provide real-time structure and content of the microbial populations in complex sample matrixes. They serve as a convenient alternative to standard culture techniques which are prone to underestimate microbial concentrations due to sub-optimal culture conditions or false interpretation of relative abundance due to preferential selection by the artificial culture media and incubation conditions. This is especially true since several works reported that bacteria can enter the viable-but non-culturable (VBNC) state under arrays of environmental stresses, and can only be observed by culture-independent methods [1–3]. Among these techniques, real-time PCR, PCR-Denaturing Gradient Gel Electrophoresis (DGGE),

Terminal Restriction Fragment Length Polymorphism (t-RFLP), and the recent pyrosequencing technology, all have been successfully applied for the analysis of microorganisms in wastewater [4–5], activated sludge [4,6], and biosolids [7–9]. This resulted in providing valuable information for wastewater engineers.

DNA extraction is the first step for all of these DNA-based techniques. Development of commercial DNA extraction kits greatly reduced processing time for simultaneous DNA extraction and purification of environmental samples, and resulted in high purity DNA products suitable for subsequent DNA analysis. Among these products, the extraction kits designed for soil samples are most commonly used for wastewater and biosolids samples for their ability to handle complex environmental matrixes. Biosolids are byproducts of municipal wastewater treatment which contains mostly microbial cells and their debris as a result of aerobic or anaerobic digestion. Dewatered biosolids typically contains 16–30% dry solids. Therefore, the soil extraction kit's ability to release bacterial DNA from solids makes them the primary choice for biosolids.

Despite their convenience, direct adaption of soil kits for biosolids may not provide proper recovery.

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Both the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH) and the PowerSoil® DNA Isolation Kit (Mo-Bio, Carlsbad, CA) are commonly used by researchers for DNA extraction from solid or semi-solid samples but several works reported problems for low DNA recovery when using these extraction kits and thus biased data interpretations [10–13]. Similar problems were also reported when extracting DNA from biosolids samples and therefore a combination of 3-step sequential extraction with a solvent-based purification protocol was proposed, which resulted in 3–4 times more recovery [14]. Though the protocol overcame the recovery issue, the processing time also lengthened and the requirement of using a hazardous solvent as well as an inconvenient sample handling volume (larger than 2 ml) limited its acceptance. It was discovered on one quick test that within this protocol the 3-step sequential extraction with buffers containing SDS is the major contributor to high recovery instead of subsequent solvent purification. Therefore, the objective of this paper is to further optimize a DNA extraction protocol for wastewater biosolids based on a commercial DNA kit without use of a hazardous solvent while maintaining high recovery. This work also compared potential biases to bacterial quantification of *E. coli* and qualitative analysis of bacterial diversity among the new protocol and other reference protocols with low recovery.

MATERIALS AND METHODS

Biosolids Sample Collection and Process

Mesophilic, anaerobically digested, and centrifuge dewatered Class B biosolids were collected from a local municipal wastewater treatment plant. The biosolids had a typical solid content of approximately 20%. Samples were aseptically collected right off the centrifuge biosolids discharge point and stored in sealed sample containers for 2 days at room temperature of ~22°C. Biosolids at the end of storage were first homogenized by a sterile spatula for sampling consistency and each 0.05g of samples were aliquot into the Lysing Matrix E tubes (MP Biomedicals, Solon, OH) for DNA extraction. Triplicate tubes were prepared for each extraction protocol and stored at –20°C until the day of experiment.

Solvent-Based DNA Extraction Method

A solvent-based DNA extraction protocol (i.e., sol-

vent method), which was previously demonstrated to produce high recovery, was used for comparison with the new protocol and a commercial kit [14]. In brief, 750 µl of lysis buffer [100 mmol l⁻¹ Tris-HCl, 100 mmol l⁻¹ Sodium EDTA, 1.5 mol l⁻¹ NaCl, and 1% hexadecylmethylammonium bromide (CTAB), pH 8] was added to each lysing tube and homogenized by a FastPrep® Instrument (MP Biomedicals, Solon, OH) at 5.5 speed for 30 s. Each sample tube then received 5 µl of 20 mg ml⁻¹ protease K and incubated at 55°C for 30 min, followed by the addition of 200 µl of 20% sodium dodecyl sulfate (SDS) and incubated at 65°C for 2 h with complete mixing every 30 min. Sample were then centrifuged at 14,000 g for 10 min and supernatant were collected in clean 5 ml tubes. Pellets were then re-extracted twice each with 500 µl of lysis buffer and incubated at 65°C for 10 min followed by the same centrifugation and supernatant collection. Combined supernatants were then purified by two phenol/chloroform/isoamyl alcohol extractions followed by one chloroform extraction. DNA were precipitated by 0.7 volume of ice-cold isopropanol. DNAs were then pelleted by 16,000 g centrifugation for 10 min at 4°C, washed twice with 70% alcohol, and dissolved in 400 µl of TE buffer.

Extracted DNA were first quantified by using the Quant-iT™ PicoGreen® dsDNA (Life Technologies, Grand Island, NY) with a SpectraMax M2 fluorescence microplate reader (Molecular Devices, Sunnyvale, CA) and diluted to 50 ng µl⁻¹ in TE buffer. Only 100 µl of the diluted DNA (total of 5 µg) were purified by the Promega Wizard DNA Clean-Up System (Madison, WI) to assure high recovery. Purified DNA was then quantified with the same method and loss through purification was calculated. Both purified and unpurified DNA extracts were then diluted to 1 ng µl⁻¹ in DNase/RNase free water for subsequent *E. coli* quantification by real-time PCR.

Modified DNA Extraction Kit Method

The modified DNA protocol (i.e., modified protocol) is a combination of the 3-sequential cell lysis followed by purification with the commercial kit. Overall volume of the liquid was controlled so all steps can be easily done using 1.5 or 2.0 ml microtubes and by a table-top mini-centrifuge. Each 500 µl of a modified lysis buffer (120 mmol l⁻¹ sodium phosphate buffer, pH 8.0, and 5% sodium dodecyl sulfate) was added into sample tubes and homogenized by the FastPrep® Instrument at 5.5 speed for 30 s. Samples were then

centrifuged at 14,000 g for 10 min and supernatants were collected in a clean 1.5 ml tube. The remaining pellets were re-extracted twice with 250 μ l of lysis buffer and subjected to the same centrifugation. The combined supernatants were then purified with the FastDNA™ 2 ml SPIN Kit for Soil (MP Biomedicals, Solon, OH) by precipitating proteins with 250 μ l of protein precipitation solution (PPS), 1:1 ratio of binding matrix, and 2 SEWS-M washes. To enhance DNA recovery, the final elution was done by 2 additions of 200 μ l of pre-warmed TE buffer to 55°C with a pH 8 for 1 min incubation at 55°C each. Extracted DNA were then quantified and diluted to 1 ng μ l⁻¹ in DNase/RNase free water for subsequent real-time PCR quantification of *E. coli*.

To investigate DNA recovery in each of the 3 fractions, a separate set of samples were extracted side-by-side with separate collections for each of the 3 fractions (1st extract, 2nd extract, and 3rd extract). DNAs in the 3 fractions were then separately purified, quantified, and diluted for subsequent analysis.

Commercial DNA Extraction Kit Method

A set of samples were also extracted using the commercial kit, FastDNA™ 2 ml SPIN Kit for Soil (MP Biomedicals, Solon, OH), per the recommended protocol by the manufacturer (i.e., soil kit). The key difference for the commercial kit and the modified protocol was that the commercial kit only suggested a single cell lysis using 1 ml of kit-provided lysis buffer and shearing at the speed of 6.0 and 40 s. Since the commercial kit also suggested an option to use other alternative methods in the final DNA elution, the same modification was used with two 200 μ l of pre-warmed 55°C TE buffer instead of the kit-provided DNase free water. To compare the difference of the lysis buffer provided by the kit and the one used in the modified method, another set of samples were extracted with the identical procedure except using the modified lysis buffer (soil kit + MLB). All extracted DNA were quantified and diluted in DNase/RNase free water for subsequent *E. coli* quantification by real-time PCR.

Real-Time PCR Quantification of *E. coli*

Copies of *E. coli* DNA in each sample were quantified by using the previously developed real-time PCR protocol targeting on the *gadAB* gene with a forward primer 5'-GCG TTG CGT AAA TAT GGT TGC CGA-3' (*gadrt-1*) and a reverse primer 5'-CGT CAC AGG

CTT CAA TCA TGC GTT-3' (*gadrt-2*) which result in a 305 bp PCR product [14]. The Stratagene MX3005P rt-PCR system (La Jolla, CA) was used for quantification with each reaction containing 12.5 μ l of the Brilliant SYBRs Green QPCR Master Mix (Stratagene, La Jolla, CA), 30 nmol l⁻¹ of reference dye (Rox), 0.5 mmol l⁻¹ of each primer, and 10 μ l of the 1 ng μ l⁻¹ sample DNA. All were diluted to a final volume of 25 μ l with DNase/RNase free water. The PCR program contained a 10 min initial denaturation at 95°C followed by 40 cycles each of denaturation at 95°C for 30 s, annealing at 59°C for 1 min, and extension at 72°C for 30 s. Known quantities of standard *E. coli* DNA from 2 to 1,300 copies (cp) each in 10 ng of background *Pseudomonas putida* DNA was analyzed side-by-side as an external DNA standard for the quantification. A final melting curve analysis for PCR products was also performed for the QA/QC check.

bTEFAP Pyrosequencing and Bacterial Diversity Analysis

Purified DNA were analyzed for their bacterial composition and diversity using the bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) by the Research and Testing Laboratory (Lubbock, TX) as described previously using Gray28F 5'-TTTGATCNTGGCTCAG and Gray519r 5'-GTNTTACNGCGGCKGCTG [15]. The initial sequencing library was generated by amplifying each 100 ng of purified DNA using the PCR mix and the Hot Start Plus tag polymerase (Qiagen, Valencia, CA). Amplification included 94°C initial denaturation for 3 min, followed by 35 cycles of 94°C denaturation for 30 seconds, 60°C annealing for 40 seconds, 72°C extension for 1 min, and a final extension at 72°C for 5 min. Tag-encoded FLX amplicon pyrosequencing analyses were performed using a Roche 454 FLX instrument with Titanium reagents based on RTL protocols (www.researchandtesting.com).

All resulting sequences for quality check were processed through denoising and chimera checking prior to taxonomic identification. USEARCH [16] was used for denoising by clustering sequences at a 1% divergence. Any cluster that does not contain at least two member sequences is removed and a second clustering at a 5% divergence is again performed. Chimera sequences were removed with UCHIINE in the de novo mode [17]. Finally, any sequence with failed sequence reads; with low quality tags, primers, or ends; and less than 250 bp in length was removed from the library. Taxonomic identifications for remaining sequences

were conducted by first clustering into OUT (operational taxonomical unit) clusters with 96.5% identity using USEARCH and queried against a database of high quality sequences derived from NCBI.

Bacterial composition for each of the samples were analyzed at species level with species identification at < 3% divergence to well characterized 16S sequences. Species richness, evenness, the Shannon-Wiener Diversity Index, the Chao-1 species richness estimator, and the Sørensen Similarity Index were calculated using a Microsoft Excel Spreadsheet.

Statistical Analysis

Data comparisons were performed by using the IBM SPSS Statistics 20 where one-way ANOVA was first used to identify differences among protocols and Tukey's multiple comparisons were used to compare paired sample means at critical value set at 0.05.

RESULTS

Quantitative DNA and *E. coli* Recovery

Close to 3,000 $\mu\text{g g}^{-1}$ DS (dry solids) of DNA was recovered by the previously developed solvent method that was shown to have high DNA recovery within the typical range of biosolids observed in our laboratory (Table 1). The new modified protocol using 3 sequential extractions combined with a DNA kit for DNA purification yielded 86% ($2539 \pm 18 \mu\text{g/g DS}$) of what was recovered by the solvent method ($2941 \pm 31 \mu\text{g/g DS}$). This means a 14% loss from this purification change. This modified protocol also yielded more than 7 times the extracted DNA compared to the commercial soil DNA kit ($347 \pm 13 \mu\text{g/g DS}$) which recovered only 12% of the solvent method. Interestingly, using the same soil kit but replaced with the modified lysis

buffer containing high amounts of SDS increased the yield by almost 5 times ($1689 \pm 18 \mu\text{g/g DS}$). However, it still recovered only 57% of the solvent method. This shows that in addition to inefficient DNA extraction by 1 sample shearing the lysis buffer provided by the manufacturer is also ineffective in releasing DNA from biosolids.

To verify if different DNA extraction methods may bias microbial enumeration using molecular methods copies of *E. coli* DNA were quantified using real-time PCR. Results showed that significant underestimation can happen if only 1 extraction (the two soil kit methods) is used ($p < 0.05$) and as high as 1.5-log loss was observed when the kit-provided lysis buffer was used. Loss from purification using the commercial soil kit was not significant compared to the solvent method ($p > 0.05$) when 3 extractions were used. It was also discovered during real-time PCR runs that all extracted samples have slopes around threshold cycles parallel to those of the standard *E. coli* DNA except for unpurified solvent-extracted DNA. This shows that despite the high recovery from solvent-based method there is a potential of carryover of PCR inhibitors if not further purified. Additional QA/QC with melting curve analysis at the end of PCR runs confirmed that all resulting PCR products have identical melting point with those of the *E. coli* standards.

When further analyzing the DNA recovery in the 3 sequential extraction fractions using commercial soil kits for purification results indicated that only 47% of DNA was recovered by the first extraction and as much as 34% and 19% from the second and third extractions were observed (Figure 1). The first extraction in this analysis is lower than that of the single-extraction method (57%) since a lower quantity of buffer was used to allow purification of the combined extracts to be conducted in a 2 ml microtube. This result also implies that a potential 4th extraction may benefit the

Table 1. Amount of Total DNA and *E. coli* in Biosolids Recovered using 4 Extraction Protocols.

Extraction Methods ^a	DNA ($\mu\text{g/g DS}$)	Percent DNA Recovery Compared to Solvent Method	Fold Increase in DNA Recovery over Soil Kit	Log <i>E. coli</i> (cells/g DS)	Log <i>E. coli</i> (cells/ $\mu\text{g DNA}$)	Detection Limit ^e	Detection Limit ^e
						10 ng Template (log cells/ $\mu\text{g DNA}$)	100 ng Template (log cells/ $\mu\text{g DNA}$)
Solvent Method ^a	2941 ± 31	100%	8.5	6.48 ± 0.21	3.0 ± 0.2	5.47	4.47
Soil Kit ^b	347 ± 13	12%	1	5.01 ± 0.39	2.5 ± 0.4	4.54	3.54
Soil Kit + MLB ^c	1689 ± 17	57%	4.9	5.92 ± 0.09	2.7 ± 0.1	5.23	4.23
Modified Protocol ^d	2539 ± 18	86%	7.3	6.43 ± 0.11	3.0 ± 0.1	5.40	4.40

^aSolvent Method contains 3 extractions and solvent purification [14].

^bSoil kit method is direct adaption from a commercial soil kit with kit-provided buffer.

^cSoil Kit + MLB is direct adaption from a commercial soil kit with modified lysis buffer.

^dModified Protocol contains 3 extractions and a DNA kit purification.

^eDetection limits are calculated based on 1 copy of *E. coli* DNA in each PCR reaction expressed in log values.

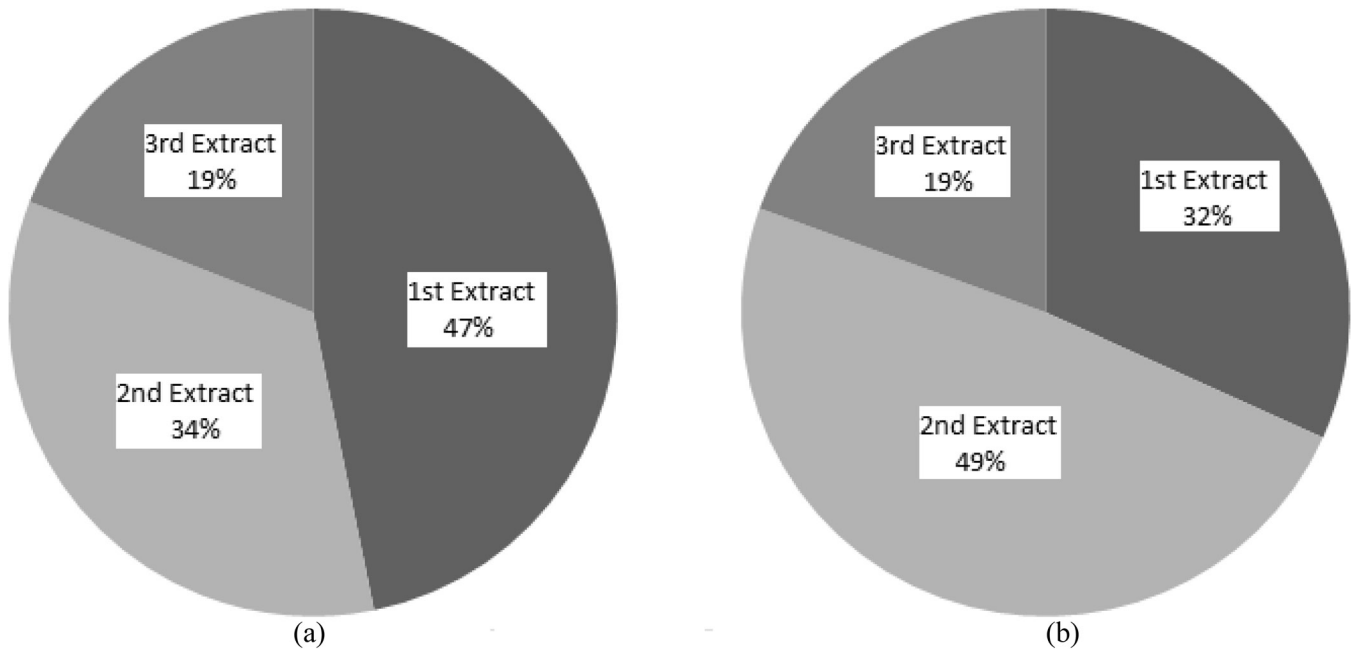


Figure 1. Percent (a) DNA and (b) *E. coli* in 3-sequential extraction fractions from dewatered biosolids.

recovery of the reflected 14% DNA loss. However, despite this loss, it does not impact the overall outcome of *E. coli* enumeration. Therefore, this current 3 extraction method is sufficient for the quantification of *E. coli* in biosolids. Addition of a 4th extract will not impact the result but instead will bring the overall volume over the capacity to be purified in a microtube.

Relative *E. coli* proportions to the background microbial population of each extraction method are presented by expressing *E. coli* concentration in per gram of background DNA. Results showed that similar proportions were observed for the two 3-extraction methods and slightly lower but significantly different ($p < 0.05$) representations of *E. coli* were observed in the two single-extraction methods (Table 1). The highest amount of *E. coli* in addition was observed to be from the second extract which has lower total DNA than the 1st extract. This means that a potential underestimation of *E. coli* abundance may result if only one single extraction is used for this specific sample. These results indicate that both microbial enumeration and community analysis may be biased if a DNA extraction method with only single extraction or with low recovery is used (Figure 1).

Qualitative Bacterial Composition and Diversity

Pyrosequencing analyses were performed on all 7 samples resulting in 4,000–12,000 identified amplicons

at an overall average read length of 390 and a standard deviation of 90. Phylogenetic analysis showed that all samples contain the same 5 dominant (> 5%) phyla including Proteobacteria, Chloroflexi, Bacteroidetes, Firmicutes, and Actinobacteria (Figure 2) which is consistent with those reported by other researchers for mesophilic anaerobic biosolids [7]. When using the kit protocol and also in the 1st extract Proteobacteria is the most abundant phylum. However, an increasing proportion of Chloroflexi was observed when samples were subjected to 2nd and 3rd extractions. Chloroflexi as a result became the most abundant phylum in the 2nd and 3rd extracts as well as those with combined 3 extractions (combined and solvent). Chloroflexi is also the most abundant phylum in the single extraction with 1 ml modified buffer sample which is likely due to its relatively higher DNA recovery than other single extraction protocols. These results indicate that Chloroflexi seem relatively difficult to extract in biosolids and thus is only seen in the most dominant phylum when a high DNA recovery method is used.

Common diversity indexes used for microbial analyses were also compared among the 7 samples at the species level. Results show species richness ranges from 224 to 311 and the Chao-1 species richness estimate ranges from 271 to 338. An increasing number of species was observed in 2nd and 3rd extracts but they were also accompanied by a decreasing Shannon-Wiener Diversity Index and species even-

Table 2. Bacterial Diversity Analyses from Species Identified by Bacterial Tag-encoded FLX Amplicon Pyrosequencing (bTEFAP).

	1st Extract	2nd Extract	3rd Extract	Modified Protocol	Soil Kit	Soil Kit + MLB	Solvent Method
Species richness	224	256	303	279	311	301	244
Chao-1 Estimate	271 ± 8	290 ± 3	323 ± 5	338 ± 7	329 ± 6	320 ± 5	298 ± 7
Shannon-Wiener Diversity	4.20	3.99	3.90	4.05	4.10	4.38	4.22
Evenness	0.78	0.72	0.68	0.72	0.71	0.77	0.77

ness. Among the 4 extraction protocols, up to 8% of differences were observed in both the Shannon-Wiener Diversity Index and for species evenness. One underlying concern of diversity analysis using data generated from pyrosequencing analysis is that it may result in variable amounts of OTUs sequenced from each sample which may likely impact the outcome of species richness, evenness, and the Shannon-Wiener Diversity Index. Random trimming of resulting OTUs down to the same level was performed by other researchers which showed that all but species richness remain similar to those of the untrimmed data [18]. Random trimming was also performed on the 7 samples and the same consistency was also observed (data not shown). When further comparing species composition similarities among the 7 samples using

the Sørensen's Similarity Index only as high as 76% similarity was observed in any paired samples (Table 3). This highest similarity is between the lowest recovery soil kit method and the highest recovery solvent method. The new 3 extraction protocol despite having the second highest recovery and the closest quantity to the solvent method only shows 68% species similarity. This shows that similar recovery of different extraction protocols may not always be interpreted to be the extraction of similar groups of bacteria in samples. Results further exemplify the importance of having identical DNA extraction protocol when attempting to compare microbial structures of samples as well as for the concern of comparing community structures across works when different extraction methods are used.

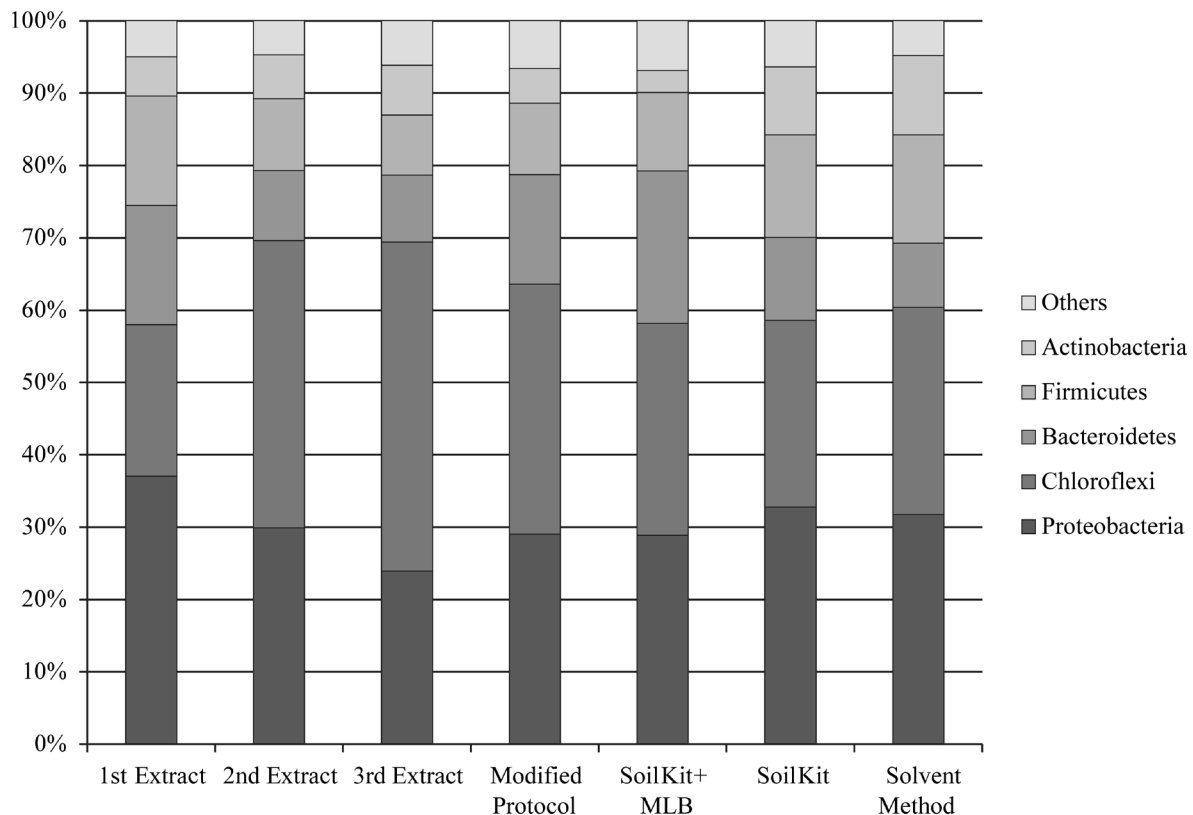


Figure 2. Phylogenetic compositions for 7 samples. All phyla with less than 5% abundance are lumped into the percent range, "Others".

Detection Sensitivity

E. coli among the 4 extraction protocols was recovered at as high as $3.0 \pm 0.2 \log \text{ cells } \mu\text{g}^{-1} \text{ DNA}$. Assuming that all microorganisms have a similar genome size as *E. coli* does at 4.6 Mb [19], this interprets to roughly 1 *E. coli* in 200,000 cells for $6.48 \log \text{ cells } \text{g}^{-1} \text{ DS}$. This also means that to detect presence of *E. coli* in pyrosequencing analysis for this sample, a minimum of 200,000 OTUs needs to be sequenced. This is also about the same level as the Class B biosolids limit for fecal coliforms in the United States. Not surprisingly, no *E. coli* was observed since only up to 12,000 OTUs were sequenced per sample. A minimum of 1 target cell per 12,000 cells or $7.7 \log \text{ cells } \text{g}^{-1} \text{ DS}$ is required in order to see presence of the bacteria under the resolution of pyrosequencing used for this work. Required OTUs will increase to 200M for the Class A limit at 1,000 fecal coliforms $\text{g}^{-1} \text{ DS}$. Therefore, selection of the extent of sequencing needs to consider the level of relative abundance of target organisms in the sample.

Low DNA recovery may also over-credit detection sensitivity in microbial quantification in addition to potential bias in quantitative enumeration of microorganism and qualitative microbial diversity comparison. Table 1 listed calculated detection limits for the 4 extraction methods if 10 or 100 ng of DNA templates were used for *E. coli* enumeration through real-time PCR. Results show that almost 1-log more sensitive detection would have been reported when the low recovery DNA extraction method is used compared to those with high DNA recovery. This difference is further increased to 2-log between high recovery with a 10 ng template and low recovery with a 100 ng template. Though detection sensitivity increased as more DNA template is used, inefficient or false DNA amplification may occur which can also bias the results. Therefore, it is recommended that works on biosolids reporting detection sensitivity lower than these values should assure the proper DNA extraction method is used.

