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# CONTENTS

## Research

# Nitrogen Removals by *Ceratophyllum Demersum* from Wastewater

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**ABSTRACT:** Ammonium removal from wastewater has been of considerable concern for several decades. This study focused on nitrogen removal by *Ceratophyllum demersum.* Treatments included raw municipal wastewater (RMW), treated municipal wastewater (TMW), and diluted fresh latex (DFL). Results showed the *Ceratophyllum demersum* reduced ammonium and nitrate more than 62% and 41.66%, respectively, from three treatments in the first six days of non-aeration. Results showed that in aeration conditions the concentration of ammonium and nitrate were increased in aquatic solution after a second and third six day period of aeration, because *Ceratophyllum demersum* uptake 100% of lead from solution.

## INTRODUCTION

ONE of the most well-understood consequences of human pollution associated with the environment results from eutrophication [8,17]. Nitrogen (N) contained in agricultural effluents and industrial wastewaters are mainly responsible for eutrophication [41]. Ammonium nitrogen is one of the essential nutrients that can lead to water eutrophication when present in excess amounts [28].

There are many different kinds of human activities that generate wastewater with large quantities of ammonium: petrochemical, pharmaceutical, fertilizer and food industries, leachates produced by urban solid waste disposal sites, and waste from pig farms. Disposal of this type of waste is a serious environmental problem because free ammonia, diluted in water, is one of the worst contaminators for aquatic life [10].

The main problem with biological treatment of high strength industrial wastewater is that high concentrations of ammonium, or nitrite, inhibit nitrification [2]. Biological nitrification-denitrification is the most common process for nitrogen removal from wastewater and especially for municipal wastewater. The nitrification process involves biological oxidation of ammonia ($NH_4$-N) to nitrite ($NO_3$-N) by two genera of autotrophic bacteria. This process is simultaneous with the removal of biodegradable organic compounds such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) by heterotrophic bacteria [4,5,21].

Nitrifying bacteria have specific growth rates much slower than heterotrophic bacteria in activated sludge processes. Nitrifying bacteria are also very sensitive to factors such as pH because of modification of the acid–base equilibrium, temperature, and toxic compounds including both heavy metals and xenobiotic organic chemicals [9,16,24].

Lead (Pb) is a major pollutant in both aquatic and terrestrial ecosystems. In many forest ecosystems in Europe and North America Pb has accumulated to high levels [12,26]. Uptake of mineral nutrients has been shown to be depressed by Pb. In *Phaseolus vulgaris*, Pb treatment lowered the Zn level in leaves. In *Cucumis sativus* seedlings, Pb decreased the uptake of K, Ca, Mg, Fe, and $NO_3$ [6,7], and in *Zea mays* the uptake of Ca, Mg, K, and P [44].

Nitrate ($NO_3^-$) contamination of groundwater is a worldwide problem. Nitrate in drinking water is regulated by environmental agencies around the world. A high concentration of nitrate may cause health problems (methanoglobanemia) in infants.

Nitrate contamination of aquifers is often linked to agricultural (chemical fertilizer, manure, and animal feed lots) and nonagricultural (wastewater and solid waste disposal) sources [39,45].

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Most nitrogen in domestic wastewater is the product of our eating habits and food preparation, body exudates washed off in the bath or shower, and products washed from clothes. Cleaning chemicals also contribute organic compounds in varying amounts. These organic compounds require microbial activity to degrade them [35].

Biological [27] and physicochemical treatment methods are conventionally used strategies for ammonium nitrogen removal. The biological process is economical for wastewater treatment, but it is often not effective for high concentrations of ammonium nitrogen removal due to a shortage of carbon sources for denitrification. Various methods of physico-chemical processes can be applied to treat high concentrations of ammonium nitrogen such as air stripping [30], ion exchange [20], membrane separation [36], and chemical precipitation [15,38,50].

Submerged aquatic vascular plants are known to absorb nutrients such as nitrogen (N) and phosphorus (P) and far in excess of their normal metabolic requirements [48]. Thus, considerable amounts of nutrients can be stored in plant dominated littoral areas of aquatic ecosystems. Nutrient uptake and storage by aquatic plants is an integral part of the biogeochemical cycle of both natural wetland ecosystems [32] and treatment wetlands [22,37]. Because some species of submerged aquatic vegetation assimilate nutrients directly from the water column, this community may play an important role in maximizing nutrient removal for treatment wetlands [14].

Iamchaturapatr *et al.* (2007) researched nutrient removals for 21 aquatic plants and said that aquatic plants could remove nitrogen and phosphate from wetlands. Additionally, the physiology of plants such as height of plant and the shape of leaves also affect overall nutrient removals in aquatic plant treatment systems.

*Ceratophyllum demersum* is a completely submersed plant and commonly seen in ponds, lakes, ditches, and quiet streams with moderate to high nutrient levels [19]. It does not produce roots. Instead it absorbs all the nutrients it requires from the surrounding water. When growing near the lake bottom it will form modified leaves which it uses to anchor to the sediment. However, it can float free in the water column and sometimes forms dense mats just below the surface [25].

*C. demersum* (*Coontail* or *hornwort*) an aquatic plant that may absorb high concentrations of most of the elements such as phosphorous and nitrogen from solutions in non-aeration conditions [11].

The aims of current study are (1) to investigate maximum nitrogen absorption by *C. demersum* during two different conditions, non-aeration during 18 days and aeration during 18 days immediately following non-aeration and (2) to investigate absorption capacity of nitrogen in aeration conditions after non-aeration conditions by *C. demersum.*

## MATERIALS AND METHODS

*Ceratophyllum demersum* is an aquatic plant found in rivers. These aquatic plants were collected from the Zayandehrud River located at Esfahan-Iran in the spring season. Plants were thoroughly washed with tap water to remove any sediment particles attached to their surfaces and then were placed in three treatments with four replications. These three treatments included monocultures raw municipal wastewater (RMW), treated municipal wastewater (TMW), and diluted fresh latex from industrial compost of Esfahan (DFL). They were poured into three pots with 4 replications (volume 6 L). Each microcosm was planted with 100 g (fresh weight) of *C. demersum*. The air temperature at Khorasgan University during this study was between 28 and 32°C. However, the temperature of wastewater plants were located in was between 24 and 26°C. Experiments were performed in an outdoor area under natural daylight for 18 days of non-aeration conditions and after the 18 days with aerated conditions. Losing volume from each pot due to evapotranspiration was compensated by adding distilled water to the original level every 6 days of the period. After 18 days non-aeration, samples for the three treatments and submerged plants were collected and parameters such as macronutrients in solution and plants were measured at the laboratory. Preliminary tests were accomplished for the three treatments (TMW, RMW, and DFL) at the beginning of the experiment and after 18 days of non-aeration conditions (Table 1).

After the first experiment which took 18 days of non-aeration conditions, air circulation was added to create an aeration condition for all treatments. The new experiment continued like the first experiment for 18 days. Samples were collected 4 times over an 18-day period, on days 0, 6, 12, and 18. At the end of the first experiment *C. demersum* was sampled for measuring absorption of macronutrients such as nitrogen. Nitrate and ammonium of samples were measured according to standard methods [23].

Collected plants were thoroughly washed in distilled water and oven dried at 80°C. Dried plant mate-

rials were powdered and wet digested in $HNO_3$:$HClO_4$ (3:1, v/v) at 70°C [49]. Total Nitrogen (TN) was determined by the Kjeldahl digestion, distillation, and titration method [34].

All data collected was analyzed with the Statistical Package for the Social Sciences software (SPSS) (version 16.0) and compared with Duncan's multiple range tests.

## RESULTS AND DISCUSSION

Table 1 shows results for the three treatments (TMW, RMW, and DFL) at the beginning and end of 18 days non-aeration.

### Investigating $NH_4^+$ and $NO_3^-$ Under Non- Aeration Condition

Foroughi *et al.* (2010) explained that ammonium $(NH_4^+)$ and nitrate $(NO_3^-)$ were removed from wastewater by Ceratophyllum demersum completely. *C. demersum* accumulated high amounts of $NH_4^+$ and $NO_3^-$ in its tissues. Concentrations of ammonium and nitrate in solutions were reduced from all treatments in the non-aeration experiment after 18 days (Table 2). According to this table, maximum percent reductions of $NH_4^+$ and $NO_3^-$ from the solutions were found after the first 6 day period.

Performing the aeration experiment after the non-aeration experiment for 18 days showed that percent reduction of ammonium and nitrate from solutions decreased. Unrooted submerged vegetation such as *C. demersum* require nutrient uptake from the water [33]. Tracy *et al.* (2003) showed that *C. demersum* is a nitrophile that can tolerate high nitrogen concentrations and has a very good removal effect on nitrogen in the water column. Aquatic macrophytes have been widely used to remove nitrogen from both wetlands and wastewater [29]. It has now been well documented in studies by Allen (1971), McRoy *et al.* (1972), Wetzel and Manny (1972a, b), and Wetzel and Allen (1972) that appreciable amounts of dissolved organic matter (DOM), including soluble nutrients, are continuously being excreted by living aquatic vascular plants. Indeed, certain researchers support the point of view that macrophytes can constitute an important part of the mechanism regulating the metabolism of an aquatic ecosystem [47].

***Table 1. Results of Three Treatments (TMW, RMW, and DFL) Under Non-aeration Conditions.***

| Parameters at the Beginning of Experiment | Treatments of Non-aeration Experiment | | |
|---|---|---|---|
| | TMW | RMW | DFL |
| TP (mg/l) | 4.48 | 13.68 | 1.2 |
| $NH_4$ (mg/l) | 90 | 135 | 60 |
| $NO_3$ (mg/l) | 60 | 60 | 90 |
| Pb (mg/l) | 0.58 | 0.59 | 0.58 |
| COD (mg/l) | 260 | 664 | 728 |
| **Parameters at the End of Experiment** | **TMW** | **RMW** | **DFL** |
| TP (mg/l) | 0.53 | 1.15 | 0.21 |
| $NH_4$ (mg/l) | 16.66 | 29.16 | 16.11 |
| $NO_3$ (mg/l) | 26.19 | 27.5 | 25.55 |
| Pb (mg/l) | 0 | 0 | 0 |
| COD(mg/l) | 64.5 | 152.75 | 189.5 |
| pH | 7.83 | 7.9 | 7.52 |

TP = total phosphorus, $NH_4$ = ammonium nitrogen, $NO_3$ = nitrate nitrogen, Pb = lead, COD = chemical oxygen demand.

***Table 2. Ammonium and Nitrate Reduction for Three Treatments (TMW, RMW, and DFL) Under Non-aeration Conditions.***

| | Ammonium Reduction (%) | | |
|---|---|---|---|
| **Parameters** | **First 6 Days** | **Second 6 Days** | **Third 6 Days** |
| RMW | 62.96 | 55 | 33.33 |
| TMW | 69.44 | 54.54 | 20 |
| DFL | 62.5 | 40 | 1.26 |
| | **Nitrate Reduction (%)** | | |
| **Parameters** | **First 6 Days** | **Second 6 Days** | **Third 6 Days** |
| RMW | 66.66 | 26.66 | 9.09 |
| TMW | 41.66 | 38.57 | 6.97 |
| DFL | 69.44 | 13.45 | 3.57 |

### Investigating $NH_4^+$ and $NO_3^-$ Under Aeration Conditions

*C. demersum* continued its life in aeration conditions for 18 days after living in non-aeration conditions. Results from the new conditions indicate that when all treatments were subjected to aeration conditions, ammonium concentration of solutions continued to decrease for the first 6 days after the aeration experiment (Figure 1). However, after 12 days and 18 days in the aeration experiment, all three treatments (TMW, RMW, and DFL) showed that concentrations of $NH_4^+$ were increasing instead of decreasing (Figure1).

Figure 2 indicates variations of $NO_3^-$ in all treatments (RMW, TMW, and DFL) under aeration conditions. Detection of $NO_3$–N in the plant cultures indicated that nitrification occurred in these treatments, whereby ammonia was oxidized to $NO_3$–N by nitrify-

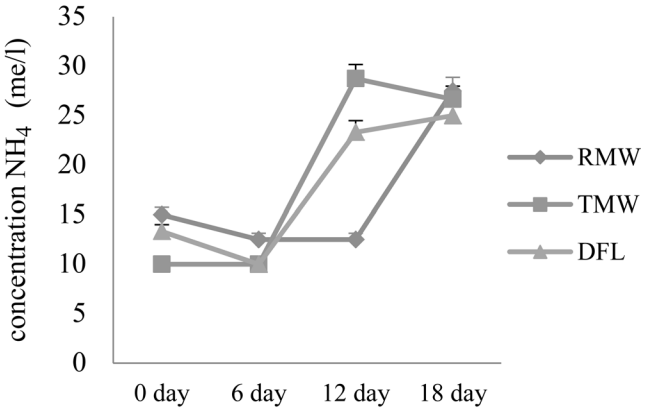


***Figure 1.*** *Ammonium variation for three treatments (RMW, TMW, and DFL) under non-aeration conditions.*

ing bacteria [42]. Evaluating the results of $NH_4^+$ and $NO_3^-$ variations in treatments showed that the releasing amount of $NO_3^-$ in aquatic solution were higher than the releasing amount of $NH_4^+$ in aquatic solution. One assumption that may demonstrate why different amounts are released between $NO_3^-$ and $NH_4^+$ is that nitrification converts ammonium to nitrate.

## Nitrogen Concentration in Plants Under Non-Aeration and Aeration Conditions

Potential removal of nitrogen by plants depends upon growth rate and tissue nitrogen content. Nitrogen (N) content of plants varies. It depends on factors such as plant species, plant age, and the parts which samples are excised from the plants. Initial absorption tests to determine nitrogen by *Ceratophyllum demersum* under non-aeration conditions showed that this plant could absorb high concentrations of nitrogen from treatments. However, after 18 days under aeration conditions the capacity of *C. demersum* to remove nitrogen from aquatic solution declined (Figure 3).

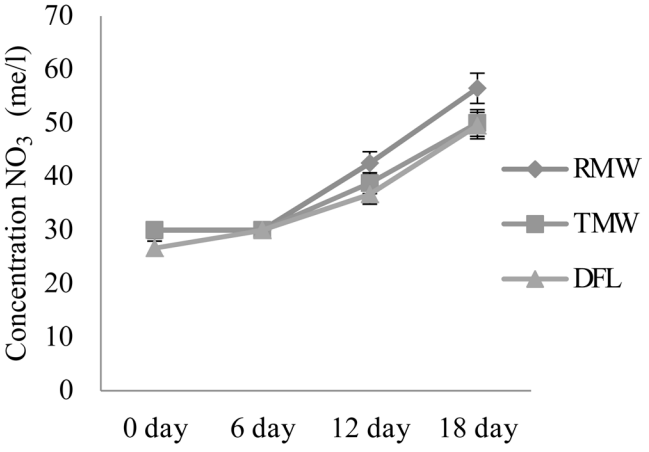


***Figure 2.*** *Nitrate variation for three treatments (RMW, TMW, and DFL) under aeration conditions.*

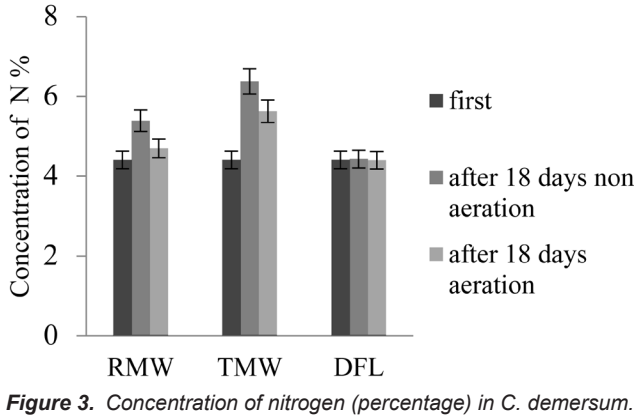


***Figure 3.*** *Concentration of nitrogen (percentage) in C. demersum.*

There are some assumptions that could explain why after 18 days of aeration *C. demersum* has a low uptake rate of nitrogen. Godbold and Kettner (1991) revealed that lead absorption by plant reduces uptake and transportation for some nutrients. Saygideger *et al.* (2004) indicated that particularly at a pH of 5.0 and 9.0 reduction in the uptake and transportation of nitrogen to plants occurred. Another argument that could support this situation is the age of the aquatic plant. Young plants, rather than older ones, are efficient in removing nutrients. If plants were not harvested at an appropriate time, nutrients from the plants leach back into the water [3].

## CONCLUSIONS

In this study, nitrogen was removed from all treatments under non-aeration conditions. However, creating aeration conditions after non-aeration conditions indicates that concentrations of nitrogen in aquatic solution increased because aquatic plants released nitrogen into solution. Possible reasons plants release nitrogen are (1) plants become old and nutrients from plants are leached back to solution and (2) in all treatments plants absorb high concentrations of Pb during the 18 days of non-aeration conditions that inhibit uptake of nitrogen during the 18 days of aeration conditions. At the same time, plants release small proportions of nitrogen into the solution causing higher nitrogen concentrations in solution. In conclusion, use of *Ceratophyllum demersum* is one of the best biological methods for refining wastewater in a short time period with high efficiency.

## REFERENCES

1. Allen, H. L., “Chemo organ trophy in epiphytic bacteria with refer-

ence to macrophyte release of dissolved organic carbon. In: Cairms JC (ed.) *The Structure and Function of Freshwater Microbial Communities*". Virgol. Polytech. Press, 1971, pp. 277–280.

2. Anthonisen, A. C., R. C. Loehr, T. B. S. Prakasan and E. G. Srineth, "Inhibition of nitrification byammonia and nitrous acid". *J Water Pollut Control Fed. 48*(5): 1976, pp. 835–52.
3. Ayyasamy, P., M. Rajakumar, S. M. Sathishkumar, K. Swaminathan, K. Shanthi, P. Lakshmanaperumalsamy and S. Lee, "Nitrate removal from synthetic medium and groundwater with aquatic macrophytes". *Des alination 242*; 2009, pp. 286–296.
4. Barth, E. F., M. B. Ettinger, B.V. Salotto, G.N. McDermott, "Summary Report on the Effects of Heavy Metal on the Biological Treatment Processes". *Journal of the Water Pollution Control Federation, 37* (1); 1965, pp. 86–96.
5. Blum, D. J. W. and R. E. Speece, "A database of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations". *Research Journal of the Water Pollution Control Federation, 63* (3), 1991, pp. 198–207.
6. Burzynski, M. "The influence of lead and cadmium on the absorption and distribution of potassium, calcium, magnesium and iron in cucumber seedlings". *Acta Physiol. Plantarum. 9*, 1987, pp. 229–238.
7. Burzynski, M. and A. Grabowski, "Influence of lead on $N0_3$—uptake and reduction in cucumber seedlings". *Acta Soc. Bot. Pol., 53*, 1984, pp. 77– 86.
8. Cooperband, L. R. and L. W. Good, "Biogenic phosphate minerals in manure: implications for phosphorus loss to surface waters". *Environmental Science and Technology 36* (23): 2002, pp. 5075–5082.
9. Daigger, G. T. and T. E. Sadick, "Evaluation of methods to detect and control nitrification inhibition with specific application to incinerator flue-gas scrubber water". *Water Environment Resources, 70* (7), 1998, pp. 1248–1257.
10. Effler, S. W., Brooks, C. M., Auer, M. T. and S. M. Doerr, "Free ammonia and toxicitycriteria in a polluted urban lake". *J Water Pollut Control Fed 62*: 1990, pp. 71–9.
11. Foroughi, M., P. Najafi, A. Toghiani and N. Honarjoo, "Analysis of pollution removal from wastewater by Ceratophyllum demersum", *African Journal of Biotechnology 9*(14): 2010, pp. 2125–2128.
12. Friendland, A. J. and A. H. JOHNSON, "Lead distribution and fluxes in a high-elevation forest in Northern Vermont". *J. Environ. Qual. 14*, 1985, pp. 332–336.
13. Godbold, D. L. and C. Kettner, "Lead influences root growth and mineral nutrition of Picea abies seedlings", *J. Plant Physiol., 139*: 1991, pp. 95–9.
14. Gumbricht, T., "Nutrient removal process in freshwater submersed macrophyte systems", *Ecol. Eng. 2*: 1993, pp. 1–30.
15. Hao, X. D., Wang, C. C., Lan, L. and van Loosdrecht, M. C. M., Struvite formation, analytical methods and effects of pH and $Ca_2$. *Water Science and Technology 58* (8): 2008, pp. 1687–1692.
16. Hockenbury, M. R. and C. P. L. Grady Jr., "Inhibition of nitrification—effects of selected organic compounds". *Journal Water Pollution Control Federation. 49* (5), 1977, pp. 768–777.
17. Holloway, J. M., R. A. Dahlgren, B. Hansen and W. H. Casey, "Contribution of bedrock nitrogen to high nitrate concentrations in stream water", *Nature 395*: 1998, pp. 785–788.
18. Iamchaturapatr, J., S. W. Yi and J. S. Rhee, "Nutrient removals by 21 aquatic plants for vertical free surface-flow (VFS) constructed wetland", *Ecological Engineering 29*: 2007, pp. 287–293.
19. Johnson, D., L. Kershaw, A. MacKinnon and J. Pojar.1995. *Plants of Western Boreal Forest and Aspen Parkland.* Lone Pine publishing, Vancouver, BC.
20. Jorgensen, T. C. and L. R. Weatherley, "Ammonia removal from wastewater by ion exchange in the presence of organic contaminants", *Water Research 37* (8): 2003, pp. 1723–1728.
21. Juliastuti, S.R., J. Baeyens, C. Creemers, D. Bixio and E. Lodewyckx, "The inhibitory effects of heavy metals and organic compounds on the net maximum specific growth rate of the autotrophic biomass in activated sludge". *Journal of Hazardous Materials, 100* (1-3), 2003, pp. 271–283.
22. Kadlec, R. H. and R. L. Knight. 1996. *Treatment Wetlands.* Lewis Publishers, Boca Raton, FL.
23. Keeney, D. R. and J. M. Bremner, "Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability", *Agron. J. 58*: 1996, pp. 498–503.
24. Kelly II, R.T., I.D.S. Henriques and N.G. Love, "Chemical inhibition of nitrification in activated sludge". *Biotechnology and Bioengineering. 85* (6), 2004, pp. 683-694.
25. Keskinkan, O., M. Z. L. Goksu, M. Basibuyuk and C. F. Forster, "Heavy metal adsorption properties of a submerged aquatic plant (*Ceratophyllum demersum*)", *Bioresour. Technol. 92*: 2004, pp. 197–200.
26. Lamersdorf, N., "Verteilung und Akkumulation von Spurenstoffen in Waldokosystemen". In: ULRICH, B. (Ed.): *Ber. Forschungszentr. Waldokosyst.* B36, Gottingen, 1988.
27. Lay-Son, M. and C. Drakides, "New approach to optimize operational conditions for the biological treatment of a highstrength thiocyanate and ammonium waste: pH as key factor", *Water Research 42* (3): 2008, pp. 774–780.
28. Liikanen, A. and P. J. Martikainen, "Effect of ammonium and oxygen on methane and nitrous oxide fluxes across sediment-water interface in a eutrophic lake", *Chemosphere 52* (8): 2003, pp. 1287–1293.
29. Maine, M. A., N. Sune, H. Hadad, G. Sanchez and C. Bonetto, "Nutrient and metal removal in a constructed wetland for wastewater treatment from a metallurgic industry", *Ecol. Eng., 26*: 2006, pp. 341–347.
30. Marttinen, S. K., R. H. Ketunen, K. M. Sormunen, R. M. Soimasuo and J. A. Rintala, "Screening of physical–chemical methods for removal of organic material, nitrogen and toxicity from low strength landfill leachates", *Chemosphere 46* (6): 2002, pp. 851–858.
31. McRoy, C. P., R. J. Barsdate and M. Nebert, "Phosphorous cycling in an eelgrass (Zostera marina L.) ecosystem", *Limnol. Oceanogr. 17*: 1972, pp. 58– 67.
32. Mitsch, W. J. and J. E. Gosselink. 2000. *Wetlands, 3rd ed.* John Wiley & Sons, Inc., New York, NY.
33. Mjelde, M. and B. A. Faafeng, "*Ceratophyllum demersum* Hampers phytoplankton development in some small Norwe Gian lakes over awide range of phosphorus concentrations and Geographic allatitude", *Fresh wat. Biol. 37*: 1997, pp. 355–365.
34. Nelson, D. W. and L. E. Sommers, "Determination of total nitrogen in plant material", *Agron. J. 65*: 1973, pp. 109–112.
35. Patterson, R. A., "Nitrogen in Wastewater and its Role in Constraining On-Site Planning in *Future Directions for On-site Systems: Best Management Practice. Proceedings of On-site '03 Conference* by Patterson, R. A., and Jones, M J (Eds). Held at University of New England, Armidale 30th September to 2nd October 2003", Published by Lanfax Laboratories Armidale. ISBN 0-9579438-1-4 2003, pp. 313–320.
36. Palma, L. D., P. Ferrantelli, C. Merli and E. Petrucci, "Treatment of industrial landfill leachate by means of evaporation and reverse osmosis", *Waste Management 22*: 2002, pp. 951–955.
37. Reed, S. C., Crites, R. D. and E. J. Middlebrooks, *Natural Systems for Waste Management and Treatment*. 2nd ed. McGraw-Hill, Inc., New York, NY., 1995.
38. Renou, S., Givaudan, J. G., Poulain, S., Dirassouyan, F. and Moulin, P., Landfill leachate treatment: review and opportunity. *Journal of Hazardous Materials 150*: 2008, pp. 468–493.
39. Rocca, C. D., Belgiorno, V. and Meric, S., Overview of in-situ applicable nitrate removal processes. *Desalination, 204*: 2007, pp. 46–62.
40. Saygideger, S., M. dogan and G. keser, "Effect of Lead and pH on Lead Uptake, Chlorophyll and Nitrogen Content of *Typha latifolia L.* and *Ceratophyllum demersum L*", *International Journal of agriculture & biology. 6*, No. 1: 2004, pp.168–172.
41. Smith, V. H., G. D. Tilman and J. C. Nekola, "Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystem", *Environ. Pollut. 100* (1-3): 1999, pp. 179–196.
42. Sooknah, R. D. and A. C. Wilkie, "Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater", *Ecological Engineering 22*: 2004, pp. 27–42.

