

Effects of temperatures and extracellular proteins on dewaterability of thermophilically digested biosolids

Jianpeng Zhou, Donald S. Mavinic, Harlan G. Kelly, and William D. Ramey

Abstract: Thermophilic processes digest sludge at high temperatures to produce Class A biosolids. Recent research work revealed that digestion temperature is the predominant factor affecting dewaterability of thermophilic biosolids. This paper presents findings of a laboratory study that investigated how various digestion temperatures affect dewaterability of digested biosolids, studied the phase partition of the substances affecting dewaterability in digested biosolids, and tested the role of extracellular proteins in affecting dewaterability. Secondary sludges were digested at 40°C, 50°C, 60°C, 70°C, or 22°C for up to 12 d. Filtrates from thermophilically digested biosolids were treated with protease and boiling. This study found that, during the first few hours of digestion, higher temperatures resulted in more rapid and more significant deterioration in dewaterability than lower digestion temperatures. Continued digestion resulted in either improved (60°C or 70°C), unchanged (40°C or 50°C), or gradually deteriorated dewaterability (22°C). The substances affecting dewaterability were primarily located in the liquid phase of thermophilically digested biosolids. Boiling treatment did not result in significant changes in dewaterability. Protease treatment of the liquid phase of thermophilic biosolids improved dewaterability by 13–19%. Such an improvement confirmed the role of proteins in affecting dewaterability.

Key words: dewaterability, thermophilic, mesophilic, digestion, biosolids, protein, protease.

Résumé : Les procédés thermophiles digèrent la boue à des hautes températures et produisent des biosolides de Classe A. La recherche récente a révélé que la température de digestion est le facteur prédominant affectant la déshydratation des biosolides thermophiles. Cet article présente les conclusions d'une étude en laboratoire qui examine comment diverses températures de digestion affectent la déshydratation des biosolides digérés, la séparation de phases des substances affecte la déshydratation dans les biosolides digérés et qui a testé le rôle des exo-protéines sur la déshydratation. Les boues secondaires ont été digérées à 40°C, 50°C, 60°C, 70°C ou 22°C pour des périodes allant jusqu'à 12 jours. Les filtrats des biosolides digérés de manière thermophile ont été traités avec des protéases et par ébullition. L'étude a découvert que, durant les premières heures de digestion, des températures très élevées engendrent une détérioration plus importante et plus rapide de la déshydratation que des températures de digestion plus basses. La digestion continue a donné lieu à une déshydratation améliorée (60°C ou 70°C), inchangée (40°C ou 50°C) ou graduellement détériorée (22°C). Les substances affectant la déshydratation étaient principalement localisées dans la phase liquide des biosolides digérés de manière thermophile. Le traitement par ébullition n'a pas généré de changements importants dans la déshydratation. Le traitement aux protéases de la phase liquide des biosolides thermophiles a amélioré la centrifugabilité de 13 à 19 %. Une telle amélioration a confirmé le rôle des protéines dans les facteurs influençant la déshydratation.

Mots clés : déshydratation, thermophile, mésophile, digestion, biosolides, protéine, protéase.

[Traduit par la Rédaction]

Introduction

Wastewater treatment facilities produce large amounts of sludge. In the Greater Vancouver area of Canada, over 20 000 dry t a⁻¹ of wastewater sludge are produced from a popula-

tion of 1.9 million people. In the United States, wastewater sludge from the 16 000 publicly owned wastewater facilities is approximately 6.8 million dry t a⁻¹ (Bastian 1997). Therefore, cost effective design and operation of sludge treatment and management systems are very important. Thermophilic sludge

Received 13 May 2002. Revision accepted 7 October 2002. Published on the NRC Research Press Web site at <http://jees.nrc.ca/> on 15 November 2002.

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Written discussion of this article is welcomed and will be received by the Editors until 31 March 2003.