DISCUSSIONS

Molecular DNA analysis both for quantification of specific microorganisms or qualitative analysis of microbial community structures have been widely used in environmental samples. However, results here show that if an inappropriate DNA extraction method is chosen a potential biased interpretation may result. Most commercial DNA extraction kits provide fast, easy, and high purity products allowing scientists to process large amounts of samples within a short period of time. None of these kits was specifically designed for biosolids extraction and direct adaptation of extraction protocols for biosolids may not provide sufficient recovery. Results presented here show that commercial kits are able to provide suitable DNA release and high quality purification. However, modification by incorporating sequential mechanical shearing and replacement with a stronger lysis buffer can greatly improve DNA extraction efficiency. It is possible with these modifications to still benefit from the convenience of a commercial kit without losing accuracy in subsequent DNA analyses. One should also note that there is not one extraction method currently known to extract 100% DNA from biosolids or any other environmental samples because it is difficult to experimentally prove true recovery. Though researchers have attempted to spike bacteria from an absent species and extract it alongside to demonstrate recovery [9,11], these spiked materials are mostly suspended single cells which are likely to be extracted more readily than biosolids bacteria that are buried deep within the flocs. Subsequent microbial diversity analysis will, therefore, be impacted by level of DNA recovery for selected extraction protocol. Consequently, our current effort is to optimize extraction protocols to obtain the highest possible recovery and to seek to better approach and reflect true composition of microorganisms in subsequent analyses. Class B biosolids of this type typically have highest DNA recovery at around 2,000–3,000 $\mu\text{g/g DS}$. Therefore, it

Table 3. Paired Sørensen Similarity Index for 7 Samples.

	1st Extract	2nd Extract	3rd Extract	Modified Protocol	Soil Kit	Soil Kit + MLB	Solvent Method
1st Extract	–	0.71	0.69	0.68	0.68	0.75	0.76
2nd Extract		–	0.73	0.76	0.72	0.74	0.72
3rd Extract			–	0.73	0.70	0.71	0.70
Modified Protocol				–	0.75	0.70	0.67
Soil Kit + MLB					–	0.70	0.69
Soil Kit						–	0.73
Solvent Method							–

would be a good practice to reexamine the protocol if recovery is severely lower than this range. The newly developed protocol was able to show 86% of those recovered from the solvent method with comparable *E. coli* enumeration results. However, these two protocols still show only 68% species similarity despite using identical sample. Cautions should therefore be taken and limit application to comparisons of samples that use the exact same sample processing protocol.

The convenience and power of pyrosequencing has drawn popular attention and use of such a technique in microbial diversity analysis and replaces methods such as denaturing gel gradient electrophoresis (DGGE) that is more labor intensive. It not only provides an overall microbial diversity profile but it also provides direct species identification and relative abundance for each species. However, it still entails some limitations. The detection sensitivity of the resolution for example is only 7.7 log cells g⁻¹ DS compared to the real-time PCR method that is at as low as 4.7 log cells g⁻¹ DS. The OTUs sequenced are still subjected to PCR amplification in addition and therefore any biases created during amplification will carry over to subsequent diversity analysis.

US EPA regulation stipulates that biosolids containing less than 2 million fecal coliforms g⁻¹ DS are considered Class B and can be applied to land providing proper site restriction [20]. Since *E. coli* is one of the dominant fecal coliforms in biosolids the data would indicate that this specific biosolids sample did not meet the Class B indicator limit when 3 sequential extractions were used whether purified by solvent or the commercial soil kit. Contradictory results by the two single extraction methods would report it as meeting Class B biosolids with bias created by the low DNA recovery on the other hand. This highlights the importance of choosing a proper DNA extraction method with good recovery to avoid false interpretation of the outcome. This report also shows that DNA based enumeration for biosolids is suitable for Class B classification only. Determination of Class A biosolids requires sensitivity as low as 1,000 cells g⁻¹ [20] which is not possible unless DNA recovery is extremely low or a DNA template is overloaded in PCR reactions. It would either way create bias in enumeration and therefore should be avoided.

CONCLUSIONS

This work overall presents a modified DNA extraction protocol for biosolids that may be completed within 3 h and results in more than 7 times the recovery of

a commercial soil kit. The method of enumeration by quantitative PCR was able to show comparable levels of *E. coli* to the high-recovery solvent method. However, dissimilar microbial species level diversity still exists among protocols. It was demonstrated here that high recovery extraction protocols can prevent biases in microbial quantification but an identical extraction method is needed for microbial community comparisons.

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Influence of Raw Sludge Quality on the Efficiency of Microaerobic Sulfide Removal during Anaerobic Digestion of Sewage Sludge

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ABSTRACT: Biogas produced by anaerobic digestion of organic substances is often polluted by hydrogen sulfide (H_2S) when organic matter containing sulfur compounds is digested. Energy use of biogas is complicated by H_2S content and it must be removed. Microaeration has recently been proven as a relatively simple and highly efficient biological method for sulfide removal regarding anaerobic digestion of biosolids. Our experience with a long term operation of the microaerobic digester confirms feasibility of highly efficient H_2S removal (about 99 %). However, a reliable control for air dosing according to raw sludge quality is a necessary condition for high and stable efficiency for microaerobic H_2S removal. It was observed that H_2S concentration in biogas varied significantly even at stable concentrations of sulfur compounds in raw sludge. Results prove that concentration of H_2S is significantly influenced not only by occurrence of sulfur compounds but also by presence of metals capable of binding to insoluble sulfide. Fe^{3+} and Al^{3+} , most commonly used phosphorus removal agents during wastewater treatment, have a completely different impact on distribution of sulfide compounds.

INTRODUCTION

WASTEWATER TREATMENT and sludge treatment are closely related technologies strongly influencing each other. In past decades research in this field was focused mainly on the problem of nitrogen return stream from sludge treatment represented by sludge liquor from anaerobic digesters [1].

Influence of wastewater treatment technological modifications on sludge quality and on the digestion process is also an equally important example of the interrelation between wastewater treatment and sludge treatment. Applied wastewater treatment technology influences the ratio between primary sludge and waste activated sludge, volatile suspended solids (VSS) content in sludge, biodegradability of sludge, rheology, and many other properties of sludge [2–3].

It is very important if there are applied chemical or physical-chemical treatment methods because technologies such as neutralization, coagulation, adsorption, membrane filtration, and phosphorus precipitation will change sludge quality considerably.

This study focused on evaluation of the effect of coagulation and phosphorus precipitation on sludge and biogas quality. Hydrogen sulfide (H_2S) presence, a problematic minor component of biogas, may especially be influenced by coagulation and in particular by choice of coagulating agents. Salts of Fe^{3+} and Al^{3+} are the most commonly used phosphorus removal agents during wastewater treatment and their behavior during anaerobic digestion will influence H_2S presence and desulfurization methods efficiency. Additionally, interactions between anaerobic biomass and heavy metals such as iron and others are largely unknown [4]. Additions of Fe^{3+} and Al^{3+} into activated sludge systems for phosphorus removal is likely to impact both efficiency of anaerobic digestion and generation of odor-causing compounds following digestion and dewatering [5].

Microaeration has recently proved to be a relatively simple and highly efficient biological method of sulfide removal regarding anaerobic digestion of biosolids [6–10]. This process is based on controlled dosing of a limited amount of air or oxygen into the digester to ensure oxidation of sulfide into elemental sulfur. Precipitated elemental sulfur is then removed from the digester together with digested sludge. Presence of oxygen does not influence the anaerobic process negatively

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if it is consumed quickly and completely. Apart from H₂S removal, microaerobic conditions also offer other advantages such as sulfide toxicity suppression and improved degradability of some recalcitrant compounds [11]. Main benefits from microaerobic H₂S removal are ability to remove H₂S inside the anaerobic reactor without requirements to build a new separate desulfurization unit and absence of additional chemicals. The aim was to evaluate influence of raw sludge quality and Fe³⁺ and Al³⁺ content in the sludge on efficiency of microaerobic sulfide removal during anaerobic digestion of sewage sludge.

Quality of Biogas Produced During Anaerobic Digestion of Sewage Sludge

Composition of biogas produced in anaerobic stabilization of sewage sludge with regard to major components methane and carbon dioxide is very stable according to past experience from plants located in Czech Republic. Typical concentration values are 63 ± 3 vol% of methane and 35 ± 3 vol% of carbon dioxide (dry gas at standard conditions). For further use of biogas H₂S is the most problematic minor component. Its concentration is usually low. However, if there is a source of sulfur compounds such as industrial wastewater or other similar source H₂S concentrations may grow up into units of grams per cubic meter of biogas [12].

Generally speaking, a certain amount of hydrogen may be found in each biogas. Concentrations above 10,000 mg/m³ are exceptional, but can occur. However, in such cases it is not just a problem of high H₂S concentration in biogas but also inhibition of methanogenic bacteria is a more serious complication. Concentration ranges of H₂S may be very broad and biogas may be classified by the following criterion:

1. biogas with negligible H₂S concentration: up to 50 mg/m³
2. biogas with low H₂S concentration: 50–250 mg/m³
3. biogas with middle H₂S concentration: 250–1500 mg/m³
4. biogas with high H₂S concentration: above 1500 mg/m³

Need to reduce concentration of hydrogen sulfide in biogas is caused mainly by corrosion problems in boilers or cogeneration units and by concentration limits of sulfur dioxide in a flue gas. Desulfurization is usually not necessary in biogas from the 1st and 2nd groups according to the above classification. In most cases de-

sulfurization is recommended for 3rd group and it is absolutely necessary for 4th group.

Amount of hydrogen sulfide released into biogas is in equilibrium with dissolved hydrogen sulfide in the liquid phase. This process depends on overall balance of sulfides in the digester. Depending on amount of dissociated sulfide, temperature, and pH, H₂S may be created and part of which passes into the gaseous phase. Concentration of free dissolved hydrogen sulfide which determines transport rate to the gaseous phase is provided by Equation (1):

$$[\text{H}_2\text{S}] = [\text{S}^{2-}] \cdot R_{\text{H}_2\text{S}} \cdot F_{\text{H}_2\text{S}} \quad (1)$$

where [S²⁻] is concentration of sulfides in sludge liquor, R_{H₂S} is the portion of dissolved sulfides (usually ranging from 0.4 to 0.6), F_{H₂S} constant depends on pH value for the dissociation constant and for temperature according to Equation (2):

$$1/F_{\text{H}_2\text{S}} = 1 + K'/10^{pH} + 0.00811(T_s - 20) \quad (2)$$

where K' is the apparent dissociation constant which in wastewater at 25°C reaches approximately 2 × 10⁻⁷ and at 20°C decreases to about 1.7 × 10⁻⁷. T_s in surface temperature in degrees Celsius. In digesters where the relative humidity is near 100% surface temperature approaches the temperature of the total digester volume [13].

Risks Associated with High Concentrations of Sulfides and Hydrogen Sulfide in Anaerobic Digesters

High concentrations of sulfide inhibit activity of methanogenic and acetogenic bacteria. Maximum concentration of sulfides tolerated by methanogens is influenced significantly by concentration of organic substrate. It was found that production of methane is hardly possible if the ratio of COD/S is less than 15 [7].

Sulfide toxicity depends on many factors such as temperature and pH. These factors effect distribution of dissolved sulfide ions between dissociated and undissociated form and distribution of undissociated form between liquid and gaseous phases. Highly toxic properties are mainly from undissociated hydrogen sulfide. It is a colorless strong-smelling gas at low concentrations (0.001%) whereas at higher concentrations it is an odorless but deadly poison (0.1%). High concentration of H₂S in biogas may cause operational problems:

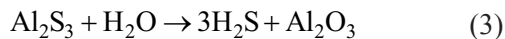
- inhibition of anaerobic processes (especially methanogenesis and acetogenesis),
- corrosion of concrete and steel (e.g., tanks, pipes, boilers),
- odor emissions, and
- sulfur dioxide emissions after combustion of biogas.

An advantage of sulfide presence on the other hand stems from their ability to immobilize heavy metals into insoluble sulfides. Sulfides are in this way removed from solution and toxicity is reduced.

Interaction of Iron and Aluminum Salts in Anaerobic Digestion

Salts of trivalent iron or aluminum are most commonly used as coagulation and precipitation agents for municipal wastewater treatment. After application complexes are formed containing hydrated oxides, phosphates, or other salts of these metals. These complex precipitates are then separated with sludge and digested in the reducing environment of the anaerobic digester. Behavior of the aluminum cation is completely different while the ferric cation reacts to ferrous sulfide which is very poorly soluble and does precipitate.

The aluminum cation may also create aluminum sulfide, theoretically, but it is very unstable in the same conditions and immediately hydrolyzes to form hydrogen sulfide [14]. This is described by the following Equation (3):



MATERIAL AND METHODS

Laboratory experiments were carried out in two identical digesters with a working volume of 10 L (Figure 1). Entering through the bottom, one of them was continuously dosed with air using a peristaltic pump with a flow rate of 1.7 L/d.

A mixture of digested mesophilic sludge from two municipal wastewater treatment plants was used as an anaerobic inoculum. The reactor was operated under mesophilic conditions at 40°C. Thickened excess activated

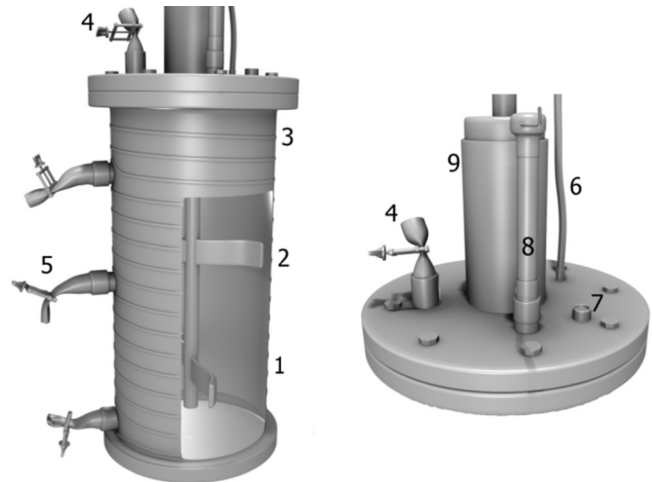


Figure 1. Scheme for reactor: 1. body of reactor, 2. paddle wheel stirrer, 3. resistance heater, 4. input pipe for substrate, 5. vessel for ORP analysis, 6. sampling point, 7. air supply, 8. thermometer, and 9. water.

sludge from a municipal wastewater treatment plant was used as the substrate. Fresh sludge was delivered in two-week intervals and slightly varying in quality.

Sludge was fed to maintain a constant volumetric loading of the reactor at $2.0 \text{ g.l}^{-1}\cdot\text{d}^{-1}$ (expressed in COD) which corresponded to a solid retention time of about 35 days. Average properties of excess activated sludge are presented in Table 1.

Sodium sulfate was added in both digesters to achieve an increase of hydrogen sulfide concentration in the biogas. Dose of sodium sulfate was adjusted to 2–3 g per day according to actual sludge quality. Analytical procedures were carried out according to Standard Methods [15]. Biogas composition and volatile fatty acids were determined by gas chromatograph GC 8000Top equipped with a heat conductivity detector HWD 800 [16].

RESULTS AND DISCUSSION

Quantity and quality of the biogas produced was monitored in both anaerobic and microaerobic digesters. See Figures 2 and 3 to observe the relationship between H_2S concentration and differing amounts of sulfates for the anaerobic and microaerobic digesters. Notice significantly lower amounts of H_2S in the microaerobic digester.

Table 1. Average Concentration and Standard Deviation of Basic Parameters of Treated Sludge (COD—Chemical Oxygen Demand, TS—Total Solids, VS—Volatile Solids).

Substrate	pH	COD [g/l]	COD _{soluble} [g/l]	TS [g/l]	VS [g/l]	VS/TS [%]
thickened waste activated sludge	7.58 ± 0.33	73.1 ± 7.5	2.39 ± 1.65	67.9 ± 6.5	48.4 ± 4.0	71.3 ± 1.8

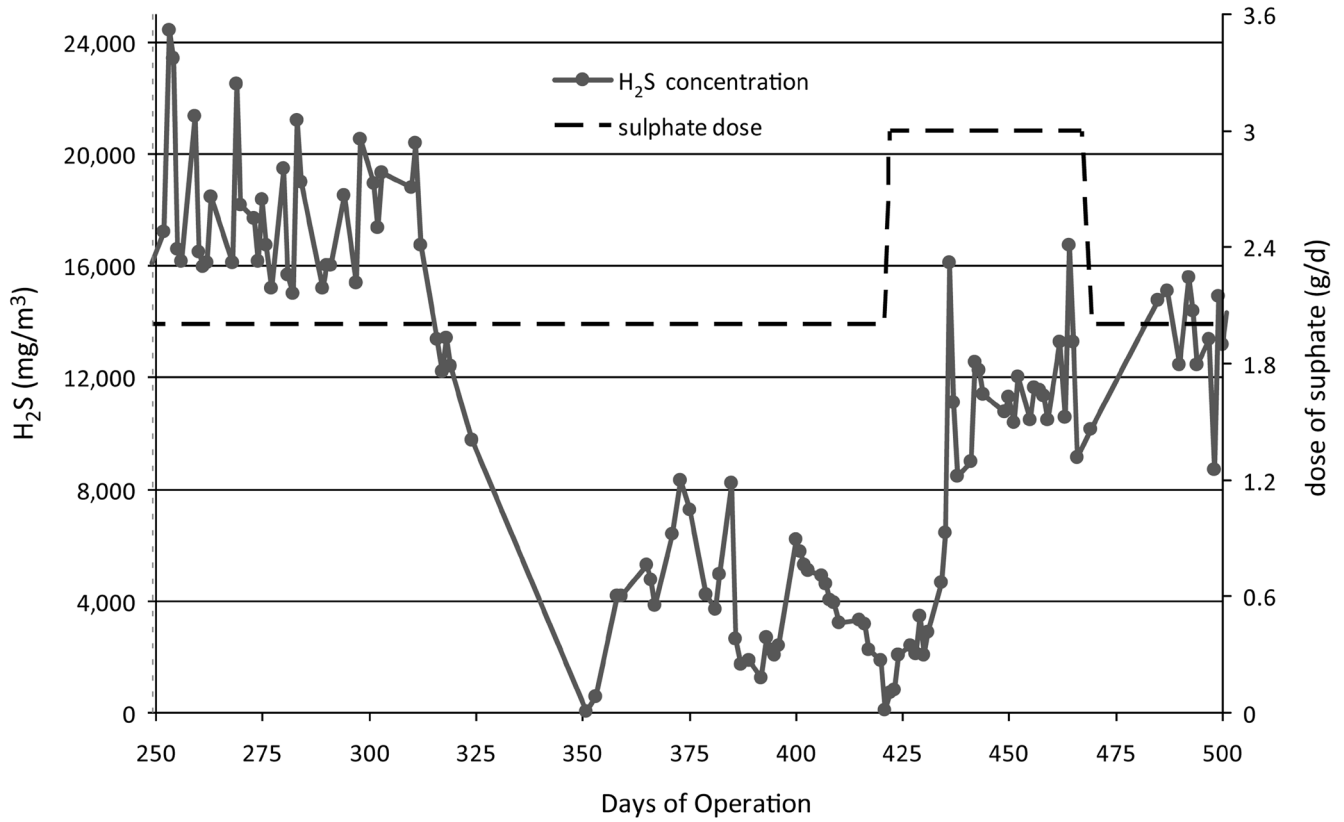


Figure 2. Relation between H₂S concentration and dosed amount of sulfates—anaerobic digester.

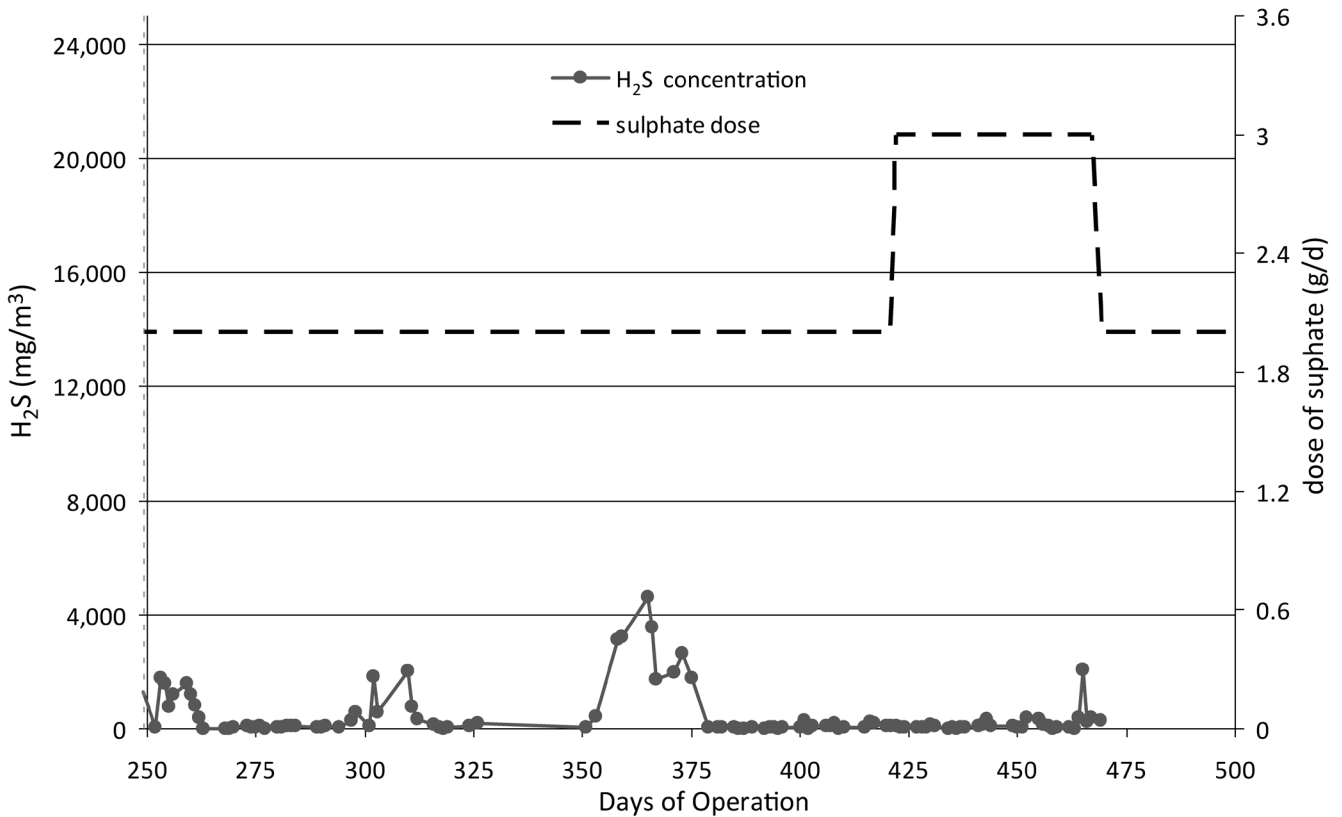


Figure 3. Relation between H₂S concentration and dosed amount of sulfates—microaerobic digester.

It was discovered that especially for anaerobic digester the concentration of hydrogen sulfide was higher as illustrated in Figure 2. It was surprising because excess activated sludge was digested and its quality was quite stable in terms of basic parameters such as total solids, volatile solids, COD, pH, and other as displayed in Table 1. Due to aforementioned variation in biogas quality, elemental composition of treated waste activated sludge with emphasis on sulfur, iron, and aluminum was evaluated.

Figures 4 and 5 display evaluation results. Figure 4 confirms that during the monitored period there were no major changes in sulfur content for treated activated sludge. Percentage of sulfur in the mineral fraction of sludge was on average 4.6% with a minimum of 3.7% and a maximum of 5.3%. It is obvious variations in percentage of sulfur can not be a reason for dramatic changes in concentration of hydrogen sulfide as displayed in Figure 2.

Monitoring of iron and aluminum percentages in treated sludge displayed in Figure 5 has brought entirely different information. Percentage of iron in the mineral fraction of sludge was extremely high because of intensive application of iron salts used in wastewater treatment.

After the 315th day of operation there appeared a significant increase in the already unusually high percentage of iron of sludge from about 30 to 45–50%. A parallel decline in percentage of aluminum content in sludge may be observed during the same period. When looking at evolution of hydrogen sulfide concentration in biogas it is possible to see that at the same time there appeared to be a steep decline in concentration. It is therefore probable that a higher concentration of iron in the sludge or replacement of aluminum with iron was the main cause of the decline. It was confirmed by results obtained after the 458th day of operation when

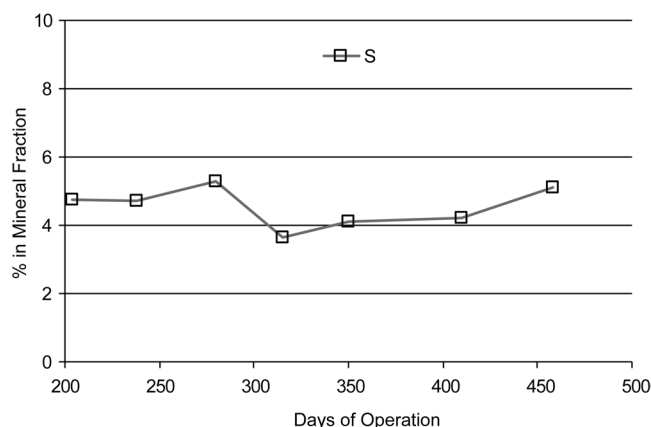


Figure 4. Course of sulfur content in treated waste activated sludge.

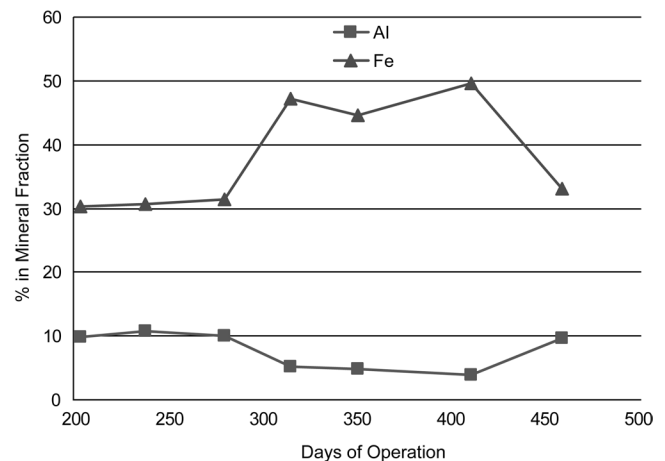


Figure 5. Course of iron and aluminum content in treated waste activated sludge.

percentage of iron returned to 33% and concentration of hydrogen sulfide in biogas started again to be extremely high.

CONCLUSION

Concentration of hydrogen sulfide in biogas produced during anaerobic stabilization of sewage sludge is significantly influenced not only by occurrence of sulfur-containing compounds but also by presence of metals capable of sulfur immobilization into insoluble sulfides.

Most commonly used coagulation and precipitation agents, Fe^{3+} and Al^{3+} , have a diametrically different impact on distribution of sulfide compounds. While the ferric cation reacts to ferrous sulfide which is poorly soluble and does precipitate, the aluminum cation creates unstable aluminum sulfide which immediately hydrolyzes and forms hydrogen sulfide.

Results suggest that at the same sulfur compounds concentration in the digester hydrogen sulfide concentration in biogas may vary by two orders of magnitude due to changes of Fe^{3+} and Al^{3+} concentrations.