43. Tracy, M., J. M. Montante, T. E. Allenson and Hough, R. A., Long-term responses of aquatic macrophyte diversity and community structure to variation in nitrogen loading, *Aquat. Bot., 77*, 2003, pp. 43–52.
44. Walker, W. M., J. E. Miller and J. J. Hassett "Effect of lead and cadmium upon the calcium, magnesium, potassium, and phosphorus concentration in young corn plants". *Soil Sci. 124*, 1977, pp. 145–151.
45. Wakida, F. T. and D. N. Lerner, "Non-agricultural sources of groundwater nitrate: a review and case study", *Water Res., 39*: 2005, pp. 3–16.
46. Wetzel, R. G. and H. L. Allen, "Functions and interactions of dissolved organic matter and the littoral zone in lake metabolism and eutrophication. In: Kajak Z. Hillbricht-Il- kowska A (ed.)", *Productivity Problems of Freshwaters,* 1972, pp. 333–347.
47. Wetzel, R. G. and B. A. Manny, "Secretion of dissolved organic carbon and nitrogen by aquatic macrophytes", *Verh. Int. Verein. Limnol. 18*: 1972, pp. 162–170.
48. Wilson, D. O., "Phosphate nutrition of the aquatic angiosperm, Myriophyllum exalbescens Fern", *Limnol. Oce- anogr. 17*: 1972, pp. 612–616.
49. Zhao, F. J., S. P. McGrath and A. R. Croslant, "Comparison of three wet digestion methods for the determination of plant sulpher by inductively coupled plasma atomic emission spectrometry (ICP-AES)", *Commun Soil Sci Plant Anal; 25*: 1994, pp. 407–18.
50. Zhang, T., L. Ding, and H. Ren, " Pretreatment of ammonium removal from landfill leachate by chemical precipitation", *Journal of Hazardous Materials 166*: 2009, pp. 911–915.

# Survival of Infectious Prions During Anaerobic Digestion of Municipal Sewage Sludge and Lime Stabilization of Class B Biosolids

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**ABSTRACT:** The objective of this study was to evaluate the fate of infectious prions during various stages of municipal waste treatment. Specifically mesophilic (35°C) and thermophilic (50°C) anaerobic digestion of sewage sludge (biosolids) and lime stabilization of Class B biosolids were evaluated. Standard scrapie cell assay which includes the ELISPOT (Enzyme Linked Immuno-Spot) reaction was utilized to quantitatively determine prion infectivity. A 4.2-$\log_{10}$ decrease was observed under mesophilic conditions after 21 days of digestion, while thermophilic digestion resulted in a 4.7-$\log_{10}$ decrease. When Class B mesophilically digested biosolids were treated with lime, a 2.9-$\log_{10}$ reduction of infectious prions was observed within the first two hours. Overall these results suggest that infectious prions are reduced significantly by either anaerobic digestion and/or lime treatment, and subsequent land application of biosolids is not a viable route of human exposure to prions.

## INTRODUCTION

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSE) are a group of neurological prion diseases of mammals which in humans include Kuru, Creutzfeldt-Jakob disease (CJD), sporadic Creutzfeldt-Jakob disease (spCJD), and variant Creutzfeldt-Jakob disease (vCJD) [1]. In animals, TSE results in scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) affecting deer, elk, and moose [1]. In the United Kingdom, vCJD was reported to cause an epidemic in the 1990's transmitted by bovine spongiform encephalopathy prions [2]. Once vCJD is orally ingested by humans they can subsequently be seen in organs such as the spleen, appendix, tonsils, and eventually the brain after being adsorbed mainly through the Peyer's patches of intestines. [3,4]. Although research has identified a number of key details about prions since that time there are still many questions remaining about its resistance to treatment processes, and its persistence in the environment. Prions have been reputed to be able to survive even the most extreme physical (e.g., irradiation, dry heat, and autoclaving) and chemical (e.g., acids, bases, alkylating agents, halogens, organic solvents, oxidizing agents, proteolytic enzymes, and more) means of disinfection [5]. Therefore, disease-causing prions, hereafter termed infectious prions and referring to any prion that is infectious, may still enter the food chain undetected, escaping barriers in place while subsequently exposing humans to infectious prions. One route of exposure through which infectious prions may enter the food chain is through animal rendering and meat processing operations of raw wastewater that may contain prion infected cattle or sheep. There is also the possibility that urine and feces contaminated infectious prions are discharged into source water by animals [6–8]. Nichols *et al.* (2009) demonstrated the infectious prions could be detected at low concentrations in water runoff and at a water treatment facility.

At many wastewater treatment (WWT) facilities anaerobic digestion is a very common treatment method to reduce mass and volume of primary sludge and activated sludge biomass production. However, recently it was reported that mesophilic anaerobic digestion did not sufficiently reduce infectious prion numbers due to the extreme resistant properties of infectious prion proteins [10]. Infectious prions have also been shown to be partitioned out into sewage sludge known as bio-

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solids generated during treatment of domestic sewage [10]. Since biosolids are routinely applied to land for agricultural uses, this could be a potential route for human and animal exposure to infectious prions.

There are a limited number of studies that have explored infectious prions in wastewater sludge [10–12]. Both Hinckley *et al.* (2008) and Kirchmayr *et al.* (2006) found that following mesophilic anaerobic digestion there is a very small reduction, if any, of infectious prions in activated sludge solids. On the other hand, samples under thermophilic conditions declined by 20–40% of initial values [11]. In contrast, Maluquer de Motes *et al.* (2008a) demonstrated in a regression model there should be a 90% reduction of infectious prions within 13 days at 20°C which is fifteen degrees lower than standard mesophilic digestion conditions.

Once biosolids are produced, they can be land applied and thereafter mixed into the top layer of soil in an agricultural field or pasture situation. Studies have shown mobility of infectious prions depends on the soil matrix, and that mobility decreases as inorganic matter content increases [13–15]. Due to high affinity of soil for prions it has been proposed that soils may be an infectious prion reservoir [16]. These observations indicated there was a potential risk of infectious prion ingestion by animals or even humans in horizontal transmission of TSE via land application of biosolids posed by grazing or from eating crops from home gardens [17].

Gale and Stanfield (2001) conducted a quantitative risk assessment for infectious prions in sewage sludge concluding that exposure to humans through vegetable crops grown by biosolids was low at $1.32 \times 10^{-9}$ infections/year. While this calculated risk to humans is acceptably low, risks to cattle are greater because they are exposed to soil over a longer time period during grazing [18]. Though this risk assessment did not directly quantify the actual quantity of infectious prions found in wastewater sludge, it indicated possibility of wastewater sludge becoming contaminated and ultimately creating a reservoir of contaminated land applied biosolids for livestock to ingest. However, more recently, Miles *et al.* (2011) utilized the standard scrapie cell assay for infectious prions to demonstrate a reduction of infectious prions within mouse brain homogenate in Class B biosolids. Specifically, a 99% reduction of infectious prions infectivity after 15 days at 37°C, and a 99.9% reduction after 10 days at 60°C were observed. To further understand the fate of infectious prions in wastewater, the objective of this study was to quantitatively evaluate survival of infectious prions in wastewater during anaerobic digestion treatment under mesophilic and thermophilic conditions. Additionally, the influence of lime treatment of Class B biosolids to produce Class A biosolids was also evaluated. Unlike similar studies that assessed presence of prions qualitatively via immunochemistry or transmission of infectious prions via animal bioassays, the standard scrapie cell assay was utilized to detect the infectivity and quantity of infectious prions in samples.

## MATERIALS AND METHODS

### Biosolid and Sludge Samples

Primary sludge and Class B biosolids were collected from the Ina Road Wastewater Treatment Plant located in Tucson, Arizona. Total suspended solids (TSS) content was 3.7% and 7% of wet weight, respectively. Anaerobic digested sludge samples were obtained from two different wastewater treatment plants. A mesophilic anaerobically digested sewage sludge sample was obtained from the Ina Road Wastewater Treatment Plant located in Tucson, Arizona. A thermophilic anaerobically digested sewage sludge sample was provided by Los Angeles County Sanitation District municipal WWTP in Whittier, CA. Total suspended solids content of the sludge samples were 1.6 and 1.4% of the wet weight for mesophilic and thermophilic anaerobically digested sewage sludge, respectively. All samples were stored at 4°C before use.

### Source of Infectious Prions

The Rocky Mountain Laboratory scrapie strain ($PrP^{Sc}$) adapted to the CD1 mouse is the source of infectious prions used in this study [20]. The infected mouse brain was homogenized at 10% w/v with a phosphate buffer saline (PBS) at pH 7.4 on ice. It was passed through needles of decreasing sizes from 18, 20, 22, 24, and 28 gauges. The mouse brain homogenate was aliquoted into 500 μL volumes and frozen at –80ºC. The mouse brain homogenate was a gift from Weissmann lab Scripps Research Institute, Jupiter, Florida.

### Extraction of Infectious Prions from Wastewater or Biosolids

Urea concentration and temperature of the extraction method from Miles *et al.* (2011) was further optimized to 2 M urea at 70°C. Thereafter, the 1 mL or 1 g of sludge or biosolids containing infectious prions was

centrifuged at 8,000 × g for 7 minutes at room temperature. The supernatant was removed and the sediment fraction was resuspended in 1 mL of 2 M urea (pH 9). Vials were vortexed and then heated at 70°C for 10 minutes. Samples were subsequently vortexed and centrifuged at 8,000 × g for 7 minutes at room temperature. The supernatant was transferred to a pre-wet centrifugal filter device to remove urea and centrifuged at 3,500 × g for 15 minutes. Thereafter, 1 mL of PBS (pH 7.4) containing 10% of penicillin/streptomycin was added to the centrifugal filter device (Amicon Ultra-15 10 kDa, Millipore, Billerica, MA). The centrifugation step was repeated two more times. The supernatant was transferred to a 1.5 mL microcentrifuge tube and PBS containing 10% penicillin/streptomycin was added to adjust the total volume to 1 mL. Samples were stored at –80°C.

**Survival of Infectious Prions during Mesophilic and Thermophilic Digestion**

Mesophically digested and thermophically digested sludge were used as inoculants for the primary undigested sludge. For each condition, 100 µL of 10% brain homogenate suspended in PBS was re-homogenized by passage through a 28-gauge needle and added to 10 mL samples (7 mL primary sludge as substrate and 3 mL anaerobic digested sludge as inocula) of sludge in a 25 mL anaerobic tube. To simulate anaerobic digestion, miniature anaerobic digestion tubes were sealed and shaken at 120 rpm on a shaking incubator (Labnet International Inc, Edison, NJ) in a dark climate-controlled room at 35 ± 2°C and 50 ± 2°C for mesophilic and thermophilic anaerobic digestion, respectively.

The efficacy of these miniature anaerobic digesters was determined by digestion activity, monitoring volatile fatty acids, and methane during anaerobic incubation. Various controls including endogenous controls lacking substrates, and controls inoculated with heat-treated sludge were utilized in each experiment. Endogenous samples lacking substrates were included to measure endogenous production of methane in order to properly account for methane formation generated during sludge digestion. Heat treated sludge samples were also prepared by autoclaving inoculum with substrates for 20 min at 121ºC for three consecutive days. This was performed to inactivate microorganisms and thereby enabling evaluation the influence of microorganisms on infectious prions. All treatments were conducted in triplicate. Digestion for all conditions was incubated for a period of 21 days and samples were collected for volatile fatty acid production, methane production, infectivity of $PrP^{Sc}$, and quantity of infectious prions for each sample after 0, 1, 2 and 3 weeks. Samples containing infectious prions were then stored at –80°C prior to further use.

**Volatile Fatty Acids and Methane Determinations**

Volatile fatty acids including acetic, propionic, and butyric acid; concentrations in liquid samples; and methane concentration from the headspace for the batch bioassays were determined by gas chromatography using an HP5290 Series II system (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector. The gas chromatograph was fitted with a Nukol fused silica capillary column (30 m length × 0.53 mm ID, Supelco, St. Louis, MO). The carrier gas was helium at a flow rate of 30 mL/min and a split flow of 84 mL/min. For volatile fatty acids measurements, the temperatures of the column, the injector port, and the detector were 140, 250, and 275°C, respectively, and the injection volume was 5 µL. For methane, the temperatures of the column, injector port, and the detector were 140, 180, and 250°C, respectively. Samples for measuring methane content (100 µL) in the headspace were collected using a pressure-lock gas tight syringe (1710RN, 100 µL (22s/2″/2), Hamilton Company, Reno, Nevada USA). Other analytical determinations (e.g., pH, total suspended solids, and more) were conducted according to Standard Methods [21].

**Lime Treatment**

Lime treatment is a common alternative for pathogen reduction in Class B biosolids to achieve Class A standards [22]. To determine survival of prions during lime treatment, 20 µL of 10% brain homogenate suspended in PBS was re-homogenized by passage through a 28-gauge needle and added to one g of Class B biosolids treated with $Ca(OH)_2$ resulting in a pH of 12.9. The lime treated biosolids were incubated for 12 hours at 52°C and samples were assayed for infectious prions after 0, 2, 8, and 12 hours. Samples were performed in triplicate. A positive control that did not include lime treated biosolids was assayed for infectious prions at the beginning of the experiment to confirm initial concentration. After each incubation period, infectious prions were immediately extracted from samples to separate from $Ca(OH)_2$ treated biosolids and stored at –80°C.

### Quantitative Analysis of Infectious Prions

Infectious prions were detected by the standard scrapie cell assay which includes in-vitro cell culture and enzyme linked immunospot assay (ELISPOT) [23]. Briefly, ten-fold serial dilutions of each sample were made in OBGS (Opti-MEM, Invitrogen, Carlsbad, CA), 9.1% bovine growth serum (HyClone, Logan, UT), 1% Penicillin- 10,000 IU/mL, Streptomycin-5mg/mL (MP Biomedicals, Solon, OH), and then 200 µL of each dilution was added to wells of a 96-well cell culture plate. Approximately 5,000 CAD2A2D5 (CAD5) cells (a gift from Weissmann lab at the Scripps Research Institute) were suspended in 90 µL of OBGS and added to the wells. After four days of incubation at 37°C with 5.0% $CO_2$ the confluent monolayer was suspended by gently pipetting up and down. Approximately 20,000 infected CAD5 cells were transferred into each well of the ELISPOT plate and subsequently the ELISPOT assay performed.

## RESULTS AND DISCUSSION

To investigate survival of infectious prions during anaerobic digestion, miniature laboratory anaerobic digester tubes were utilized to simulate performance of mesophilic and thermophilic anaerobic digestion processes. To determine efficacy of these miniature anaerobic digesters, the anaerobic digestion activity was monitored using methane production as the indicator of performance under mesophilic (35°C) and thermophilic (50°C) digestion over 21 days as shown in Figures 1 and 2, respectively. Production of methane expressed as mg chemical oxygen demand (COD) $L^{-1}_{liq}$ accounted for 75.9 ± 1.9% and 59.9 ± 3.3% of the theoretical initial COD added at the beginning of incubation under mesophilic and thermophilic conditions, respectively. In comparison, low levels of methane production were observed in either endogenous controls lacking substrates, or controls inoculated with heat treated sludge. In addition, production of volatile fatty acids was measured to determine transformation products from an anaerobic digestion process. During the incubation period, negligible volatile fatty acids (expressed as COD) accumulated under both mesophilic and thermophilic conditions (date not shown). As a result, the laboratory simulated anaerobic digesters performed well because the $CH_4$ production falls in the typical range of 50–75% with negligible volatile fatty acids accumulation observed in the biological treatment.

Consequently, to understand the role of anaerobic digestion processes on fate of infectious prions during anaerobic digestion concentration of prions were monitored under both mesophilic and thermophilic conditions for 21 day incubation periods. In the first week there was a 1.0-$\log_{10}$ decrease under mesophilic conditions, and a corresponding 5.0-$\log_{10}$ decrease under thermophilic conditions (Figure 3). After 21 days there were 4.2 and 4.7-$\log_{10}$ reduction under mesophilic and thermophilic conditions, respectively (Figure 3). Infectious prions inoculated in sterile sludge with no microbial activity under mesophilic conditions was also anaerobically incubated. Although initial concentration in this sample was lower than for other samples, which could be due to recovery or degrada-

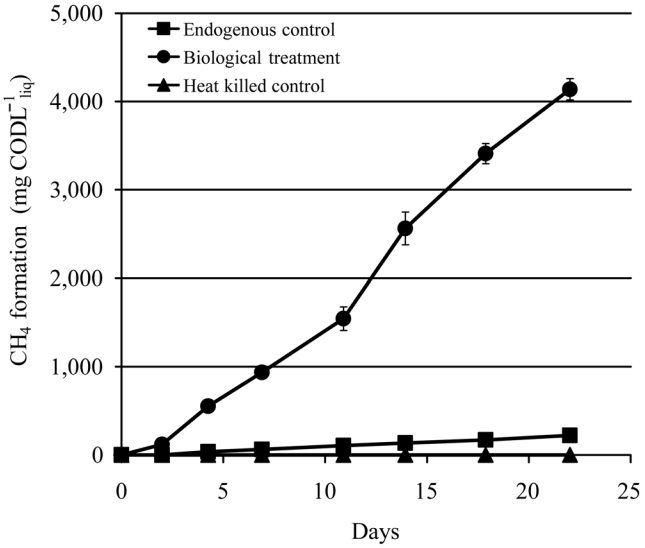


***Figure 1.*** *Efficacy of miniature laboratory anaerobic digesters as indicated by methane formation under mesophilic (35°C) conditions.*

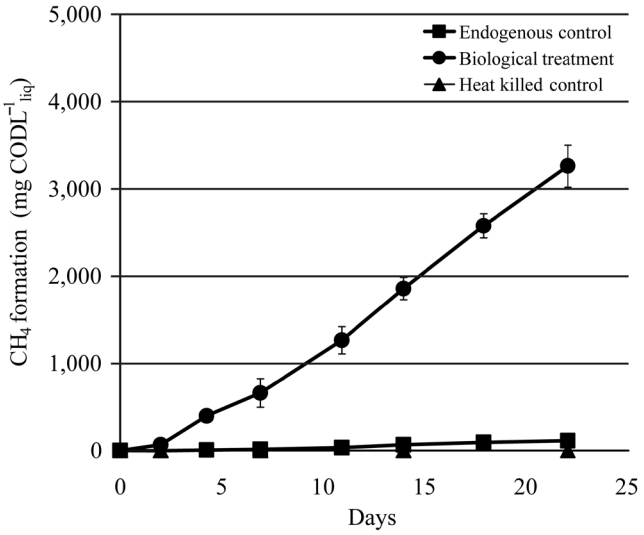


***Figure 2.*** *Efficacy of miniature laboratory anaerobic digesters as indicated by methane formation under thermophilic (50°C) conditions.*

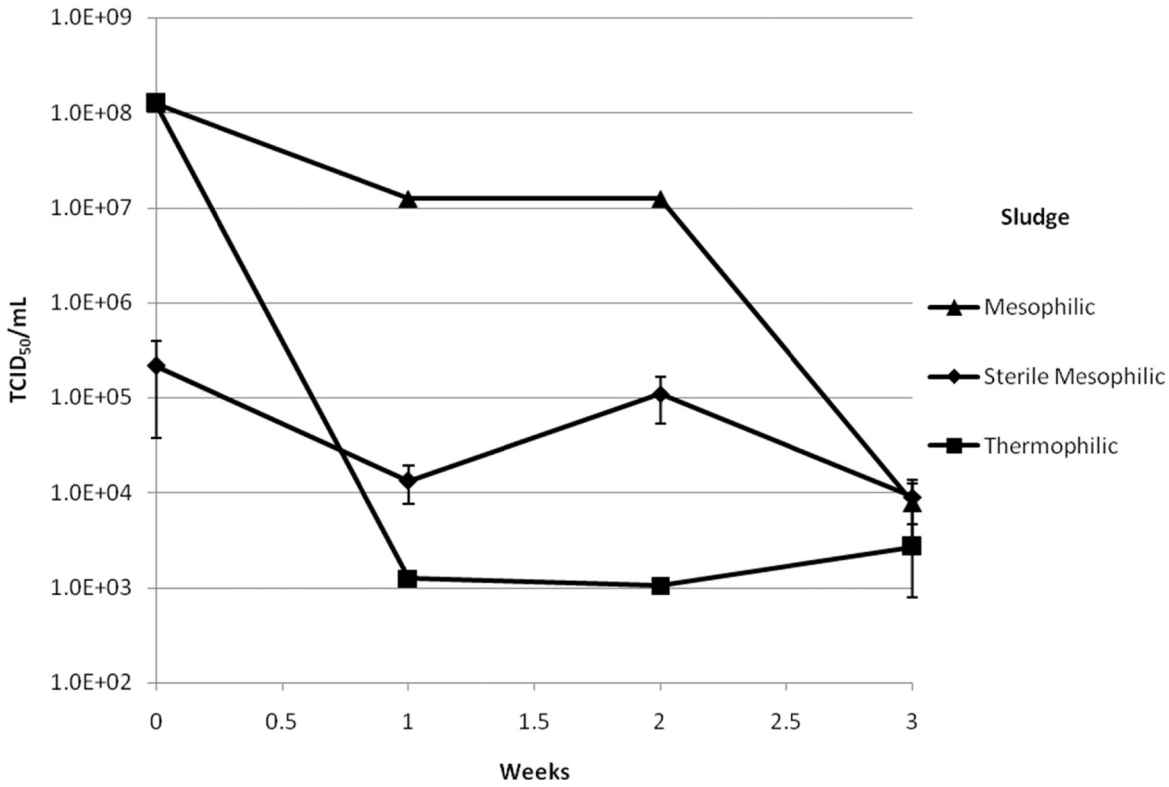


***Figure 3.*** *Reduction in infectious prions during anaerobic digestion under mesophilic and thermophilic conditions.*

tion, the sample demonstrated that there was at least a 1.4-$\log_{10}$ reduction after 21 days (Figure 3). These results suggest the difference in reduction of infectious prions during anaerobic digestion with raw and sterile wastewater could be due to microbial activity. Other studies that involved mesophilic anaerobic digestion [10–11] reported that there was very little if any reduction of infectious prions in their studies, most likely because they did not use an infectivity assay. The immunoblot method they used could only determine presence of intact prions and not infectivity. In contrast, a previous study using the Western blot method reported infectious prions were degraded by ruminal and colonic microbiota once ingested by sheep and cattle, since infectious prions were not detected in the Western blot method [24–25]. However, after further investigation via animal bioassays, Scherbel *et al.* (2006) discovered that although prions levels were undetectable by Western blot infectivity still remained when animal bioassays were conducted.