Variation in concentration of Fe^{3+} or Fe^{3+} substitution for Al^{3+} may have a significant influence on quality of biogas and especially on concentration of hydrogen sulfide and desulfurization efficiency. Desulfurization methods must be agile and able to flexibly respond to such changes.

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A Solar Sludge Drying Model: Study of Drying Phenomena—Validation of an Industrial Solar Dryer

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ABSTRACT: This paper introduces a model for solar sludge drying in order to optimize process design and operation. Several ways to model the drying kinetics are discussed. Evaporation, organic substrate biodegradation, radiative, convective, conductive, and biological thermal phenomena were modeled. The model deals with process dynamics and drying efficiency by taking into account impact of optimization means such as windrow turning frequency, air renewal, and destratification fans management. Validation was achieved on one drying cycle of an industrial unit located in north-west of France.

INTRODUCTION

WASTEWATER TREATMENT results in large quantities of sludge with high water content. Disposal of this sludge is very costly and also induces technical problems. Agricultural spreading, incineration, or landfill disposal are different methods used to manage this waste production. Before using any of these solutions, sludge must be transformed. Also, a major task remains for volume reduction by water removal. Solar drying greenhouses in this drying step become an interesting economical alternative to thermal dryers.

Veolia Water markets a solar sludge drying unit named Solia™ and adapted to treat sludge from small to medium-sized wastewater plants. The drying procedure in the greenhouse consists of sludge spreading and turning into windrow. Drying phenomenon is managed with air renewal. The process to be efficient requires knowledge of drying rate as a function of the greenhouses environment and operation. A modeling approach is a useful tool for understanding drying mechanisms and for optimizing design and build of a Solia™ unit. Despite increasing interest for solar sludge drying, models able to predict drying rate are still lacking realism [1, 2] and some coupled phenomena are not modeled.

This paper aims to develop a predictive model for

predicting solar drying efficiency in a greenhouse according to external weather conditions and operation. The drying process will be presented in the first part of the paper. Different ways are discussed for modeling drying kinetics. The model is validated on an industrial unit thanks to sensitivity analysis and kinetics identification.

PROBLEM STATEMENT

In a Solia™ unit, dewatered sludge with a dry solid content around 0.2 to 0.3 kg of solid per kg of sludge is uniformly spread over a concrete floor under a greenhouse. Sludge is shaped into windrows in order to offer a most optimized transfer area with forced air flux. This forced air renewal allows water evaporation from sludge and the transport of gas water out of the unit. Two means operate to avoid heterogeneous thermal and water concentration fields of air and sludge. Firstly, some electric fans placed inside the unit ensure homogeneous gas water concentration in surrounding air and sufficient convective transfer between air and sludge to be dried. Secondly, a robot named Solia-Mix™ regularly adds some dewatered sludge within windrows and mixes them to homogenize dryness and temperature fields (Figure 1).

Solia™ units major operating costs are due to energy consumption (i.e., air renewal and robot). Optimized design and operation could improve both process efficiency and operating costs.

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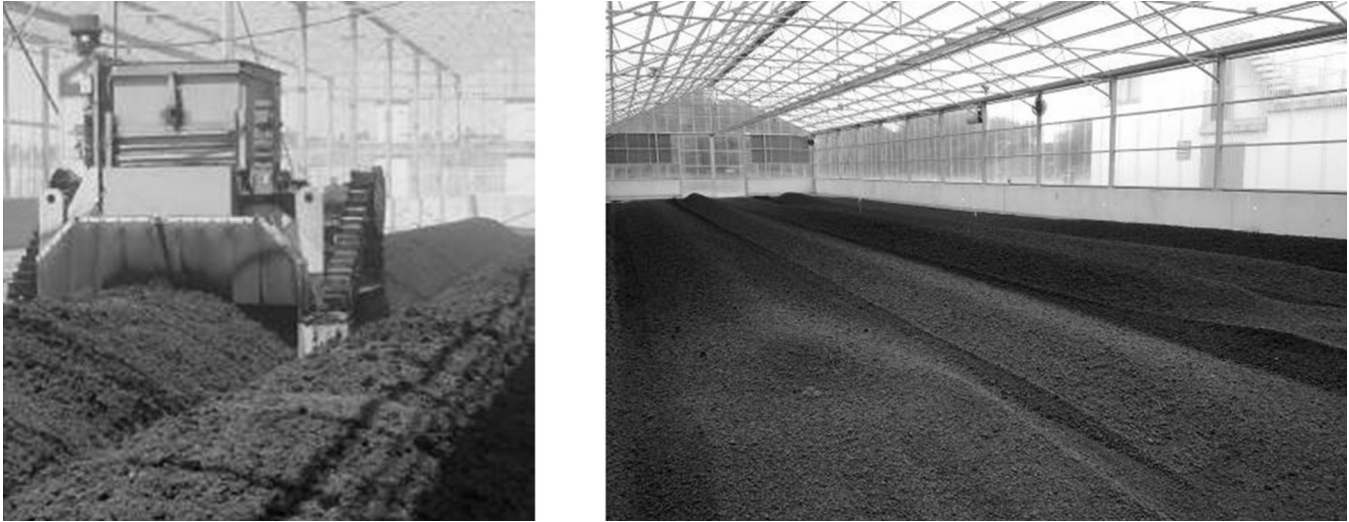


Figure 1. SoliaMix™ robot and Solia™ unit.

The model to be efficient has to take into account all coupled transfers occurring in the greenhouse. By specifying only external conditions, localization, and material properties the model should be able to predict thermal fields within sludge and air and at boundary walls according to radiative and convective phenomena as well as evaporated water and organic substrate removed from sludge (Figure 2).

Benefits of a Solia™ dryer are reduction of greenhouse surface and concentration of biodegradable organic substrate of sludge by drying waste in a windrow shape. In fact, the windrow height until 0.8 m (See Figure 1) allows for creation of thermal energy in the windrow core with a specific range of sludge dryness as in composting phenomenon. This contributes to reducing the biodegradable organic substrate. Therefore, this phenomenon also has to be modeled to predict a realistic drying behaviour.

MATERIALS AND METHODS

Industrial Unit of Solar Drying

An experimental unit located in the north-west of France has been chosen to define a reference case. Sensors have been implemented in order to feed and validate simulations: hygrometers, temperature probes, anemometers, pyranometers, and more were used. This unit is 52 m long, 4.5 m high, and 13 m wide. A drying cycle has been performed in 2011 for 6 months and online data were acquired with periodic samplings of sludge for dryness and windrow height measurements.

Modeling of Drying Kinetics

Previous works deal with modeling of drying kinetics but few of them are applied and validated on a real solar greenhouse dryer. Seginer and Bux [1,2] have focused their studies on prediction of evaporation rate by using different numerical models like neural network ones for example but without taking into account a physical approach for drying phenomenon. The idea of other specialists is to use drying experiments to adjust model parameters and define a generic concept that may be applied in the greatest number of operating conditions. Van Meel [3] and Krischer and Kast [4] proposed to represent experimental results for a specific product with a unique curve called the characteristic curve of drying (Figure 3). This curve allows for summing up experimental results acquired under different conditions of air velocity, temperature, and moisture.

Leonard [5,6] modeled drying kinetics of urban sludge by highlighting three phases of water evapora-

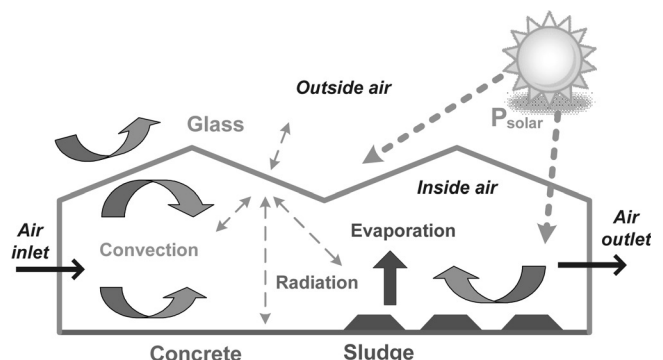


Figure 2. Transfer phenomena in a Solia™ unit.

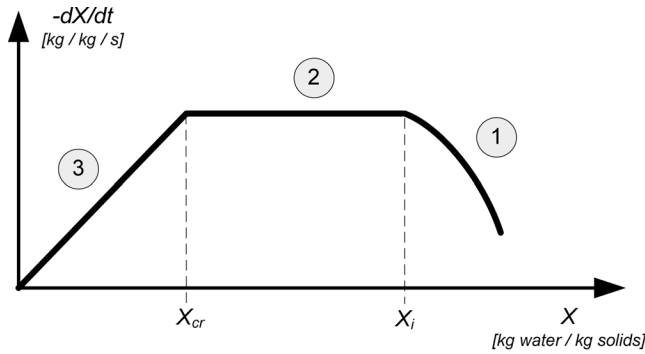


Figure 3. Theoretical curve of drying, Krischer et al. (1978).

tion from sludge. These three phases are displayed in Figure 3 and each one is delimited by water content interval. Noting X , water content of the sludge [kg water/kg solids], the water content evolution model can be expressed as follows.

Warm-up Phase

During this phase, heat and mass transfers on the sludge surface occur until an equilibrium value is reached. Sludge temperature increases until wet bulk temperature corresponding to a dry environment (zone 1) is reached. See Figure 3. Maximum water content is named initial water content, noted X_i . This period is often very short in comparison to total drying time.

First or Maximum Drying Rate

The second drying phase is often isenthalpic. Water is evaporated at a constant flux and thermal exchanges only serve to evaporate. Evaporation is assumed to occur only on the sludge surface which is supposed to be covered by a thin layer of free water. This first phase occurs for a range of water content between X_i and X_{cr} (critical water content). Thus, drying rate depends only on external conditions (i.e., air velocity, moisture, and temperature).

The first drying rate is noted $(-dX/dt)_1$; its determination can be experimental or theoretical by solving the thermal balance equation on sludge at wet bulb temperature. However, its experimental determination is not obvious for a biological product because sludge cellular walls would disturb fast migration of humidity to the sludge surface [7]. Critical water content X_{cr} may be assumed equal to initial water content, X_i . Hamadou [8] confirmed these observations with some lab-experimental tests on urban sludge.

Reduced Drying Rate

At this stage, water molecules within samples are not free but are linked with other molecules via intracellular connections. This water is termed “bound water” and water migration is then slowed down through the product. Water content is less than X_{cr} . Reduced water content X_r is then introduced and defined by Equation (1). The drying characteristic curve is now very useful because kinetics may be simply deduced from initial and equilibrium water content for every air moisture content condition. Kinetics are obtained by a standardization (Figure 4) displaying the ratio of drying rate $(-dX/dt)$ at a t instant and the first drying rate $(-dX/dt)_1$ in the same experimental conditions according to reduced water content X_r :

$$X_r = \frac{X - X_{eq}}{X_i - X_{eq}} \quad (1)$$

In Equation (1), X_{eq} is the equilibrium water content deduced from sorption isotherms which are required to characterize sludge. Some lab-tests are needed to get sorption isotherms for urban and industrial sludge (Figure 5) according to ambient air temperature. These experimental data may also be modeled. Vaxelaire [11] concluded that Oswin’s relationship [12] gives the best fit with air temperature and moisture. Reduced water content for an urban sludge may be expressed as described in [7,8] and by Equation (2):

$$X_r = \frac{X - X_{eq}}{X_{cr} - X_{eq}} \quad \text{with} \quad X_i = X_{cr} \quad (2)$$

In this way, the reduced drying rate (Figure 4) is deduced from experimental tests and must be estimated at each model resolution step.

Several approaches are available to model kinetics. Slim [9] and Hamadou [8] introduce two different approaches integrating kinetics for solar drying. The first one is a reduction of the mass transfer coefficient which is defined during the first drying rate by a Chilton-Colburn analogy. Their reduction coefficient is an identified function from experimental lab-scale data that can be difficult to extrapolate for other kinds of sludge and conditions. The second approach deduces reduced drying rate by a polynomial function $F(X_r)$ fitted on standardization of the drying characteristic curve. This last approach is also used by others authors [7, 8, 10].

Finally, drying rate can be expressed as a reduced

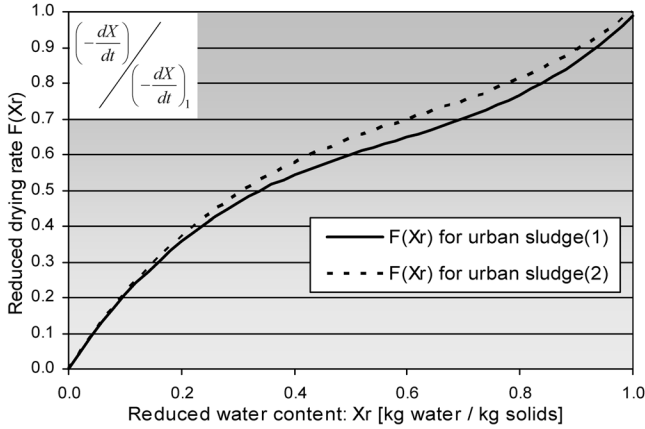


Figure 4. Standardization of drying characteristic curve (polynomial functions for reduced drying rate).

part of the first drying rate due to a polynomial function $F(X_r)$:

$$\left(-\frac{dX}{dt}\right) = \left(-\frac{dX}{dt}\right)_1 F(X_r) \quad (3)$$

Drying Modeling Concept for Solia™

The model introduces a stratified windrow of two layers since Solia™ is working until 0.8 m windrow thickness (Figure 6). The Hamadou's approach is implemented and drying mechanisms occur in the thin upper layer called windrow cover. Whereas, the windrow core is only subject to thermal exchange by conduction.

Exchange depth of windrow cover has an important role on model behaviour. However, its evaluation is difficult to determine experimentally but it is probably linked to sludge dryness (Figure 7). The exchange depth is assumed to be minimal for high water content (until 30% of dryness—stage 1) because of weak inter-

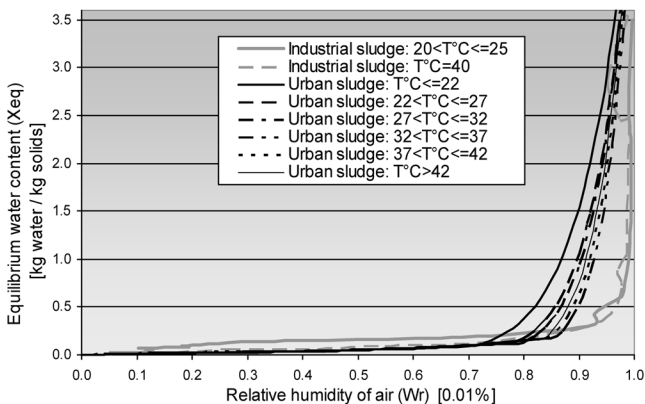


Figure 5. Water vapour sorption isotherms for urban and industrial sludge.

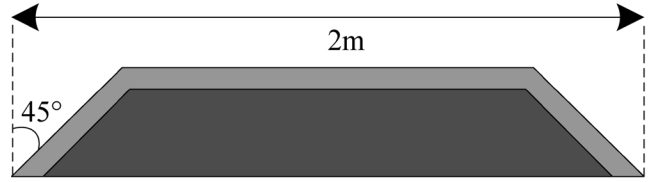


Figure 6. Windrow section.

stitial space between sludge grains. The second stage in Figure 7 displays depth increasing with sludge drying. This variation is assumed to be linear until a maximum exchange depth corresponding to a saturation of air flux penetration in the media is reached.

This work is also dedicated to overcoming difficulty managing layer transfers during numerical solver iterations. Exchange depth of sludge takes place in mass and thermal balances and has a significant effect on model prediction capability. Volume varies and a transfer volume is generated as the thin layer dries or gets wet between two iterations. A mass mixing law is then applied to layers after numerical resolution of ordinary differential equations to uniform layers dryness and temperature.

Sludge density is determined by several experiments according to sludge dryness. Three phases highlight its evolution which is modeled by three continuous functions. Increasing of mixture density after 40% of dryness can be experimentally explained by size of sludge grains which become finer with dryness evolution and mixing.

To be representative, our model has to take into account the specific thermal production term that occurs in a specific range of sludge dryness and due to aerobic biological activity it occurs during organic waste composting. This phenomenon allows governing biodegradable organic substrate and drying evolutions

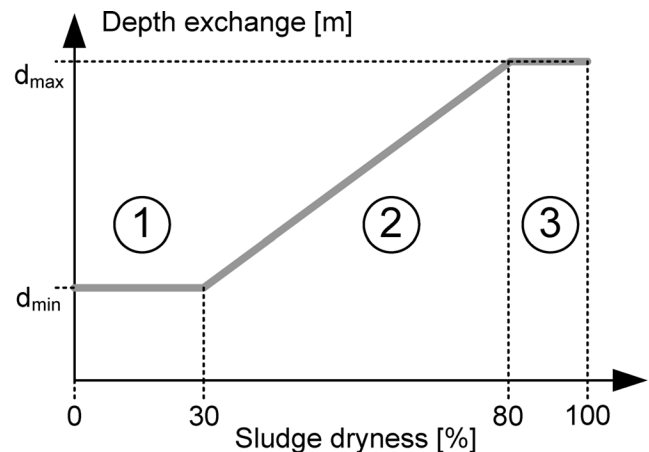


Figure 7. Exchange depth of windrow cover according to dryness.

which are linked to heat emission issued from sludge biological activity. Capou *et al.* [14] developed a biodegradation model and the approach here consists of some simplifications of the model. Oxygen contribution is not implemented because windrow turning and convection into sludge are assumed to induce sufficient oxygen concentration for sludge layers. This assumption could be improved in future work because oxygen concentration is certainly reduced in the windrow core between two turnings. A simplified biodegradation model is based on a first order hydrolysis of organic substrate. Microbial growth and death kinetics were neglected.

The model is provided in Equation (4),

$$\frac{dX_{os,i}}{dt} = -\frac{\gamma_{T,i} p_{W,i}}{\zeta_i} X_{os,i} \quad (4)$$

where X_{os} is the organic substrate content [kg OS/m³ sludge] and i indicates windrow layer (cover or core). γ_T and p_W are saturation terms of organic substrate biodegradation, respectively, due to temperature and humidity effects :

$$\gamma_{T,i} = \frac{(T_i - T_{\max,i})(T_i - T_{\max,i})^2}{(T_{opt,i} - T_{\min,i})[(T_{opt,i} - T_{\min,i})(T_i - T_{opt,i}) - (T_{opt,i} - T_{\max,i})(T_{opt,i} + T_{\min,i} - 2T_i)]} \quad (5)$$

with $T_{\min,i} < T_i < T_{\max,i}$.

$$p_{W,i} = \begin{cases} 0 & \text{if } \frac{X_{H_2O,i}}{1 - X_{H_2O,i}} \leq X_{\min,i} \\ \frac{\frac{X_{H_2O,i}}{1 - X_{H_2O,i}} - X_{\min,i}}{X_{\max,i} - X_{\min,i}} & \text{if } X_{\min,i} < \frac{X_{H_2O,i}}{1 - X_{H_2O,i}} < X_{\max,i} \\ 1 & \text{if } \frac{X_{H_2O,i}}{1 - X_{H_2O,i}} \geq X_{\max,i} \end{cases} \quad (6)$$

where X_{H_2O} is water content of sludge [kg water/kg sludge].

The heat production rate is expressed in each windrow layer as :

$$J_{T,i} = \frac{460000}{M_{O_2}} \left(\frac{\gamma_{T,i} p_{W,i}}{\zeta_i} \frac{1 - Z_a}{Z_a} X_{os,i} \right) \quad (7)$$

Experiments have demonstrated a heat production gradient at different depths. Table 1 introduces the observed temperature variations according to sludge dryness during a night. Temperatures are significantly increased during May and June thanks to a heat emission

linked to biological activity. This production appears between 30% and 65% of sludge dryness.

Drying phenomena are supposed to occur only in windrow cover. Both windrow layers are subject to biological activity and thermal exchanges. The following mathematical expression is implemented for windrow core heat balance :

$$\rho_{Bo}(s_{Core})C_{pBo}V_{Core}\frac{dT_{Core}}{dt} = \frac{\lambda_{Bo}A_{Core-Cover}}{(H_{Cover} + H_{Core})/2}(T_{Cover} - T_{Core}) + \frac{\lambda_{Bo}A_{Ground-Cover}}{H_{Core}/2}(T_{Ground} - T_{Core}) + V_{Core}J_{T,Core} \quad (8)$$

The windrow cover heat balance is expressed by Equation (9):

$$\rho_{Bo}(s_{Cover})C_{pBo}V_{Cover}\frac{dT_{Cover}}{dt} = [P_{Bo}a_{Bo}\tau_v + h_{Bo}(T_a - T_{Bo})]A_{Bo} + \sum_{\substack{j \\ \text{walls} \\ \neq Bo}} R_{j-Bo} + m_{S,Cover}\frac{dX_{Cover}}{dt} + \frac{\lambda_{Bo}A_{Core-Cover}}{(H_{Cover} - H_{Core})/2}(T_{Cover} - T_{Core}) + V_{Cover}J_{T,Cover} \quad (9)$$

Then, the first drying rate is theoretically determined by the previous thermal balance at wet bulb temperature:

$$\left(\frac{dX_{cover}}{dt}\right)_1 = -\frac{1}{m_{S,Cover}Lv} \left[[P_{Bo}a_{Bo}\tau_v + h_{Bo}(T_a - T_h)]A_{Bo} + \sum_{\substack{j \\ \text{walls} \\ \neq Bo}} R_{j-Bo} + \frac{\lambda_{Bo}A_{Core-Cover}}{(H_{Cover} - H_{Core})/2}(T_{Core} - T_{Cover}) + V_{Cover}J_{T,Cover} \right] \quad (10)$$

Another particularity of the Solia™ process is the addition of fresh sludge and the frequent mix of windrows. Thus, our stratified model has to take into account sludge mixing and its effects on drying efficiency. An algebraic resolution is developed for each layer variable (temperatures, water contents, and organic substrate contents) with respect to an ideal mass mixing law.

Thermal Fields and Air Mass Balance

First step for modeling is to validate all thermal fields. Jung *et al.* [13] have developed a model focused on thermal exchanges and concluded that sludge temperature is highly sensitive to model parameters and water evaporation. At the written stage model parameters are coming from literature or are experimentally determined.

Only external conditions are set up and the model solved thermal balances for each boundary wall (glass,

Table 1. Temperature Variation According to Sludge Dryness.

A night of	Sludge Dryness	Temperature Variation during a Night (°C)	
		Sludge Surface	Windrow Core
March	29%	-3	0
April	29%	-2	-2
May	34%	+1	+5
June	44%	-5	+6
July	72%	-10	-15

sludge, concrete) and for internal air as well as water evaporation from sludge and its transport in the unit. Absorbed solar power for each face should be set up thanks to a database for example.

Thus, the thermal balance for upper glass (i.e., greenhouse roof) taking into account solar power and convective and radiative exchanges is expressed by:

$$\rho_{V\text{sup}} V_{V\text{sup}} C_{pV\text{sup}} \frac{dT_{V\text{sup}}}{dt} = [P_{V\text{sup}} a_{V\text{sup}} + h_{V\text{sup}} (T_a - T_{V\text{sup}}) + h_{\text{ext}} (T_{\text{ext}} - T_{V\text{sup}})] A_{V\text{sup}} + \sum_{\substack{j \\ \text{walls} \\ \neq V_{\text{sup}}}} R_{j-V_{\text{sup}}} \quad (11)$$

where R_{i-j} are radiative exchanges terms of the boundary walls. Two walls at different temperatures exchange a radiative flux of [17]:

$$R_{1-2} = \frac{\sigma(T_1^4 - T_2^4)}{\frac{1-\epsilon_1}{A_1\epsilon_1} + \frac{1}{A_1F_{12}} + \frac{1-\epsilon_2}{A_2\epsilon_2}} \quad (12)$$

In the same way the thermal balance on internal air takes into account these exchanges but also water evaporation contribution:

$$\rho_a V_a C_{pa} \frac{dT_a}{dt} = \left[\begin{aligned} &h_{Be} A_{Be} (T_{Be} - T_a) + h_{Bo} A_{Bo} (T_{Bo} - T_a) + h_{V\text{sup}} A_{V\text{sup}} (T_{V\text{sup}} - T_a) \\ &+ \sum_{i=1}^4 h_{V\text{lati}} A_{V\text{lati}} (T_{V\text{lati}} - T_a) - \rho_a Q C_{pa} (T_a - T_{\text{ext}}) - m_{S,\text{Cover}} L_v \frac{dX_{\text{Cover}}}{dt} \end{aligned} \right] \quad (13)$$

Water vapour mass balance includes air renewal and water evaporation from the windrow cover. It is expressed by Equation (14):

$$\rho_a V_a \frac{dW_a}{dt} = \rho_a Q (W_{\text{ext}} - W_a) - m_{S,\text{Cover}} \frac{dX_{\text{Cover}}}{dt} \quad (14)$$

with defined by Equation (3).

Modeling Approach

The modeling approach is useful for reaching a long time scale representative of a drying cycle and to finely describe phenomena occurring within sludge (e.g., heat exchanges, evaporation, biological activity, and more). Dynamics of these phenomena are described and improve understanding of how best to optimize the process operation. A systemic approach is displayed in Figure 8.

Benefits of this approach consist of simulating experimental tests, comparing scenarios to online measurements, and predicting process behaviours according to external conditions.

The model is able to predict all thermal fields, internal air moisture, sludge dryness, and windrow height. Thus, it is possible to evaluate drying efficiency and also evaporation evolution in different climatic conditions. The temperature calculation for the roof al-

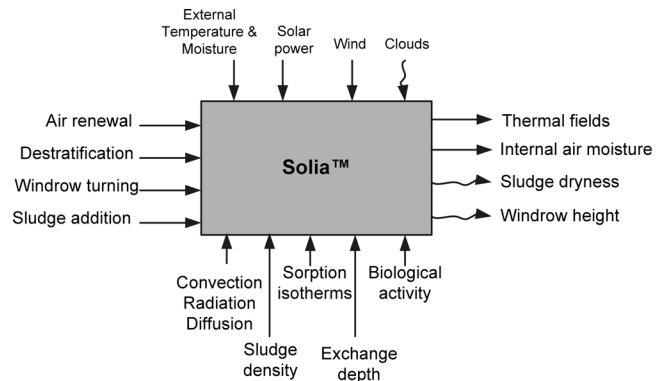


Figure 8. Functional bloc for systemic approach applied on a solar drying unit.

allows one to estimate greenhouse heater requirements to compensate for heat losses. Knowledge of internal air temperature and moisture allows one to estimate air renewal requirements in the greenhouse.

Internal air is to be homogeneously mixed. Temperature and water content of the product are supposed to be uniform. The windrow mixing is instantaneous in the whole greenhouse space although SoliaMix™ robot needs 20 minutes to achieve a cycle. Ordinary differential equations are solved by Euler's solver.

RESULTS AND DISCUSSION

Model Validation

A drying cycle of six months has been achieved from February with online measurements acquisition and periodic analysis of sludge dryness, windrow height, and organic substrate content. These data have primordial importance related to observing drying efficiency and for judging good agreement of model predictions. Model inputs presented in Figure 8 are provided by online measurements to simulate experimental scenarios and for computing temperature profiles, organic substrate concentration, windrow height, and dryness.

Temperatures and air moisture dynamics are observed during several days whereas windrow dryness

and height dynamics are more representative for the whole drying cycle. Data are in good agreement on Figure 9. Air extraction or destratification fans are stopped. An encouraging point is notable, windrow mixing effects are well predicted when core temperature prediction is in a practical range. Otherwise, the thermal balance on internal air gives accurate results both in magnitude and dynamics even when destratification fans are stopped. Electric fan operation is modeled by reducing convective exchanges. Mass balance on internal air is an important indicator which relates to process drying efficiency. Results were considered quite good and may be seen in Figure 10.

Finally, Figure 11 presents sludge dryness and windrow height evolutions with comparison to analysed samplings. These model predictions are encouraging for forecasting drying efficiency and process design. Windrow height decreases faster experimentally and maybe due to lack of accuracy for bulk density estimation and maintenance events (access opening to the greenhouse = non modeled air leakage).

Model Application

One application or case has been simulated to illustrate use of the model for designing a solar sludge process. This case aims to design a SOLIA™ process

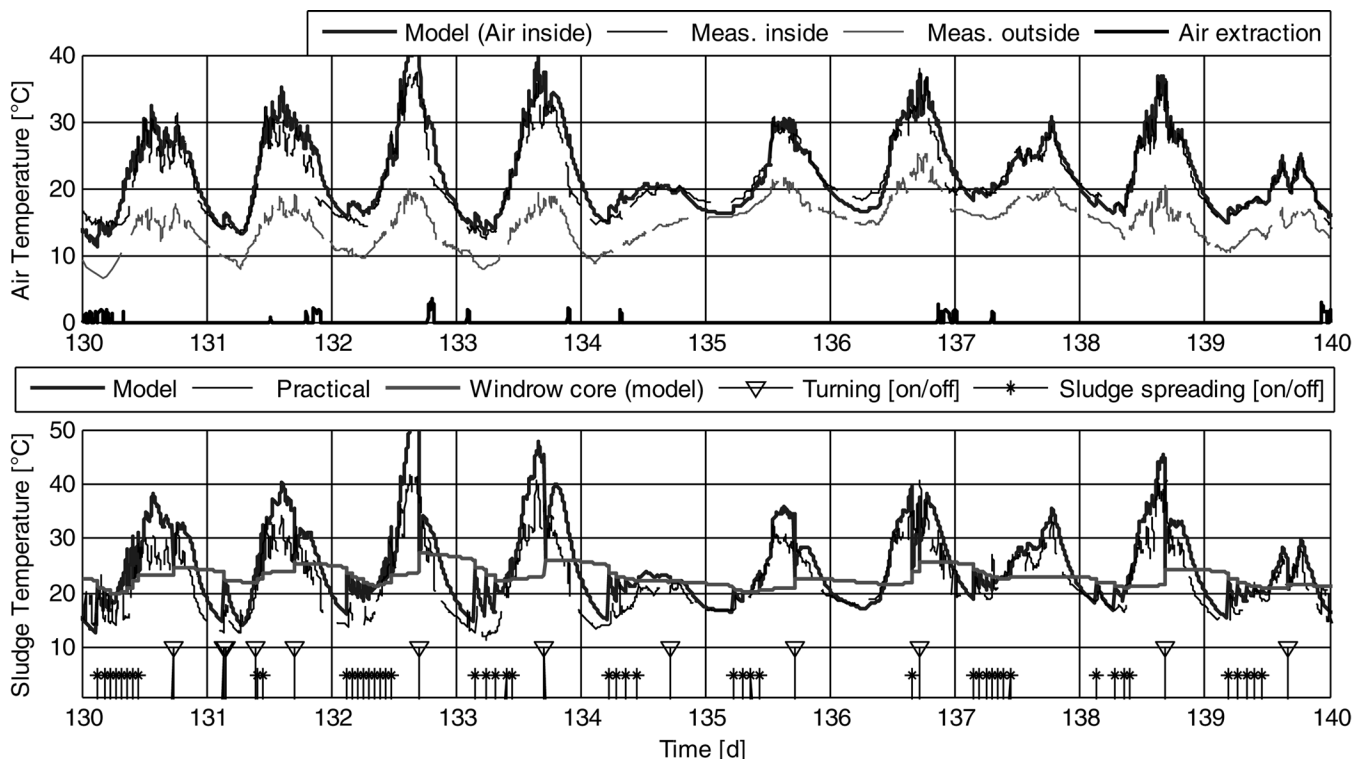


Figure 9. Comparison of thermal fields.

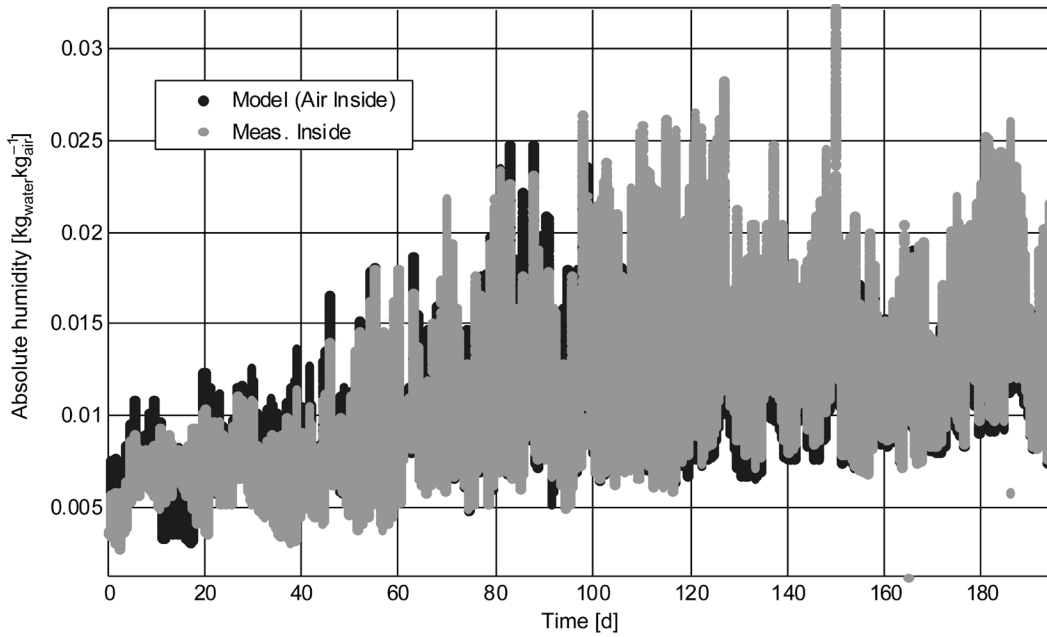


Figure 10. Comparison of internal air moisture.

in Poland with an optimum greenhouse surface and an intelligent operation. Thus, weather data, produced amount of dehydrated sludge by waste water treatment, and sludge spreading frequency are model inputs. Drying cycle starts in September. Model predictions are presented in Figure 12. Constraints are finding a good ratio for quantity of dewatered sludge per m^2 in order to reach a specified final dryness and not to exceed the

maximal value of windrow height for an efficient use of the Soliamix™ robot. The greenhouse surface and air renewal operation are main optimization methods used to find the smallest surface needed to treat sludge.

Sludge dryness decreases the sixtieth day (start of November) and reaches a minimum at 45% at the 150th day or end of January which is maintained until February. Windrows reach a maximum height of almost

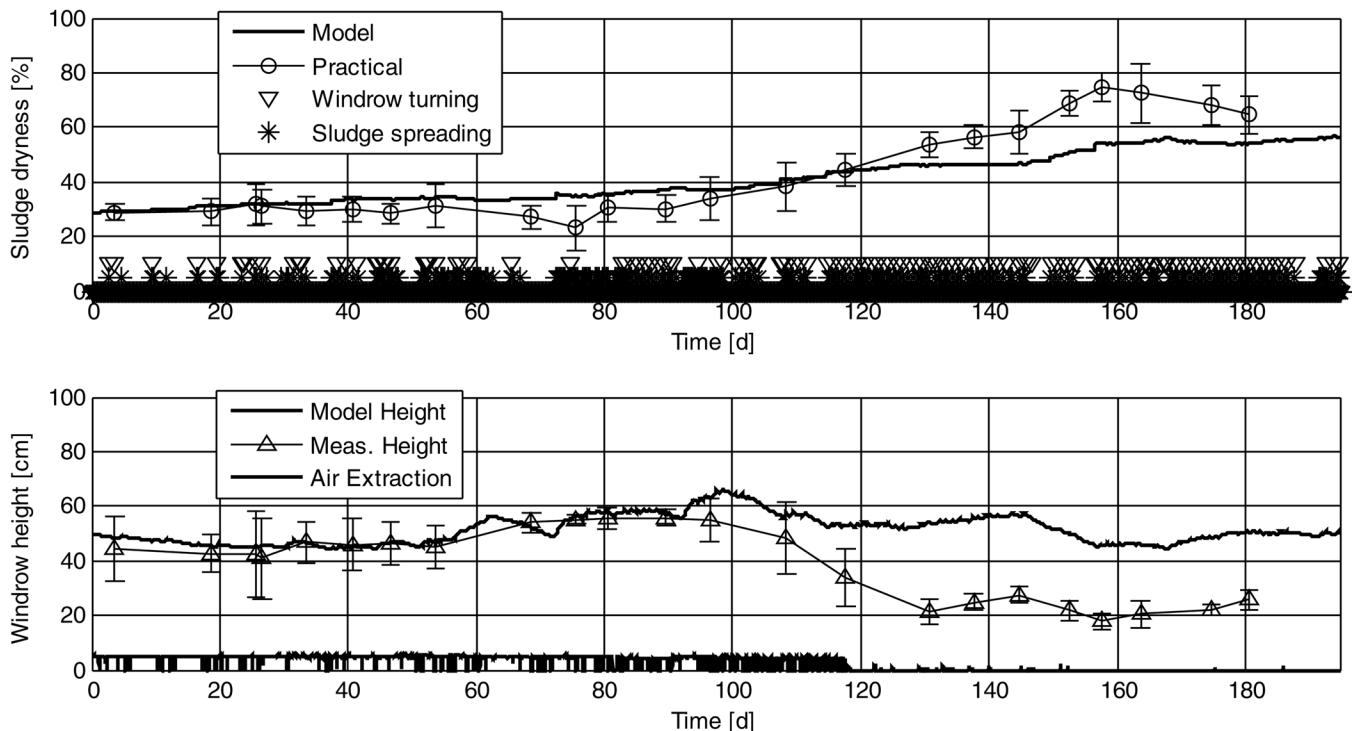


Figure 11. Comparison of model and experiment of sludge dryness and windrow height during a drying cycle of 2011 in north-west of France.

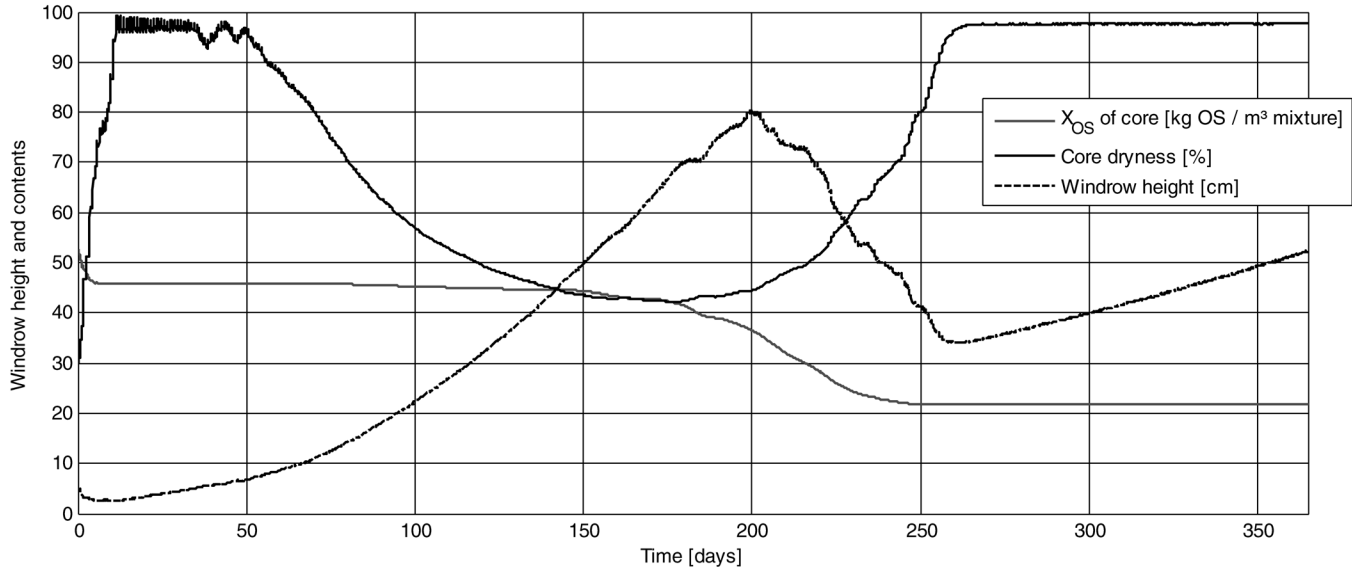


Figure 12. Evolution of sludge dryness, windrow height, and organic substrate content.

80 cm with contact of the SoliaMix™ robot at 85 cm during March around the 200th day where biological activity is acting until the end of April at the 240th day when organic substrate content is decreasing. Then, the drying rate is more efficient which allows getting dried sludge at the end of April up to 70% dryness. Sludge may be disposed of and used for agricultural nutrient spreading as an example of use.

CONCLUSION

This work introduces an appropriate model for planning and evaluating solar drying with sludge in a windrow shape. The model includes drying and biodrying kinetics. The model has been validated thanks to measurement recordings on an industrial unit. Model predictions are quite good and encouraging for making design and operating optimisations. A reproducibility test is forecasted to validate kinetics on another industrial unit. This numerical tool is now available in a relevant domain and may be used for operation and optimization objectives according to its location. The general approach of drying kinetics may also be relevant for solar drying of other materials.

NOTATIONS

Parameters

- a = Solar absorption coefficient [-]
- A = Area [m²]
- C_p = Specific heat [J.kg⁻¹.K⁻¹]
- F = Vision factor between walls [-]

- $F()$ = Reduced drying rate [-]
- H = Depth of layer [m]
- h = Convective exchange coefficient [W.m⁻².K⁻¹]
- J = Heat production rate [J.m⁻³.s⁻¹]
- L_v = Latent heat of water vaporisation [J.kg⁻¹]
- M = Molar mass [kg.mol⁻¹]
- m = Mass [kg]
- p = Kinetic constant of organic substrate biodegradation for humidity [-]
- P = Solar radiation (normal to wall) [W.m⁻²]
- Q = Air flow rate [m³.s⁻¹]
- R = Radiative heat [W]
- s = Sludge dryness [%]
- t = Time [s]
- T = Temperature [K]
- V = Volume [m³]
- v = Wind velocity [m.s⁻¹]
- W = Absolute air humidity [kg water.m⁻³]
- X = Concentration [kg water.kg⁻¹ dry solids] or [kg OS.m⁻³ sludge] or [kg water.kg⁻¹ sludge]
- Z = Kinetic constant of heat production from biological activity [-]

Abbreviations

- 0D = Zero-dimensional
- 3D = Three-dimensional

Greeks letters

- ϵ = Infrared emissivity [-]
- ρ = density [kg.m⁻³]

- τ = Solar transmission coefficient [–]
 σ = Stefan-Boltzmann's constant [$\text{W}\cdot\text{m}^{-2}\cdot\text{K}^{-4}$]
 λ = Thermal conduction coefficient [$\text{J}\cdot\text{m}^{-1}\cdot\text{K}^{-1}\cdot\text{s}^{-1}$]
 ζ = Time constant of organic substrate biodegradation [s]
 γ = Kinetic constant of organic substrate biodegradation for temperature [–]

Indices

- I = First or maximum drying rate
 a = Air
 Bo = Sludge
 Be = Concrete
 $Core$ = Sludge layer of windrow
 $Cover$ = Sludge layer of windrow
 $Core-Cover$ = Interface between core and cover layers
 cr = Critical
 ext = External
 eq = Equilibrium
 g = Gaz
 h = Wet
 H_2O = Water
 i = Initial or indices the windrow layer
 (core or cover)
 ini = Initial
 min = Minimal
 max = Maximal
 opt = Optimal
 os = Biodegradable organic substrate
 r = Reduced
 S = Solids
 T = Thermal
 v = Vapour
 V = Glass
 $Vsup$ = Glass roof
 W = Air humidity

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Effects of Biosolids Treatment Processes on Nitrogen Cycling under Various Tillage Practices

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ABSTRACT: Biosolids are typically injected or incorporated into soils by tillage. However, little research has been conducted on the effects of biosolids on nitrogen (N) availability under no tillage for crop production. We conducted a three-year field study to investigate the effects of lime-stabilized (LS) and anaerobically digested (AD) biosolids on N availability in a corn (*Zea mays* L.)-soybean (*Glycine max* L.) rotation under conventional tillage (CT) and no-tillage (NT) practices. Research was established on an Orangeburg loamy sand (*Fine-loamy, kaolinitic, thermic Typic Kandudults*) during 2009-2011 in Sussex County, VA. Results showed that both LS and AD biosolids increased spring soil nitrate N, plant tissue N at silking, post-season corn stalk nitrate N, grain yield, and total soil N by the end of the growing season. The “book” factors employed to estimate ammonia volatilization and organic nitrogen mineralization for the calculation of plant available N resulted in equally accurate predicted agronomic N rates for both LS and AD biosolids. This means that the same N availability factors can be used to calculate agronomic application rates for both CT and NT practices in coarse-textured soils of the Mid-Atlantic Coastal Plain.

INTRODUCTION

LAND APPLICATION of biosolids has been demonstrated to be a safe and effective means for recovery of plant nutrients (mainly N and P) [1–3]. Increased crop yields have been obtained with land application of biosolids [4–6]. Land application of biosolids also replenishes valuable organic matter, while simultaneously improving soil structure, water retention, nutrient capacity, and microbiological properties [7–10].

Biosolids are traditionally incorporated into soil or injected directly below the surface to reduce odors, runoff risk, and soil compaction resulting from the application [11,12]. No-till practices have been increasingly implemented for row crop production [13–15]. Incorporation of biosolids into soil increases N availability for loss through rapid microbial decomposition, while no-tillage generally results in slower biosolids decomposition and a net increase in soil organic C [16,17]. Field studies throughout the eastern United States have demonstrated that long-term N conservation and C storage can be increased through the land application of biosolids under continuous no-tillage practices [18–20]. However, few studies report the short-term

effects of tillage management on biosolids N availability and C accumulation under no-tillage practices for row crops, especially in the Mid-Atlantic region.

The method of biosolids application, the biosolids processing methods used, and the environmental properties (temperature, moisture, and aeration) control biosolids plant available N (PAN). $\text{NH}_4\text{-N}$ can be lost via NH_3 volatilization when biosolids are applied on a soil surface [21,22] resulting in a reduction of available N and lower yields [4]. Incorporation has been shown to produce more mineralized biosolids N compared to surface-applied biosolids [23,24]. $\text{NO}_3\text{-N}$ generated from mineralization and nitrification is more easily lost through leaching when biosolids are incorporated into soil [4,25,26].

Type of biosolids treatment processes can also influence PAN [27–29]. Organic N in lime-stabilized biosolids may mineralize to a greater extent than anaerobically digested biosolids during the first season after application [25,30]. Anaerobically digested biosolids contain significant proportions of $\text{NH}_4\text{-N}$, which can be more vulnerable to loss through ammonia volatilization during and immediately after application and incorporation [27,28,31].

Edaphic factors (i.e., soil temperature, moisture, and aeration) also influence biosolids PAN [32,33]. Leaching potential is greater in coarse-textured soils possess-

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ing high hydraulic conductivity [12, 34, 35]. Mineralization rates are generally greater in coarse textured soils due to greater aeration [36].

Both soil and plant tissue testing diagnostic tools can be used to evaluate the supplying capacity of N sources such as biosolids for corn. The pre-sidedress soil nitrate test (PSNT) has proven useful for assessing available soil N sufficiency when corn is 25 to 30 cm tall [37]. The ear leaf N test at silking provides reliable assessment of corn plant N sufficiency/deficiency [38] that can be used to interpret tillage and biosolids effects on PAN. The end-of-season corn stalk nitrate test (CSNT) provides post-season evaluation of N status because corn plants store excessive $\text{NO}_3\text{-N}$ in the bottom of stalks that can be re-mobilized upon crop needs [39,40]. Corn grain yield is an excellent indicator of N availability if all other potentially limiting growth factors are optimal [41]. End-of-season soil properties such as soil C and N contents can be used to measure accumulation of soil organic matter among various soil building processes (e.g., no-till) [42], and the plant available water (PAW) capacity can provide direct information about soil aggregate and soil water infiltration [43].

We proposed measuring these indicators to evaluate biosolids PAN for corn growth under various tillage practices. Objectives were to compare biosolids types (i.e., lime-stabilized and anaerobically digested biosolids) and tillage practices (i.e., conventional tillage and no-tillage) on short-term N availability in a corn-soybean rotation in the Mid-Atlantic Coastal Plain.

MATERIALS AND METHODS

Experimental Site

This study was conducted on a commercial farm in Sussex County, VA on an Orangeburg loamy sand (*Fine-loamy, kaolinitic, thermic Typic Kandiuults*) whose grain yield potential is estimated to be 8.80 Mg ha^{-1} (Virginia Department of Conservation and Recreation [44]).

Experimental Design

A split-plot design with tillage type randomly assigned to the main plot and fertility treatment randomly assigned to subplots was implemented. Each treatment was replicated four times resulting in a total of 48 experimental plots per each crop rotation. Each

experimental plot had an area of $9.1 \text{ m} \times 3.7 \text{ m}$ (33.5 m^2). Two tillage practices were employed: conventional tillage (CT) and no-tillage (NT). Conventional tillage consisted of disking to a depth of 10 cm, and no-tillage consisted of seed drilling directly into the undisturbed soil through the stubble remaining from a previously harvested crop. The six fertility treatments were made up of four fertilizer N rates ($0\times$, $0.5\times$, $1\times$, $1.5\times$ agronomic N rate), an agronomic N rate of each lime-stabilized (LS; Blue Plains Wastewater Treatment Plant, DC: http://www.epa.gov/owm/septic/pubs/alkaline_stabilization.pdf), and anaerobically digested (AD; Alexandria Sanitation Authority, VA: <http://epa.gov/OWM/mtb/multi-stage.pdf>) biosolids. The agronomic N rate for corn grain of 156 kg ha^{-1} for the Orangeburg soil was reduced by either 51 kg N ha^{-1} (following peanut) or 23 kg N ha^{-1} (following soybean) each year based on residual N availability of previous legume crop [44].

Corn in a corn (*Zea mays* L.)-soybean (*Glycine max* L.) rotation was used as a bioassay crop to assess biosolids N availability at two adjacent sites on the farm. Crop rotations were corn in 2009, soybean in 2010, and corn in 2011 for site I and corn in 2010 and soybean in 2011 for site II so that corn was planted every year of the field experiment on the Orangeburg soil. Site I and II corn crops were preceded by peanuts in 2008 and corn in 2009, respectively.

Biosolids and Fertilizer Application

Biosolids application rates were calculated using previous biosolids analyses. Nutrient rates applied were calculated each year from analyses of biosolids actually applied. Ten subsamples were randomly collected from the biosolids stockpiled at the site, placed on ice, and sent to a commercial laboratory (A&L Eastern Laboratories) for property analysis. Analyses performed included total solids (SM-2540G), total Kjeldahl N (SM-4500-TKN), ammonium-N (SM-4500-NH₃) [45], phosphors (SW-846-6010C), potassium (SW-846-6010C) [46], and other macro/micro nutrients.

Fresh biosolids were weighed in the field, applied to the surface of each plot, and uniformly raked. Commercial urea N fertilizer [$\text{CO}(\text{NH}_2)_2$] was applied to non-biosolids applied plots by hand before planting corn. According to soil testing results, the non-biosolids applied plots received the same rates of supplemental basal inorganic P ($\text{NH}_4\text{H}_2\text{PO}_4$) and all experimental plots received the same rates of K (KCl) fertilizers [47].

Conventional tillage treatments were disked each spring prior to planting corn to incorporate biosolids and fertilizers into soil. A field cultivator was used following disking to further mix the material and to prepare a seedbed. Corn (*Zea mays* L. Pioneer 31G71) was planted in mid-April each year with a row spacing of 91 cm and a seeding rate of 69,300 kernel ha⁻¹ resulting in a plant population of 63,000 plant ha⁻¹. Soybean (*Glycine max* L. Pioneer 95M82) was rotated on the plots in 2010 (study site I) and 2011 (study site II), respectively, at a seeding rate of 334,000 seed ha⁻¹ resulting in a plant population of 304,000 plant ha⁻¹. Pest and weed control was implemented according to standard Virginia Cooperative Extension recommendations [48]. This included a pre-plant roundup herbicide-glyphosate before planting a 7.8 kg ha⁻¹ Counter TM insecticide for corn in seed furrow at planting and a 0.14 kg ha⁻¹ Karate TM insecticide for soybean at moth egg threshold in August.

Estimated biosolids PAN was calculated using Virginia regulatory “book” values for ammonia-N volatilization and organic N mineralization factors [44] (<http://www.dcr.virginia.gov/documents/StandardsandCriteria.pdf>). Ammonia-N availability factor for LS biosolids for the first year after application is 0.75 for conventional tillage and 0.25 for no-tillage. Ammonia-N availability factor for AD biosolids for the first year after application is 0.85 for conventional tillage and 0.50 for no-tillage. Organic N mineralization factor for both types of biosolids for the first year after application is 0.3 for both tillage practices. Biosolids PAN was calculated as the sum of expected mineralized organic N and non-volatilized ammonium/ammonia.

Sampling and Analysis

Soil Routine Test

Soil cores with a diameter of 1.9 cm were randomly collected each autumn from 2008 to 2011 from 0–15 cm and 15–30 cm depths at the two study sites (2008). Within every treatment plot from 2009–2011 samples were air-dried, ground to pass a 2 mm sieve, and sent to Virginia Tech Soil Testing Laboratory for routine soil test analysis of Mehlich 1 extractable P, K, Ca, Mg, and pH [49].

Pre-sidedress Soil Nitrate Test

When corn plants were 25–30 cm tall in spring, ten

soil cores with a diameter of 1.9 cm were collected from 0–15 cm and 15–30 cm depths in each plot for pre-sidedress soil nitrate test (PSNT) analysis and as an in-season indicator of available soil inorganic N [37,50,51]. All samples were stored on ice and transported to the laboratory where they were extracted with 2 M KCl for nitrate (NO₃-N) and ammonium (NH₄-N) analysis [52] by flow injection analysis using a Lachat 8000 (Lachat Instruments, US).

End-of-season Soil N, C and Plant Available Water

Ten soil cores with a diameter of 1.9 cm were collected from a 0–15 cm depth in each plot after harvest each autumn. Samples were air-dried and ground to pass a 0.5 mm sieve for total soil N analysis by dry combustion using a Vario Max CNS macro elemental analyzer (Elementar, GER).

Two soil cores from each replication of the conventionally tilled 1× N fertilizer plots, LS biosolids plots, and AD biosolids plots at study site I were sampled after corn was harvested in 2011. Bulk density samples were collected with a drop hammer using the core method [53]. Cores were air-dried and weighed to calculate bulk density based on moisture correction and ground to pass a 0.5 mm sieve for soil organic C analysis by dry combustion using a Vario Max CNS macro elemental analyzer (Elementar, GER). C stock was calculated from bulk density and soil organic C concentration. Plant available water capacity was determined on bulk density soil cores using a pressure plate method [43]. The volume of water held by soil under 33 kPa of pressure (field capacity) minus the volume of water held at 1,500 kPa of pressure (wilting point) was considered plant available water capacity and was reported as % water in soil (v/v) on an oven-dried basis.