Kirchmayr *et al.* (2006) found a decrease by 20–40% of that detected by an immnoblot assay during thermophilic anaerobic digestion. A decrease of infectious prions was also seen in Maluquer de Motes *et al.* (2008a) using a western blot assay, and a regression model estimated a 99% reduction of infectious prions ranging between 22.4 and 51.2 days of incubation at 20°C. In another statistical model, Maluquer de Motes *et al.* (2008b) calculated a possible 99% reduction of infectious prions by ELISA and western blot assays in 63 to 82 days in raw wastewater and also at 20°C.

Lime treatment is a common method for reducing pathogenic microorganisms in biosolids [22]. In this study, the initial concentration at $1.2 \times 10^5$ $TCID_{50}$/mL of infectious prions decreased by 2.9-$\log_{10}$ within the first two hours of lime treatment (pH of 12.9 at 52°C) to $1.4 \times 10^2$ $TCID_{50}$/mL. In sample hours two through twelve, infectious prions were below the limit of detection. Although this method suggests that it is effective as demonstrated by the marked reduction, it does not take into consideration the mobility of prions once biosolids are applied to land. Ma *et al.* (2007) suggested that common disposal practices such as a lime treatment that is applied to landfills would elevate pH increasing the mobility of infectious prions due to increased pH and a decrease in ionic strength. Reduction due to lime treatment seen in this current study however, resulted in at least a 99% decrease which reduces risk considerably. For example, Maluquer de Motes *et al.* (2008b) estimated that if wastewater was contaminated with one infected cow at a slaughterhouse the concentration would be lower than $0.6–26 \times 10^{-4}$ cattle

oral $ID_{50}$ per liter. Risk decreases as water travels to the treatment plant.

Additionally, when biosolids are mixed with soil when applied to land, studies have shown that prions adsorb to soil thus reducing mobility [13,15,28]. Jacobson *et al.* (2009) conducted a study evaluating potential for transport of infectious prions in biosolids, composting materials, and soil. Results show that infectious prions could in fact be transported in biosolids and composting material, but were retained in quartz sand and fine-textured burial soil [13]. Similarly, Jacobson *et al.* (2010) showed that infectious prions were not mobilized in five characteristically different soils with high sand or silt contents. Adsorption may also depend on the matrix where it has been shown that adsorption depends on infected tissue concentration that enters the soil [28]. Low tissue concentration appears to adsorb freely, whereas high tissue concentration results in competition with other proteins decreasing their affinities to the mineral content within the soil matrix [28]. Thus, these studies suggest that if infectious prions in biosolids survive lime treatment mobility of infectious prions can be significantly reduced in the presence of soil.

## CONCLUSION

This study demonstrates normal holding time for anaerobic digestion at mesophilic and thermophilic temperatures results in a significant decrease of infectious prions within 21 days. Reduction occurred rapidly at a higher thermophilic incubation temperature of 50°C. It also appears microorganisms may influence prion reduction during anaerobic digestion since sterile wastewater resulted in only a modest decrease when compared to non-sterilized raw wastewater. Finally, lime treatment of Class B biosolids was shown to significantly reduce infectious prions within 2 hours. Hence, these data suggest there is a reduction of infectious prions during wastewater treatment and that land application of biosolids is not a likely route for human prion exposure.

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## REFERENCES

1. Prusiner, S.B. (ed.) 2004. *Prion Biology and Diseases*. Cold Spring Harbor Laboratory Press, Woodbury, New York.
2. Bruce, M.E., R.G. Will, J.W. Ironside, I. McConnell, D. Drummond, A. Suttie, L. McCardle, A. Chree, J. Hope, C. Birkett, S. Cousens, H. Fraser and C.J. Bostock. 1997. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature 389*:498–501.
3. Aguzzi, A. 2003. Prions and the immune system: A journey through gut, spleen, and nerves. *Adv. Immunol. 81*:123–171. APHA, AWWA and WEF (2005) Standard methods for the Examination of Water and Wastewater, 21st ed. American public health association, Washington, D.C.
4. Kimberlin, R.H. and C.A. Walker. 1989. Pathogenesis of scrapie in mice after intragastric infection. *Virus Res. 12*:213–220.
5. Taylor, D.M. 2000. Inactivation of transmissible degernerative encephalopathy agents: A review. *Veterinary Journal 159*:10.
6. Gonzalez-Romero, D., M.A. Barria, P. Leon, R. Morales and C. Soto. 2008. Detection of infectious prions in urine. *FEBS Lett. 582*:3161–3166.
7. Murayama, Y., M. Yoshioka, H. Okada, M. Takata, T. Yokoyama and S. Mohri. 2007. Urinary excretion and blood level of prions in scrapie-infected hamsters. *J. Gen. Virol. 88*:2890–2898.
8. Safar, J.G., P. Lessard, G. Tamguney, Y. Freyman, C. Deering, F. Letessier, S.J. Dearmond and S.B. Prusiner. 2008. Transmission and detection of prions in feces. *J. Infect. Dis. 198*:81–89.
9. Nichols, T.A., Pulford, B., Wyckoff A.C., Meyerett, C. Michel, B., Gertig, K., Hoover, E.A., Jewell, J.E., Telling, G.C., Zabel, M.D. 2009. Detection of protease-resistant cervid prion protein in water from a CWD-endemic area. *Prion 3*:171.
10. Hinckley, G.T., C.J. Johnson, K.H. Jacobson, C. Bartholomay, K.D. McMahon, D. McKenzie, J.M. Aiken and J.A. Pedersen. 2008. Persistence of pathogenic prion protein during simulated wastewater treatment processes. *Environ. Sci. Technol. 42*:5254–5259.
11. Kirchmayr, R., H.E. Reichl, H. Schildorfer, R. Braun and R.A. Somerville. 2006. Prion protein: Detection in 'spiked' anaerobic sludge and degradation experiments under anaerobic conditions. *Water Sci. Technol. 53*:91–98.
12. Maluquer de Motes, C., M.J. Cano, J.M. Torres, M. Pumarola and R. Girones. 2008a. Detection and survival of prion agents in aquatic environments. *Water Res. 42*:2465–2472.
13. Jacobson, K.H., S. Lee, D. McKenzie, C.H. Benson and J.A. Pedersen. 2009. Transport of the pathogenic prion protein through landfill materials. *Environ. Sci. Technol. 43*:2022–2028.
14. Johnson, C.J., K.E. Phillips, P.T. Schramm, D. McKenzie, J.M. Aiken and J.A. Pedersen. 2006. Prions adhere to soil minerals and remain infectious. *PLoS Pathog. 2*:e32.
15. Jacobson, K.H., S. Lee, R.A. Somerville, D. McKenzie, C.H. Benson and J.A. Pedersen. 2010. Transport of the pathogenic prion protein through soils. *J. Environ. Qual. 39*:1145–1152.
16. Ma, X., C.H. Benson, D. McKenzie, J.M. Aiken and J.A. Pedersen. 2007. Adsorption of pathogenic prion protein to quartz sand. *Environ. Sci. Technol. 41*:2324–2330.
17. Leita, L., F. Fornasier, M. De Nobili, A. Bertoli, S. Genovesi and P. Sequi. 2006. Interactions of prion proteins with soil. *Soil Biol. Biochem 38*:1638.
18. Gale, P. and G. Stanfield. 2001. Towards a quantitative risk assessment for BSE in sewage sludge. *J. Appl. Microbiol. 91*:563–569.
19. Miles, S.L., K. Takizawa, C.P. Gerba and I.L. Pepper. 2011. Survival of infectious prions in Class B biosolids. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 46*:364–370.
20. Chandler, R.L. 1961. Encephalopathy in mice produced by inoculation with scrapie brain material. *Lancet 1*:1378–1379.

21. APHA, AWWA and WEF (2005) *Standard methods for the Examination of Water and Wastewater, 21st ed.* American public health association, Washington, D.C.
22. U.S. Environmental Protection Agency. 1994. *A Plain English Guide to the EPA Part 503 Biosolids Rule.* Office of Wastewater Management, Washington, D.C.
23. Mahal, S. P., C. A. Demczyk, E. W. Smith, Jr., P. Klohn, and C. Weissmann. 2008. The standard scrapie cell assay (SSCA) and the scrapie cell assay in end point format (SCEPA). p. 49. *In* A.F. Hill (ed.) Prion protein protocols. Methods in molecular biology. Humana Press, Totowa, NJ.
24. Jeffrey, M., L. Gonzalez, A. Espenes, C.M. Press, S. Martin, M. Chaplin, L. Davis, T. Landsverk, C. MacAldowie, S. Eaton and G. McGovern. 2006. Transportation of prion protein across the intestinal mucosa of scrapie-susceptible and scrapie-resistant sheep. *J. Pathol. 209*:4–14.
25. Scherbel, C., R. Pichner, M.H. Groschup, S. Mueller-Hellwig, S. Scherer, R. Dietrich, E. Maertlbauer and M. Gareis. 2006. Degradation of scrapie associated prion protein (PrPSc) by the gastrointestinal microbiota of cattle. *Vet. Res. 37*:695.
26. Scherbel, C., R. Pichner, M.H. Groschup, S. Mueller-Hellwig, S. Scherer, R. Dietrich, E. Maertlbauer and M. Gareis. 2007. Infectivity of scrapie prion protein (PrPSc) following in vitro digestion with bovine gastrointestinal microbiota. *Zoonoses Public. Health. 54*:185-190.
27. Maluquer de Motes, C., S. Simon, J. Grassi, J.M. Torres, M. Pumarola and R. Girones. 2008b. Assessing the presence of BSE and scrapie in slaughterhouse wastewater. *J. Appl. Microbiol. 105*:1649–1657.
28. Saunders, S.E., J.C. Bartz and S.L. Bartelt-Hunt. 2009. Influence on prion strain on prion protein adsorption to soil in a competitive matrix. *Environ. Sci. Technol. 43*:5242.

# Laboratory Determination of Selected Properties of Activated and Return Activated Sludge

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**ABSTRACT:** Results are presented from laboratory measurements of viscosity and density of activated and return activated sludge. Solubility of oxygen in sludge liquor was measured and results were compared to solubility of oxygen in pure water. Viscosity measurements show that sludge containing up to 1% dry solids can be considered a Bingham fluid and sludge with dry solids content above 1% should be described using the Herschley-Buckley model. Sludge density is only slightly higher than water density and with increasing dry solids content increases negligibly. The amount of air dissolved in sludge liquor can be calculated using Henry's Law constants for water because the solubility of oxygen in water and sludge liquor differs minimally.

## INTRODUCTION

DESIGN of technology-oriented processes should be based on specific material properties that are being processed in facilities. Regarding wastewater treatment design, a problem is that some features of wastewater or sludge are not available. Properties of pure water or designer experiences are used often. This paper describes properties of activated sludge (AS) and return activated sludge (RS) measured for needs of a flotation unit design and its CFD model.

Flotation as a separation technology was originally used for processing mined ore and rock and later found its use in water treatment and wastewater treatment plants. Solids or a liquid are divided by flotation from a liquid that is usually water based on differences in density (e.g., sedimentation). Unlike sedimentation, the separated lighter particles rise to the surface where they are skimmed off. In the case of particles with higher density the establishment of agglomerates with bubbles of gas is necessary. Diameter of 40 to 100 μm is recommended [1] and these "microbubbles" are formed in various ways which divide flotation types into the following catergories [2]:

- mechanical flotation (air is blown into a flotation tank using aeration systems like a perforated grate),
- dissolved air flotation (air is dissolved in liquid under high pressure and a subsequent decrease in atmospheric pressure is eliminated in the form of very fine bubbles),
- vacuum flotation (flotation similar pressure when the liquid is saturated with air at atmospheric pressure and then reduced and vacuum released from the liquid fine air bubbles),
- electroflotation (bubbles of gas, mostly oxygen, are released at the appropriate electrode), and
- biological and chemical flotation (gas bubbles are created by the activities of microorganisms or chemical reactions).

Flotation of activated or returned activated sludge can be used at wastewater treatment plants (WWTP) for its thickening versus gravity or mechanical thickening. Dissolved air flotation is the most common type of flotation used for wastewater treatment. The air can be dissolved directly in the sludge or in a recirculation fluid (i.e., usually sludge liquor). The pressure is recommended to be 0.2 to 0.5 MPa to obtain bubbles of a diameter of 40 to 100 μm [2,3].

Within the frame of a Ministry of Industry and Trade of Czech Republic project, "Innovative Approach toward Cleaning of Waste Water—KUNST Flotation Unit" (searched with companies KUNST, s.r.o., and SIGMAINVEST), a new flotation unit with innovative components was designed. The pilot scale unit (see Figure 1) was realized at a WWTP in the town of Hranice which is why sludge samples were collected there. In addition to flotation unit design, measured

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***Figure 1.*** *Pilot flotation unit.*

data where used to CFD model the designed flotation tank and especially for density of sludge and diameter of flocs.

## MATERIALS AND METHODS

Samples of activated sludge and returned activated sludge were taken from the WWTP in Hranice and then stored in a refrigerator for a maximum of four days at 5°C. For determination of total solids (TS) influence samples were decanted and then diluted with a sludge liquor prepared from strain off.

The density was measured by density bottles (50 ml) from 5 to 30°C. Viscosity was measured using a rheometer RheolabQC with a measuring system CC39 and tempered chamber. Viscosity was measured from 5 to 30°C and at a shear rate up to 1000 $s^{-1}$. Diameter of sludge flocs was determined using an optical microscope Padim. Pictures of sludge flocs were scanned by digital camera Moticam and evaluated using Motic Images Plus software. Solubility of oxygen in sludge liquor was measured using a WTW MultiLine 3420 with a probe FDO 925. Total solids were determined by drying at 105°C. Suspended solids (SS) were determined using vacuum filtration with a glass fibres filter with pores of 1.0 μm and drying at 105°C. Volatile solids (VS) were determined by combustion at 550°C.

## RESULTS AND DISCUSSION

Approximately ten samples of AS and RS were taken from the period of January 2011 to April 2012. Average total solid and volatile solids of samples are provided in Table 1. Average values measured at the WWTP in Hranice during 2011 are also presented.

### Density

Density of sludge samples with the above mentioned TS content is only slightly higher than the density of water. This can be seen in Figure 2.

Total solids influence on density of activated and returned activated sludge was determined using samples with content of TS at 0.63, 0.83, 1.06, and 1.29 wt % in samples of AS and at 0.66, 0.86, 1.08, and 1.61 wt %

***Table 1. Content of TS and VS in Sludge Samples.***

| | Activated Sludge (wt %) | Returned Sludge (wt %) |
|---|---|---|
| Total solids (collected samples) | 0.35 ± 0.05 | 0.68 ± 0.17 |
| Volatile solids (collected samples) | 60.60 ± 2.95 | 63.10 ± 3.59 |
| Total solids—average of 2011 | 0.34 ± 0.04 | 0.76 ± 0.12 |
| Volatile solids—average of 2011 | 63.25 ± 6.60 | 66.38 ± 3.63 |

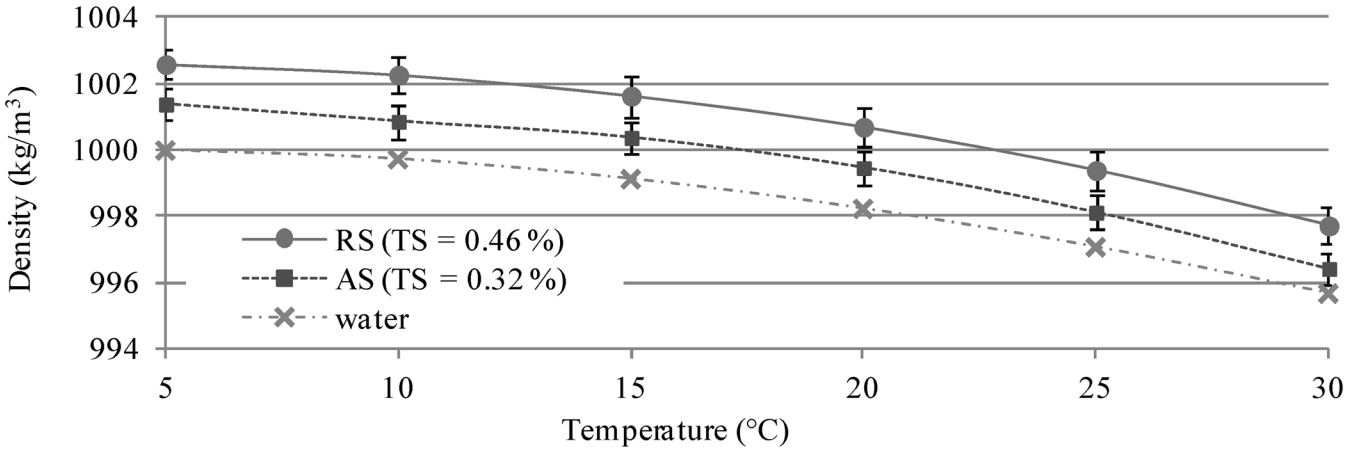



***Figure 2.*** *Density of AS (TS 0.32 wt %) and RS (TS 0.46 wt %).*

in RS samples. Density was measured at 10 and 20°C. It may be seen in Figure 3 that influence of TS is in the presented range.

Operational data from the WWTP in Hranice show TS content does not exceed 0.44 wt % for AS or 1.15 wt % for RS.

**Viscosity**

Sewage sludge is generally considered a non-Newtonian fluid with yield stress. The Bingham model [4,5,6], the Herschel-Buckley model [4,5], or the Oswald model [4] is usually used for activated sludge behaviour description. Behaviour of non-Newtonian fluids is described by rheograms where dependence of shear stress on shear rate is given. The equation for viscosity calculation is as follows:

$$\eta = \frac{\tau}{\dot{\gamma}} \qquad (1)$$

Viscosity of sludge depends on temperature and total solids content. Viscosity decreases with increasing temperature. Viscosity increases with increasing TS content. It may be found in the literature sludge with TS up to 2 wt % behaving as a Newtonian fluid. Yet, Figure 4 and Figure 5 display a slight dependence of apparent viscosity on shear rate for sludge with TS less than 1 wt %.