Corn Ear Leaf Total N

Ten corn ear leaf samples from each plot were randomly collected at the early silking (R1) stage [38], dried at 65°C, and ground in a Wiley mill to pass a 0.5 mm sieve for determination of nitrogen each July. Total Kjeldahl N (TKN) of these plant samples was determined colorimetrically by flow injection analysis using a Lachat 8000 (Lachat Instruments, US).

End-of-season Corn Stalk Nitrate Test

End-of-season corn stalk NO₃-N was determined

Table 1. Chemical Properties of Soils at Study Site Prior to Treatment Applied in October of 2008.

Depth (cm)	pH	Mehlich 1 P	K (mg kg ⁻¹)	Ca (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	C (g kg ⁻¹)
0–15	6.67	14	73	442	45	1	5.6	34
15–30	6.55	9	57	399	43	0.9	3.8	28

to evaluate the adequacy (or excess) of the N fertility program for the current growing season before harvest. Corn segments (20 cm) were taken 15 cm above-ground from ten plants in each plot. Stalk segments were cut, dried at 65°C, and ground in a Wiley mill to pass a 0.5 mm sieve for analysis of NO₃-N content via electrode analysis [39,40].

Corn Grain Yield

Corn ears were hand-picked at 6.1-m of the two center rows for each plot. They were then dried at 65°C and shelled to obtain grain yield estimates (0.155 g moisture g⁻¹ dry weight) each September.

Statistical Analysis

The split-plot design [54] was implemented using a mixed model procedure using the Statistical Analysis System (SAS) 9.2 [55]. Analysis Of Variance (ANOVA) and Least Significant Difference (LSD) measurements were applied at a level of 0.05 to compare differences between treatment means (e.g., corn yield). The N variable responses to treatments and treatment interactions were determined separately by year be-

cause the corn crop rotated annually between sites. The relationship between soil or plant tissue N level and fertilizer N application rate was evaluated with linear and quadratic regression analyses using the PROC REG procedure in SAS 9.2 [55]. A randomized complete block design was used to statistically analyze soil C stocks and plant available water capacity obtained in November of 2011.

RESULTS AND DISCUSSION

Experimental Site

Soil properties from samples obtained from 0–15 cm and 15–30 cm depths at the two sites prior to treatments are displayed in Table 1. The pH values prior to treatment were above 6.2 and no additional liming was required. Soil pH values were 6.5 following addition of the AD and fertilizer treatments and increased to 7.1 with LS biosolids. Despite an initially adequate soil pH, the resulting pH from LS biosolids should not have altered plant essential nutrient availability. Soil testing (i.e., Mehlich I extractable) indicated a need for P and K that was provided by biosolids and/or supple-

Table 2. Monthly Temperature and Precipitation Means During 2009–2011 and 30-Year Average for the Study†.

Month	Temperature (°C)				Precipitation (mm)			
	30-year Mean	2009	2010	2011	30-year Mean	2009	2010	2011
Jan	2.8	2.8	1.9	1.4	108	48.3	113	39.1
Feb	4.5	4.8	1.6	5.7	84.8	15.5	72.6	33.5
Mar	9.1	7.9	10.1	8.7	119	139	110	112
Apr	14.2	14.6	15.9	15.7	87.9	354.8	37.6	47.0
May	19.2	20.1	20.5	19.7	110	140	98.6	83.6
Jun	23.8	24.1	26.3	24.8	103	128	101	175
Jul	26.0	24.4	27.3	26.8	115	59.7	36.1	143
Aug	24.6	26.2	26.2	25.3	113	134	89.2	234
Sep	21.1	20.4	23.3	22.2	112	143	192	226
Oct	14.3	14.0	15.6	14.6	88.9	46.7	91.9	70.6
Nov	9.4	11.1	8.9	11.2	79.2	222	25.9	154
Dec	4.6	3.8	0.2	7.3	74.2	223	84.6	47.0
Annual	14.5	144	14.8	15.3	1195	1334	1053	1365

†The data were obtained from the U.S. Department of Commerce National Climatic Data Center Stony Creek 2 N weather station, Stony Creek, VA (36°58'N/77°24'W and 32.0 m (105') above s/l). <http://www.ncdc.noaa.gov/oa/ncdc.html>

Table 3. Attributes of Blue Plains Lime-stabilized and Alexandria Anaerobically Digested Biosolids Applied at Study Sites in the Springs of 2009 to 2011.

Attribute	Lime-stabilized Biosolids			Anaerobically Digested Biosolids		
	2009	2010	2011	2009	2010	2011
Solids (g kg ⁻¹)	421	322	335	282	263	271
Volatile solids (g kg ⁻¹)	546	629	609	627	635	642
Total Kjeldahl N (g kg ⁻¹)	30.4	42.2	40.1	60.2	76.5	56.8
NH ₄ -N (g kg ⁻¹)	1.1	2.6	2.3	17.2	21.4	14.7
Organic N (g kg ⁻¹)	29.3	39.6	37.7	42.8	55.1	42.1
Phosphorus (g kg ⁻¹)	10.2	10.9	13.6	37.5	33.5	35.9
Potassium (g kg ⁻¹)	1.8	2.0	2.3	1.5	1.7	1.7
Sulfur (g kg ⁻¹)	5.9	4.5	4.6	10.4	9.4	9.7
Calcium (g kg ⁻¹)	127	101	124	25.3	20.4	22.1
Magnesium (g kg ⁻¹)	3.0	2.5	2.4	3.5	3.3	3.1
pH	12.4	12.2	12.3	8.2	8.3	8.8
Calcium Carbonate Equivalent (CCE) (g kg ⁻¹)	238	166	108	–	–	–

mental fertilization [47]. Total organic C concentration was typical for coarse-textured Virginia Coastal Plain soils. The low nutrient and C contents of the coarse-textured soils made application of organic residuals (i.e., biosolids) a desirable practice.

Monthly temperature and precipitation data for the study period are displayed in Table 2. Mean temperature during the growing season (April–August) was 21.9°C, 23.2°C, and 22.5°C for three years, respectively. Total precipitation during the growing season (April–August) was 498 mm, 363 mm, and 683 mm for three years, respectively. Plots were irrigated in 2009 twice between June and July with applications of 2 cm of water to supplement rainfall and prevent crop failure due to drought. Plots were irrigated in 2010 four times between June and August with applications of 2 cm of water.

Biosolids Properties

Attributes for the two biosolids are provided in Table 3. The AD biosolids contained almost twice as much TKN and a higher proportion of NH₄-N than the LS biosolids. The AD biosolids also contained higher concentrations of P and S with no calcium carbonate equivalent (CCE) and a lower Ca and pH than LS biosolids. The target and estimated actual biosolids PAN applied during 2009–2011 are displayed in Table 4. Target agronomic N rate was not achieved because actual N composition of the material varied in analyses used to calculate application rates. Application rates for biosolids for NT were higher than for CT accounting for N volatilization loss. Therefore, application rates of CCE and nutrients other than N were higher for NT than CT treatments.

Table 4. Target and Actual Nitrogen (estimated) from Lime-stabilized and Anaerobically Digested Biosolids Applied at Study Sites in the Springs of 2009 to 2011.

Rate	Tillage	Lime-stabilized Biosolids			Anaerobically Digested Biosolids		
		2009	2010	2011	2009	2010	2011
Target PAN† (kg ha ⁻¹)	CT‡	106	157	135	106	157	135
	NT§	106	157	135	106	157	135
Actual biosolids (dry Mg ha ⁻¹)	CT	10.2	11.6	8.0	4.8	5.3	4.4
	NT	10.9	12.8	8.7	6.0	6.9	53.7
Actual organic N (kg ha ⁻¹)	CT	90	137	90	62	89	56
	NT	95	152	98	71	114	73
Actual NH ₄ ⁺ -N (kg ha ⁻¹)	CT	8.0	23	14	77	97	56
	NT	3.0	8.0	5.0	51	73	42
Actual PAN (kg ha ⁻¹)	CT	98	160	104	133	186	112
	NT	98	160	103	128	187	115

†PAN = plant available nitrogen, ‡CT = conventional tillage, and §NT = no tillage.

Soil Properties

Pre-sidedress Soil Nitrate N

Statistical results and measured values from pre-sidedress soil nitrate tests (PSNT) are presented in Table 5 and 6 and in Figure 1(a). Table 5 displays that only fertility treatments significantly influenced PSNT values. Tillage did not affect spring soil $\text{NO}_3\text{-N}$ levels in any fertility treatment plots, likely because organic and ammonium N were not differentially mineralized and/or nitrified under the various tillage systems in the coarse-textured Coastal Plain soils. This result is consistent with Meisinger, *et al.* [56].

The response of soil $\text{NO}_3\text{-N}$ concentration to an increasing fertilizer N rate followed a similar pattern according to similar slopes for regression lines in 2010 and 2011. The slope in 2009 was reduced by a higher volume of rainfall which likely increased leaching loss of N beyond the zone of sampling. The $1\times$ fertilizer N rate resulted in a $\text{NO}_3\text{-N}$ concentration at or above the critical level of 20 mg kg^{-1} [37] for corn production in 2010 and 2011 but not in 2009. These responses demonstrate susceptibility to a loss of N due to leaching in the coarse-textured Coastal Plain soils when receiving sufficient precipitation in early May [57]. There were no significant fertility treatment differences in soil $\text{NH}_4\text{-N}$ (data not shown) for all three years. This is likely because ammonium was rapidly converted to nitrate through nitrification in the coarse-textured soils [56].

The biosolids maintained soil $\text{NO}_3\text{-N}$ concentrations within a range of $21\text{--}45 \text{ mg kg}^{-1}$ during 2009–2011. According to PSNT diagnostic criteria, both biosolids types provided sufficient $\text{NO}_3\text{-N}$ for optimal corn production necessitating no additional N [37]. Both biosolids types showed lower soil $\text{NO}_3\text{-N}$ concentrations than the $1\times$ fertilizer N rate in 2010 with soil $\text{NO}_3\text{-N}$ lower in AD (24 mg kg^{-1}) rather than in LS biosolids (43 mg kg^{-1}). This phenomenon was consistent with the U.S. Environmental Protection Agency's (U.S. EPA) [30] recommendation that annual first-year organic N mineralization rate is higher for LS (30%) than AD (20%) biosolids. Gilmour, *et al.* [28] by contrast determined that there is no difference in mineralization rate between lime stabilized and digested biosolids, and climate plays a greater role in mineralization rate than biosolids processing treatment. Rainfall in April and May of 2010 were considerably below average which may have reduced microbially facilitated N transformations such as mineralization and nitrification in the biosolids. This could have decreased the

$\text{NO}_3\text{-N}$ concentration released by both biosolids types during this period.

Lower $\text{NO}_3\text{-N}$ concentrations were possibly due to leaching loss occurring in coarse-textured soil under conditions of high soil moisture which reduced fertilizers and biosolids PAN simultaneously in spring of 2009. Al-Kaisi and Kwaw-Mensah [58] reported that PSNT on a Kenyon loam soil was decreased by deep leaching of $\text{NO}_3\text{-N}$ due to above normal rainfall in early April. These findings indicate a need to reassess N availability while using PSNT for interpreting biosolids PAN in the coarse-textured Coastal Plain soils where leaching loss of $\text{NO}_3\text{-N}$ may be greater than estimation.

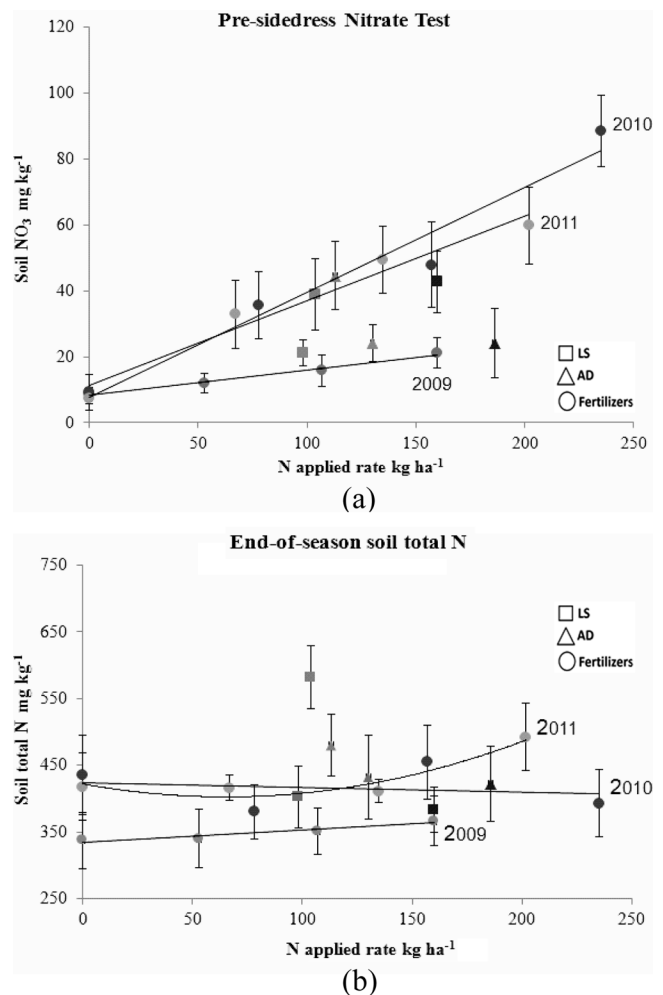


Figure 1. (a) Effect of fertilizer N rate and estimated biosolids plant available N on spring soil pre-sidedress nitrate-N test under conventional tillage and no-tillage systems during 2009–2011 (LS = lime stabilized biosolids and AD = anaerobically digested biosolids). (b) Effect of fertilizer N rate and estimated biosolids plant available N on fall soil total N concentration under conventional tillage and no-tillage systems during 2009–2011 (LS = lime stabilized biosolids and AD = anaerobically digested biosolids).

Table 5. P Values from ANOVA[#] for Soil Properties and Crop Measurement during the 2009–2011 Period.

Year	Source	PSNT [†]	ELN [‡]	Grain Yield	CSNT [§]	Fall Soil N
		P value				
2009	Tillage	0.785	0.852	0.509	0.162	0.039*
	Fertility	< 0.0001*	< 0.0001*	< 0.0001*	0.016*	0.000*
	Tillage*Fertility	0.096	0.131	0.164	0.065	0.336
2010	Tillage	0.086	0.707	0.484	0.770	0.436
	Fertility	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	0.037*
	Tillage*Fertility	0.057	0.351	0.085	0.957	0.357
2011	Tillage	0.107	0.002*	0.011*	0.092	0.137
	Fertility	< 0.0001*	< 0.0001*	< 0.0001*	0.043*	< 0.0001*
	Tillage*Fertility	0.071	0.257	0.495	0.144	0.368

[†]PSNT = Pre-sidedress Nitrate Test, [‡]ELN = Corn Ear Leaf N, [§]CSNT = Corn Stalk Nitrate Test, and [#]ANOVA = Analysis of variance.

* Significant level $\alpha = 0.05$.

End-of-season Soil N, C and Plant Available Water

Statistical results and measured values from the end-of-season soil N are presented in Table 5 and 6 and in Figure 1(b). Table 5 displays no interaction effects between tillage and fertility treatment. There were significant differences between the two types of tillage as well as with fertility treatments. Tillage only influenced end-of-season soil total N concentration in 2009 ($p = 0.04$) with a higher level under NT (388 mg kg^{-1}) than CT (355 mg kg^{-1}) (Table 6). Increased tillage intensity decreased soil capacity to immobilize and conserve mineral N [18]. Gallaher and Ferrer [59] reported that no-tillage resulted in 20% more total Kjeldahl N than conventional tillage at the 0 to 5 cm soil depth range in a 3-year study. However, this finding is inconsistent with that of Al-Kaisi, *et al.* [60] who reported that tillage effects on soil total N were negligible at the end of 3 years of tillage practices. Short-term effects of tillage management on soil N dynamics are often complex and variable.

End-of-season soil total N concentration showed either a linear (2009 and 2010) or quadratic (2011) in-

crease with increasing fertilizer N rates during three years [Table 7 and Figure 1(b)]. Soil total N concentration was not greatly increased due to fertilizers and/or biosolids application. Soluble fertilizer N was likely rapidly assimilated by plants or leached through the soil resulting in lower residual N levels from fertilizers than from biosolids. Moreover, the sufficient volume of early-spring rainfall in 2009 may have increased leaching losses of $\text{NO}_3\text{-N}$ which reduced soil N availability.

Soil N concentration was higher where biosolids were applied than where commercial fertilizers were applied in 2009 and 2011. Biosolids contain recalcitrant N which remains in organic form in soil for a long time after application. The slowly available organic N pool is made up of resistant N compounds that take months or years for complete decomposition [61]. The 2011 field site also received biosolids application in 2009 and the double applications led to the highest soil total N concentration among the three years. The warmer temperature in 2010 might have increased biosolids mineralization rate resulting in lower biosolids residual N for the field site.

End-of-season soil C stocks in 2011 ($p = 0.06$) were decreased in conventionally-tilled soils in the following order: LS biosolids (4.2 Mg ha^{-1}) > AD biosolids (3.6 Mg ha^{-1}) = $1 \times$ N rate (3.5 Mg ha^{-1}). Biosolids application increased soil C stocks at the end of the three-year study because biosolids added C inputs into soils. Other research has shown that short-term added biosolids increased soil C accumulation [62,63]. However, such an increase in soil C accumulation cannot be regarded as sequestration as C sequestration is unlikely to occur in the Coastal Plain soils following a few biosolids applications [64]. The warm temperature and great aeration of Coastal Plain soils accelerate decom-

Table 6. Mean Concentrations and p-values of Fall Soil Total N in 2009, Corn Ear Leaf N in 2011, and Grain Yield in 2011 Under Various Tillage Practices.

Year	Tillage		p-value
	Conventional Tillage	No-tillage	
Fall Soil N, 2009 (mg kg^{-1})	355	388	0.039
ELN [†] , 2011 (g kg^{-1})	23.2	21.2	0.002
Grain yield, 2011 (Mg ha^{-1})	9.3	10.9	0.011

[†]ELN = Corn Ear Leaf N.

Table 7. Regression Equation†, R², and p-value for Soil or Crop N Response as Function of N Applied Rate.

Dependent variable	Year	Regression Equation	R ²	p-value
PSNT‡ (mg kg ⁻¹)	2009	$y = 0.077x + 8.2893$	0.9864	< 0.0001
	2010	$y = 0.3423x + 6.6251$	0.9411	< 0.0001
	2011	$y = 0.2577x + 11.29$	0.9636	< 0.0001
Fall soil N (mg kg ⁻¹)	2009	$y = 0.0002x + 0.3339$	0.9141	0.0001
	2010	$y = -7E-05x + 0.4238$	0.0389	0.037
	2011	$y = 0.0046x^2 - 0.5952x + 422.07$	0.9092	0.004
ELN§ (g kg ⁻¹)	2009	$y = -0.0001x^2 + 0.0675x + 16.208$	0.9406	< 0.0001
	2010	$y = -0.0003x^2 + 0.129x + 13.483$	0.9935	< 0.0001
	2011	$y = -0.0002x^2 + 0.1052x + 13.757$	0.9986	< 0.0001
CSNT# (mg kg ⁻¹)	2009	$y = 0.0137x^2 - 1.1719x + 334.01$	0.9608	0.016
	2010	$y = 0.0433x^2 - 3.8906x + 440.08$	0.9963	< 0.0001
	2011	$y = 0.0091x^2 - 0.4822x + 414.45$	0.9922	0.043
Grain yield (Mg ha ⁻¹)	2009	$y = -0.0002x^2 + 0.053x + 10.29$	0.9805	< 0.0001
	2010	$y = -9E-05x^2 + 0.0306x + 2.7929$	0.9458	< 0.0001
	2011	$y = -0.0002x^2 + 0.071x + 5.1754$	0.9189	< 0.0001

†Dependent variable (y) = crop or soil response, Independent variable (x) = N applied rate, ‡PSNT = Pre-sidedress Nitrate Test, §ELN = Corn Ear Leaf N, and #CSNT = Corn Stalk Nitrate Test.

position of soil C and induce a negative C balance [36]. Additionally, biosolids N inputs increase plant residues and improve microbial activity which depletes the labile SOC pool [65–67].

Biosolids application increased volumetric soil plant available water (PAW) ($p = 0.074$) in the conventionally-tilled soils in the following order: LS biosolids ($52.4 \text{ cm}^3/100 \text{ cm}^3$) > AD biosolids ($45.8 \text{ cm}^3/100 \text{ cm}^3$) = $1 \times \text{N rate}$ ($44.4 \text{ cm}^3/100 \text{ cm}^3$). The increase in soil organic matter as measured by soil organic C likely increased soil water holding capacity [68].

Crop Responses

Corn Ear Leaf N

Statistical results and measured values from corn ear leaf N are presented in Table 5 and 6 and in Figure 2(a). Table 5 displays that both tillage and fertility treatments significantly affect corn ear leaf N concentrations. Tillage practices in 2011 were significant for ear leaf N ($p < 0.01$) with an ear leaf N concentration higher under CT (23.2 g kg^{-1}) than under NT (21.2 g kg^{-1}) (Table 5 and 6). Although PSNT values were unaffected by tillage systems, tillage may have promoted mineralization of previously grown soybean stubble (2010) as well as biosolids residual N in 2009 and 2011 which appeared to increase with tillage and thus improve crop N absorption and assimilation.

Corn ear leaf N concentration increased with fertilizer N rate and followed a similar quadratic pattern

across all three years [Table 7 and Figure 2(a)]. Variability in weather among the 2009–2011 seasons did not influence fertilizer-induced corn ear leaf N concentration.

Both biosolids maintained higher ear leaf N concentrations than $1.5 \times$ fertilizer N rate during the rainy 2009 season. LS biosolids gave higher ear leaf N concentration than AD biosolids in two of the three years of the study (2010 and 2011). This is possibly due to higher mid-season soil N maintenance. LS biosolids have a higher proportion of slowly released organic N than AD biosolids which could be recalcitrant and not leach through soils. AD biosolids revealed lower ear leaf N concentration than $1 \times$ fertilizer N rate in 2010 and 2011 but higher in 2009 when soluble fertilizer N would have been more likely to leach under the high rainfall.

Although PSNT showed that spring soil $\text{NO}_3\text{-N}$ may have been lost through leaching during high volumes of rainfall in 2009, the corn ear leaf N concentration response to various rates and sources of N did not vary considerably over the three years.

Corn Stalk Nitrate N

Statistical results and measured values from corn stalk nitrate N are presented in Table 5 and 6 and in Figure 2(b). Table 5 displays that there was no significant difference of corn stalk $\text{NO}_3\text{-N}$ concentrations between conventional tillage and no-tillage during the growing seasons of 2009–2011 (Table 5). Tillage practices did

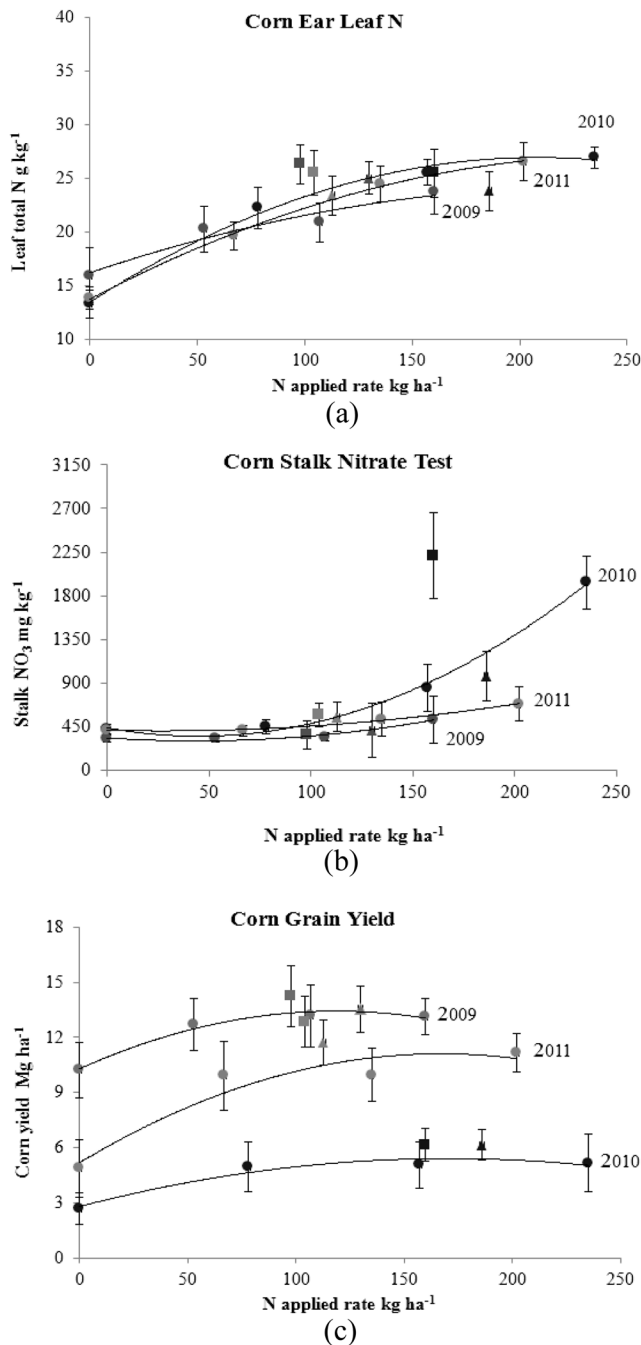


Figure 2. (a) Effect of fertilizer N rate and estimated biosolids plant available N on corn ear leaf N concentration under conventional tillage and no-tillage systems during 2009–2011 (LS = lime stabilized biosolids and AD = anaerobically digested biosolids). (b) Effect of fertilizer N rate and estimated biosolids plant available N on end-of season corn stalk nitrate-N test under conventional tillage and no-tillage systems during 2009–2011 (LS = lime stabilized biosolids and AD = anaerobically digested biosolids). (c) Effect of fertilizer N rate and estimated biosolids plant available N on corn grain yield (on a basis of 15.5% of moisture) under conventional tillage and no-tillage systems during 2009–2011 (LS = lime stabilized biosolids and AD = anaerobically digested biosolids).

not affect NO₃-N concentrations accumulation at the bottom of the corn stalks indicating that plants may have absorbed and utilized the same amounts of N under these two types of tillage managements.

There was a positive quadratic relationship between corn stalk NO₃-N concentration and fertilizer N rate [Table 7 and Figure 2(b)]. Corn stalk NO₃-N concentration remains steady and does not increase with N applied rate until maximum yield is reached [69]. Optimum levels of corn stalk NO₃-N occur in the range of 450–2000 mg kg⁻¹ [51,70–72]. The upper boundary was never attained by any of the fertilizer treatments but rather by LS biosolids in 2010. In fact, insufficient N (CSNT < 450 mg kg⁻¹) diagnosis was more often indicated than excessive N throughout the three years of the study. This was likely due to application of less than target PAN rates, high crop biomass accumulation, and/or nitrate-leaching rainfalls.