Ratkovich *et al.* [7] summarized the published data and showed sludge behaviour is usually studied in a wild range of shear rates at temperatures of 20 or 25°C and with a wild range of TS content. Most common ranges for shear rates are from 0 to 100 $s^{-1}$ or from 0 to 1000 $s^{-1}$. Here shear stress (apparent viscosity) was measured in the range of shear rates from 0 to 1000 $s^{-1}$. The most important area is from 0 to 100 $s^{-1}$ from an operational point of view. Measured data are divided into two graphs for each type of sludge. Graphs in Figures 4 and 5 show the effect of temperature on sludge viscosity. Graphs in Figures 6 and 7 show the effect

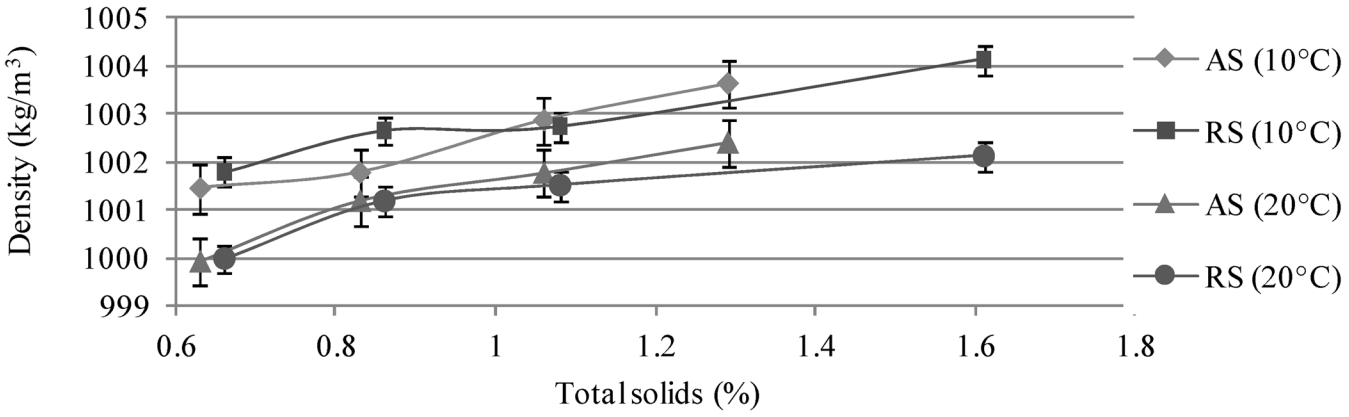



***Figure 3.*** *Dependence of density of AS and RS on the TS content.*

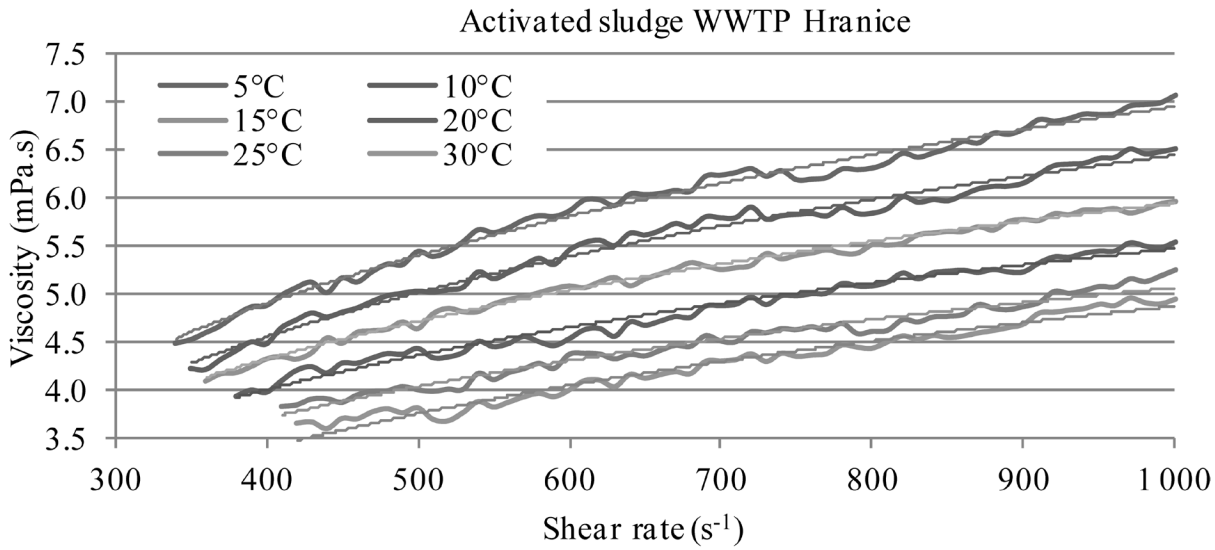



***Figure 4.*** *Dependence of apparent viscosity of AS (TS 0.32 wt %) on shear rate and temperature.*

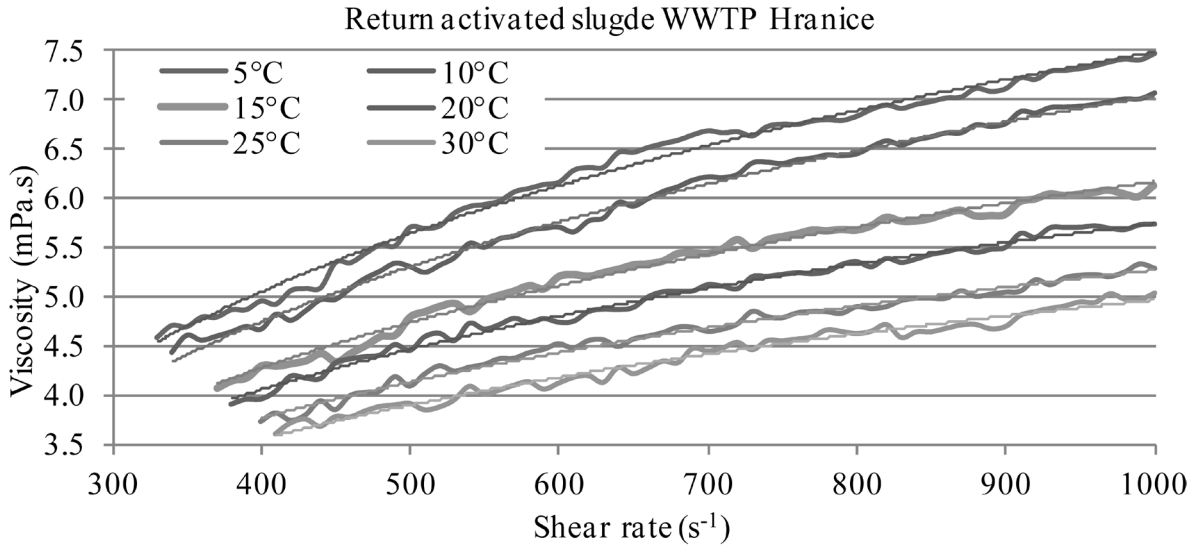



***Figure 5.*** *Dependence of apparent viscosity of RS (TS 0.46 wt %) on shear rate and temperature.*

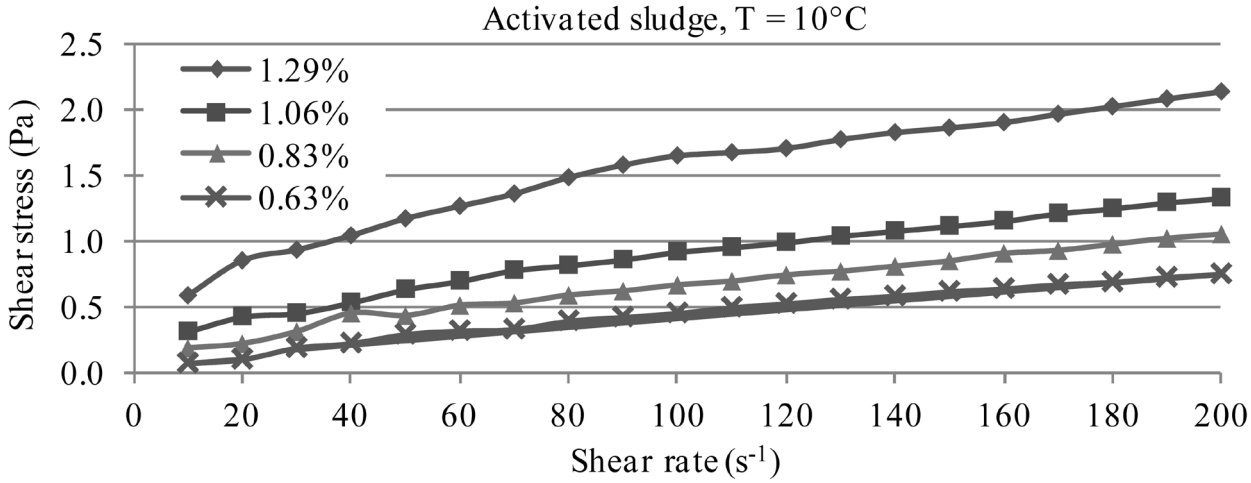



***Figure 6.*** *Dependence of shear stress of AS on shear rate and TS at 10°C.*

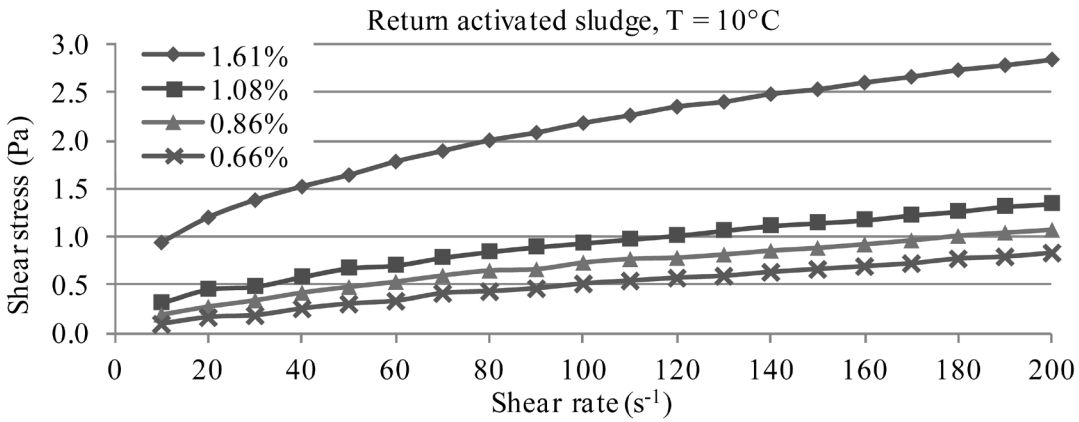



***Figure 7.*** *Dependence of shear stress of RS on shear rate and TS at 10°C.*

of TS content and are focused on an important area of shear rates.

The effect of a TS content up to 1 wt % on sludge viscosity is only negligible. This may be seen in Figures 6 and 7. Apparent viscosity of AS and RS is approximately the same for the same TS content.

Design parameters for the pilot scale flotation unit allow the shear rates in the pipes to range from 10 to 100 $s^{-1}$. The difference between the Newtonian fluid and the sludge samples is more noticeable in the lower range of shear rates as seen in Figures 6 and 7. Samples with TS less than 1 wt % can be described by a Bingham model with linear Equation (2). Samples with TS more than 1 wt % are better described by a Herschel-Buckley model with the power law Equation (3).

$$\tau = \tau_0 \eta_p \dot{\gamma} \tag{2}$$

$$\tau = \tau_0 + K(\dot{\gamma})^n \tag{3}$$

where $\tau$ (Pa) is shear stress, $\tau_0$ (Pa) is yield stress, $\eta_p$ (Pa.s) is plasticity (Bingham´s viscosity), $\dot{\gamma}$ ($s^{-1}$) is shear rate, $K$ is the consistency coefficient, and $n$ is the power-law index. A good agreement of aforementioned models and measured data is seen in Figure 8. Sludge

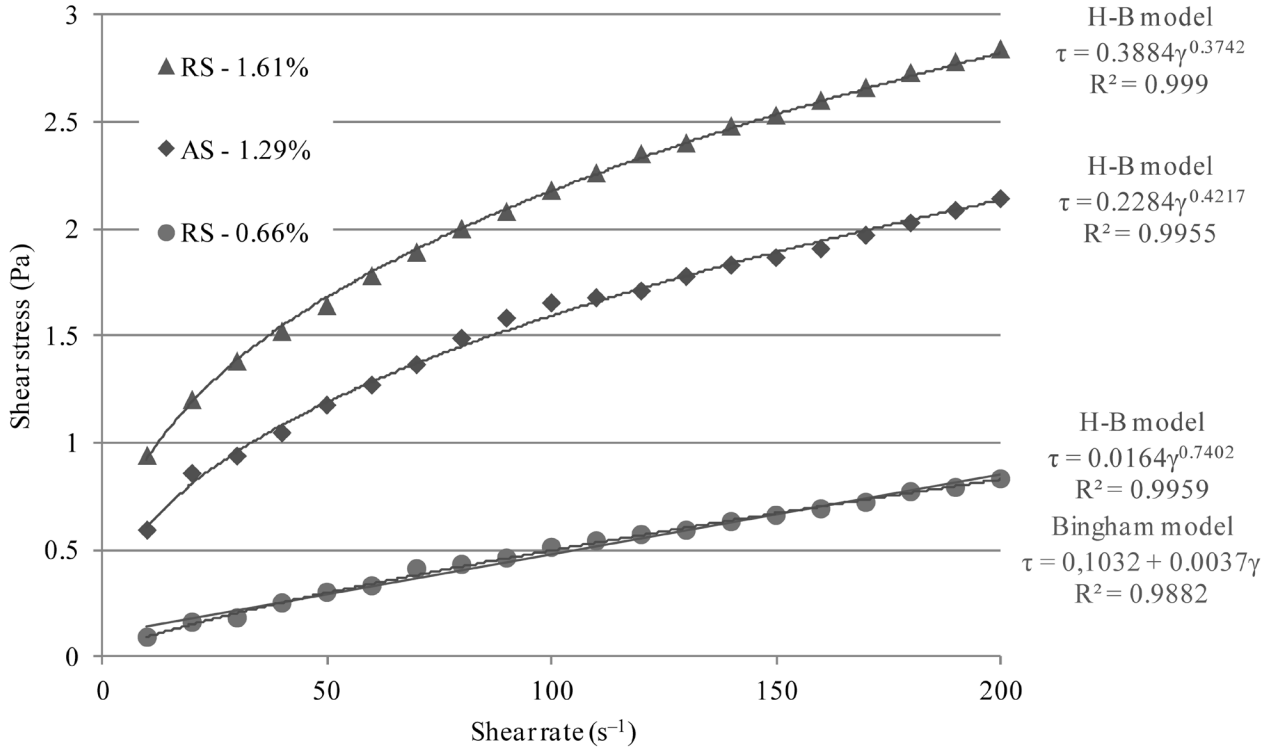
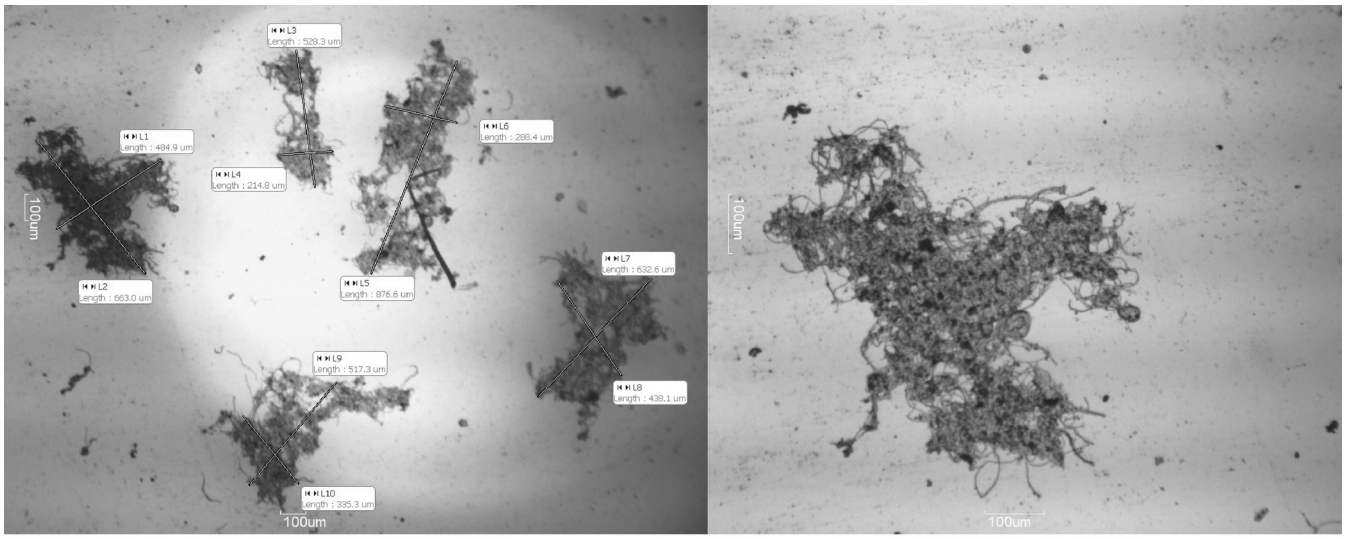



***Figure 8.*** *Mathematical models for behaviour of sludge samples at low shear rates.*




(a) (b)

***Figure 9.*** *Flocs of AS: (a) magnification 40x and (b) magnification 100x (floc dimensions 673 x 465 μm).*

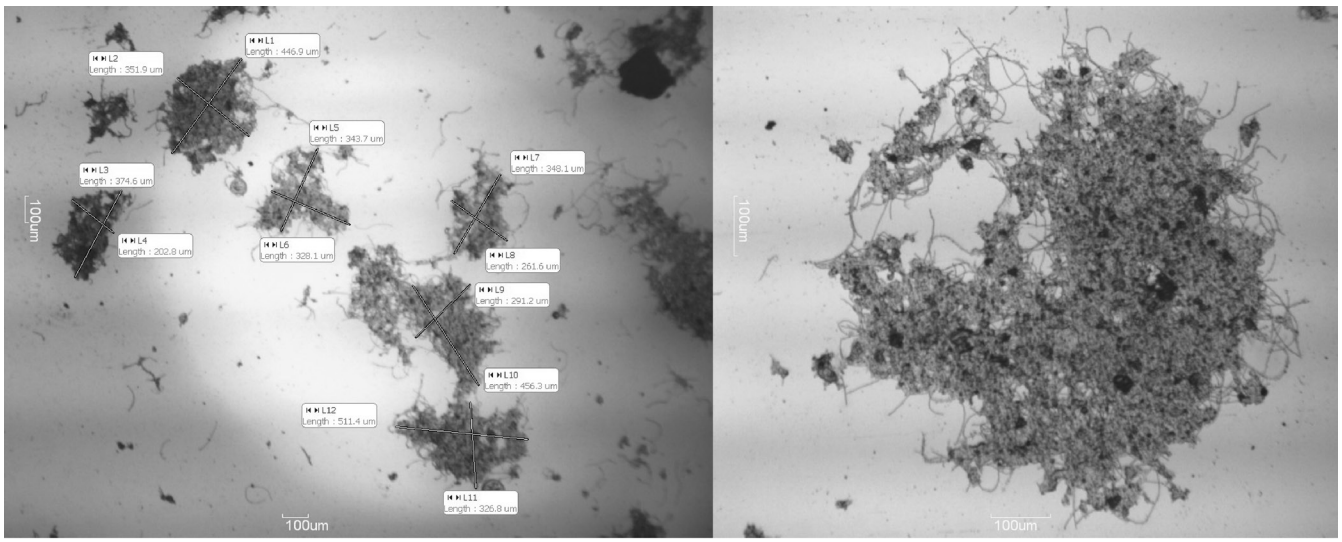
samples of AS and RS with TS more than 1 wt % are described using a Herschel-Buckley model. Samples with TS less than 1 wt % (return activated sludge with 0.66 wt %) are described both with the Bingham and the Herschel-Buckley models. It is possible to see the Bingham model is less accurate than the Herschel-Buckley, but for technical calculations it is sufficient.

## Sludge Flocs Size

The size of sludge flocs was important information regarding preparation of the CFD model for the flotation unit. For this purpose, micro photos of sludge flocs were taken and size was measured using the software Motic Images Plus. Average dimensions of activated sludge flocs were 600 × 400 μm and shape was usually rectangular. Average dimensions were 350 × 250 μm for return activated sludge flocs with shape being circular more often. Pictures of the sludge flocs are seen in Figures 9 and 10.

## Solubility of Oxygen in the Sludge Liquor

Solubility of gases in liquids is described by Henry´s law, see Equation (4), and it depends on temperature and pressure.




(a) (b)

***Figure 10.*** *Flocs of RS: (a) magnification 40x and (b) magnification 100x (floc diameter 720 μm).*

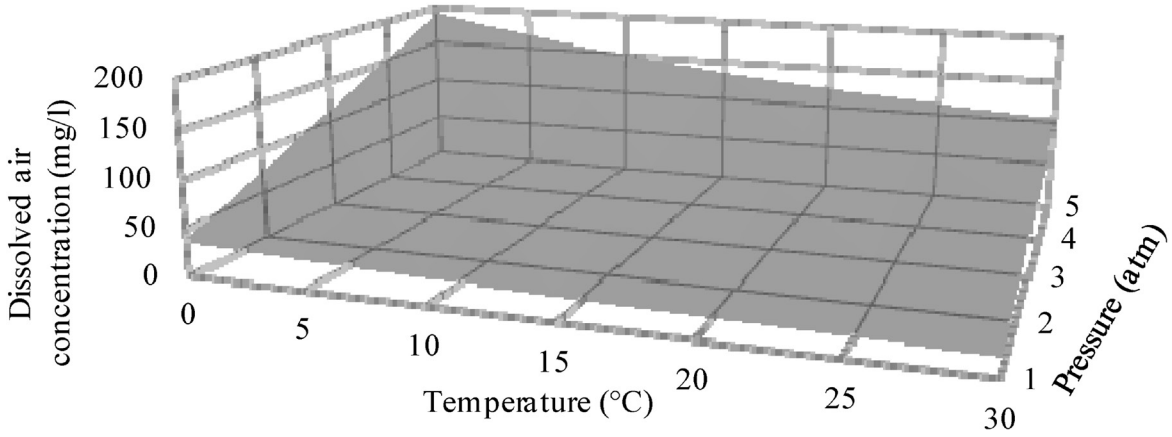



***Figure 11.*** *Solubility of air in water calculated based on tabulated values of $K_H$.*

$$x_i = \frac{p_i}{K_{H,i}} \tag{4}$$

where $x_i$ (–) is the mole fraction of gas dissolved in a solution, $K_{H,i}$ (Pa) is Henry´s constant for a couple solvent-gas, and $p_i$ (Pa) is partial pressure of gas above the solution.

Solubility of air in water was calculated for several pressures and temperatures based on data published in [8]. It was considered a simplified air composition of 21 vol % $O_2$ and 79 vol % $N_2$. Graphical representation for these results are seen in Figure 11.

Sludge liquor produced by flotation is used as a recycle stream saturated by air. The liquor contains both suspended solids (SS) and dissolved solids (DS) and may decrease concentration of dissolved gas within the liquor. This is why dissolved oxygen was measured in sludge liquor from activated and return activated sludge and pure water. Results of measurements are shown in Figure 12. The measurement was taken at 98 kPa. It is obvious that SS and DS decrease solubility of oxygen only negligibly. The activated sludge liquor contains 26 mg/l of SS and 450 mg/l of DS and return activated sludge liquor contains 43 mg/l of SS and 470 mg/l of DS. A bigger deviation is clear at higher temperatures, but because the temperature of sludge at the WWTP in Hranice is from 10 to 20°C the solubility of air in sludge liquor can be calculated based on data tabulated for pure water.

## CONCLUSION

Only some properties of activated and return activated sludge with total solids content less than 1 wt % can be substituted by pure water properties. Samples of sludge from the WWTP in Hranice (CZ) were studied for the purpose of flotation unit design and its CFD model preparation.

The biggest difference is between rheological be-

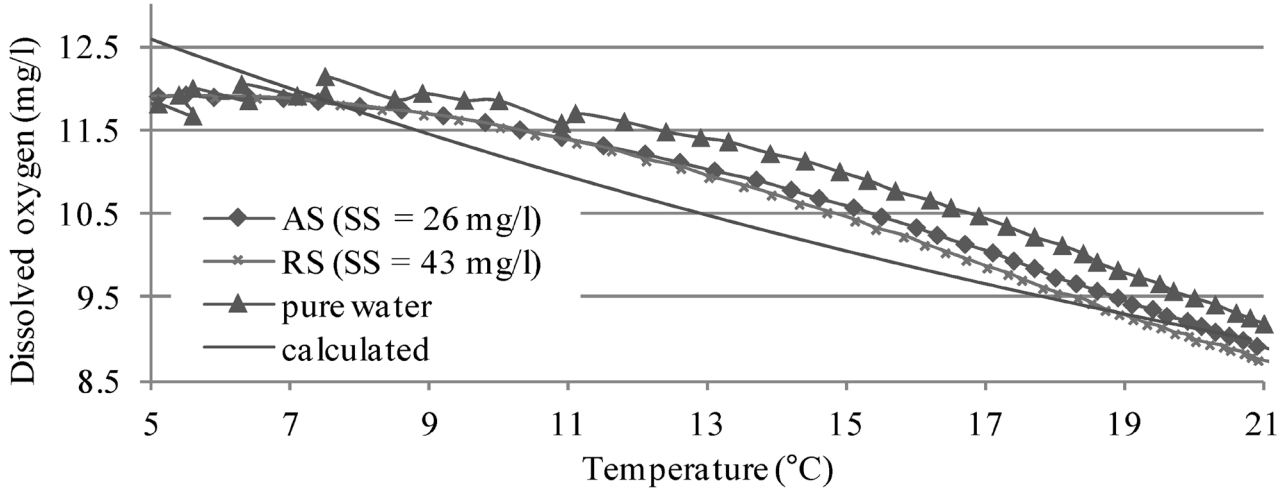



***Figure 12.*** *Dissolved oxygen measured in AS liquor and RS liquor and measured and calculated in pure water (at the pressure 98 kPa).*

haviour of water and sludge. Although, TS content was only about 0.3 wt % for AS or 0.8 wt % for RS. Sludge must be described using the Bingham or the Herschel-Buckley model. Its apparent viscosity is higher than viscosity of water. Regarding density, substitution of density of sludge with density of water for technical calculations (e.g., pump design) is possible. Accurate data should be used for CFD model development.

In addition to sludge properties, the solubility of oxygen in sludge liquor was measured. The solubility of oxygen is the same in the sludge liquor from both AS and RS and in pure water at low temperatures (i.e., about 10°C). Increasing temperature only slightly reduces the amount of oxygen dissolved in sludge liquor versus water. Therefore, to calculate the amount of dissolved air in sludge liquor data tabulated for water may be used.

## AKNOWLEDGEMENT

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## REFERENCES

1. Edzwald, J.K. 1995. Principles and applications of dissolved air flotation. *Wat. Sci. Tech.,* vol. 31, no 3-4. 1–23.
2. Wang, L. K., Shammas N. K. Selke, W.A. and Aulenbach, D.B. 2010. *Handbook of Environmental Engineering, Volume 12: Flotation Technology. 1nd ed.* The Humana Press Inc., USA.
3. Wang, L. K., Hung, Y.-T. and Shammas N. K. 2005. *Handbook of Environmental Engineering, Volume 3: Physicochemical Treatment Processes. 2nd ed.* The Humana Press Inc., USA.
4. Xia, M., Wang. Z., Wu, Z., Wang, X., Yhou, Z. and Lu, J. 2009. Simulation and assessment of sludge concentration and rheology in the process of waste activated sludge treatment. *Jour. of Envir. Sci.*, vol. 21. 1639–1645.
5. Guibaud, G., Dollet, P., Tixier, N., Dagot, C. and Baudu, M. 2004. Characterisation of the evolution of activated sludges using rheological measurements. *Process Biochemistry,* vol. 39. 1803–1810.
6. Mikkelsen, L.H. 2001. The shear sensitivity of activated sludge: Relations to filterability, rheology and surface chemistry. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* vol. 182. 1–14.
7. Ratkovich, N., Horn, W., Helmus, F.P., Rosenberger, S., Naessens, W., Nopens, I. and Bentzen, T.R. 2013. Activated sludge rheology: A critical review on data collection and modelling. *Water Research,* vol. 47. 463–482.
8. Perry, R.H. 1997. *Perry's chemical engineers' handbook. 7th ed.* McGraw-Hill, USA.

# Ozonation and Thermal Pre-Treatment of Municipal Sewage Sludge—Implications for Toxicity and Methane Potential

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**ABSTRACT:** The aim of this study was to determine effects on methane potential and overall sludge quality from two different sludge pre-treatment technologies (ozonation high/low dosage and thermal treatment 55/70°C). In general both treatments produced increased methane potential. Thermal treatment resulted in higher chemical oxygen demand (COD)-solubilisation, while the highest volatile fatty acids (VFA) increase was obtained with ozonation. Sludges had inhibiting effects in a barley seed germination assay and a yeast oestrogen screen both before and after pre-treatment, but inhibition was reduced by ozone treatment and digestion. No statistical significant reduction in concentrations of included pharmaceuticals could be observed.

## INTRODUCTION

IMPLEMENTATION of the European urban wastewater directive (91/271/EEC) yields more sludge as connectivity to sewer systems increases and treatment further generates sludge in EU member countries. Today the common sludge management paradigm involves reduction of volume by thickening and dewatering and at many places also stabilization by anaerobic digestion. The anaerobic digestion step not only reduces organic content by degradation but also contributes to energy production in form of biogas that may be used for heating, electricity, or as fuel to replace fossil fuels. Today about 50% reduction of volatile solids during anaerobic digestion of sewage sludge is possible with traditional mesophilic digestion. However, it has been shown that increased degradation and biogas production may be achieved using pre-treatment methods that degrade particulate matter and hardly biodegradable compounds [1,2,3]. Studies have also shown that some sludge treatment methods could affect toxic compounds in a positive way leading to improved sludge quality [4,5,6]. As a result the possibility for nutrient recirculation by using sludge as a fertilizer will increase and risk of bringing toxic compounds to farmlands will decrease. The sewage sludge directive (86/278/EEC) focuses on protection of the environment when sludge is used in agriculture and also encourages use of sludge. It is also acknowledged that sewage sludge has valuable agronomic properties (86/278/EEC).

Many studies have focused on technologies for increased biogas production and reduced sludge volume [1,2] but not together with effects on toxicity of the final sludge. The aim of this study was therefore to determine which pre-treatment had both beneficial effects on methane potential and overall sludge quality assessed using chemical analyses and bioassays. Different pre-treatment technologies, ozonation with high and low dosage and thermal treatment at 55 and 70°C, were therefore applied to municipal sewage sludges from two wastewater treatment plants (WWTPs) in a comparative study. Sludges were mixes of primary sludge (PS) and waste activated sludge (WAS) in different proportions and with different characteristics.

## MATERIALS AND METHODS

### Sludges and Treatments

Sewage sludges were collected from two different municipal wastewater treatment plants in Southern Sweden. First, there was Öresundsverket (Oer) in Helsingborg with 130,000 *person equivalents* (PE) + industries connected and operated by NSVA with biological phosphorus removal (i.e., no precipitation chemicals). The second was Sjölundaverket (Sj) in Malmö with

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*Table 1. Characteristics of Raw Sludges and Inoculums Used for Digestion Experiments.*

| Substrate | pH | $COD_{fil}$ (mg/l) | TS | VS | Acetate (mg/l) | Propionate (mg/l) | Biosludge | Primary Sludge |
|---|---|---|---|---|---|---|---|---|
| Öresund- raw sludge | 5.77 | 1,710 | 4.5% | 77% | 426 | 297 | 70% | 30% |
| Sjölunda – raw sludge | 5.07 | 2,960 | 4.0% | 82% | 954 | 662 | 10% | 90% |
| Inoculum | 7.60 | 903 | 2.4% | 60% | < 20 | <20 | | |

fil = filtered (Munktell 1002, 110 mm, 6–10 µm)

300,000 PE connected and operated by VA SYD with phosphorus removal by pre-precipitation with $FeSO_4$.

Raw sludge was in both cases a mix of waste activated sludge and primary sludge. Inoculum used in the biogas test was digested sludge from the Sjölunda wastewater treatment plant. Characteristics for the sludges are seen in Table 1.

Thermal treatments were conducted by placing sludge in bottles in a heated water bath at controlled temperatures. Sludges were heated up to 55 or 70°C for 30 min and were then kept at 55 ± 1°C for 6 hours or at 70 ± 1°C for 1 hour. Ozonation was performed in two dosages using an ozone generator (O3-Tech model 1330) and an ozone analyser (Ebara Jitsugyo Co model EG-2001R). Gas flow rate was set to a low of 17 l/h and a high of 36 l/h with an ozone low concentration of 85 g/m$^3$ and ozone high concentration of 130 g/m$^3$. The time needed to obtain 20 mg $O_3$/g TS and 125 mg $O_3$/g TS was 25 min and 45 min, respectively. The lowest dosage of 20 mg $O_3$/g TS was also tested in [4]. Equipment was limited to treating batches of 700 ml sludge at the time. Three batches were therefore treated and then mixed together for each treatment and each sludge type. Raw sludge was kept at 20°C while the other treatments where occurring.

Samples were also taken out for heavy metal analysis and bioassays. They were frozen before transportation and thawed before analysis.

## Physical and Chemical Analyses

Total solids (TS) and volatile solids (VS) were determined by weighing samples before and after drying at 105 ± 1°C for 48 h and ignition at 550°C for 2 h. The pH was measured with a PHM 210 Standard pH meter. Ammonium ($NH_4$) and chemical oxygen demand (COD) were determined spectrophotometrically using a DR Lange, Lasa 100, LCK 303 ($NH_4$) and LCK 114 (COD) after centrifugation (Centronix Microcentrifuge, Model RPM × 1000) and filtration (Cellulose filter Munktell 1002 with filtering rate at 250 ml/min). Propionate and acetate (filtrated samples) were determined by GC (Agilent 6850 Series) using a HP-FFAP column (30 m/0.53 mm/1 µm). Samples for metal analysis were dried at 105°C for 48 h and then digested by a $HNO_3/H_2O_2$ mixture according to EPA 3050. Extracts were analysed by ICP-OES. Methane production was monitored by a GC (Agilent 6850 series equipped with flame ionisation detector (FID) and a column 30 m length/0.32 mm diameter/0.25 µm film).

## Bioassays

For the barley seed germination inhibition test sludge samples were centrifuged at 2,000 rounds per min (rpm) for 10 min, decantered, and the sedimented sludge was dried for 48 h at 50°C. Dried sludge was mortled with sand (washed and dried sand 00; 0.32–0.71 mm) in the ratio of < 10% sludge and > 90% sand in triplicates. Samples were moisturized with demineralised water (6.25 ml/100 g) and germination inhibition was assessed according to US EPA seed germination test [7] using barley (*hordeum vulgare*) seeds and incubated for 7 days at 20 ± 0.5°C. Ecological (KRAV-labelled) planting soil was included as a negative control (triplicates) and 8 controls of 100% sand were included.

For yeast oestrogen screen (YES) sludges were freeze-dried and 0.5 g aliquots were extracted sequentially by methanol and acetone and subsequently subjected to silica gel cleanup according to [8] before transfer into ethanol. Ethanol extracts were utilised for YES [9] where genetically modified yeast cells were incubated for 3 days at 32°C, and the response was measured as absorbance at 540 and 630 nm. A reagent blank and sample blank were included, and the calibration curve ranged from 0.03 µg/l to 60 µg/l. Spiked samples of oestradiol (E2) +6 µg/l were included.

## Methane Potential Tests

Methane potential for the sludges was tested in triplicate using the laboratory-scale anaerobic batch method described in [10]. The test was performed in 2 L glass bottles containing an amount of test substrate

representing 40% of the total volatile solids in each bottle as well as 400 ml of inoculum. The reactors were kept at mesophilic temperature of 35°C and methane production was monitored by a gas chromatograph until gas production ceased. The method provides an easy-to-operate and fast means of measuring methane potentials in test substrates. Reference substrate in the form of cellulose was used to test function of the inoculums used in the test.

### Pharmaceutical Residues

All sludges were freeze-dried and 0.1 g (dry weight) sample aliquots were used for extraction to which internal and surrogate standards were added before extraction. Sequential extraction was performed using ethyl acetate and methanol (1:1) followed by methanol and water (7:3) with 5% triethylamine. Samples were homogenized for four minutes at 42,000 oscillations per minute using a Mini Beadbeater (Biospec. Bartlesville, USA) with zirconium beads and then centrifuged at 14,000 rpm for 10 min. This protocol was done for both eluent mixtures and supernatants were combined, evaporated to 20 µL, and reconstituted in 1 ml water and acetonitrile (95:5 mixture) with 0.1% formic acid.

All pharmaceuticals were analyzed with the same methodology as reported in [11]. In short, a triple stage quadrupole MS/MS TSQ Quantum Ultra EMR (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela LC pump (Thermo Fisher Scientific, San Jose, CA, USA) and a PAL HTC autosampler (CTC Analytics AG, Zwingen, Switzerland) were used as the analytical system. Twenty µL of the sample was loaded onto a Hypersil GOLD aQ TM column (50 mm × 2.1 mm ID × 5 µm particles, Thermo Fisher Scientific, San Jose, CA, USA) preceded by a guard column (2 mm × 2.1 mm i.d, 5 µm particles) of the same packing material and from the same manufacturer.

Both heated electrospray (HESI) and atmospheric pressure photoionization (APPI) in positive and negative ion modes were used for ionization of target compounds. The same method was used to investigate the fate of APIs in wastewater treatment by [12] and [13] and a full method evaluation and detailed description is given in [11].

## RESULTS AND DISCUSSION

Results from measurements of pH, TS, phosphorus, metals and trace elements in all samples before and af-

***Table 2. Sample Matrix, Sample Number, Pre-treatment Type, and Results from Measurement of pH, TS, and P.***

| | WWTP | Sludge Type | Pretreatment | pH | TS (%) | VS (% of TS) | P (% of TS) | COD filt (mg/L) | $NH_4$-N (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Oer | Raw | None | 5.59 | 5.52 | 77.2 | 2.1 | 1710 | n.d |
| 2 | Sj | Raw | None | 5.15 | 4.86 | 82.4 | 1.1 | 2960 | n.d |
| 3 | Oer | Raw | Thermal 55°C | 5.82 | 5.78 | 76.8 | 2.2 | 8310 | n.d |
| 4 | Oer | Raw | Thermal 70°C | 5.73 | 5.43 | 77.6 | 2.3 | 8660 | n.d |
| 5 | Oer | Raw | Ozone (low) | 5.59 | 6.58 | 77.4 | 2.1 | 3350 | n.d |
| 6 | Oer | Raw | Ozone (high) | 5.50 | 6.15 | 76.7 | 2.3 | 5600 | n.d |
| 7 | Sj | Raw | Thermal 55°C | 5.34 | 5.05 | 78.8 | 1.2 | 7890 | n.d |
| 8 | Sj | Raw | Thermal 70°C | 5.29 | 6.50 | 82.9 | 1.2 | 6970 | n.d |
| 9 | Sj | Raw | Ozone (low) | 5.19 | 4.98 | 81.7 | 1.2 | 5180 | n.d |
| 10 | Sj | Raw | Ozone (high) | 4.95 | 6.52 | 81.6 | 1.4 | 6570 | n.d |
| 11 | Sj | Inoculum | Inoculum/None | 7.66 | 2.35 | 60.1 | 3.3 | 903 | n.d |
| 12 | Oer | Digested | Thermal 55°C | 7.71 | 2.51 | 53.8 | 3.5 | 1890 | 1525 |
| 13 | Oer | Digested | Thermal 70°C | 7.74 | 2.07 | 55.6 | 3.8 | 1850 | 1590 |
| 14 | Oer | Digested | Ozone (low) | 7.73 | 2.60 | 57.1 | 3.6 | 1760 | 1450 |
| 15 | Oer | Digested | Ozone (high) | 7.69 | 2.77 | 53.0 | 3.6 | 1640 | 1410 |
| 16 | Sj | Digested | Thermal 55°C | 7.76 | 2.58 | 52.3 | 3.4 | 1520 | 1460 |
| 17 | Sj | Digested | Thermal 70°C | 7.56 | 2.99 | 57.7 | 3.4 | 1500 | 1490 |
| 18 | Sj | Digested | Ozone (low) | 7.72 | 2.91 | 55.6 | 3.4 | 1480 | 1450 |
| 19 | Sj | Digested | Ozone (high) | 7.68 | 2.72 | 53.7 | 3.4 | 1490 | 1425 |
| 20 | Sj | Digested | Inoculum/Dig. | 7.75 | 2.61 | 56.3 | 3.5 | 2020 | 1300 |
| 21 | Oer | Digested | None | 7.67 | 3.30 | 59.7 | 3.7 | 1630 | 1450 |
| 22 | Sj | Digested | None | 7.56 | 2.54 | 55.9 | 3.2 | 1650 | 1430 |

n.d. – not determined

*Table 2. Heavy Metal and Trace Elements (mg/ kg TS) in All Samples Analysed.*

| | WWTP | Sludge Type | Pretreatment | As | Cd | Co | Cr | Cu | Mn | Ni | Pb | Se | Si | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Oer | Raw | None | 5.3 | 0.5 | 2.7 | 11.6 | 406 | 193 | 31.1 | 11.8 | <QL | 169 | 476 |
| 2 | Sj | Raw | None | 3.5 | 1.1 | 2.9 | 9.8 | 315 | 112 | 19.4 | 46.2 | n.d. | 114 | 490 |
| 3 | Oer | Raw | Thermal 55°C | 4.4 | 0.6 | 2.6 | 13.4 | 436 | 201 | 43.8 | 15.5 | <QL | 143 | 527 |
| 4 | Oer | Raw | Thermal 70°C | 6.7 | 0.6 | 3.5 | 12.3 | 407 | 196 | 28.3 | 12.8 | 1.1 | 128 | 495 |
| 5 | Oer | Raw | Ozone (low) | 4.4 | 0.5 | 3.3 | 13.3 | 429 | 193 | 32.3 | 16.0 | 1.5 | 97.9 | 514 |
| 6 | Oer | Raw | Ozone (high) | 9.2 | 0.6 | 2.9 | 13.8 | 445 | 204 | 28.6 | 15.0 | 1.1 | 122 | 531 |
| 7 | Sj | Raw | Thermal 55°C | 3.3 | 1.2 | 3.3 | 10.1 | 330 | 115 | 38.0 | 50.1 | <QL | 166 | 509 |
| 8 | Sj | Raw | Thermal 70°C | 7.8 | 1.0 | 3.2 | 9.9 | 311 | 114 | 18.6 | 46.6 | 1.8 | 101 | 480 |
| 9 | Sj | Raw | Ozone (low) | 2.8 | 1.1 | 2.9 | 8.5 | 322 | 94.9 | 10.1 | 48.6 | <QL | 80.2 | 472 |
| 10 | Sj | Raw | Ozone (high) | 2.8 | 1.3 | 2.7 | 10.7 | 361 | 79.1 | 11.7 | 55.2 | 0.9 | 66.6 | 494 |
| 11 | Sj | Inoculum | Inoculum/None | 4.8 | 1.1 | 8.1 | 22.4 | 543 | 326 | 20.5 | 33.7 | n.d. | 130 | 733 |
| 12 | Oer | Digested | Thermal 55°C | 7.2 | 1.1 | 6.7 | 22.3 | 590 | 333 | 18.1 | 31.7 | n.d. | 124 | 770 |
| 13 | Oer | Digested | Thermal 70°C | 11.3 | 1.1 | 7.5 | 23.6 | 597 | 342 | 24.6 | 32.0 | n.d. | 153 | 793 |
| 14 | Oer | Digested | Ozone (low) | 7.1 | 1.1 | 7.4 | 22.9 | 591 | 338 | 18.1 | 33.8 | 1.1 | 101 | 770 |
| 15 | Oer | Digested | Ozone (high) | 9.9 | 1.1 | 7.3 | 22.2 | 595 | 343 | 17.6 | 32.1 | n.d. | 118 | 785 |
| 16 | Sj | Digested | Thermal 55°C | 9.7 | 1.4 | 8.3 | 24.4 | 612 | 342 | 17.5 | 49.3 | n.d. | 90.2 | 851 |
| 17 | Sj | Digested | Thermal 70°C | 8.6 | 1.4 | 8.1 | 23.0 | 614 | 335 | 17.9 | 53.1 | n.d. | 118 | 864 |
| 18 | Sj | Digested | Ozone (low) | 5.5 | 1.3 | 7.3 | 23.3 | 598 | 332 | 16.4 | 48.2 | <QL | 160 | 837 |
| 19 | Sj | Digested | Ozone (high) | 5.8 | 1.3 | 8.3 | 24.2 | 596 | 340 | 18.3 | 46.0 | <QL | 142 | 827 |
| 20 | Sj | Digested | Inoculum/Dig. | 9.0 | 1.1 | 8.4 | 23.4 | 564 | 342 | 18.7 | 36.6 | <QL | 134 | 770 |
| 21 | Oer | Digested | None | 6.1 | 1.0 | 7.4 | 21.9 | 597 | 344 | 17.5 | 34.2 | <QL | 112 | 799 |
| 22 | Sj | Digested | None | 11.4 | 1.2 | 7.7 | 21.7 | 561 | 318 | 16.1 | 46.4 | n.d. | 117 | 800 |

n.d < 0.3; <QL < 0.9

ter pre-treatment and anaerobic digestion are presented in Tables 2 and 3 together with sample numbers.

For metals and trace elements it is clear that anaerobic digestion significantly reduced the TS content and thus the calculated metal concentration (mg/kg TS) increases accordingly.

## Sludge Treatment

All treatments resulted in increased levels of dissolved COD for both sludge types (Figure 1). The relative increase in dissolved COD was higher for all treatments of sludge from Öresundsverket. It could be seen that thermal treatment resulted in higher hydrolysation, measured as dissolved COD, than ozonation.

The VFA-content (as acetate + propionate) was also measured before and after the treatment (Figure 1). Results show that both acetate and propionate levels increase for both sludge types after all kinds of treatment which is logical since some of the dissolved COD consists of VFA. Similar to the COD increase, the relative increase of VFA is higher after treatment of sludge from Öresundsverket. In contrast to COD which increased the most after thermal treatment, the highest VFA increase is obtained when treating sludge with the highest dose of ozone. This was seen for both sludge types.

## Methane Potential

In total the batch digestion experiment was conducted over a 43 day period. Measurement of accumulated methane produced in relation to organic content in volatile solids (VS) after 43 days is presented in Figure 2 for both sludges. All treatments except one result in higher methane potentials (6–14% higher) compared to untreated sludge in both cases, Oer and Sj. Ozonation with a high dose of Sj-sludge resulted in a lower methane potential than without treatment. This might be a result of oxidation by ozone of easily degradable organic matter or that ozonation of organic matter might lead to degradation products that could inhibit methane production.