Both biosolids maintained higher corn stalk NO₃-N concentrations than 1x fertilizer N rate during the 2009–2011 period. LS biosolids maintained a higher corn stalk NO₃-N concentration than AD biosolids in 2010 but slightly lower than AD in 2009 and equal to AD biosolids in 2011. Higher estimated actual PAN from AD versus LS biosolids did not lead to constantly higher plant tissue N concentration. The excessive corn stalk NO₃-N concentration with LS biosolids in 2010 (2212 mg kg⁻¹) was consistent with early season high N assimilation followed by biomass-reducing drought [69].

Because CSNT provides a direct measure of N sufficiency and deficiency for plant growth during the growing season these measurements provide evidence that sufficiency of N for plant growth has been obtained in 2010–2011 but not in 2009.

Corn Grain Yield

Statistical results and measured values from corn grain yield are presented in Table 5 and 6 and in Figure 2(c). Table 5 shows no occurrence of interaction effects between tillage and fertility treatments, but tillage significantly ($p < 0.05$) affected corn grain yield in 2011 after the study had been in NT for the longest time. A higher corn grain yield under no-till rather than under conventional tillage (Table 5 and 6) in Coastal Plain soils has been reported by other scholars [15,18]. The typical explanation for such advantages of NT over CT is that improved water availability and water use efficiency may have increased corn grain yields under no-till [73]. No-till decreases soil bulk density and in-

creases water infiltration and water holding capacity consequently resulting in increased plant growth and crop yields [20,74].

Grain yields increased with increasing fertilizer N rate and surpassed the expected yield for this soil (8.80 Mg ha⁻¹; Virginia Department of Conservation and Recreation, 2005) in two of the three years of the study (2009 and 2011) [Figure 2(c)]. The lowest yields occurred during the droughty 2010 season in which the 1.5× fertilizer N rate attained a yield of 5.14 Mg ha⁻¹.

LS biosolids resulted in higher yields than AD biosolids due to greater PAN as supported by PSNT, the corn ear leaf N test, and CSNT. Higher yields with LS biosolids may have been due to maintenance of higher PAN concentrations later in the season from a potentially mineralizable resource or from some other benefits provided by organic materials [75]. Both biosolids types produced higher yields than 1.5× fertilizer N rate in 2009–2011 justifying biosolids as a valuable inorganic fertilizer replacement.

Biosolids appeared to significantly increase corn grain yields compared to inorganic fertilizer following repeated applications in 2011). This is possibly due to a multiplicative effect of biosolids biostimulants and nutrients on crop drought stress tolerance [75] and/or increase in soil PAW and soil C accumulation. Such yield-increasing effects under drought conditions have been reported [68,75].

CONCLUSIONS

Surface-applied and unincorporated biosolids should be treated the same as biosolids incorporated into the soil by tillage for the purposes of estimating plant available N using mineralization and volatilization factors employed in the Mid-Atlantic U.S.. Biosolids PAN was not affected by tillage practices management on this very coarse-textured soil containing little organic matter. Both lime-stabilized biosolids and anaerobically digested biosolids were equally capable of providing the PAN required for optimal corn grain yield. Furthermore, additional evidence is provided that biosolids use provides a yield advantage over inorganic fertilizers under repeated application. Causes of this effect may be a result of improved soil physical properties (e.g., plant available water) or a plant biostimulant effect.

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Combination of Hydrogen Sulphide Removal from Biogas and Nitrogen Removal from Wastewater

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ABSTRACT: The aim was to verify effect of sulphides on the course and effectiveness of autotrophic and heterotrophic denitrification by non-adapted activated sludge. Joint application of organic substrate and sulphides indicates heterotrophic and autotrophic denitrification does not run simultaneously, but the organic substrate is used preferably and only after exhaustion of organic substrate autotrophic denitrification with sulphide proceeds. Results offer a possible solution for wastewater with a lack of organic carbon as well as for some sources of dissolved sulphides available especially from external desulphurization of biogas.

INTRODUCTION

BIOLICAL DENITRIFICATION is an effective and economical method of nitrate removal from wastewaters. However, heterotrophic denitrifying bacteria require organic carbon as an energy source and an electron donor and effectiveness of the process is dependent on a sufficient amount of readily biodegradable substrates (lower volatile fatty acids, ethanol, or methanol at C/N = 7–9) [1]. Some types of wastewater have a low C/N ratio which limits complete denitrification. It is necessary in such cases to supply external organic carbon and this fact negatively influences operational costs and production of excess sludge.

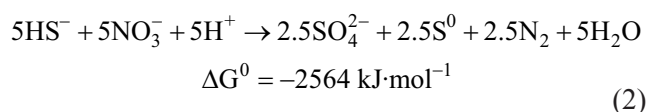
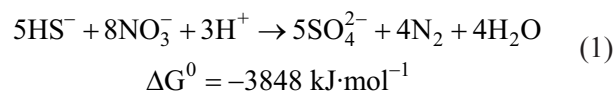
When insufficient amounts of organic carbon occur and some reduced inorganic compounds are present autotrophic denitrification could run as an alternative to heterotrophic denitrification. Studies on autotrophic denitrification have been divided into two main fields, denitrification with hydrogen or reduced forms of sulphur. A much more applicable approach is autotrophic denitrification with a reduced form of sulphur compounds as an electron donor [2].

Reduced forms of inorganic sulphur (S^{2-} , S^0 , $S_2O_3^{2-}$, and SO_3^{2-}) are used by some chemolithotrophic denitrifying bacteria simultaneously reducing nitrate to nitrogen [3]. Because autotrophic bacteria use inorganic substances such as CO_2 as a carbon source no external organic carbon needs to be added. Moreover, it produced two to three times less excess sludge [4].

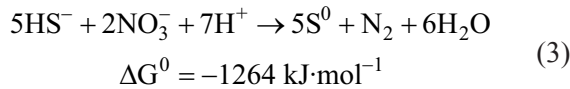
An additional benefit from this process results from removal of reduced sulphur compounds which are also pollutants and need to be treated. These compounds, especially the most reduced forms—sulphides and hydrogen sulphide, due to their toxicity, odour, and corrosion properties cause serious environmental problems. The first step of biogas desulphurization is absorption of hydrogen sulphide to liquid forming sulphides that may be treated by chemical or biological methods.

Biological removal of sulphides is mediated by sulphur oxidizing bacteria. These bacteria need electron acceptors, oxygen (*Thiobacillus*, *Thiothrix*, *Beggiatoa*) or nitrate (*Thiobacillus denitrificans*, *Thiomicrospira denitrificans*) [5]. Products from biooxidation of reduced sulphur compounds are no longer harmful to the environment. Elementary sulphur as a solid can be removed completely from the system. Dissolved sulphates can be discharged into recipients. Additionally, biological methods for sulphides removal compared to chemical and physico-chemical methods (e.g., precipitation, ion exchange, or stripping) [6,7] are less expensive and without wastes requiring disposal.

A mathematical description for anoxic sulphide oxidation using nitrate as an electron acceptor is provided in the following Equations (1), (2), and (3) [8].



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According to standard Gibbs free energy, the reaction [Equation (1)] produces sulphate as an end product with the highest energy yield. Reactions in Equations (2) and (3) lead to elemental sulphur as a partial or a full end product and is not favourable to bacteria. It is evident the molar ratio of nitrates to sulphides (N/S) is very important and some studies highlight its controlling role in the process [9].

METHODS

Experiments were carried out in double-jacket glass cells tempered to 20°C mixed with a magnetic stirrer (Figure 1). Excess activated sludge from the Prague Central Wastewater Treatment Plant was used for the experiments. It was aerated overnight to reach a level of endogenous respiration. Before experiment commenced, aeration was stopped and oxygen concentration decreased to zero. A volatile suspended solids (VSS) [$\text{g}\cdot\text{L}^{-1}$] concentration was always set to 5 $\text{g}\cdot\text{L}^{-1}$ except for experiments in which the influence of sulphide concentration was studied and concentrations of VSS were adjusted to 1, 2, 3, and 4 $\text{g}\cdot\text{L}^{-1}$.

Liquid medium used in the experiments was prepared from drinking water with addition of potassium nitrate, sodium sulphide, and in the case of heterotrophic denitrification with ethanol as a source of organic carbon. The reaction mixture was continually mixed and samples were taken at regular intervals. Samples were analyzed for pH, chemical oxygen demand



Figure 1. Double-jacket glass tempered cells for denitrification experiments.

(COD), concentration of nitrites, nitrates, sulphides, and sulphates. Specific denitrification rate ($r_{\text{N-NO}_x^-}$) and specific rate of reduction of nitrate to nitrite also known as denitratation rate ($r_{\text{N-NO}_3^-}$) are volumetric rates of decline of oxidized forms of nitrogen related to VSS concentration measured here as $\text{mg}\cdot(\text{g}\cdot\text{h})^{-1}$.

Doses of sulphide and nitrate in different experiments were calculated according to stoichiometry of autotrophic denitrification with oxidation of sulphide to sulphate as the final product [Equation (1)]. In this equation the molar ratio of N/S is equal to 1.6.

Concentration of nitrite was measured spectrophotometrically with an amide of sulphanilic acid and NED-dihydrochloride. Determination of nitrate concentration was also spectrophotometrically measured with 2,6-dimethylphenol. COD [$\text{g}\cdot\text{L}^{-1}$] was determined with potassium dichromate semimicromethod [10]. Sulphides were determined by iodometric titration with sodium thiosulfate using starch as an indicator [11]. Sulphate concentration was determined with a capillary isotachophoretic analyser EA 202A.

RESULTS AND DISCUSSION

Effect of Sulphide Loading of Biomass

Influence of different molar ratios of N/S on autotrophic denitrification was studied in two series of experiments. The aim was to find out concentration of sulphide which inhibits the process and what type and amount of intermediates are accumulated. Determination of endogenous rates $r_{\text{N-NO}_x^-}$ and $r_{\text{N-NO}_3^-}$ was carried out with no sulphide addition. Values of $r_{\text{N-NO}_x^-}$ and $r_{\text{N-NO}_3^-}$ have been determined in other experiments with addition of different doses of sulphides with respect to endogenous denitrification rate. Seen in Figure 2 are examples of autotrophic denitrification with two different molar ratios of N/S, 4.6 and 1.1. Since the stoichiometric molar ratio is 1.6 [Equation (1)], the amount of sulphide was a limiting factor for the denitrification process [Figure 2(a)]. Secondly, one was an excess of sulphide in the system [Figure 2(b)].

It is clear from Figure 2 that autotrophic denitrification, especially its first step of reduction of nitrates to nitrites is supported by a higher dose of sulphide. Table 1 summarizes $r_{\text{N-NO}_x^-}$ and $r_{\text{N-NO}_3^-}$, nitrite accumulation and sulphide biomass loading. Values of $r_{\text{N-NO}_x^-}$ and $r_{\text{N-NO}_3^-}$ are presented after subtraction of endogenous rate.

The first step of denitrification is significantly accelerated with increasing sulphide concentration. While

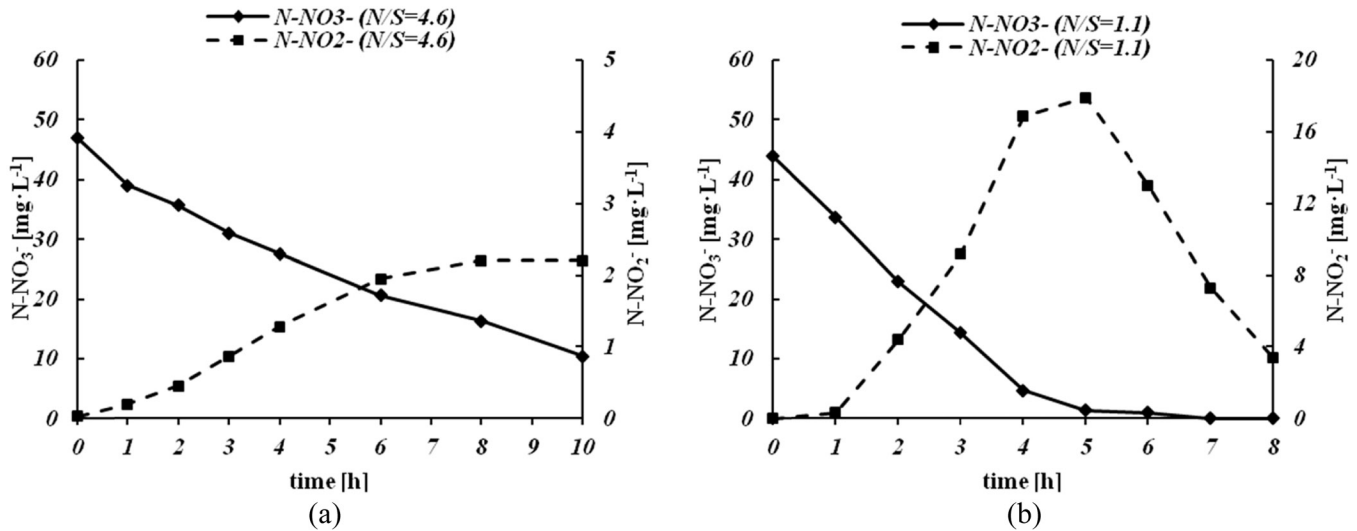


Figure 2. Course of autotrophic denitrification with a different molar ratio of N/S, (a) N/S = 4.6, (b) N/S = 1.1.

rate of nitrate reduction to nitrite increased with highest dose of sulphide approximately 8-times compared with lowest sulphide dose. Denitrification rate in the same case increased only 3-times. This also reflects relative accumulation of nitrite which with an increase of sulphides concentration changed from 5% to 50%. Experimental results show increasing sulphide concentration has a positive effect on denitrification within the range of concentrations used here. A question remains regarding whether higher sulphide loading of biomass could have an inhibitory effect on denitrification through significant accumulation of nitrite.

Purpose of following experiments was to study denitrification at higher biomass loading rates by nitrate and sulphide. Experiments were carried out with a constant stoichiometric ratio of N/S = 1.6 but with a different initial concentration of nitrate nitrogen. Results are summarized in Table 2. Values of $r_{N-NO_2^-}$ and $r_{N-NO_3^-}$ are presented after subtraction of endogenous rate.

Results confirmed that denitrification rate gradually decreased and eventually was inhibited when the limit of sulphide concentration or sulphide loading of biomass is

exceeded. Comparison of denitrification and denitrification rates show highest sulphide biomass loading strongly inhibits biomass activity and denitrification rates are lower than the endogenous one. Inhibition is more significant for the second step of denitrification (i.e., reduction of nitrite). The experiment with the highest sulphide concentration of $286 \text{ mg}\cdot\text{L}^{-1}$ illustrates that about half of initial nitrates were reduced to nitrite but this nitrite nitrogen was not further removed and remains in the system. During a 24 hour period only 5% of the total initial concentration of nitrogen was removed.

Effect of Sulphide Concentration

Experiments were carried out with the same sulphide loading of biomass but with different absolute concentrations of sulphide. From previous results the question remained whether denitrification rate is affected by absolute concentration of sulphide in the liquid or from sulphide loading of biomass. In each individual experiment sulphide and nitrate biomass loadings were the same but with different absolute concentration of sulphide. The molar ratio was N/S =

Table 1. Results of Experiments with Different Sulphide Concentrations and Constant Concentration of $N-NO_3^-$ ($50 \text{ mg}\cdot\text{L}^{-1}$).

N/S ratio	4.6	2.3	1.1	0.6
S^{2-} [$\text{mg}\cdot\text{L}^{-1}$]	25	50	100	200
$r_{N-NO_2^-}$ [$\text{mg}\cdot(\text{g}\cdot\text{h})^{-1}$]	0.19	0.42*	0.52*	0.61
$r_{N-NO_3^-}$ [$\text{mg}\cdot(\text{g}\cdot\text{h})^{-1}$]	0.23	0.74*	1.34*	1.81
Accumulation of NO_2^- [%]	4.68	28.8*	45.4*	50.1
S^{2-} [$\text{mg}\cdot\text{g}^{-1}$] [$S^{2-}\cdot\text{VSS}^{-1}$]	5	10	20	40

*Average of duplicates.

Table 2. Results of Experiments with Different Concentrations of Nitrate and with a Constant Molar Ratio of N/S = 1.6.

$N-NO_3^-$ ratio	100	150	200
S^{2-} [$\text{mg}\cdot\text{L}^{-1}$]	143	214	286
$r_{N-NO_2^-}$ [$\text{mg}\cdot(\text{g}\cdot\text{h})^{-1}$]	0.38	0.15	-0.33
$r_{N-NO_3^-}$ [$\text{mg}\cdot(\text{g}\cdot\text{h})^{-1}$]	1.40	0.78	0.33
Accumulation of NO_2^- [%]	60.9	63.8	41.4
S^{2-} [$\text{mg}\cdot\text{g}^{-1}$] [$S^{2-}\cdot\text{VSS}^{-1}$]	28.6	42.8	57.2

Table 3. Results of Experiments with Different Concentrations of Sulphides and a Constant Molar Ratio of N/S = 1.1.

	10	20	30	40
N-NO ₃ ⁻ [mg·L ⁻¹]	10	20	30	40
S ²⁻ [mg·L ⁻¹]	21.5	42.9	64.4	85.9
VSS [g·L ⁻¹]	1	2	3	4
r _{N-NO₂⁻} [mg·(g·h) ⁻¹]	0.94	1.23	1.49	1.26
r _{N-NO₃⁻} [mg·(g·h) ⁻¹]	1.58	2.16	2.54	2.83
Accumulation of NO ₂ ⁻ [%]	34.7	31.6	27.5	32.5
S ²⁻ [mg·g ⁻¹][S ²⁻ ·VSS ⁻¹]	21.5	215.	21.5	21.5

1.1; concentrations of VSS were 1, 2, 3 and 4 g·L⁻¹; and initial concentrations of N-NO₃⁻ were 10, 20, 30, and 40 mg·L⁻¹, respectively. The course of denitrification in all experiments was more or less the same. Only r_{N-NO₂⁻} and r_{N-NO₃⁻} slightly differed. Results are summarized in Table 3.

Despite the fact biomass loading of sulphides was identical in all experiments resulting denitrification rates are different. Consistent with previous results a more significant increase of rate of nitrate reduction to nitrite with increasing concentrations of sulphide occurred. Increase of the total denitrification rate was slower even as highest sulphide concentration decreased. Results suggest that a more significant effect on r_{N-NO₂⁻} and r_{N-NO₃⁻} results from absolute concentration of sulphide in liquid rather than from sulphide loading of biomass.

Effect of Sulphide Concentration on Heterotrophic Denitrification

Influence of sulphides on heterotrophic denitrifica-

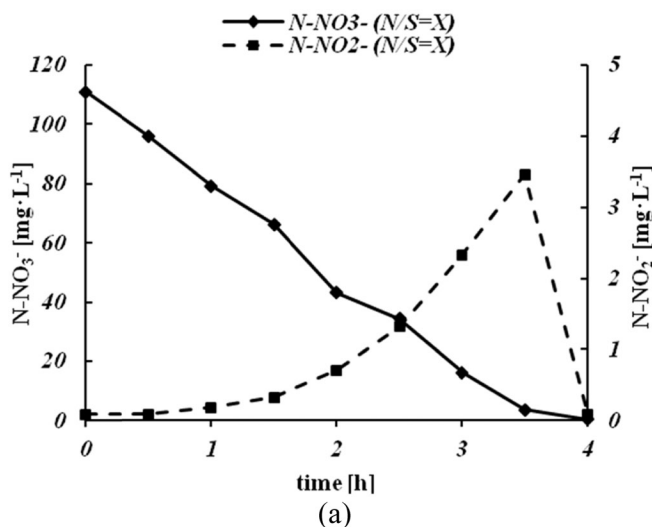


Table 4. Results of Experiments with Different Doses of Sulphide (Molar Ratio of N/S = X, 3.2, 1.6, and 0.8; 210% Stoichiometric Dose of Organic Substrate).

Molar ratio N/S	X	3.2	1.6	0.8
S ²⁻ [mg·L ⁻¹]	0	71.5	143	286
Dose EtOH* (% of stoichiometry)	210	210	210	210
r _{N-NO₂⁻} [mg·(g·h) ⁻¹]	5.94	3.50	1.55	0.32
r _{N-NO₃⁻} [mg·(g·h) ⁻¹]	6.14	4.80	3.08	1.50
Accumulation of NO ₂ ⁻ [%]	3.12	23.6	46.7	74.1
S ²⁻ [mg·g ⁻¹][S ²⁻ ·VSS ⁻¹]	0	14.3	28.6	57.1

* EtOH is ethanol.

tion was monitored. A source of organic carbon was also added as an addition to sulphides. Initial concentration of N-NO₃⁻ in all experiments was 100 mg·L⁻¹. To ensure a sufficient amount of organic substrate for complete denitrification the sample was dosed with COD in a ratio to N of approximately of 6:1. Dose of ethanol corresponded to 210% of stoichiometry. The first experiment was carried out without sulphides. These were fed in the other experiment in molar ratios (N/S) of 3.2, 1.6, and 0.8.

Examples from two selected results are presented in Figure 3. Figure 3(a) displays denitrification with no sulphide (N/S = X) and Figure 3(b) displays course of denitrification with a molar ratio of N/S = 1.6.

It is evident in Figure 3 that the addition of sulphide significantly slowed down the process of heterotrophic denitrification. Determined rates r_{N-NO₂⁻} and r_{N-NO₃⁻} for each molar ratio N/S are listed in Table-4.

It is obvious that any addition of sulphide caused inhibition of denitrifying sludge non-adapted to sulphides. Even at 50% of the stoichiometric sulphide dose and at a sulphide concentration of 70 mg·L⁻¹, the

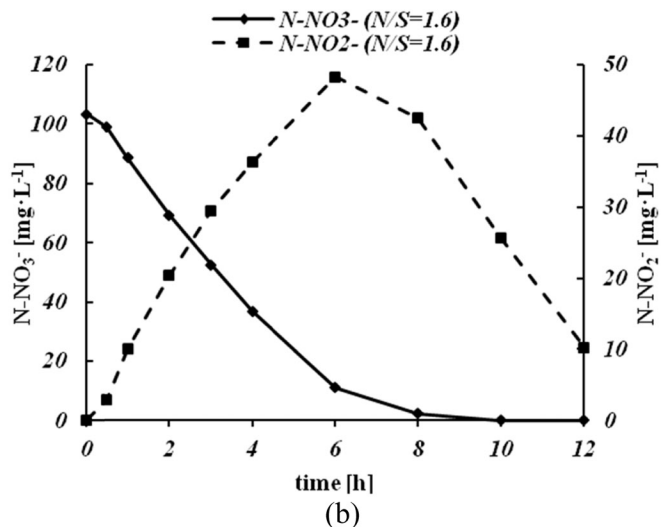


Figure 3. Course of heterotrophic denitrification based on amount of sulphide in liquid, (a) N/S = X and (b) N/S = 1.6.

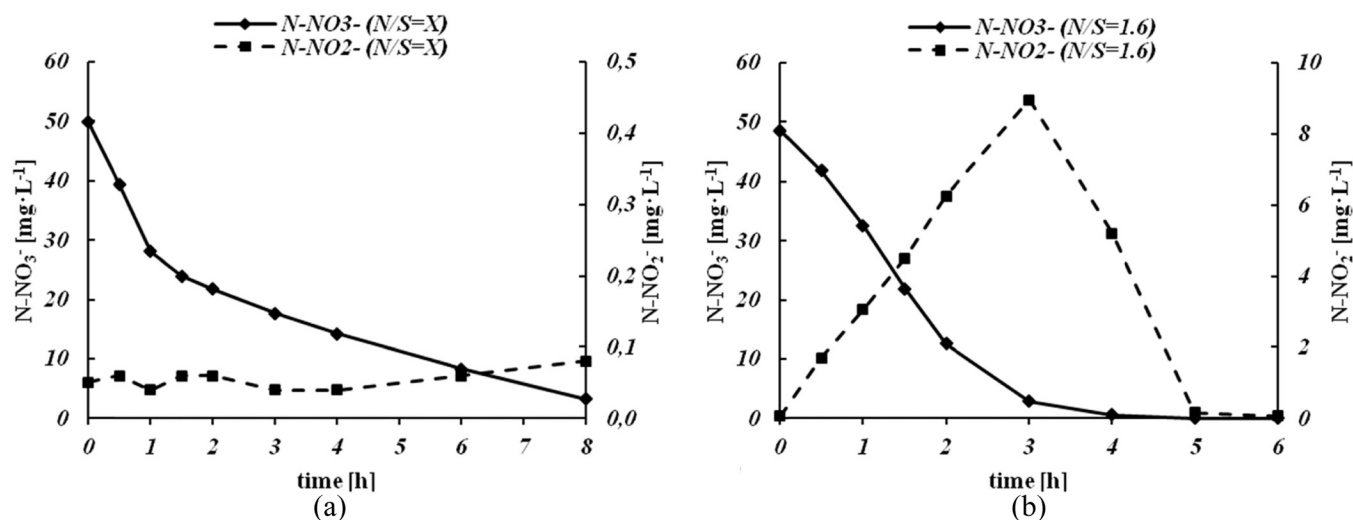


Figure 4. Results of experiments with a stoichiometric dose of organic substrate and an additional sulphide dose of $N/S = 1.6$ (a) $N/S = X$ and (b) $N/S = 1.6$.

denitrification rate was slower at 41%. Above the sulphide concentration of $200 \text{ mg}\cdot\text{L}^{-1}$ the denitrification process slowed down completely and the denitrification rate was reached at the endogenous level. Comparison of denitrification rates, rates of nitrate reduction to nitrite, and rates of nitrite accumulation shows a significant inhibitory effect of sulphide that may be seen mainly in the second step of denitrification (i.e., nitrite reduction).

Denitrification with Sulphide and Organic Substrate

Previously, the series of experiments studied influence of a stoichiometric or smaller amount of organic substrate and a stoichiometric amount of sulphide on effectiveness of denitrification. Initial concentration of nitrate nitrogen was $50 \text{ mg}\cdot\text{L}^{-1}$. One of the experiments was carried out as a reference without sulphide. Comparison in Figure 4 of denitrification in experiments with a stoichiometric dose of organic substrate and different ratios of N/S is displayed in Figure 4(a) ($N/S = X$; no sulphide dose) and Figure 4(b) ($N/S = 1.6$).

Denitrification proceeded rapidly from the beginning where an exact stoichiometric amount of organic substrate was available to microorganisms without addition of sulphide. However, since not only is denitrification consuming the organic substrate, it was exhausted in about half the time and denitrification rate then slowed considerably and reached an endogenous rate. In further experiments and on the contrary denitrification rate was slower at the beginning due to sulphide inhibition of denitrification but after exhaus-

tion of organic substrate sulphides served as electron donors and a complete denitrification occurred earlier. Summary results for all experiments are presented in Table 5. For denitrification rate and rate of nitrate reduction to nitrate two numbers were evaluated. The first values correspond to rates during the first two hours of experimental duration without sulphide addition. ($N/S = X$) correspond only to rate in the first hour. The other values represent rates for the entire experimental duration.

A stoichiometric dose of organic substrate is insufficient for complete heterotrophic denitrification as expected. If there is no other source of electrons after exhaustion of organic substrate the process significantly slows down. When sulphides are added the slowdown after a depletion of organic carbon is not so significant and autotrophic denitrification may start. It is interesting to compare rates in the first and last experiments which appeared identical. Even for a very small amount of organic substrate (25% of stoichiometric dose) sulphides are able to replace organic carbon to the extent that the final denitrification rate and denitrification rate

Table 5. Results of Denitrification with Different Doses of Ethanol and a Molar Ratio of $N/S = 1.6$.

Molar ratio N/S	X	1.6	1.6	1.6
S^{2-} [$\text{mg}\cdot\text{L}^{-1}$]	0	71.5	71.5	71.5
Stoichiometry EtOH [%]	100	100	50	25
r_{N-NO_x} [$\text{mg}\cdot(\text{g}\cdot\text{h})^{-1}$]	4.34–1.16	2.99–1.94	2.25–1.31	2.00–1.18
r_{N-NO_3} [$\text{mg}\cdot(\text{g}\cdot\text{h})^{-1}$]	4.34–1.17	3.60–3.05	2.63–1.84	2.29–1.57
Accumulation of NO_2^- [%]	0.16	18.4	21.5	21.9
S^{2-} [$\text{mg}\cdot\text{g}^{-1}$] [S^{2-} -VSS ⁻¹]	0	14.3	14.3	14.3

* EtOH is ethanol.

with 100% only organic substrate and without addition of sulphide are comparable to $1.16 \text{ mg} \cdot (\text{g} \cdot \text{h})^{-1}$ and $1.18 \text{ mg} \cdot (\text{g} \cdot \text{h})^{-1}$, respectively.

CONCLUSIONS

The aim was to verify effect of sulphides on the course and effectiveness of autotrophic and heterotrophic denitrification by non-adapted activated sludge and to explore the possibility of utilization of autotrophic denitrification with sulphides in real conditions without sludge adaptation.

In experiments without organic substrate it has been shown that sulphide addition has a positive effect on removal rate of oxidized forms of nitrogen. However, the positive effect was observed only to a certain limit of sulphide concentration in the liquid estimated at $200 \text{ mg} \cdot \text{L}^{-1}$. When the sulphide concentration is exceeded a significant amount of nitrite is accumulated in the medium and no nitrogen was practically removed from the system.

During heterotrophic denitrification with excess of organic substrate all sulphide concentrations in the tested range caused inhibition of the process. Denitrification rate slowdown was already caused by concentration of sulphide at around $50 \text{ mg} \cdot \text{L}^{-1}$.

In the case of autotrophic denitrification there also occurred a significant accumulation of nitrite at a concentration exceeding $200 \text{ mg} \cdot \text{L}^{-1}$ and the denitrification rate fell below the endogenous level.

If a joint application of organic substrate and sulphides was used a slight inhibition of denitrification occurred at the beginning. However, if after the exhaustion of organic substrate the sulphides are available in the medium slowdown of denitrification was not observed. This indicates that heterotrophic and autotrophic denitrification do not run together but the organic substrate is used preferably and only after ex-

haustion of organic substrate autotrophic denitrification with sulphide. Results offer a possible solution for wastewater with a lack of organic carbon as well as for some sources of dissolved sulphides available especially from external desulphurization of biogas.

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Degradation of Hydroxytyrosol in Olive Oil Mill Wastewaters using Thermosensitive Zinc Phthalocyanine—Modified Titanium Dioxide

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ABSTRACT: Zinc phthalocyanine-modified titanium dioxide, ZnPc/TiO₂ was used as a photocatalyst for degradation. The photocatalyst showed enhanced activity for degradation of a hydroxytyrosol (Hy) present in olive oil mill wastewater. Photodegradation of Hy was found to be enhanced by the ZnPc/TiO₂ photocatalyst under illumination with solar light compared to nonmodified TiO₂ as a photocatalyst. Enhancement is attributed to cooperative functions for two components of the photocatalyst, zinc phthalocyanine and TiO₂ semiconductor for generation of •OH radicals. Reaction intermediate components were identified by gas chromatography–mass spectrometry (GC–MS).

INTRODUCTION

WASTEWATERS from olive oil mills (OMW) may create pollution due to high concentrations of phenols and polyphenols [1,2], which are moderately toxic and may inhibit biological treatment [3]. Low-molecular mass phenolic compounds such as hydroxytyrosol, tyrosol, p-hydroxyphenyl acetic acid, p-coumaric acid, and caffeic acid are usually present in OMW together with catechol-melanin polymers [4].

OMW usually has a low pH, high electrical conductivity, and high concentrations of free polyphenols because olive pulp esters are liberated. Glycoside hydrolysis takes place during oil extraction. OMW wastewater has BOD (biochemical oxygen demand) in the range of 12–63 gL⁻¹ and COD (chemical oxygen demand) in the range of 80–200 gL⁻¹. These values are approximately 200–400 times higher than in typical municipal sewage [5].

It is not surprising research efforts have been directed towards development of efficient treatment technologies for reduction of such polluting residues in OMW. Advanced oxidative processes (AOPs) have been suggested as an alternative for environmental remediation [6–17].

It has been recently demonstrated that semiconduct-

ing materials, which are mediating photocatalytic oxidation of organic compounds, may be an alternative to conventional methods for removal of organic pollutants in wastewater [10,12]. A variety of semiconductor powders (e.g., TiO₂ and ZnO) acting as photocatalysts have already been used.

When TiO₂ was used as a photocatalysis relatively high activity occurred under ultraviolet (UV) light which exceeds a band-gap energy of 3.0 or 3.2 eV [7, 8] for rutile or anatase crystalline phase, respectively. It has been noted that use of sunlight, indoor light, or light from another artificial source has been receiving greater attention [17,18].

Attachment of dyes to the TiO₂ surface is a convenient use of solar light for sensitization of photocatalytic degradation reactions. Several attempts utilizing this method have been made by attaching dyes including Rose Bengal, chlorophylls, porphyrins, phthalocyanines, or a ruthenium bipyridyl complex [19].

Metallophthalocyanines (MPc) have many characteristics which suggest they might be useful as sensitizers for semiconductor electrodes. These characteristics include the following: they absorb light strongly in the visible region [20], it is possible to vary redox potentials by changing the central metal ion [21], they are very stable and insoluble in water [22], and they often exhibit semiconducting behavior [21].

Literature indicates they have been used as photosensitizers for treatment of pollutants in wastewater

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[23]. A major compound of OMW is Hy, so this study was limited to evaluation of this pollutant. TiO₂ particles were coated with zinc phthalocyanine (ZnPc) for studies on photocatalytic decomposition of hydroxytyrosol. Hydroxytyrosol is a compound occurring in OMW by way of irradiation via solar light. Sensitization by zinc phthalocyanine (ZnPc) is of interest for a number of reasons. These ZnPc photosensitizing dyes, when excited by photons of lower energy allow for injection of electrons from these species to the conduction band of the semiconductor increasing concentration for charge carriers. Electrons in turn may be transferred to reduce organic acceptors adsorbed onto the catalyst surface. Thus, photocatalyst composites containing a photosensitizing dye associated with the photoactive semiconductor have in general improved photocatalytic activity.

MATERIALS AND METHODS

Titanium dioxide, P25, was kindly provided by Degussa Hüls. Other materials including Phthalic anhydride ($\geq 99\%$), ammonium chloride ($\geq 99.5\%$), urea (95%), ammonium molybdate (99.98%), zinc acetate ($\geq 99\%$), ethanol ($\geq 99.5\%$), nitrobenzene ($\geq 99.0\%$), methanol ($\geq 99\%$), dimethylformamide (DMF) (99.8%), dimethylsulfoxide (DMSO) ($\geq 99.7\%$), NaOH ($\geq 98\%$), and H₂SO₄ (95.0–98.0%), pro analysis were purchased from Sigma-Aldrich. *N,O*-Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was obtained from Pierce.

Synthesis of ZnPc

Phthalic anhydride (4.32 g, 16 mmol) ammonium chloride (0.47 g, 9 mmol), urea (5.8 g, 97 mmol), ammonium molybdate (0.34 g, 0.03 mmol), and zinc acetate were thoroughly mixed. The mixture was added and slowly heated to 180°C nitrobenzene (10 mL).

The mixture was heated for 6 hours at 180°C. The crude product obtained was a dark solid cake. The product was treated in a Soxhlet extraction apparatus with methanol for 24 hours. The product was finally heated under reflux in 20 mL of ethanol for 4 hours. It was then cooled, filtered, and dried in an oven at 120°C for 12 hours.

ZnPc Characterization

The UV–vis absorption spectrum of ZnPc is displayed in Figure 1. Notice two groups of absorption

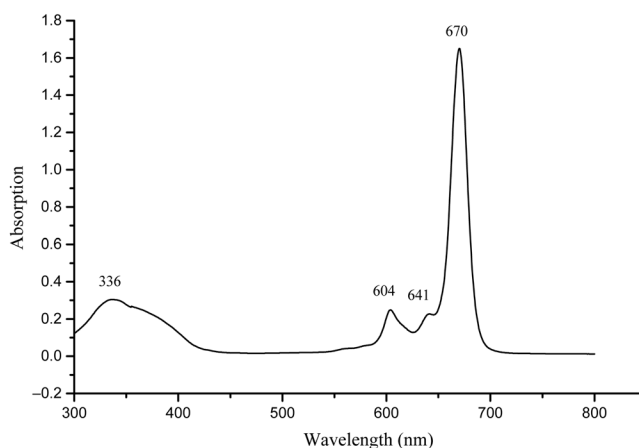


Figure 1. UV-Vis spectrum of ZnPc in DMF solution at room temperature.

bands at 550–750 nm and at 300–450 nm are present [24,25,26].

Preparation of ZnPc/ TiO₂ Composite Catalyst

Composites were prepared by coating TiO₂ particles with zinc phthalocyanine [13,26,27] dissolved in a mixture consisting of 50% dimethylsulfoxide, 20% ethanol, and 30% dimethylformamide at 60°C. The required amount of TiO₂ (P25, Degussa) covering compositions of 2.5% (m/m of ZnPc) was gradually added to the dye solution while stirring and heating. This resulted in a suspension with a homogeneous appearance. The suspension was maintained, stirred, and heated until almost complete solvent evaporation. The material presenting a creamy consistency was washed several times with distilled water with vigorous stirring to remove residues and remaining organic solvent. Finally, the composite was dried at a temperature between 70 and 80°C for 24 hours.

Analyte Characterization

X-ray powder diffraction patterns of TiO₂ and different composites result in peaks which are due only to anatase and rutile phases (figure not shown) suggesting ZnPc is adsorbed onto the semiconductor surface. This agrees with results based on scanning tunneling microscopy using different metal phthalocyanines which concluded this class of compounds lies flat on the semiconductor surface [28].

Equipment and Light Source

A recirculating photocatalytic reactor was construct-

ed and is displayed in Figure 2. The reaction vessel consists of a small cylinder constructed of Pyrex glass. It is connected to a reactor of 25 cm in length with a glass cylinder of 5 cm in diameter. The side is covered by aluminum foil and the top surface is transparent glass. A Masterflex pump (Vernon Hills, IL) was used to circulate the solution.

Analytical Methods

Two techniques were used for identification of reaction intermediate components. They included high pressure liquid chromatography (HPLC) and gas chromatography coupled with mass spectrometry (GC/MS).

High Performance Liquid Chromatography (HPLC)

Control of hydroxytyrosol decomposition, as well as tentative identification of intermediates and final product have been realized using an HPLC equipped with a UV-VIS detector series Acem 9000 (190–460 nm). A C-18 reverse phase column was used (ZORBAX XDB) with a length of 150 mm and a diameter of 4.6 mm. Used mobile phase was a mixture of water—

acetonitrile H_3PO_4 89.9:10:0.1 with a flow rate of 0.75 mL min^{-1} . Tentative identification of sub-products and final reaction products was based on either retention time or UV spectra at $\lambda = 210 \text{ nm}$.

Gas Chromatography Coupled with Mass Spectroscopy (GC/MS)

GC–MS analysis was performed using a HP model 5975B inert mass-selective detector. The mass spectra for the silylated analytes were obtained via electron impact ionization (EI) at 70 eV. The MS detector transfer line was maintained at 280°C and tuning was performed on a daily basis with perfluorotributylamine (PFTBA) with masses m/z 69, 219, and 502.

The GC was equipped with a capillary DB-5MS column of 30 m length, 0.25 mm i.d., and 0.25 μm film thickness (Agilent Technologies, J&W Scientific Products, USA). The carrier gas was helium and injected at a 1 mL min^{-1} flow rate. The oven temperature program was 1 min at 100°C , then ramped from 100 to 260°C for 4 min, and finally 10 min at 260°C . The chromatograph was equipped with a split/splitless injector in split mode. The split ratio was 100:1. One hundred microlitres of bis(trimethylsilyl)acetamide was added to

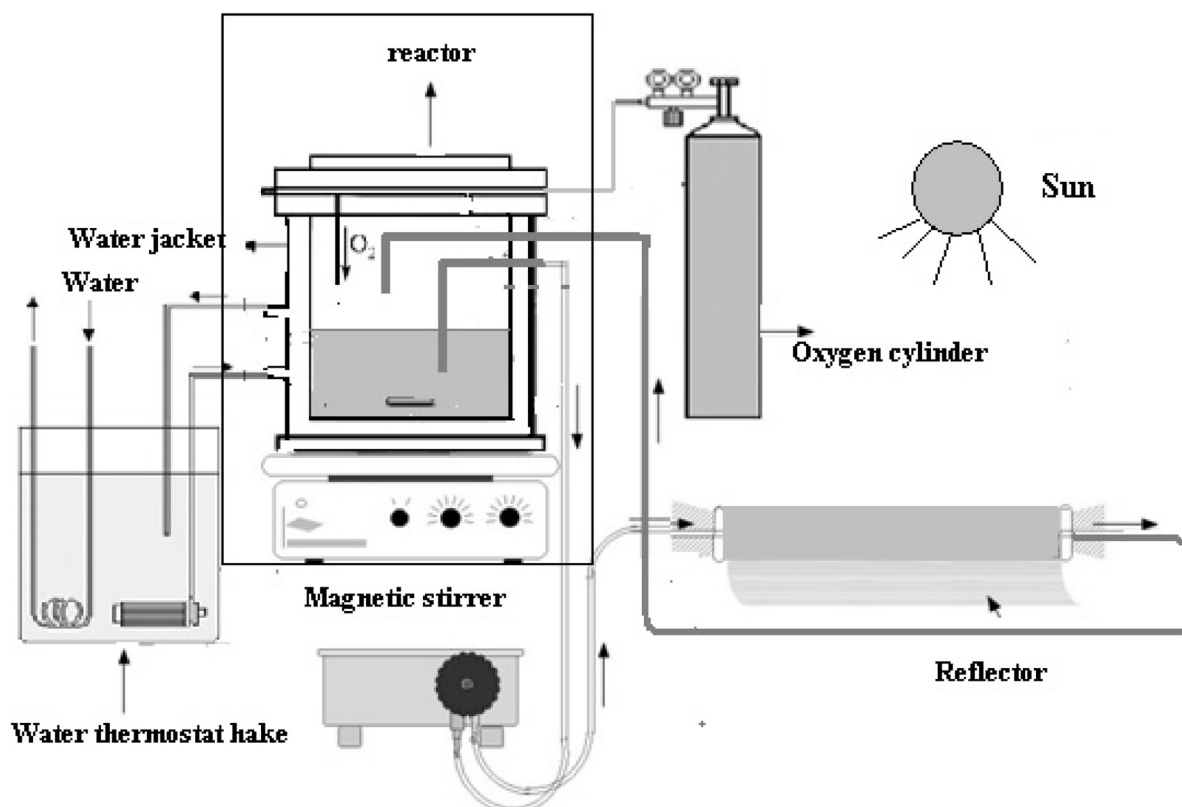


Figure 2. Schematic diagram of the solar photocatalytic reactor system.

100 μL of ethyl acetate extract from hydroxytyrosol solution. The final solution was incubated for 60 min at 80°C .

Experimental Procedure

Hydroxytyrosol at a purity around 90% and a major compound of OMW was prepared in our laboratory after chromatographic purification of ethyl acetate extract from OMW using a silica gel (Lichroprep RP; 25–40 mm) column according to methods in literature [29]. Identity of hydroxytyrosol and its trimethylsilylated derivative was confirmed using GC-MS. Purity of hydroxytyrosol was further confirmed with HPLC.

Under solar light irradiation, ZnPc/TiO₂ composites (1 g) were added in low-molecular mass. Hydroxytyrosol (250×10^{-3} mol/L) was circulated continuously with O₂ at a flow rate of 300 mL min⁻¹. Temperature of irradiated solutions was constant at 30°C using a cooling bath. Before light irradiation, the catalyst was stirred first in the dark to establish an adsorption/desorption equilibrium. At predetermined time intervals, small aliquots (2 mL) were withdrawn and filtered (0.45 μm Millipore filter) to remove catalyst particles. The pH of the filtrate was then adjusted to a suitable value. These aliquots were used for monitoring the degradation progress and for analyzing intermediate compounds. HPLC and GC/MS were used in conventional mode for monitoring the degradation progress.

All solutions were prepared with double distilled water. Proper amounts of 0.1 mol/L NaOH or 0.1 mol/L H₂SO₄ were used to adjust suitable pH values. The hydroxytyrosol concentration was measured using a UV-Visible Spectrophotometer Shimadzu 1650PC. A calibration plot based on Beer–Lambert's Law was established by relating absorbance to concentration.

Degradation rate was calculated using Equation (1),

$$\text{Degradation rate (\%)} = \left(\frac{1 - C_i}{C_0} \right) \times 100 \quad (1)$$

where C_0 is initial concentration of hydroxytyrosol (mol/L) and C_i is instant concentration at sample at time t (mol/L). Photocatalytic reaction kinetics for the photocatalyst may be expressed using the Langmuir–Hinshelwood (L–H) model [31].

When initial concentration of hydroxytyrosol is lower the reaction rate may be expressed as displayed in Equation (2) [32]:

$$\ln(C_i/C_0) = -k_{app}t \quad (2)$$

where k_{app} is the apparent pseudo-first-order reaction rate constant and t is reaction time. A plot of $\ln(C_i/C_0)$ versus t will yield a slope of $-k_{app}$.

Experiments under solar irradiation were performed in July of 2010 from 12:00 P.M. to 4:00 P.M. Irradiation was measured using a radiometer (PMA 2100 Solar Light Co.) with sensor positioned horizontally. Irradiation measured varied from 15 to 20 W m⁻².

RESULTS AND DISCUSSION

Effect of Initial Concentration of Hydroxytyrosol

The initial concentration of the pollutant in water is one of the most important parameters to consider and has to be analyzed as it may affect treatment process efficiency. The experiment was carried out by varying initial concentration of hydroxytyrosol from 50 to 200 mgL⁻¹ as a result. It may be observed that normalized concentration (i.e., concentration at time t divided by initial concentration) of the Hy decreased with time (Figure 3). Increase in the initial concentration of Hy reduced degradation efficiency of the catalyst. A possible explanation for this behavior is that as initial concentration of Hy increases the path length of photons entering solution decreases. The reverse effect is observed for low concentrations. This thereby increases the amount of photon absorption by a catalyst of lower concentration. The Hy molecule rather than the catalyst may absorb a significant amount of solar light and may also reduce catalytic efficiency.

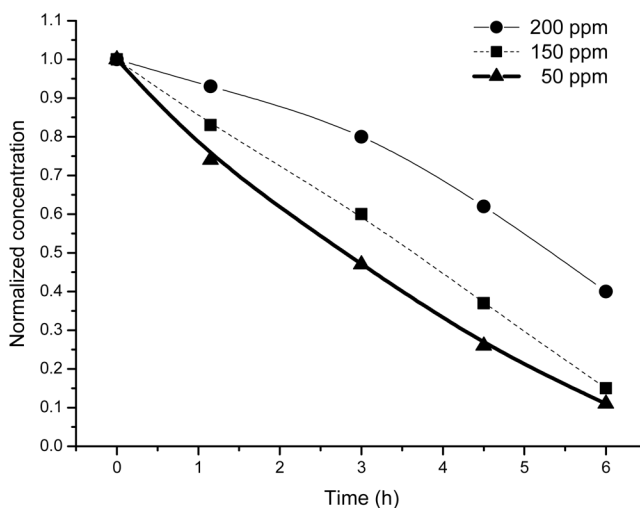


Figure 3. Normalized concentration profile at different initial concentrations.

Effect of Catalyst, Solar Light, and O₂ on Photocatalytic Oxidation of Hydroxytyrosol

The effect from a catalyst, solar light, and O₂ on photocatalytic oxidation of hydroxytyrosol is displayed in Figure 4. Approximately 10% of total hydroxytyrosol was adsorbed on ZnPc/TiO₂ under irradiation of solar light for 6 h without O₂ (Figure 4, curve 1). About 20% of total hydroxytyrosol was gradually oxidized after 6 h with catalyst and O₂ (Figure 4, curve 2) or with solar light and O₂ (Figure 4, curve 3). Upon solar light irradiation for 6 h in the presence of catalyst and O₂ more than 97 % of total hydroxytyrosol was degraded (Figure 4, curve 4). Evidently, under conditions of solar light and O₂ hydroxytyrosol may be efficiently catalyzed by ZnPc/TiO₂.

Effect of pH on Photocatalytic Oxidation of Hydroxytyrosol

Figure 5 displays the effect of pH on photocatalytic oxidation of hydroxytyrosol in the presence of ZnPc-TiO₂ under solar light irradiation. Obviously, the degradation reaction may proceed over a wide range of pH values. Conversion of hydroxytyrosol changed with solution pH. 72% conversion of hydroxytyrosol at pH 3, 65% at pH 5, and 32% at pH 7 were observed within 6 h of irradiation with an initial solution containing hydroxytyrosol ($250 \times 10^{-3} \text{ molL}^{-1}$). However, conversion of hydroxytyrosol increased with an increase of

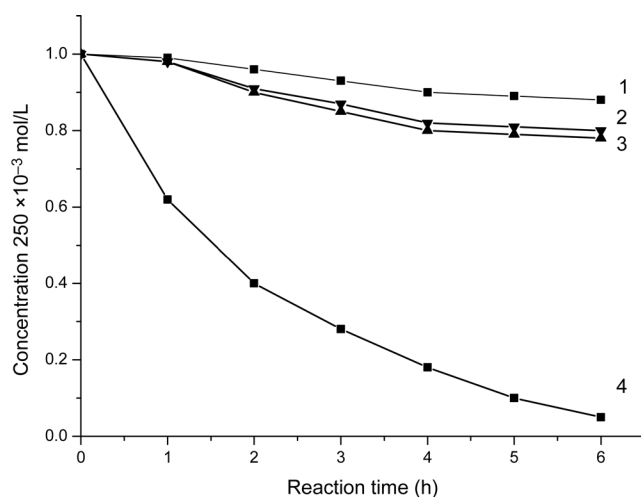


Figure 4. Effect of catalyst, visible light, and O₂ on photocatalytic oxidation of total hydroxytyrosol: (1) with PcZn/TiO₂ and visible light irradiation and without O₂, (2) with PcZn/TiO₂ and O₂ and without visible light irradiation, (3) with visible light irradiation and O₂ and without ZnPc/TiO₂, and (4) with ZnPc/TiO₂ and O₂ and with visible light irradiation. All reactions proceed at pH of 11, with 1g PcZn/TiO₂, [hydroxytyrosol] = $250 \times 10^{-3} \text{ mol/L}$, and $T = 30^\circ\text{C}$.

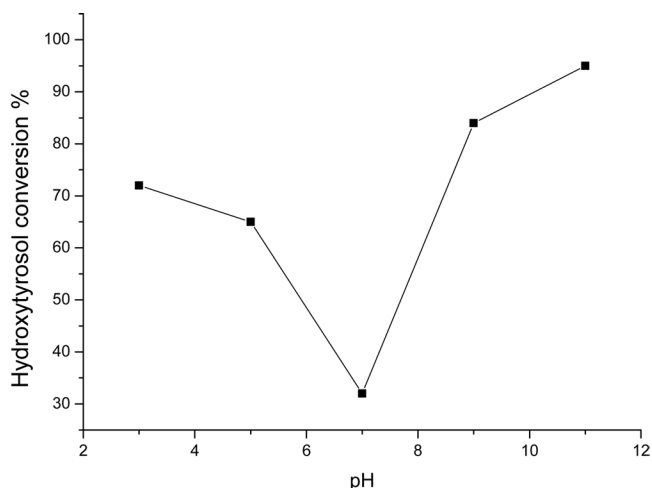


Figure 5. Effect of pH on photocatalytic oxidation of hydroxytyrosol. Experimental conditions: 1 g of PcZn/TiO₂ and [hydroxytyrosol] = $250 \times 10^{-3} \text{ mol/L}$.

pH when the reaction was conducted in alkaline media. The conversion rate of hydroxytyrosol at the same irradiation time was 84% at a of pH 9 and 95.1% at a pH of 11. Increasing activity at higher pH levels is attributed to increasing concentration of hydroxytyrosol ions.

Therefore, phenol is less reactive than hydroxytyrosol in the photo-oxidation process [32]. The reason conversion of hydroxytyrosol decreases with an increase of pH at pH < 7 will be further investigated.

Effect of Co-existing Anions on the Degradation of Hydroxytyrosol

Figure 6 presents effects of some anions on degradation of hydroxytyrosol. Surprisingly, presence of NO₃⁻ accelerated the degradation reaction of hydroxytyrosol. When adding $0.01 \text{ mol L}^{-1} \text{ NaNO}_3$ the *k_{app}* value reached $74.44 \times 10^{-3} \text{ min}^{-1}$ while addition of $0.01 \text{ mol L}^{-1} \text{ NaCl}$ decreased the *k_{app}* value to $5.23 \times 10^{-3} \text{ min}^{-1}$. Presence of carbonates may also strongly decrease degradation efficiency by scavenging hydroxyl radicals.

MECHANISM OF PHOTOCATALYTIC OXIDATION OF HYDROXYTYROSOL

Figure 7 displays the HPLC chromatogram for samples analyzed for photocatalytic oxidation of hydroxytyrosol. Four principal peaks were identified by comparing retention time to those of previously established standards. Retention times at $\lambda = 210 \text{ nm}$ used in the HPLC analysis for identification of initial, intermediate, and final products are provided in Table 1. Identifi-

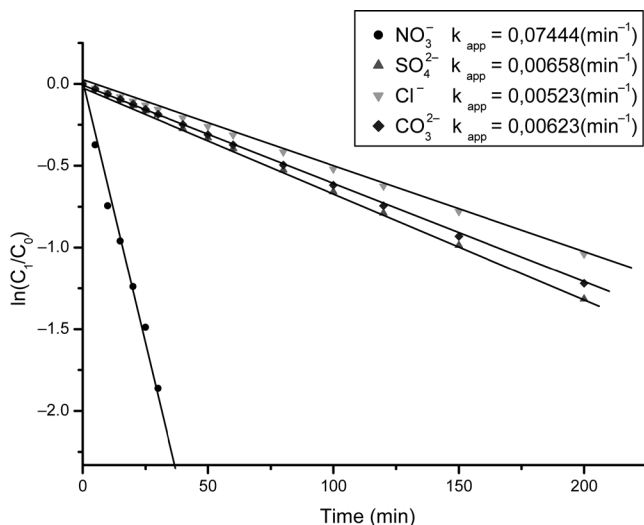
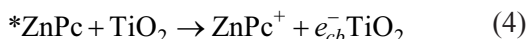


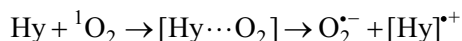
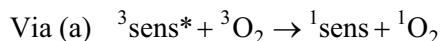
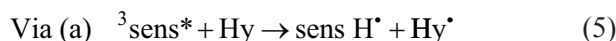
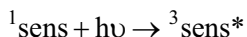
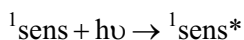
Figure 6. Influence of co-existing anions on degradation rate of hydroxytyrosol. ZnPc/TiO₂ amount was 1 g, [hydroxytyrosol] = 250 × 10⁻³ mol/L, and pH = 11.

ties of oxalic acid (A), formic acid (B), maleic acid (C), and muconic acid (D) are provided in Figure 7.