## Bioassays

All sludges inhibited barley seed germination at the mixing ratio < 10, whereas negative control stimulated germination during the same conditions. Combination of pre-treatment and anaerobic digestion, especially ozonation (Oer 14 low and Oer 15 high), was found

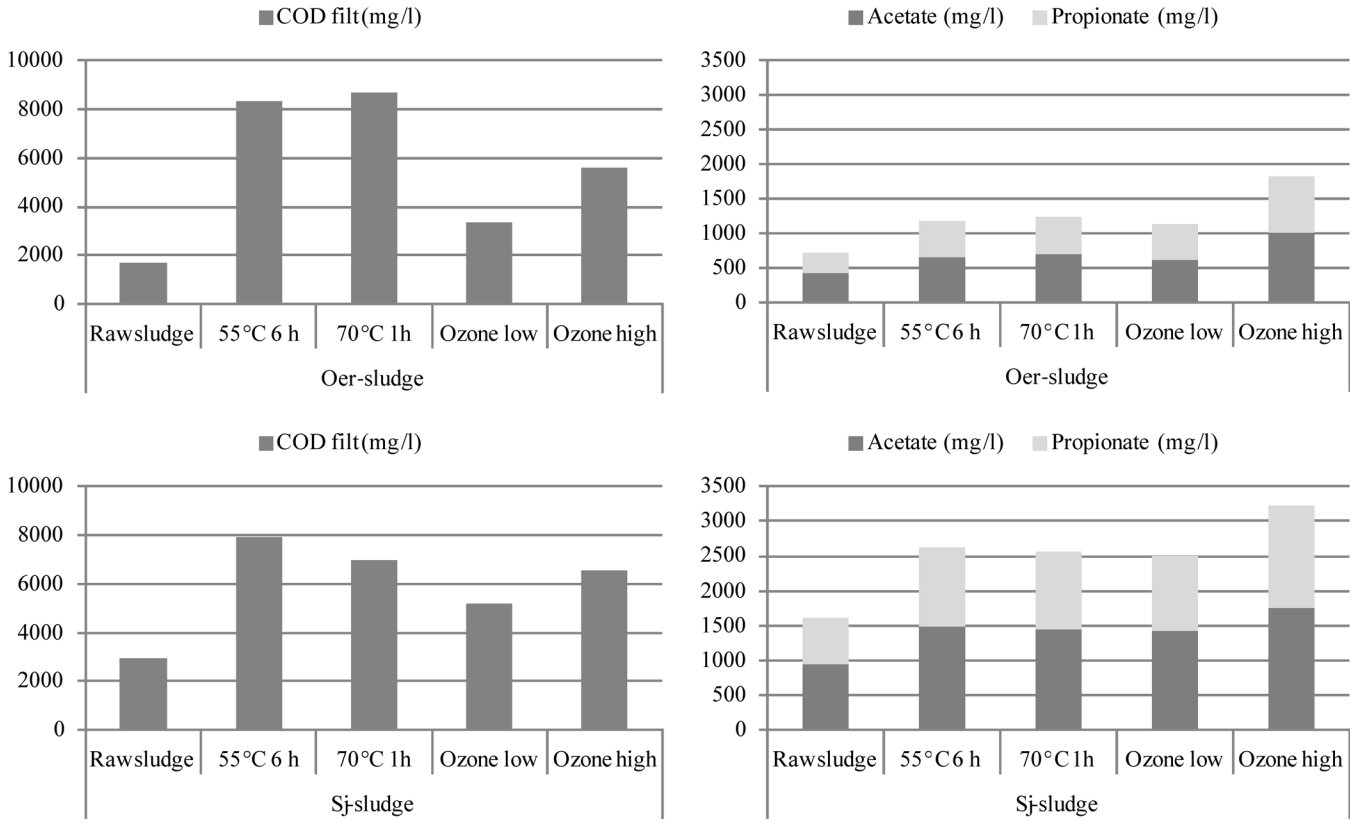


***Figure 1.*** *Dissolved COD and VFA (Acetate and Propionate in mg COD/l) in samples before (raw sludge) and after treatment.*

favourable for sludge from Öresundsverket as inhibition was reduced and growth enhanced (Figure 3). Inhibition from the Sj-sludge (Sjölundaverket) was only slightly reduced by treatments. High variation within triplicates was seen for some of the samples, especially Sj8 (an outlier according to Grubbs test).

Sludge extracts spiked with 6 µg/l of E2 did not result in the same positive response as the standards. Thus, the extracts contained unknown substances that inhibited estrogenic responses. Therefore, no conclusions of the estrogenic effect from the sludge on the yeast can be drawn. Inhibition was substantially larger for Sj than for Oer so Sj is considered more toxic but this is not an end-point in the test [15].

## Reduction of Pharmaceutical Residues

Many of the 101 pharmaceutical substances analysed in the sludges before pre-treatment, after pre-treatment, and after anaerobic digestion in batch tests were not considered detectable since their concentrations were below the limits of quantification. Substances occurring in highest concentration in both Oer- and Sj-sludge before any pre-treatment were performed and are presented in Figure 4.

Reduction of substances occurring in highest concentration in untreated sludge after pre-treatment was calculated from data on concentrations before and after pre-treatment and by considering eventual changes in TS content. However, no statistical significant reduction in concentration of pharmaceuticals in general could be seen after pre-treatment. Calculated reductions of substances occurring in highest concentration before ozonation are seen in Figure 5. As seen there are results that indicate reduction. There is also negative

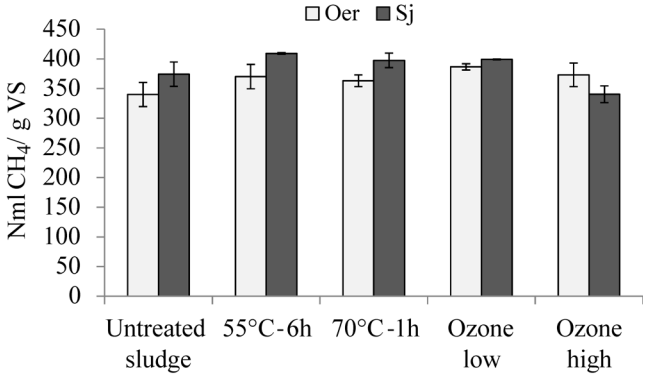


***Figure 2.*** *Accumulated methane potential after 43 days of digestion in a batch laboratory experiment. (Average values of triplicate reactors with standard deviation indicated.)*

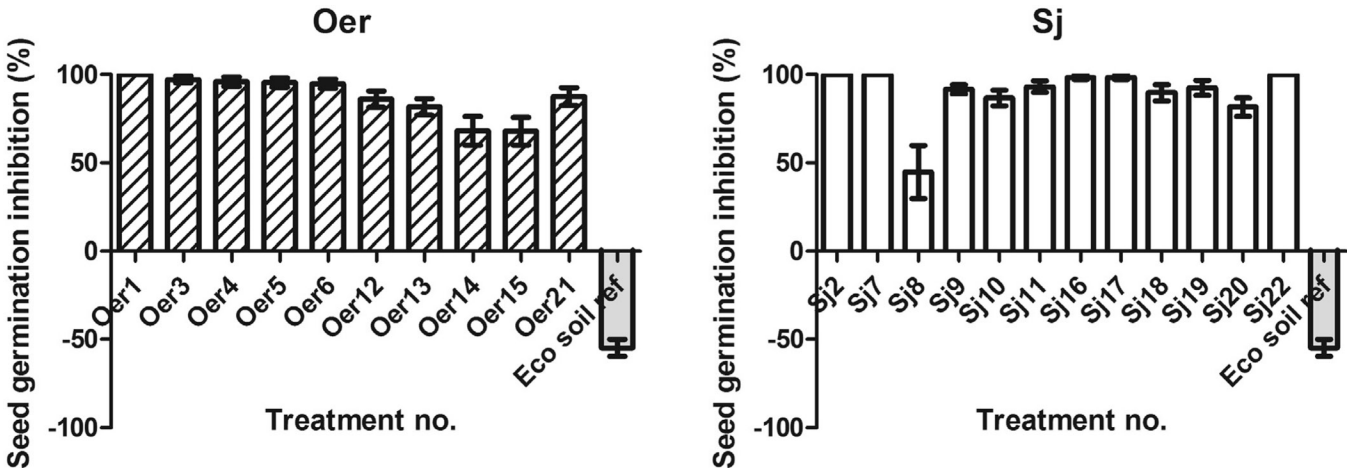


***Figure 3.*** *Germination inhibition. Inhibition normalised to TS.*

reduction (higher concentration after pre-treatment) which could to some extent be explained by deconjugation [14]. However, this also indicates there are uncertainties in the analysis method and that sludge is a complex matrix. No conclusions regarding influence of the anaerobic treatment can be drawn since concentrations of pharmaceuticals are low and tested sludges are mixed with a considerable amount of inoculum.

## CONCLUSIONS

Different pre-treatment technologies: (1) ozonation with high and low dosage and (2) thermal treatment at 55 and 70°C were applied to municipal sewage sludges. Both ozone treatment and thermal treatment create increased methane potential for municipal sewage sludges. Thermal treatment results in higher COD-solubilisation while the highest VFA increase is obtained with ozonation.

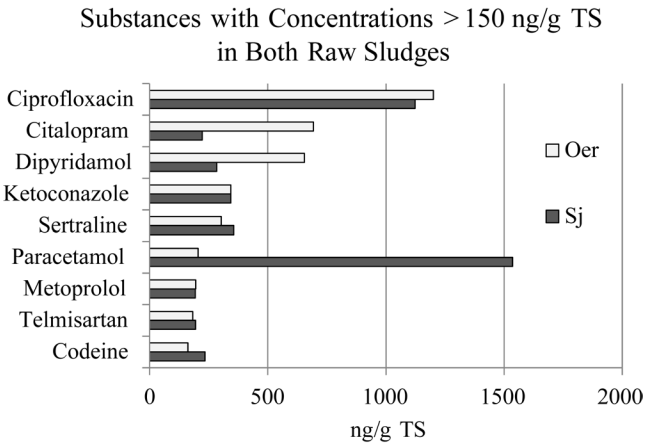


***Figure 4.*** *Substances occurring in highest concentration in both sludges before treatment.*

Also, sludges had inhibiting effects in a barley seed germination assay and a yeast oestrogen screen both before and after pre-treatment. Inhibition was reduced by ozone treatment and further by anaerobic digestion.

Finally, no statistical significant reduction in concentration of pharmaceuticals could be seen after pre-treatment. Some pharmaceuticals could even be detected in higher levels after pre-treatment.

## ACKNOWLEDGEMENTS

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## ABBREVIATIONS

Sj = Sludge from Sjölunda wastewater treatment plant (Malmö)
Oer = Sludge from Öresundsverket wastewater treatment plant (Helsingborg)
PS = primary sludge
WAS = Waste activated sludge

## REFERENCES

1. Carrère, H., Dumas, C., Battimelli, A., Batstone, D. J., Delgenès, J. P., Steyer, J. P., Ferrer, I. (2010) Pretreatment methods to improve sludge

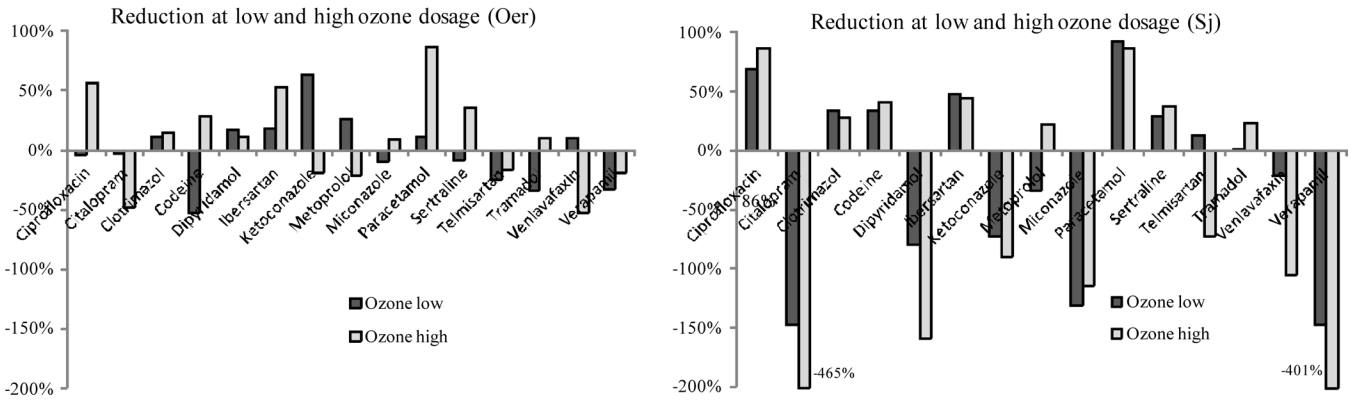


***Figure 5.*** *Reduction of pharmaceuticals during ozonation for substances occurring in highest concentrations for Oer and Sj- sludges.*

anaerobic degradability: A review. *Journal of hazardous materials,* 11/2010, Volume 183, Nr 1-3, pp. 1–15.

2. Bordeleau, É. L., Droste, R. L. (2011) Comprehensive review and compilation of pretreatments for mesophilic and thermophilic anaerobic digestion. *Water Science and Technology,* Volume 63, Nr 2, pp. 291–296.
3. Khanal, S.K., Grewell, D., Sung, S., van Leeuwen, H.J. (2007) Ultrasound Applications in Wastewater Sludge Pretreatment: A Review. *Critical Reviews in Environmental Science and Technology,* Volume 37, Nr 4, pp. 277–313.
4. Carballa, M., Manterola, G., Larrea, L., Ternes, T., Omil, F., Lema, J.M. (2007) Influence of ozone pre-treatment on sludge anaerobic digestion: Removal of pharmaceutical and personal care products. *Chemosphere,* Volume 67, Issue 7, April 2007, pp. 1444–1452.
5. Bernal-Martinez, A., Carrère, H., Patureau, D., Delgenès, J-P. M(2007) Ozone pre-treatment as improver of PAH removal during anaerobicdigestion of urban sludge. *Chemosphere* 68:6, pp.1013–1019.
6. Werker, A. G., Carlsson, M., Morgan-Sagastume, F., Le, M. Son, Harrison, D.(2007) Full scale demonstration and assessment of enzymic hydrolysist pre-treatment for mesophilic anaerobic digestion of municipal wastewater treatment sludge. *Proceedings of the Water Environment Federation, WEFTEC 2007*: Session 61–70, pp. 5406–5423(18).
7. United States Environmental Protection Agency (1996) Ecological Effects Test Guidelines, OPPTS 850.4200 Seed Germination/Root Elongation Toxicity Test, EPA 712–C–96–154
8. Ternes, T. A.; Andersen, H. R.; Gilberg, D.; Bonerz, M. (2002) Determination of Estrogens in Sludge and Sediments by Liquid Extraction and GC/MS/MS. *Analytical Chemistry 74*, pp. 3498–3504.
9. Routledge EJ. and Sumpter JP (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environmental Toxicology and Chemistry, 15* (3), pp. 241–248.
10. Hansen, T. L., Schmidt, J. E., Angelidaki, I., Marca, E., Jansen, J. la C., Mosbæk, H., Christensen, T. H. (2004) Method for determination of methane potentials of solid organic waste, *Waste Management, 24*, pp. 393–400.
11. Grabic R, Fick J, Lindberg RH, Fedorova G, Tysklind M. (2012) Multiresidue method for determination of trace levels of 100 pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry. *Talanta, 100*, pp 183–195.
12. Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., J. La Cour Jansen, H.R. Andersen, (2011) Determination of sorption of seventy-five pharmaceuticals in sewage sludge, *Water Research, 45*, pp. 4470–4482.
13. Hey, G., Ledin, A., la Cour Jansen, J., and Andersen, H.R. (2012) Removal of pharmaceuticals in biologically treated wastewater by chlorine dioxide or peracetic acid. *Environmental Technology, 33*(9), pp. 1041–1047.
14. Falås, P., la Cour Jansen, J., Ledin, A., Andersen, H. R. (2012) Occurrence and reduction of pharmaceuticals in the water phase at Swedish wastewater treatment plants. *Water Science and Technology,* Vol. 66, No. 4, 2012, pp. 783–791.
15. Routledge, EJ and JP Sumpter. (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ. Toxicol. Chem. 15*, 241–248.

# Particle Size Analysis and Ultrasound Pre-treatment of Sludge

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**ABSTRACT:** The objective of this work was to measure the modification taking place in particle size distribution during the course of batch digestion experiments of primary and secondary sludge and also to evaluate the effect of ultrasound pre-treatment on secondary sludge. Sewage sludge used in this study was obtained from the WWTP of León. Secondary sludge was pre-conditioned applying ultrasound at different energy ranges in order to investigate behavior of sludge in the anaerobic biodegradation process with respect to particle size and pretreatment. Pre-treatment of secondary sludge resulted in a decrease of mean particle size and enhanced biogas production at sonication with 2500 kJ/Kg ST when compared to digestion results obtained from secondary sludge. Application of an elevated range of energy resulted in a decrease of biogas production.

## INTRODUCTION

TODAY, one of the most important issues considering environmental and economic factors is management of sludge. Concern society is experiencing for this issue is related to the large amount of sludge produced daily from municipal wastewater treatment plants. Actually, there are several processes for sludge handling: anaerobic digestion, incineration, composting, disinfection, thickening, and thermal drying.

Anaerobic digestion is a technique usually used as an efficient technology for treatment of sludge. It plays an important role in transforming organic matter into biogas which can be valorised for obtaining energy and also it is capable of reducing the amount of solids and pathogens originally presented in the sludge [1].

Sludge can comprise primary sludge which is separated from wastewater during pre-settling and biological excess sludge from the activated sludge system (denoted also as secondary sludge) [2]. Secondary sludge is usually difficult to digest because it is mainly composed of microorganisms and organic and inorganic compounds agglomerated in polymeric substances. This results in the limiting rate of the hydrolysis step [3] and after this first step these complex structures are subsequently converted into simple compounds.

Pre-treatment of secondary sludge might be required to increase digestibility, minimise time needed for digestion, and maximise biogas production. Various alternative methods have been proposed to improve anaerobic digestion of sludge including chemical (addition of acids and surfactants) [4], thermal [5–7], and mechanical methods like ultrasonication. This method has been reported to be an efficient process for sludge disintegration and thus increases biodegradability [8]. Some authors have studied application of pre-treatment with ultrasounds with specific energies ranging from 1,000 to 10,000 kJ/kg TS obtaining as a result increased biogas production [3,9–11]. Ultrasound pretreatment leads to breakdown of flocs, cell walls, and bacterial membranes enhancing hydrolysis of volatile solids in the sludge [10].

Various studies have reported ultrasonication has different effects. It may enhance biogas production. However, if it is too intense many fine particles are produced that may not be beneficial for sludge conditioning.

During the digestion process changes can occur that influence fundamental sludge characteristics like particle size or organic solids content which directly affect biodegradability of sludge over time. The present study was conducted to assess variation in particle size distribution and investigate the effect of ultrasonic conditioning during sludge digestion.

## MATERIAL AND METHODS

Sewage and digested sludge used was obtained from the WWTP of the city of León. Primary and secondary sludge (PS and SS) were used as substrates for digestion experiments under batch conditions. Chemical characteristics of the sludge are seen in Table 1.

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*Table 1. Solid Content of Sludge Used.*

| | TS (g/l) | VS (g/l) |
|---|---|---|
| PS | 25.5 | 17.9 |
| SS | 18.2 | 13.9 |
| SS_2500 | 19.4 | 15.4 |
| LS_5000 | 19.7 | 15.4 |

## Pre-treatment Experiments

Disintegration by ultrasound of secondary sludge was performed with an ultrasonic processor UP400S (Dr. Hielscher, Germany) operating at a nominal power of 300 W and 24 kHz. The sonotrode had a diameter of 22 mm [10]. Samples of SS (500 ml) were prepared in Erlenmeyer flasks of 1,000 ml and mixed prior to sonication. Applied ultrasonication energy (Es) is given by:

$$\mathrm{Es} = \frac{P \times t}{V \times \mathrm{TS}_0} \quad (1)$$

Where $P$ is ultrasonic power, $t$ is application time, $V$ is sample volume, and $\mathrm{TS}_0$ is initial concentration of TS. This parameter was controlled in our experiments by adjusting ultrasonication time [11]. Sonication was performed for 2 min (SS_2m) and 4 min (SS_4m) corresponding to different energy inputs ranging 2,500–5,000 kJ/Kg ST.

## Batch Digestion

Batch experiments were performed to determine biochemical gas potential of substrates used in this study. Experiments were carried out until cessation of gas production was observed. Batch reactors (Erlenmeyer flasks of 250 mL) were filled with inoculum and a corresponding amount of substrate in order to attain the desired proportion of VS between substrate and inoculum. Tap water was added to complete a 250mL volume in all batch reactors.

Two reactors were used for measuring gas production and composition. A batch reactor containing only inoculum was used as a blank. Biogas produced by this reactor was subtracted from the corresponding tests. Temperature of digestion was 34°C, this being

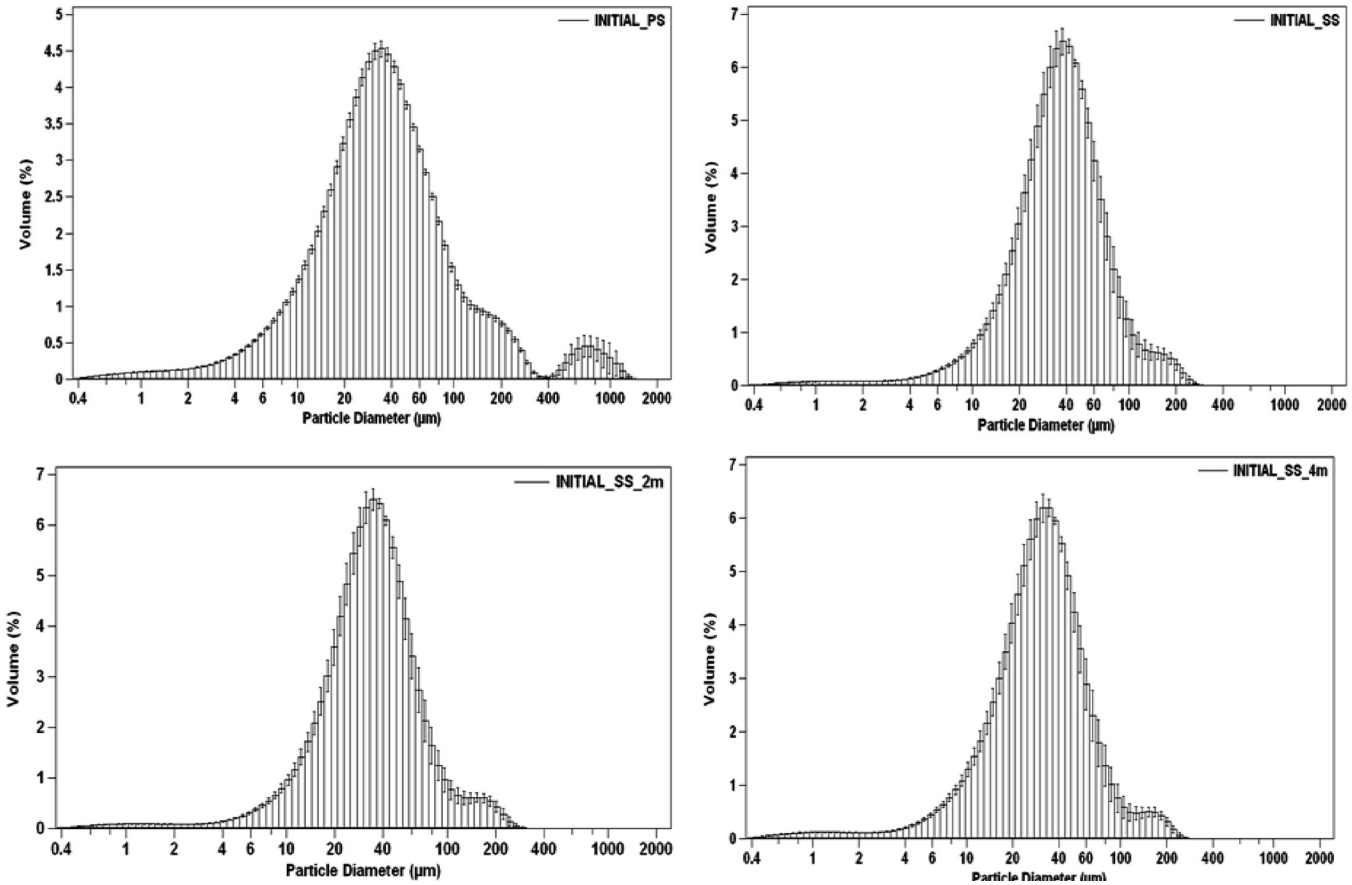


*Figure 1. Particle size distribution of sludge initial samples.*

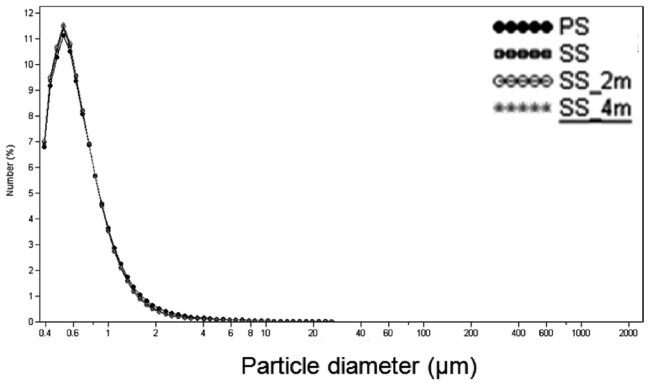


***Figure 2.*** *Number based particle size distribution of sludge before anaerobic digestion.*

controlled by a water bath. Agitation was provided by means of magnetic stirrers. Gas volumes were measured using bottle gasometers and corrected to standard temperature and pressure (STP), 0ºC and 760 mmHg, respectively.

The proportion of VS between inoculum and substrate for this experiment was 1:1. Digestion systems were denoted according to the substrate used, either PS or SS.

### Analytical Techniques

TS, VS, and pH were determined in accordance with APHA Standard Methods (1989) [12] and were regularly monitored during the digestion process.

Composition of biogas was analysed using a gas chromatograph (Varian CP3800 GC) equipped with a thermal conductivity detector. A packed column (HayeSep Q 80/100; 4 m) followed by a molecular sieve column (1 m) was used to separate $CH_4$, $CO_2$, $N_2$, $H_2$, and $O_2$. Carrier gas was helium, and columns were operated at a pressure of 331 kPa and at a temperature of 50°C [13].

### Particle Size Analysis

Particle size analysis was carried out using a Laser Diffraction Particle Size Analyser LS 13 320 Beckmann Coulter (stirrer speed = 350; pump speed = 500; and ultrasonic = off). Ror analysis each sample was diluted in tap water before being analysed 10 times [14].

## RESULTS

Figure 1 shows particle size distribution (PSD) by volume between 0.45–2,000 µm for PS and SS as well as for pre-treated SS samples.

Peak particle size for volume distribution was in the range at 5–100 µm. However, the PSD of the PS sample also showed a pronounced peak at 300–1300 µm. In the case of pre-treated SS samples with ultrasonication, it was observed that peak distribution of particles is directed towards smaller particle sizes when energy of ultrasonication is increased. Area corresponding to the base of the curve is increased with an increase in sonication energy applied to the sample. The widening of the base of the peak corresponding to the PSD as expressed in percentage of volume may indicate that disruption of the cell is taking place and thus obtaining a different fraction of variable size.

PSD of samples represented by number are seen in Figure 2. The majority of particles are in the range of 0.4–10 µm. According to other authors [15], it is around the size of a single bacteria cell. No significant differences are observed from this graphic. Thus, it indicates that although size of particles is similar in all samples, particles of greatest size report a significant fraction when considering the volumetric fraction figure.

Table 2 shows a comparative particle size analysis of secondary sludge after sonication at two different energy inputs. From this table it is observed that the increase in sonication energy results in smaller particles being obtained in the sludge and hence lower d10, d50, and d90 values were obtained. This values indicate that 10, 50, and 90% of particles measured were less than or equal to sizes stated. Results confirm that the application of the ultrasonication process results in a breakdown of flocs decreasing the average size of floc. In addition, the decrease of the size of particles is related to an increase in superficial area. This increase

***Table 2. Volume Statistics of Secondary Sludge (Calculations from 0.375 µm to 2000 µm).***

| | Amount (%) | Mean (µm) | S.S.A ($cm^2$/mL) | d10 (µm) | d50 (µm) | d90 (µm) |
|---|---|---|---|---|---|---|
| SS | 100 | 44.2 ± 3.32 | 2845 ± 156 | 14.8 ± 0.732 | 36.6 ± 2.23 | 78.5 ± 7.96 |
| SS_2 m | 100 | 41.5 ± 3.26 | 3136 ± 190 | 13.5 ± 0.77 | 33.5 ± 2.89 | 73.6 ± 7.51 |
| SS_4 m | 100 | 37.4 ± 3.03 | 3704 ± 224 | 11.2 ± 0.65 | 30.4 ± 2.02 | 66.9 ± 7.11 |

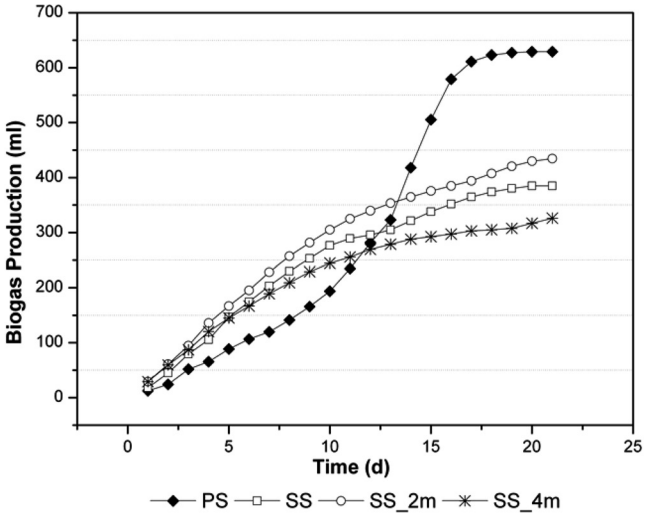


***Figure 3.*** *Gas production from batch digestion test of sludge.*

should be related to a greater area for microbial attack now being available which should speed up microbial degradation.

Results obtained from batch digestion assays are presented in Figure 3 for PS and SS systems. When considering the degradation pattern of PS, an initial slow rate of gas production which is significantly increased about day 10 of digestion is observed. The wide base of PSD obtained from this PS sample may indicate that although large volume particles may present a complex degradation pattern, most of these particles are finally degraded resulting in high biogas production.

Ultrasonic pre-treatment did not produce a significant increase in biogas production. Destruction of flocs and reduction of mean average size did not result in a high increase of methane yield. The value reported was only 13% higher than that obtained from digestion of secondary sludge. Sonication of too intense of energy produced a disintegration of flocs in many fine particles which was not beneficial to biological degradation of this sludge decreasing biogas yield 15%.

Seen in Figure 4, variation in PSD is experienced by different samples during the course of digestion. Modifications observed from the PS system indicate a decrease in content of those particles with higher diameter. Application of pre-treatment to SS led to formation of particles that although with a higher specific

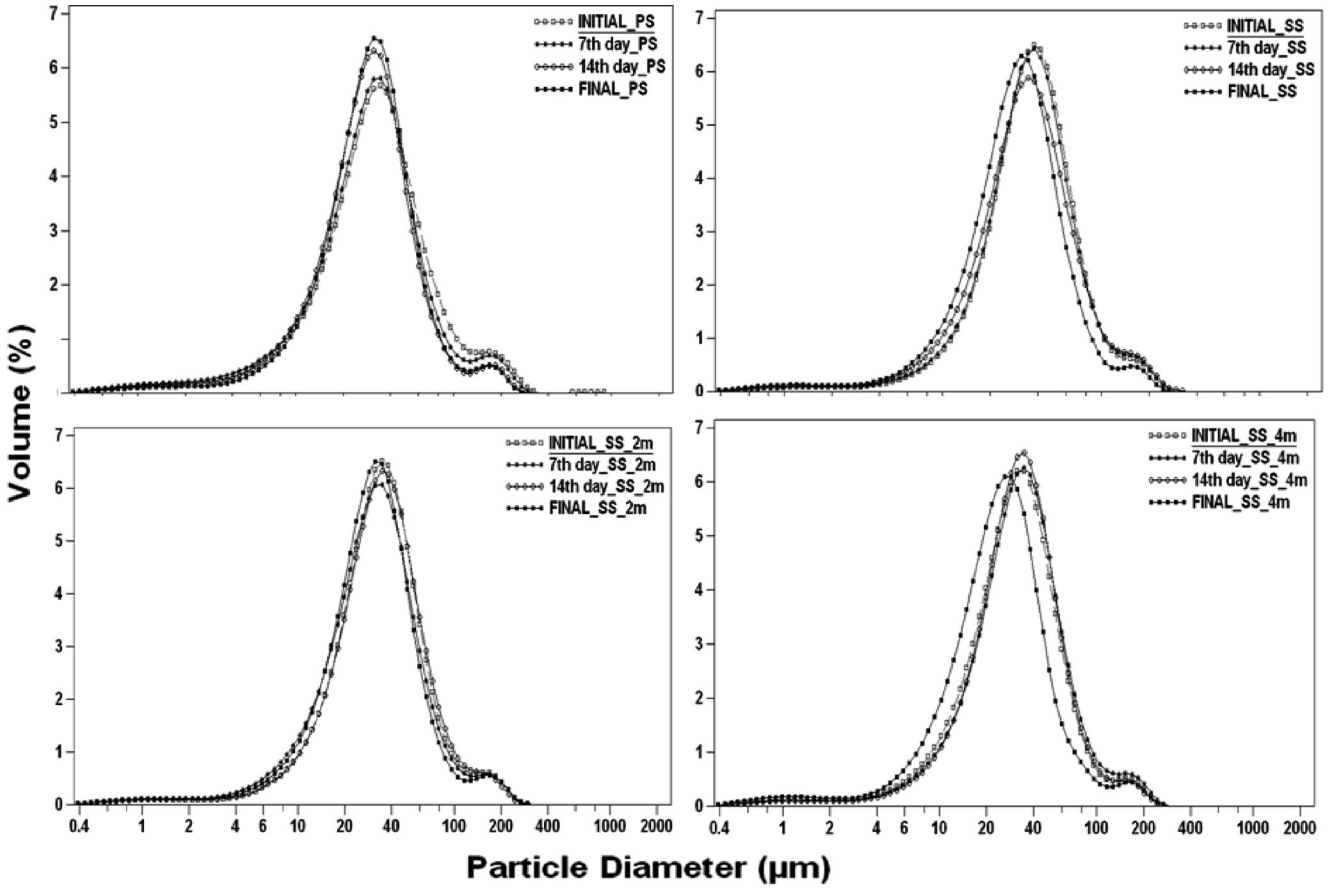


***Figure 4.*** *PSD obtained at different digestion times for samples.*

***Table 3. Volume Statistics of Secondary Sludge (Calculations from 0.375 µm to 2000 µm).***

| | Mean (µm) | S.S.A ($cm^2/mL$) | d10 (µm) | d50 (µm) | d90 (µm) |
|---|---|---|---|---|---|
| **Primary Sludge** | | | | | |
| Initial | 44.7 ± 2.82 | 3508 ± 92.95 | 11.0 ± 0.27 | 32.6 ± 0.93 | 85.9 ± 3.74 |
| 7th day | 39.8 ± 1.27 | 4202 ± 136.1 | 9.20 ± 0.34 | 30.3 ± 1.06 | 73.9 ± 2.49 |
| 14th day | 35.9 ± 1.57 | 4123 ± 157.8 | 9.83 ± 0.42 | 28.7 ± 1.15 | 62.9 ± 2.87 |
| Final | 36.8 ± 1.99 | 3714 ± 153.4 | 11.3 ± 0.45 | 29.9 ± 1.29 | 63.7 ± 4.48 |
| **Secondary Sludge** | | | | | |
| Initial | 44.2 ± 3.32 | 2845 ± 156.0 | 14.8 ± 0.73 | 36.6 ± 2.23 | 78.5 ± 7.96 |
| 7th day | 43.9 ± 1.20 | 2873 ± 77.11 | 14.3 ± 0.37 | 35.8 ± 0.89 | 79.3 ± 2.19 |
| 14th day | 43.0 ± 5.24 | 3328 ± 230.9 | 12.4 ± 0.80 | 33.7 ± 2.68 | 81.0 ± 14.28 |
| Final | 36.3 ± 1.57 | 3755 ± 152.4 | 11.1 ± 0.44 | 29.6 ± 1.26 | 64.6 ± 3.26 |
| **SS_2m** | | | | | |
| Initial | 41.5 ± 3.26 | 3136 ± 190.4 | 13.5 ± 0.77 | 33.5 ± 2.11 | 66.9 ± 7.51 |
| 7th day | 38.7 ± 1.76 | 3117 ± 136.1 | 11.1 ± 0.41 | 30.8 ± 1.24 | 72.9 ± 3.53 |
| 14th day | 41.6 ± 5.08 | 3182 ± 238.6 | 13.2 ± 0.90 | 33.9 ± 2.89 | 67.4 ± 13.44 |
| Final | 37.9 ± 2.5 | 3578 ± 174.9 | 11.8 ± 0.53 | 30.2 ± 1.58 | 55.2 ± 5.41 |
| **SS_4m** | | | | | |
| Initial | 37.4 ± 3.03 | 3704 ± 224.9 | 11.2 ± 0.65 | 30.4 ± 2.02 | 66.9 ± 7.11 |
| 7th day | 40.3 ± 1.46 | 3326 ± 105.9 | 12.2 ± 0.37 | 32.4 ± 1.04 | 72.9 ± 2.83 |
| 14th day | 38.2 ± 2.78 | 3390 ± 171.4 | 12.5 ± 0.57 | 31.8 ± 1.69 | 67.4 ± 6.36 |
| Final | 31.3 ± 0.88 | 4695 ± 115.4 | 8.86 ± 0.22 | 24.5 ± 0.60 | 55.2 ± 1.85 |

surface were not available to microorganisms when energy was applied too intense. In this sense, modifications experienced in PSD over the course of digestion are the less significant.

Table 3 presents results from digestion tests at different times over the course of the degradation process. From this table it was observed that there is an increase in surface area with time of digestion for all samples. It is of particular relevance that decrease is experienced by these samples as the digestion process takes place. Evolution of the size of the particles may have an important influence on availability of these particles to microorganisms.

## CONCLUSION

Pre-treatment of secondary sludge with ultrasounds show a decrease of particle size and also enhances biogas production at sonication energy of 2,500 kJ/Kg ST. It was demonstrated that particle size plays a relevant role in the availability of this material to microbial degradation when compared with results obtained from secondary sludge samples. Application of an elevated range of energy resulted in a decrease in biogas production.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Appels, L., Baeyens, J., Degreve, J., Dewil, R., "Principles and potential of the anaerobic digestion of waste-activated sludge", *Prog. Energ. Combust.,* Vol. 34, 2008, pp. 755–781.
2. Luostarinen, S., Luste, S., Sillanpää, M., "Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant", *Bioresourc. Technol.,* Vol. 100, 2009, pp.79–85.
3. Rajan, B., Nakhla, G., Ray, M., "Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge", *Waste Manage.,* Vol. 32, 2012, pp. 542–549.
4. Chen, Y.G., Yang, H.Z., Gu, G.W., "Effect of acid and surfactant treatment on activated sludge dewatering and settling", *Water Res.,* Vol. 35, 2001, pp. 2615–2620.
5. Appels, L., Degrève, J., Bruggen, B.V.D., Impe, J.V., Dewil, R. "Influence of low temperature thermal pre-treatment on sludge solubilization, heavy metal release and anaerobic digestion", *Bioresource Technol.*, Vol. 101, no. 15, 2010, pp. 5743–5748.
6. Climent, M., Ferrer, I., Baeza, M.M., Artola, A., Vázquez, F., Font, X., "Effects of secondary sludge pre-treatment on biogas production under thermophilic conditions", *Chem. Eng. J.,* Vol. 133, 2007, 335–342.
7. Gavala, H.N., Yenal, U., Skiadas, I.V., Westermann, P., Ahring, B.K., "Mesophilic and thermophilic anaerobic digestion of primary and sec-

ondary sludge: effect of pre-treatment at elevated temperature". *Water Research.* Vol. 37, 2003, pp. 4561–4572.

8. Tiehm, A., Nickel, K., Zellhorn, M., Neis, U., "Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization", *Water Res.,* Vol. 35, 2001, pp. 2003–2009.
9. Khanal, S.K., Grewell, D., Sung, S., Leeuwen, J.V., "Ultrasound applications in wastewater sludge pretreatment: a review", *Crit. Rev. Environ. Sci. Tech.,* Vol. 37, 2007, pp. 277–313.
10. Braguglia C., Gianico A., Mininni G., "Laboratory-scale ultrasound pre-treated digestion of sludge: Heat and energy balance", *Bioresourc. Technol.* Vol. 102, 2011, pp. 7567–7573.
11. Feng, X., Deng, J., Lei, H., Bai, T., Fan, Q., Li, Z., "Dewaterability of waste activated sludge with ultrasound conditioning", *Bioresourc. Technol.* Vol. 100, 2009, pp. 1074–1081.
12. APHA, AWWA, WPCF (1989) Standard methods for the examination of water and wastewater. In: Diaz de Santos SA (ed) *American Public Health Association, American Water Works Association and Water Pollution Control Federation,* Madrid, Spain.
13. Cuetos, M.J., Gómez, X., Otero, M., Morán, A., "Anaerobic digestion of Solid slaughterhouse waste (SHW) at Laboratory scale: influence of co-digestion with the organic fraction of municipal solid waste (OFMSW)". *Biochem. Eng. J.* Vol. 40, 2008, pp. 99–106.
14. Houghton, J., Burgess, J., Stephenson, T., "Off-line particle size analysis of digested sludge", *Water Res.,* Vol. 36, 2002, pp. 4643–4647.
15. Wu, J., Jiang, X., Wheatley, A., "Characterizing activated sludge process effluent by particle size drsitribution respirometry and modelling". *Desalinisation* Vol. 249, 2009, pp. 969–975.

# Trace Metallic Element Behaviour in Regards to Sludge Characteristics Prior to Land Application

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**ABSTRACT:** Trace metallic element behaviour was investigated for activated, thickened, digested, and composted sludge. Cadmium and copper competitive biosorption and desorption were carried out during batch tests. Isotherms were modelled to determine sorption parameters in relation with sludge characteristics. Metals sorption/desorption behaviour were related with biochemical sludge composition. Indeed, sorption was more effective and desorption less effective for digested and composted sludge which presented an important part of humic substances and polysaccharide compounds responsible for interaction with the metallic element. Although cadmium and copper were concentrated in digested sludge, the composting process reduces metals bioavailability thanks to metal complexation with humic substances.

## INTRODUCTION

In recent years development of analytical devices led to detection of organic and mineral micropollutants in different compartments of the environment such as in waste or natural waters. Removal of these compounds in a wastewater treatment plant is not complete. About 80% of micropollutants were removed by conventional activated sludge plants, but about two-thirds of removed substances were mainly transferred to sludge [1]. Sludge sorption was due to hydrophobic interactions, cation exchange, cation bridging, surface complexation, and hydrogen binding [2]. Micropollutant sludge sorption is highly dependent upon their physico-chemical characteristics. Major routes for sludge management are land application, composting, incineration, and landfill [3]. However these routes are subject to various environmental, social, and economic problems, especially for application of sewage sludge as fertilizer to agricultural land. As protection against potential adverse effects of sewage sludge constituents, such as heavy metals, the European Union Directive 91/271/EEC plans to limit the amount of micropollutants released to the environment by sewage sludge disposal [3]. Metals defined by the Water Framework Directive as priority substances are generally found in sludge at levels below 15 mg/kg of dry matter for cadmium and mercury and from 5 to 250 mg/kg of dry matter for nickel and lead [4]. However, metals content depends on type of wastewater.

One major problem hindering the disposal of excess sludge is heavy metals content which is of great concern due to their high toxicity if rejected in the environment. 70 to 90% of metals contained in wastewater are retained on sludge during treatment [5,6]. However, distribution varies according to the nature of the metal. Cadmium and copper were chosen in this work because they are among metals defined by the Water Framework Directive and found in sludge at 1 to 10 mg/kg of dry matter and 100 to 1,000 mg/kg of dry matter for cadmium and copper, respectively [1]. Sorption of heavy metals onto sludge depends on their constituents which are mainly proteins, carbohydrates, and phenolic compounds which contain functional groups such as carboxyl, hydroxyl, and amine. These functional groups are responsible for binding of metals ions. Therefore, identification of these compounds and the number of binding sites is of interest for understanding the way metals interact with sludge before landspreading.

Application of organic wastes to farmland has increased over the years as it contributes to the preservation of the environment and results in an improvement of chemical, biochemical, and physical properties of soil. Although, there is an increased risk of soil and

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ground water being contaminated by pollutants. Sewage sludge may contain heavy metals whose presence in soil may reduce enzyme activities and affect microbial communities in soil. They accumulate in soil and are taken up by crop plants posing a health hazard. The purpose of this study is to assess potential hazards linked to landspreading in relation to sludge origin and characteristics. Sorption and desorption tests were performed with cadmium and copper on different kinds of sludge to compare their behavior in regards to sludge composition.

## METHODS

### Sludge Samples

Activated, thickened, and digested sludge samples were obtained from the municipal wastewater treatment plant (WWTP) of the city of Limoges (France, 285,000 inhabitant-equivalent, influent composed of 85% v/v domestic, and 15% v/v slaughterhouse wastewater) and stored for a maximum duration of 24 h at 4°C before use. Composted sludge was obtained from the composting platform of Berneuil in Limousin which collects sewage sludge from the WWTP of Limoges. Average characteristics of samples were as follows in Table 1.

### Chemical and Biochemical Composition

Sludge characteristics measurements were triplicated. Measurements of total and volatile solids were performed on total sludge after desiccation at 105°C for 24h (total solids) followed by 2h at 550°C (volatile solids). They also were done on centrifugation pellets (6,000 g, 20 min, 4°C) which allowed for the determination of total suspended and volatile suspended solids (TSS and VSS) according to the normalized method [7]. Standard deviation for triplicate samples was below 2%.

Chemical Oxygen Demand (COD) was measured with cuvette tests (HACH LANGE). Polysaccharides were determined using the colorimetric method of Dubois *et al.* [8]. Proteins and humic like substances were determined using the method of Lowry *et al.* [9] modified by Frølund *et al.* [10]. These measurements were done on both total and soluble fractions of sludge. The soluble fraction is defined here as the fraction resulting from filtration of the samples through a cellulose nitrate membrane of 0.45 µm pore size. For all colorimetric methods used in this study the standard deviation for triplicate samples was 1–8% and 5–20% for soluble and total fractions, respectively.

***Table 1. Characteristics of Sludge Samples.***

| Sludge | Total Solids (TS) | Volatile Solids/total Solids (VS/TS) |
|---|---|---|
| Activated sludge | 3.5 g/L | 67% |
| Thickened sludge | 51.4 g/kg | 74% |
| Digested sludge | 31.5 g/L | 64% |
| Composted sludge | 427.3 g/kg | 61% |

### Determination of Ionizable Functional Groups and Acidity Constants (pKa)

In order to assess chemical differences and evolution during stages of sludge treatment, potentiometric titrations were carried out with an automatic titrator Metrohm 716 Titrino on the different sludge samples to determine pKa values and proton binding site concentrations for both floc surface and soluble phase. Data interpretation using a non-electrostatic model of proton adsorption was performed using the software PROTOFIT [11] which allowed for both number and acidity constant of the different functional groups to be determined. Sample preparation and measurements were done in accordance with protocols describe in Laurent *et al.* [12].

### Cadmium and Copper Biosorption Tests

Cadmium and copper behavior towards activated, thickened, digested, and composted sludge was evaluated by batch biosorption tests conducted at a constant pH level of 7 as described in other studies [12,13]. As total solids concentrations were different for each sludge sample and in order to compare the behavior of metals, the same total solid concentration was used for each sample (i.e., the lowest from all). Sludge samples were diluted with 0.02 M NaCl [14]. Sorption isotherms were obtained using 50 mL of sludge sample spiked with seven different initial metal concentrations ranging from 0 to 1 mg/L and 0 to 50 mg/L for Cd and Cu, respectively. The bottles were then shaken at 180 rpm on a rotary shaker at ambient temperature. Once equilibrium was reached, the sludge suspension was centrifuged and then filtered through a cellulose nitrate membrane of 0.45 µm pore size. The filtrate was acidified with a few drops of concentrated $HNO_3$ and stored at 4°C until analysis.