Electronic and photocatalytic properties of zinc phthalocyanine dyes have been widely studied [33]. Solar light intensity should be able to photoexcite TiO₂/ZnPc composites generating e⁻/h⁺ pairs in both species and consequently favoring electron injection from ZnPc excitons to the TiO₂ conduction band. See Equations (3) and (4) [34].



According to mechanisms proposed by Matsuura and Saito [35] who have extensively studied this may be easily explained through hydrogen abstraction by primary formation of a phenoxyl radical which may be produced either by a triplet excited sensitizer (via a) and/or by singlet oxygen (via b). See Equation (5).



Based on results and from the literature [35], the proposed mechanism of enhancement of photocatalytic oxidation of hydroxytyrosol is displayed in Fig-

Table 1. Retention Times of Compounds Used in HPLC Analysis.

Compounds	Retention time (min)
Oxalic acid (A)	1.88
Formic acid (B)	2.40
Maleic acid (C)	3.55
Muconic acid (D)	8.65

ure 8. Triplet oxygen (³O₂) was transformed to singlet oxygen (¹O₂) by triplet-triplet energy transfer when the ZnPc was excited by solar light. Ortho-diphenol was oxidized by ¹O₂ with formation of ortho-quinones [36]. Aliphatic carboxylic compounds such as oxalic acid, formic acid, maleic acid, and muconic acid were formed from further oxidation of hydroxytyrosol by ³O₂ and ¹O₂. Some of the hydroxytyrosol was mineralized to CO₂ and H₂O.

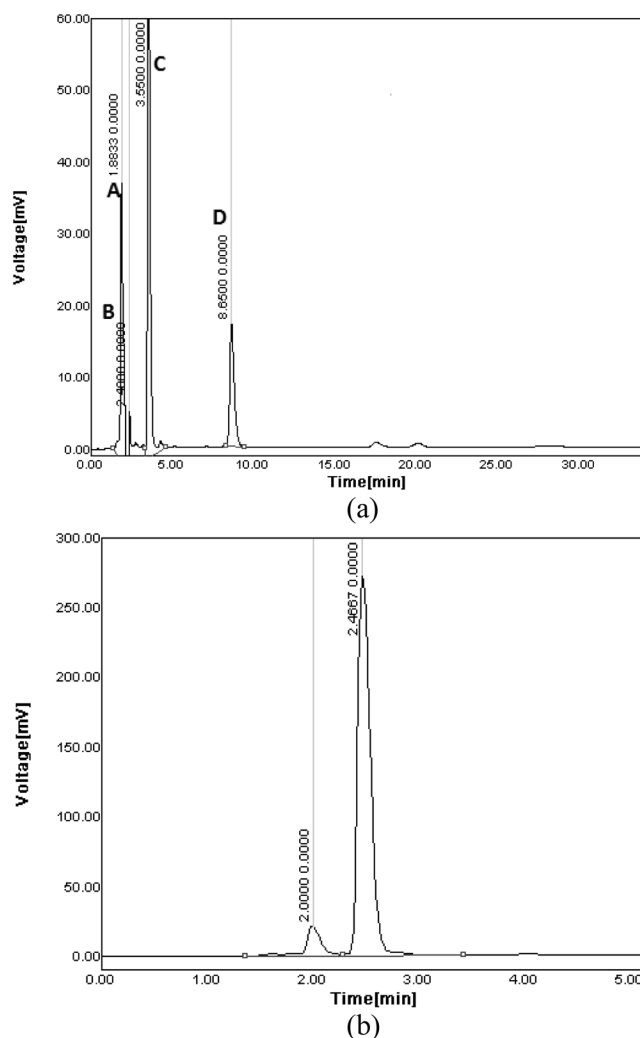


Figure 7. Chromatogram of hydroxytyrosol decomposition.

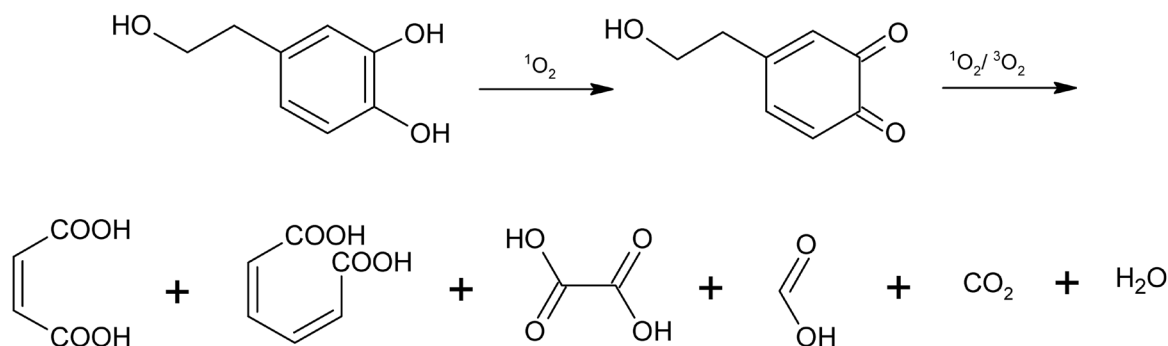


Figure 8. Proposed mechanism of photocatalytic oxidation of hydroxytyrosol.

CONCLUSION

It was discovered that presence of the catalyst zinc phthalocyanine modified titanium dioxide ZnPc/TiO_2 under a solar light source and increased degradation of the hydroxytyrosol compound existing in OMW. Degradation efficiency of the substrate decreases with an increase of concentration of the substrate. Experiments have also suggested that upon solar light irradiation for 6 h in the presence of a catalyst and O_2 more than 97% of total hydroxytyrosol was degraded. Effect of co-existing anions on degradation reaction kinetics was investigated. Identified products are intermediates of degradation hydroxytyrosol (oxalic acid, formic acid, maleic acid, and muconic acid) which were identified by HPLC and GC/MS. Finally, conversion of hydroxytyrosol also increased with an increase of pH. A mechanism for enhancement photocatalytic oxidation of hydroxytyrosol is possible.

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Developments of European Standardisation on Sludge: Guidelines for Good Practice

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ABSTRACT: Need for a safe utilisation/disposal of sludge is well recognised by European Countries which consider development of standardized characterization and management procedures to properly perform sludge operations necessary. To this purpose, CEN established the TC308 whose main objectives are to contribute to development of European Directives on sludge, give orientation to producers/users on how to meet legislation requirements, and give a larger diffusion to the standards thus favouring the global market. Within this framework, many standard methods for sludge characterization have been published together with Guidelines for good management practice whose content is outlined in this paper.

INTRODUCTION

SLUDGE MANAGEMENT is a critical issue facing modern society due to rapid increase in its production as a result of extended sewerage, new work installations, and up-grading of existing facilities. Characterization is an important step in sludge management. This is recognized by European Union Countries which consider it necessary to develop standardized characterization procedures for properly performing sludge operations and to correctly comply with legal requirements.

The European Committee for Standardization (CEN) established Technical Committee 308 (TC308) whose main tasks are production of standards for chemical, biological, and physical characterization of sludge, of guidelines for good practice, or Technical Reports for different methods of sludge use and disposal and for operational practices. These documents are intended to (1) harmonise sludge practices across Europe, (2) promote and enable sustainable development, (3) support production and revision of European Directives relevant to sludge, and (4) support European stakeholders and provide orientation to producers and users on how to meet legislation requirements.

Scope of CEN/TC308 includes sewage sludge and all other sludge types potentially having similar adverse environmental effects. CEN/TC308 also cooper-

ates with other CEN and ISO programmes. Work of CEN/TC308 has been organized in 3 working groups respectively dealing with the (1) standardization of methods for determining chemical, biological, and physical sludge parameters (WG1); (2) preparation of guidelines of good practice for different options of sludge use and disposal (WG2); and (3) preparation of documents on current and future needs in sludge management (WG3). Present status of work is reported in CEN/TC308 [1,2].

Further, to improve comparability of standards developed by different CEN/TCs for measuring the same parameters in different contexts and thus avoiding unnecessary duplication of work, CEN/TC308 promoted development of the “Horizontal” project with the objective of developing “horizontal and harmonised European standards” in the fields of sludge, treated bio-waste, and soils.

CEN/TC308 DOCUMENTS

CEN/TC308 documents include standard methods and guidelines or Technical Reports. They are extensively discussed in the following.

Standard Methods

Regarding agricultural use of sewage sludge following the European Directive 86/278/EEC, whose update is expected for many years but not yet available, determination of nutrients and so-called pollutants (heavy

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metals and organic substances) has been one of the basic goals of CEN/TC308. Methods for determination of heavy metals via aqua regia digestion and for that of phosphorus and nitrogen compounds were developed as well as for sampling, water content, dry residue, and organic carbon content. Work on determination of organic micropollutants covered development of methods for PAH (polycyclic aromatic hydrocarbons), PCB (polychlorinated biphenyls), NP/NPE (nonylphenol and its ethoxylates), LAS (linear alkylsulfonates), phthalates, and AOX (adsorbable organic halogenated substances).

Another important aspect regarding management of sludge is that relevant to evaluation of its biological stability because it strongly influences sludge handling (e.g., risks of development of bad odours, effective acceptability in a landfill, and possible reuse for environmental purposes). Regarding the aforementioned reasons, development of a standardised procedure for evaluating biological stability of sludge has just started.

Evaluation of physical properties is also of great importance as this knowledge allows for prediction of sludge behaviour when handled and submitted for almost all treatment, storage, and utilization/disposal operations. Regarding these properties, standards for determination of Capillary Suction Time (CST, also useful for qualitative evaluation of sludge centrifugability) [3,4], specific resistance to filtration, compressibility, settleability, thickenability, calorific value, and drainability (to evaluate sludge suitability to be thickened by means of a draining process) have been published.

Physical consistency is another physical parameter of fundamental importance as it strongly affects almost all treatments, utilization, and disposal operations (e.g. pumping, transportation, dewatering, drying, and land-filling) [5]. A technical report on this subject has been published [6] and standards relevant to determination

of flowability through an Extrusion tube viscometer and to that of solidity through a Vane shear apparatus have been submitted for the formal approval procedure which includes carrying out of round-robin tests for evaluating repeatability/reproducibility of measurements. However, alternative validation procedures must be considered when circulation of samples may involve alteration of characteristics thus avoiding reliable comparison of results or when large quantity samples are needed like in the case of most physical parameters. One could consist in examination of “synthetic sludge” samples to be on-site prepared on the basis of a defined recipe and ingredients (a technical report CEN/TR 16394 has been prepared). Another could involve circulation of “analysts” and not of “samples” thus allowing analysts from participating laboratories to meet in a common location close to a place where samples are collected. A validation procedure for physical parameters has been adopted by CEN/TC308 [7] and successfully applied when needed.

Guidelines for Good Practice

Seen in Table 1, Guidelines for good practice for different methods of sludge use and disposal published by CEN/TC308 are listed according to year of publication. If not differently stated, guidelines are applicable to sludges from urban wastewater treatment plants, treatment plants for industrial wastewater similar to urban wastewater, and water supply treatment plants.

Further, a basic scheme is necessary for deciding on which sewage sludge use/disposal options to choose. Relevant CEN/TC308 guidance documents have been drafted and are seen in Figure 1. Contents of guidelines or technical reports published by CEN/TC308 are summarized in the following.

Table 1. List of Published Guidelines for Characterization of Sludges (updated January 2012).

EN 12832: 1999 (known as Guide 1)	Utilisation and disposal of sludges—Vocabulary (under review)
CR 13846: 2000	Guide to preserve and extend sludge utilization and disposal routes
CR 13983: 2003 (known as Guide 5)	Good practice for sludge utilization and land reclamation
CR 13767: 2004 (known as Guide 6)	Good practice for sludge incineration with or without grease and screenings (new edition in preparation, merging with CR 13768, Guide 13)
CR 13768: 2004 (known as Guide 7)	Good practice for combined incineration of sludges and household waste (new edition in preparation, merging with CR 13767, Guide 13)
CR 15126: 2005 (known as Guide 8)	Good practice for the landfill of sludge and sludge treatment residues
CEN/TR 15473: 2007 (known as Guide 9)	Good practice for sludges drying
CEN/TR 15584: 2007	Guide to risk assessment especially in relation to use and disposal of sludges
CEN/TR 15809: 2008 (known as Guide 10)	Characterization of sludges—Hygienic aspects—Treatments
CEN/TR 13097: 2010 (known as Guide 4)	Good practice for sludge utilisation in agriculture
CEN/TR 13714: 2010 (known as Guide 2)	Good practice for sludges management in relation to use or disposal

Guide 1 (EN 12832:1999)

The first guide dealt with sludge “Vocabulary” to ensure uniformity of expression in all European Countries. This guide is now under systematic review but to avoid any possible contradiction revision will be completed only when the general “Glossary of wastewater engineering terms” in preparation by CEN/TC 165 is published. All terms and definitions in the Glossary above at that time will be adopted by CEN/TC308 unless it is necessary to introduce new specific concepts or terms not currently contained in the glossary.

Evolution regarding the term “sludge” within this framework often replaced by the term “biosolids” has to be considered. It is true that sludge disposal industry

has great difficulty convincing the public that a material with an ugly name such as “sludge” could actually be beneficial so the term “biosolids” was introduced. However, this does not change the substance. The name change, although useful as a public relations tool, could create misunderstandings if wrongly used. A similar evolution of the terminology has also not found a wide acceptance in other languages mainly due to translation difficulties.

Therefore, it should be time to definitely clarify the word “sludge” has to be used when speaking of solids within a wastewater treatment plant and out of it when it is disposed of without any utilization. We could revert to using “biosolids” only during the moment the solids in whatever form are destined for some form of beneficial use.

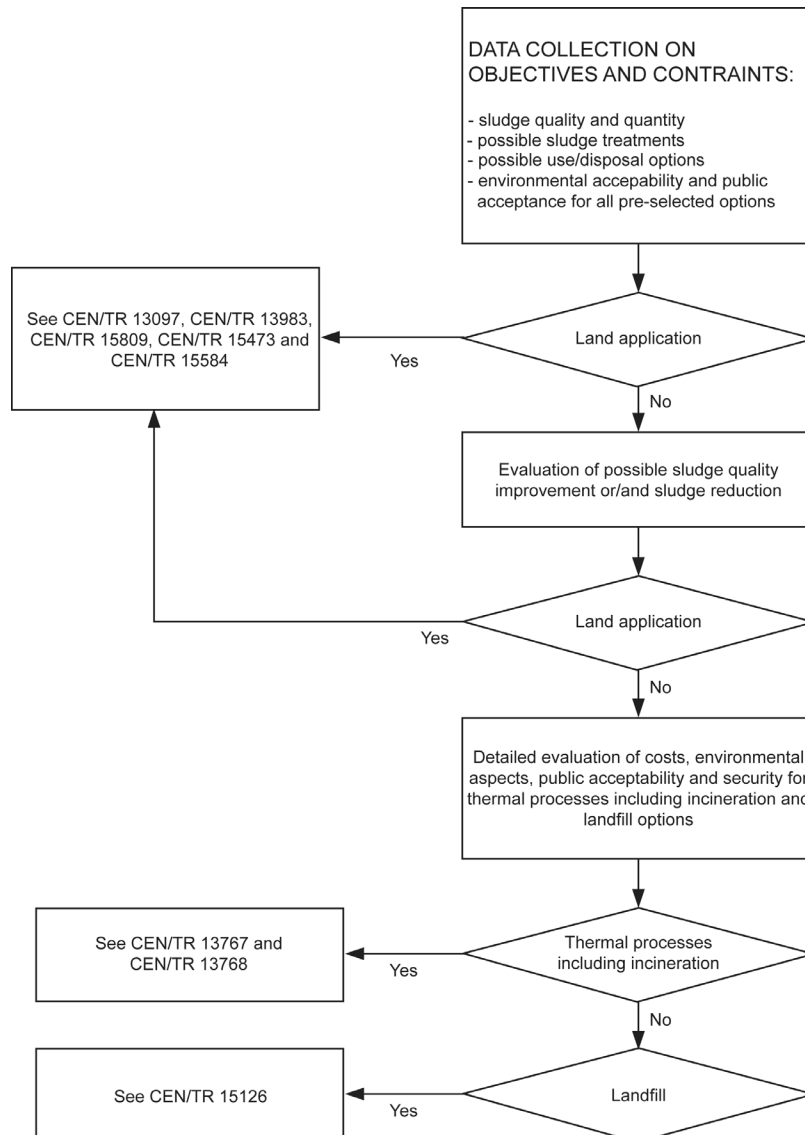


Figure 1. Basic scheme for deciding applicability of CEN/TC 308 Guidelines.

Guide 2 (CEN/TR 13714:2010)

The purpose of this guide is to outline management of sludge both upstream and downstream of the treatment process to ensure it is suitable for available outlets. The guide refers to all types of sludges covered by CEN/TC 308 including those from treating industrial wastewater similar to urban wastewater and from water treatment supply plants. This document gives recommendations for good practice but existing national regulations remain in force.

Considering likely quality of sludges, it should be noted that municipal wastewater sludge is composed of materials that have already been disposed of and are consequently likely to be more variable than many industrial sludges that arise from sourced materials or water treatment sludges arising from surface water or groundwater.

This guide considers management of sludges against a waste hierarchy, management of sludge quality, and an optional evaluation process for determining available methods. Sludge quality is central to the development of good practice for sludge production in relation to its final destination (use or disposal). Sludge quality depends on composition of upstream materials and type of treatment including post-treatment storage. As a general rule a sludge of high quality is likely to be acceptable to a large range of outlets given greater operational flexibility. High quality sludges are likely to be suitable for those outlets associated with maximum sustainability and minimum environmental pollution.

Consistency of different sludge properties is a critical aspect of sludge quality and of the ability to determine its end destination. Therefore, standard methods should be used when available to measure quality parameters of sludge.

Guide 4 (CEN/TR 13097:2010)

This Guide describes a good practice for use of sludge in agriculture where permitted. It is applicable to all sludges that may be used on land as a source of plant nutrients, soil improver, and/or amendment for crop production.

Despite differences in statutory controls between sewage sludge and other sludges, use of all types of sludge should follow good practice to maximise benefits for crops or soils; to minimise potential risks of environmental contamination; and adverse impacts on plant, animal, and human health to ensure sustainability, energy efficiency, and cost-effectiveness.

Sludge producers should be aware that if a sludge is used as a fertilising or alkaline amendment, national or European fertiliser or liming regulations may apply. The document assumes an evaluation of sludge utilisation has already been made and a decision was made that use of sludge within a land spreading policy is the best option. For evaluation and decisions for use of sludges other documents have been developed (see CR 13714, CR 13846).

Many countries and/or local administrations have regulations and/or standards and/or codes of practice applicable to the use of some of the types of sludge that are within the scope of this guide. However, it cannot and does not attempt to summarise or take account of these regulations because of their very wide range. It is thus essential this document is read in the context of conditions that prevail locally.

Guide 5 (CEN/TR 13983:2003)

This guide helps one to indicate what sludge utilisation within reclamation programmes of disturbed land aiming to address in a general qualitative way key issues which will determine in each particular case whether, how much, and which type of sludge may be used. Status of a technical report for this document has been chosen because most of its content is not completely in line with practice and regulation for each member state.

Because of the wide range of reclamation sites where sludge use as a soil ameliorate or source of plant nutrients is beneficial, different potential final uses of these sites and recommendations for applications should be considered on a site by-site basis. It is far beyond the scope of these guidelines to describe all possible situations and individual ways in which sludge may be used. The aim is to address in a general qualitative way key issues which determine in each particular case whether, how much, and which type of sludge may be used. Planning considerations are emphasised due to the fact a general scheme can be adopted as a common procedure in nearly all situations.

Guides 6 (CR 13767: 2004) and 7 (CR 13768: 2004)

Purpose of original guides 6 and 7 is to describe a good practice for sludge incineration and co-incineration, respectively, to ensure safe and economical operations. The status of technical report also in this case has been chosen because most of their content is not completely in line with practice and regulation in each

member state. Recommendations for a good practice are given but existing national regulations concerning the subject remain in force. In particular, the purpose of guide 6 is to describe a good practice for sludge incineration to ensure a safe and economical operation. The main goals are to:

- describe principal design parameters relevant to different process schemes;
- assess operating procedures able to perform optimal energy consumption, emissions control, and equipment durability;
- provide responsible authorities with well-established and easily applicable protocols for control purposes; and
- promote diffusion of this practice and favouring the formation of a public opinion consensus.

Potential advantages of high temperature processes include reduction of volume and mass of sludge, destruction of toxic organic compounds if present, and energy recovery. Priority should be given to reduction of pollutants at the origin and to recover if technically and economically feasible valuable substances (phosphorous and potassium) in sludge and derived products. This guide is not applicable to co-incineration of sludge and other wastes either urban or hazardous and for use of sludge in cement kilns.

Guide 7 constitutes a framework within which the combined incineration process may be proposed in addition to and/or as a substitution for field spreading, waste site disposal (landfilling), specific incineration, or any other process. Combined incineration should abide by the European Directives and should comply with relevant regulations and recommendations in force within each member state to reduce as far as possible negative effects on the environment such as pollution of air, ground, surface, underground waters, and on human and animal health. This concern therefore relates to pre-treatment of sludge in plants, transfer of material to a treatment centre, destruction process, treatment of gaseous discharge into the atmosphere, future of different by-products stemming from combustion, and treatment of liquid effluents potentially resulting from the process.

Priority should be given also in this case to reduction of pollutants at the origin and or to recovery of valuable substances (e.g. phosphorus) in sludge and derived products if technically and economically feasible. Regarding a process and company quality approach, relevant issues are:

- exploiting operational data and statutory inspections carried out;
- rendering the process reliable, optimising and perpetuating it, and guaranteeing a permanent development; and
- maintaining a climate of confidence between authorities, sludge producers, transporters, incineration plants, and waste disposal site operators allowing services be provided on a contractual basis.

When necessary a distinction can be made between existing facilities and new incineration plants. Considering that increasing energy costs and needs for development of sustainable energy production have resulted in a growing application not only of sludge incineration but also for other thermal processes it has been decided that it is best to unify and update the above guides by considering in a new guide (numbered as Guide 13) all options (e.g. incineration, gasification, pyrolysis) available for thermally processing sludge. Possibility of recovering phosphorus out of the ashes is playing a fundamental role.

Within this framework it is also to be considered that European legislation in this field is now deeply changing. In particular, existing regulations on thermal treatment of waste especially directives on incineration of wastes (2000//76/CE) and on integrated pollution prevention and control (2008/1/CE) have been merged in the recent Directive 2010/75/EU on industrial emissions.

Regarding European regulation, an incineration plant is dedicated for thermal treatment of wastes with or without recovery of combustion heat generated. This includes incineration by oxidation of waste as well as other thermal treatment processes such as pyrolysis, gasification, or plasma processes in so far as substances resulting from treatment are subsequently incinerated. This is not applicable if gases resulting from the thermal treatment of waste (pyrolysis or gasification) are purified to such an extent that they are no longer a waste and cause emissions no higher than those resulting from burning of natural gas. Incineration of liquid and solid waste whether hazardous or non-hazardous is covered by this directive whose deadline for its full applicability has been set for January of 2014.

Guide 8 (CR 15126:2005)

This technical report describes a good practice for disposal of sludges and sludge treatment residues into a landfill where national regulations permit. All rec-

ommendations from this document constitute a framework within which the landfilling process may be proposed as a substitution for field spreading or in addition for specific or combined incinerations or for any other process.

This document should be read in context of the requirements of Directive 1999/31/EC. Landfill of waste applies to the landfill of sludge and any other relevant regulations, standards, and codes of practice that may prevail locally within member states.

Guide 9 (CEN/TR 15473:2007)

This Guide gives guidance on (1) drying processes, (2) characteristics of dried sludge products, and (3) recycling or disposal of dried sludge products from urban wastewater treatment plants. Sludges of other origin like sludge from water supply or industrial treatment plants are not exactly in the scope of this guide. However, handling of most of those sludges will comply to a large extent with advice given in this guide.

Status of this document as a technical report has been chosen because much of its content is not completely in line with practice and regulations for each member state. Therefore, this document gives recommendations for good practice concerning drying of sludges. However, existing national regulations remain in force.

Various directives will apply to thermally dried sludge products depending on the use to which they are to be ascribed including Directive 86/278/EEC for recycling to land, Directive 1999/31/EC for disposal to a landfill, Directive 2000/76/EC for incineration and energy recovery, and Directive 94/9 for equipment intended for use in potentially explosive atmospheres. This document should be read in the context of the requirements of these directives and any other relevant regulations, standards, and codes of practice that may prevail locally within member states.

Guide 10 (CEN/TR 15809:2008)

Guide 10 provides guidance on good practice for hygienic aspects of use of sludges on land. It concerns microbiological life as an important part of the management of sludge in accordance with environmental requirements (human, animal, and plant health). It applies to all utilisations of sludge in the environment (e.g., agriculture supplement, land reclamation, cover for landfills, manufacturing of soil, and more). It also provides information about existing treatment processes for meeting hygienic requirements.

Status as a technical report in this case has been chosen because most of its content is not completely in line with practice and regulations for each member state so existing national regulations remain in force.

This technical report is applicable to sludge produced by urban wastewater treatment plants and systems. Sludges of other origin are not exactly in the scope of the document but handling of most of these sludges will comply to a large extent with advice provided in this document.

Guide 11 (WI 308069) (in publication)

It is recognized everywhere that sludge management is a major problem regarding water and wastewater treatment as it can account for up to 50% of total operational costs. Effectiveness and cost of sludge treatment and disposal operations are strongly affected by volume and consequently by water content or solids concentration. Therefore, thickening and dewatering are important steps in the total sludge processing work flow and have serious impact on subsequent operations.

This guide now submitted for the formal approval procedure describes good practices for sludge conditioning, thickening and dewatering, technical and operational aspects, and characterization methodologies.

This Report is applicable for sludges from urban wastewater treatment plants, treatment plants for industrial wastewater similar to urban wastewater, and water supply treatment plants. This document may also be applicable to sludges of other origin.

CONCLUSIONS

A number of requirements such as guide and/or limit values are contained in sludge regulations but methods for determining respective parameters are often not described. Therefore, a definition for standardized procedures and methods are a necessary support for sludge management as they allow not only prediction of the behaviour of sludge when handled and submitted to different management operations but also correct fulfillment of legal requirements, comparison and consistency of application, and improvement of stakeholder and public confidence.

To provide necessary support for development of European Directives directly or indirectly involving sludge, the European Committee for Standardization (CEN) established Technical Committee 308 (TC308) whose scope is standardization of methods and proce-

dures employed for sludge characterization and production of guidelines for good practice.

CEN/TC308 activities have been addressed regarding production of (1) standardised methods for evaluation of chemical, biological, and physical properties/parameters and (2) several Guidelines of good practice and Technical Reports on different aspects of sludge management.

Contents of Guidelines of good practice dealing with sludge vocabulary, management in relation to use or disposal, utilization in agriculture, utilization for land reclamation purposes, incineration and co-incineration, landfilling, drying, hygienic aspects, and thickening and dewatering have been outlined and may provide useful information to those dealing with sludge and/or biosolids.

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