Desorption tests were performed on centrifugation

pellets obtained with sorption tests for each sludge. Pellets were resuspended with 0.02 M NaCl then bottles shaken at 180 rpm on a rotary shaker at ambient temperature. Once equilibrium was reached, the sludge suspension was centrifuged then filtered through a cellulose nitrate membrane of 0.45 µm pore size. The filtrate was acidified with a few drops of concentrated $HNO_3$ and stored at 4°C until analysis.

Cd and Cu concentrations were determined in the filtrate of both sorption and desorption tests by a graphite furnace atomic absorption spectrometer (FAAS) (Varian 880Z). Particulate Cd and Cu concentrations at equilibrium ($Q_{eq}$) were determined by differences between the aqueous added and the residual equilibrium concentrations ($C_{eq}$) according to Equation (1). Relative standard deviation was always below 5% when measuring metal concentrations by FAAS.

$$Q_{eq} = \frac{Ci - C_{eq}}{TS} \quad (1)$$

With $Q_{eq}$ = particulate metal concentration (mg/g TS), Ci = added metal concentration (mg/L), $C_{eq}$ = aqueous metal concentration (mg/L), and TS = sludge total solids concentration (g/L).

## RESULTS AND DISCUSSION

### Sludge Biochemical Properties

Biochemical composition (polysaccharides, proteins, and humic substances) of both soluble and particulate fractions was measured in the different sludge sample.

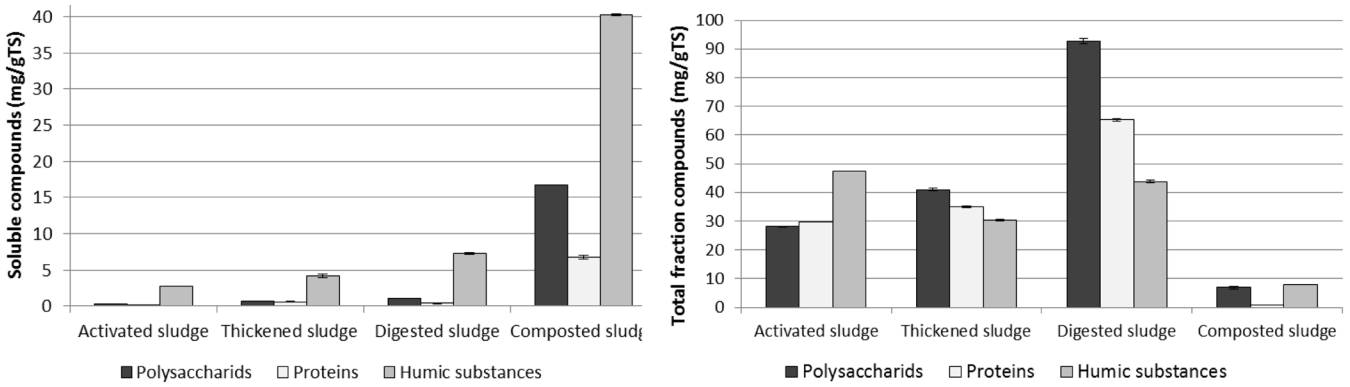
Figure 1 showed that the soluble fraction was mainly constituted of humic substances. However, for the total fraction the part of polysaccharides and proteins increased during sludge treatment until digestion from 30 to 90 mg/g of TS and 30 to 70 mg/g of TS for polysaccharides and proteins, respectively. The part of humic like substances and polysaccharides was more important in composted sludge probably due to composting process characteristics. The part of humic like substances and polysaccharides was more important in composted sludge probably due to composting process characteristics.

Metal behaviour during sludge treatment is linked to specific interactions between active sites in the sludge matrix and metallic species in solution. Functional groups involved in metal biosorption were characterized by acid-base properties. Amine, carboxyl, hydroxyl, and phosphate groups originating from proteins, polysaccharides, and phospholipids are responsible for the sorption. According to the pH of the medium these groups are able to dissociate and generate a negative surface charge on the sludge. This leads to attraction and interaction of positively charged metallic species.

### Assessment of Soluble and Particulate Functional Groups

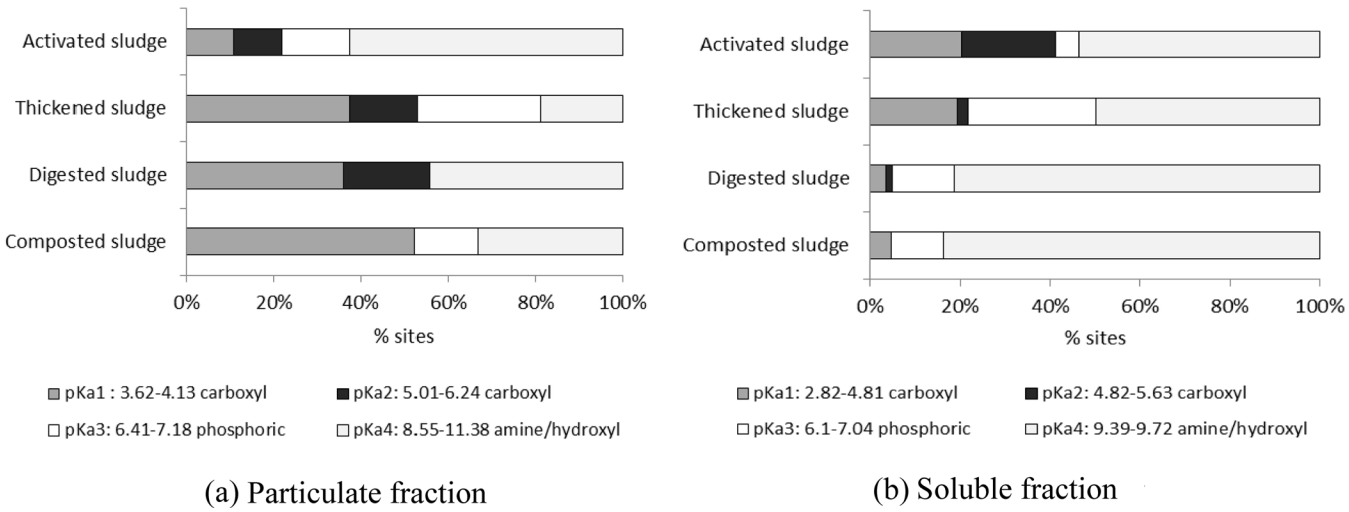
Potentiometric titration data were modeled with PROTOFIT software which allowed for the determination of acid-base properties for both particulate and soluble fractions of sludge. According to the typical pKa values of functional groups present on the bacterial surface [15], pKa values obtained via PROTOFIT using a four acidic sites model were linked to the nature of functional groups and are presented in Figure 2.

In soluble and particulate fractions, activated, thickened, and digested sludge showed four pKa values: 3.62–4.13 ($pKa_1$), 5.01–6.24 ($pKa_2$), 6.41–7.18 ($pKa_3$),




(a) Soluble fraction (b) Total fraction

***Figure 1.*** *Biochemical composition of soluble (a) and total (b) fraction of activated, thickened, digested, and composted sludge.*




(a) Particulate fraction (b) Soluble fraction

***Figure 2.*** *Relative contributions of the four sites concentrations associated with their pKa values to the total site concentration for particulate (a) and soluble (b) fraction of sludge.*

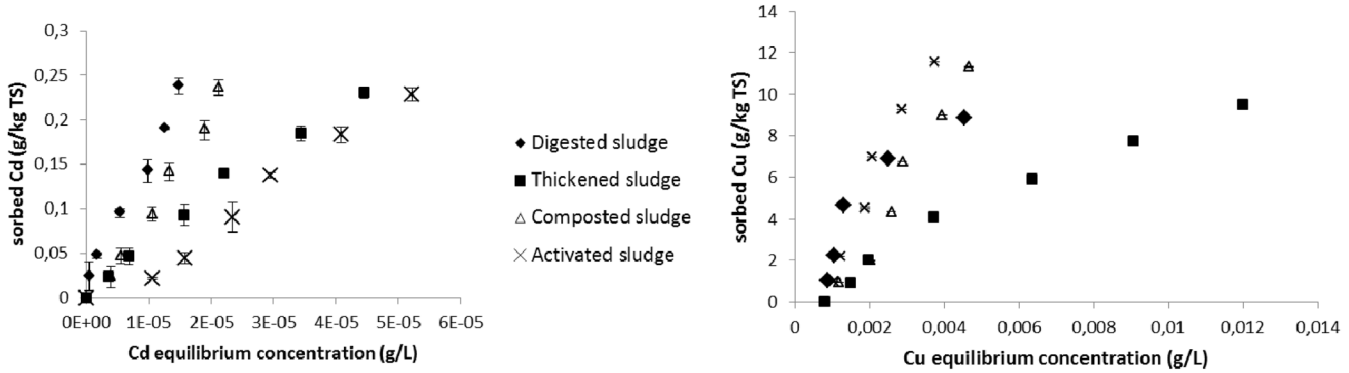
and 8.55–11.38 ($pKa_4$). Each pKa values can be assimilated into a group of components: $pKa_1$ and $pKa_2$ to a carboxylic group, $pKa_3$ to a phosphoric group, and $pKa_4$ can be attributed to amine and/or hydroxyl groups.

In the soluble fraction of thickened, digested, and composted sludge, amine and hydroxyl groups were predominant from 50 to 80%. For the activated sludge soluble fraction, the part of the carboxyl group was about the same as the part of amine and/or hydroxyl groups. However, in the particulate fraction of thickened, digested, and composted sludge carboxyl groups were predominant. In the particulate fraction of activated sludge, the part of amine and hydroxyl groups was more important than the part of carboxyl groups. Carboxyl groups can be linked to proteins, humic like substances, and uronic acids. Amine groups are mainly present in proteins whereas hydroxyl groups originate essentially from polysaccharides and humic like substances. Consequently, the observed composition of particulate and soluble fractions was consistent with biochemical composition previously established.

## Metals Sorption and Isotherms Modeling

Sorption isotherms for the four different sludge samples for cadmium and copper are represented in Figure 3. Isotherms were drawn based on equilibrium cadmium and copper concentration. All experiments were carried out at pH = 7 and initial concentration ranged from 0 to 1 mg/L and from 0 to 50 mg/L for cadmium and copper, respectively. Each of the sludge samples were spiked with both cadmium and copper solution to reach 10 fold the regulation at the maximum with each metal analyzed separately.

For cadmium, sorption was the most effective on digested sludge and composted sludge compared to activated and thickened sludge. Indeed for the first one




(a) Cadmium sorption (b) Copper sorption

***Figure 3.*** *Comparison of cadmium (a) and copper (b) sorption isotherms for each type of sludge. Sludge concentration was 4 g/L.*

the maximum released concentration was 0.021 mg/L compared to 0.052 mg/L for the second one at 1 mg/L of spiked cadmium concentration (0.23 g/kg TS sorbed Cd). For copper, sorption was the most effective on digested, composted, and activated sludge.

Given the shape of sorption isotherms, different models have been tested to determine different parameters. Freundlich and Langmuir models fitted well with cadmium sorption but not with copper. Therefore, another model, the Langmuir-Freundlich model [16] described by Equation (2) has been used for cadmium and copper sorption.

$$Q_{eq} = Q_{max} \frac{LC_{eq}^{n}}{1 + (LC_{eq})^{n}} \quad (2)$$

where $Q_{eq}$ = sorbed metal concentration at equilibrium (mg/kgTS), $Q_{max}$ = limited adsorption capacity (mg/kg), $C_{eq}$ = total aqueous metal concentration (mg/L) at equilibrium, and L = affinity constant (L/mg).

This model was chosen because it is adapted for competitive sorption and has an ability to predict sorption behavior at the saturation limit. Model parameters were determined from experimental data by a non-linear regression algorithm and are presented in Tables 2 and for cadmium and copper, respectively.

The main useful information provided by these parameters was the maximum adsorption capacity. In the case of cadmium sorption, Qmax reached the highest values for digested and composted sludge. However, for copper sorption, composted sludge showed the lowest maximum adsorption capacity.

This result could be linked to the high humic like content in the soluble phase of the composted sludge. As reported in the literature [17, 18, 19], Cu has a high binding ability with humic acids. This complexation to soluble organic matter probably limited copper sorption onto the particulate fraction of composted sludge.

Globally, metals were concentrated during digestion and composting process. These results were in accordance with studies of Karvelas *et al.* [6] and Liu *et al.* [20] which showed on the one hand that high levels of metals were found in digested sludge due to reduction of dry matter during the process and on the other hand that metals distribution during the composting process depends on different parameters such as organic matter content. Moreover humic substances mainly consisting of humic acids and fulvic acids are known for their significant influence on mobility and phyto-availability of heavy metals in soils [21] and generally binding of metal ions to humic acids reduces their bioavailability, mobilization, and transport capability in soil [17]. Results showed that it was possible to predict metal binding ability from sludge sample biochemical characteristics.

## Metals Desorption

Metal ability to desorb from sludge was assessed in order to quantify potential metal release when sludge was used for agricultural land for example. Figure 4 displays desorption efficiency of cadmium and copper for activated, thickened, digested, and composted sludge.

Cadmium desorption efficiency showed the highest value for activated sludge (until 2% against 0.4% for digested and composted sludge). Copper desorption efficiency decreased when initial concentration of copper

***Table 2. Cadmium Biosorption Parameters for Sludge Samples.***

| Sludge | Model | Parameters | | $R^2$ |
|---|---|---|---|---|
| Activated sludge | Langmuir-Freundlich | L | 21.6 | 0.99 |
| | | $Q_{max}$ | 4820.9 | |
| | | n | 1.8 | |
| Thickened sludge | Langmuir-Freundlich | L | 10.1 | 0.99 |
| | | $Q_{max}$ | 804.3 | |
| | | n | 1.03 | |
| Digested sludge | Langmuir-Freundlich | L | 0.75 | 0.98 |
| | | $Q_{max}$ | 10618 | |
| | | n | 0.84 | |
| Composted sludge | Langmuir-Freundlich | L | 2.79 | 0.99 |
| | | $Q_{max}$ | 9985 | |
| | | n | 1.24 | |

***Table 3. Copper Biosorption Parameters for Sludge Samples.***

| Sludge | Model | Parameters | | $R^2$ |
|---|---|---|---|---|
| Activated sludge | Langmuir-Freundlich | L | 0.46 | 0.99 |
| | | $Q_{max}$ | 2683 | |
| | | n | 3.08 | |
| Thickened sludge | Langmuir-Freundlich | L | 0.11 | 0.99 |
| | | $Q_{max}$ | 7812 | |
| | | n | 1.32 | |
| Digested sludge | Langmuir-Freundlich | L | 0.69 | 0.96 |
| | | $Q_{max}$ | 4437 | |
| | | n | 2.26 | |
| Composted sludge | Langmuir-Freundlich | L | 0.32 | 0.98 |
| | | $Q_{max}$ | 874.6 | |
| | | n | 3.42 | |

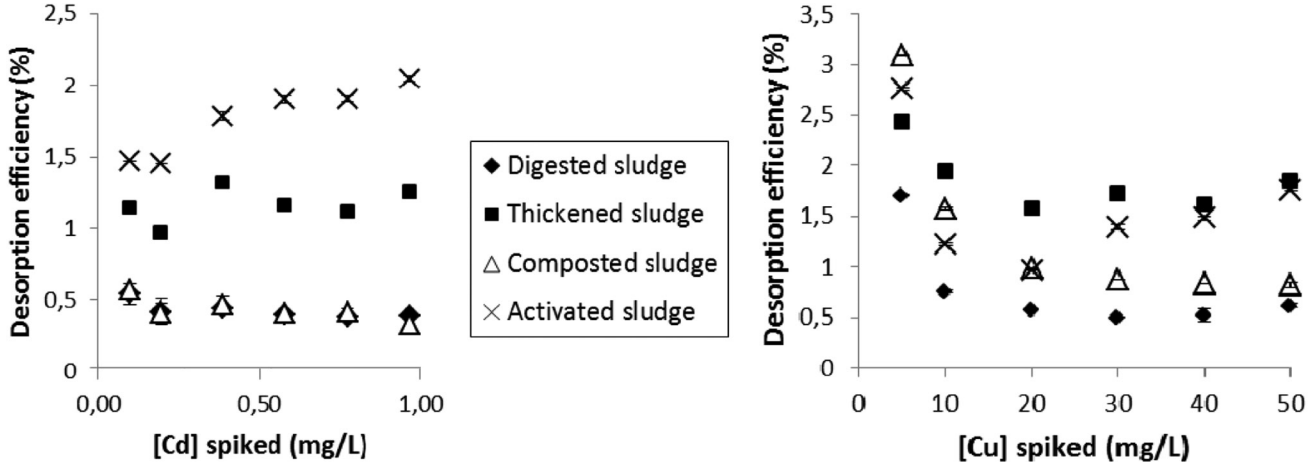



***Figure 4.*** *Comparison of cadmium (a) and copper (b) desorption efficiency for each kind of sludge.*

spiked in the sludge and for the highest copper concentration used in this study desorption was less effective with digested and composted sludge. Results were in line with those obtained for sorption because sludge samples for which sorption was most effective are those for which desorption was less effective. Metal desorption from digested and composted sludge was less effective so bioavailability was reduced. Although desorption efficiency was higher for activated and thickened sludge, the sludge at this stage of treatment would not be used for agriculture in this state. Further stabilization treatment such as composting could be realized before land application. These results were encouraging for the potential to recycle sewage sludge safely.

## CONCLUSION

Sorption and potential release of heavy metals in different sludge samples collected at different locations of sludge treatment in relation with their biochemical characteristics was evaluated. Soluble fractions of sludge samples mainly constituted of humic like substances. However, polysaccharides and proteins were predominant in total fractions except for composted sludge which contained more humic substances. Different metal sorption capacities and desorption efficiencies were observed between the four sludge samples. Indeed, cadmium sorption was the most effective on digested and composted sludge but copper sorption onto composted sludge showed the lowest maximum adsorption capacity due to high humic content. Moreover, metals desorption from digested and composted sludge was less effective and therefore bioavailability was reduced.

Results clearly demonstrated that an increase of humic substances in composted sludge increased binding ability of metal ions, particularly copper, to humic acids. This could reduce their bioavailability, mobilization, and transport in soil when sludge is used for land application.

## REFERENCES

1. Choubert, J.M., Pomies, M., Martin-Ruel, S., Coquery, M., "Influent concentrations and removal performances of metals through municipal wastewater treatment processes", *Water Science and Technology,* Vol. 63, No. 11, 2011, pp. 1967–1973.
2. Tolls, J., "Sorption of veterinary pharmaceuticals in soils: a review", *Environmental Science & Technology,* Vol. 35, No. 17, 2001, pp. 3397–3406.
3. Spinosa, L., "Evolution of sewage sludge regulations in Europe", *Water Science & Technology,* Vol. 44, 2001, pp. 1–8.
4. EPA, "Targeted National Sewage Sludge Survey", 2009, EPA-822-R-08-014.
5. ADEME, 1995, Les micropolluants métalliques dans les boues résiduaires des stations d'épuration urbaines. Paris: ADEME Editions, 209 p.
6. Karvelas, M., Katsoyiannis, A., Samara, C., "Occurrence and fate of heavy metals in the wastewater treatment process", *Chemosphere,* Vol. 53, 2003, pp. 1201–1210.
7. AFNOR, NF T 90–105, Qualité de l'eau - dosage des matières en suspension – méthode par centrifugation, Association française de normalisation, Paris, 1997.
8. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., "Colorimetric method for determination of sugars and related substances", *Analytical Chemistry,* Vol. 28, 1956, pp. 350–356.
9. Lowry, E.H., Rosebrough, N.J., Lewis Far, R.A., Randall R.J., "Protein measurement with the folin phenol reagent", *Journal of biological chemistry,* Vol. 193, 1951, pp. 265–275.
10. Frølund, B., Palmgren, R., Keiding, K., Nielsen, P.H., "Extraction of extracellular polymers from activated sludge using a cation exchange resin", *Water Research,* Vol. 30, No 8, 1996, pp. 1749–1758.
11. Turner, B.F., Fein, J.B., "Protofit: a program for determining surface protonation constants from titration data", *Computers & Geosciences,* Vol 32, 2006, pp. 1344–1356.
12. Laurent, J., Pierra, M., Casellas, M., Dagot, C., "Fate of cadmium in activated sludge after changing its physicochemical properties by thermal treatment", *Chemosphere,* Vol. 77, 2009, pp. 771–777.
13. Laurent, J., Casellas, M., Dagot, C., "Heavy metals biosorption on disintegrated activated sludge: description of a new equilibrium model", *Chemical Engineering Journal,* Vol. 164, 2010, pp. 63–69.

14. Pan, B., Huang, P., Wu, M., Wang, Z., Wang, P., Jiao, X., Xing, B., 2012. "Physiochemical and sorption properties of thermally-treated sediments with organic matter content", *Bioresource Technology,* Vol. 103, 2012, pp. 367–373.
15. Hong, Y., Brown, D.G., "Cell surface acid-base properties of Escherichia coli and Bacillus brevis and variation as a function of growth phase, nitrogen source and C:N ratio", *Colloids and Surfaces B: Biointerfaces,* Vol. 50, No. 2, 2006, pp. 112–119.
16. Limousin, G., Gaudet, J.P., Charlet, L., Szenknect, S., Barthès, V., Krimissa, M., "Sorption isotherms: A review on physical bases, modeling and measurement", *Applied Geochemistry,* Vol.22, No. 2, 2007, pp. 249–275.
17. Plaza, C., Brunetti, G., Senesi, N., Polo, A., "Molecular and quantitative analysis of metal ion binding to humic acids from sewage sludge and sludge-amended soils by fluorescence spectroscopy", *Environmental Science & Technology,* Vol. 40, 2006, pp. 917–923.
18. Xiong, X., Li, Y.X., Yang, M., Zhang, F.S., Li, W., "Increase in complexation ability of humic acids with the addition of ligneous bulking agents during sewage sludge composting", *Bioresource Technology,* Vol. 101, 2010, pp. 9650–9653.
19. Kang, J., Zhang, Z., Wang, J. J., "Influence of humic substances on bioavailability of Cu and Zn during sewage sludge composting", *Bioresource Technology,* Vol. 102, 2011, pp. 8022–8026.
20. Liu, Y., Ma, L., Li, Y., Zheng, L., "Evolution of heavy metal speciation during the aerobic composting process of sewage sludge", *Chemosphere,* Vol. 67, 2007, pp. 1025–1032.
21. Laborda, F., Bolea, E., Gorriz, M.P., Górriz, M.P., Martín-Ruiz, M.P., Ruiz-Beguería, S., Castillo, S., "A speciation methodology to study the contributions of humic-like and fulvic-like acids to the mobilization of metals from compost using size exclusion chromatography-ultraviolet absorption-inductively coupled plasma mass spectrometry and deconvolution analysis", *Analytica Chimica Acta,* Vol. 606, 2008, pp. 1–8.