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Table 1. Composition of reconstituted samples.

| Sample ID | Solid phase (pellet) | Liquid phase |
|--------------------------|----------------------|-----------------------|
| A (thermophilic control) | Thermophilic | Thermophilic filtrate |
| B | Thermophilic | Distilled water |
| C | Thermophilic | Mesophilic filtrate |
| D (mesophilic control) | Mesophilic | Mesophilic filtrate |
| E | Mesophilic | Distilled water |
| F | Mesophilic | Thermophilic filtrate |

digestion is a process that digests sludge at high temperatures and produces Class A biosolids. Such a final product of high quality can be beneficially reused in non-restricted applications as parkland soil conditioners or as agricultural and forest fertilizers. Therefore, nutrients are recovered and costs of the final disposal can be reduced. Full-scale experience has revealed, however, that thermophilically digested biosolids exhibit poorer dewaterability than mesophilically digested biosolids, as defined by the need for 3 to 10 times higher dosages of polymer to condition, than mesophilically digested biosolids (Burnett et al. 1997; Kelly et al. 2000).

Recent research work has revealed that digestion temperature is the predominant factor affecting dewaterability of thermophilic biosolids (Zhou et al. 2001). It appears there is a correlation between dewatering properties and extracellular polymeric substances (EPSs consist primarily of proteins and polysaccharides) that were produced during digestion processes (Houghton et al. 2000; Murthy et al. 2000a, 2000b; Zhou et al. 2001). However, the following questions remain unanswered: (1) is there an opportunity to optimize process temperatures for improved dewaterability of thermophilically digested biosolids? (2) within the matrix of thermophilic biosolids, where are the substances that affect dewaterability primarily located? Are they in the solid or in the liquid phase, or in both phases? (3) is there any evidence to prove the role of EPSs in affecting dewaterability?

The objectives of this laboratory study were (1) to investigate how various temperatures affect dewaterability of thermophilically digested biosolids, (2) to identify location of substances affecting dewaterability, and (3) to confirm the role of extracellular proteins in affecting dewaterability.

Materials and methods

Experimental facilities and operation

Three bench-scale batch aerobic sludge digesters (5 L each) were used. Duplicate digesters were placed in a waterbath and were operated at either 40°C, 50°C, 60°C, or 70°C; the third was operated at ambient room temperature (about 22°C). Air diffusers provided fine bubble aeration and mixing (same airflow rate to each digester at 11 volume of air per volume of sludge per hour). Prior to each sampling, the contents in each digester were thoroughly mixed manually, and the volume of evaporated water was replaced with distilled water. The feed sludge

was thickened waste-activated sludge (100% secondary sludge) from Lulu Island Wastewater Treatment Plant (WWTP) of the Greater Vancouver Regional District, which has a trickling-filter solids contact biological treatment process. Wastewater influent to Lulu Island WWTP is predominantly municipal sewage. Collected sludge contained approximately 4.5% total solids (TS) and was diluted with tap water to about 2.5% TS, as the feed to the bench-scale digesters.

Experimental analysis

Dewaterability was measured as capillary suction time (CST) at room temperatures of about 22°C, according to Standard Methods 2710G (APHA et al. 1998), and was reported as CST and specific CST (SCST). Specific capillary suction time is calculated by dividing the measured CST by its respective TS concentration. Batch operation of digestion resulted in progressive solids reduction in digested sludge. Reporting dewaterability in SCST allows the dewaterability of samples having various solids concentrations to be compared. High SCST indicates poor dewaterability. The concentrations of TS were measured in duplicates, according to Standard Methods 2540G (APHA et al. 1998).

To measure extracellular proteins in the liquid phase of sludge samples, each sample was first centrifuged at 10 000 × g for 20 min and then was filtered through Fisher G-6 filter papers (pore size of 1.2 μm). Such centrifugation and filtration treatment normally can remove most of bacteria (having the size of *E. coli* or larger) from the liquid phase of sludge samples. Proteins in the filtrate (the liquid phase) were measured by using the Lowry assay with bovine serum albumin as the standard (Lowry et al. 1951).

Locating substances affecting dewaterability

Thermophilic samples were digested at 60°C for 24 h, while mesophilic samples were digested at 22°C for 6 h. These different treatments in digestion produced two groups of samples with different dewaterability. One sample of each was centrifuged at 10 000 × g for 20 min, then filtered to become liquid (filtrate) and solid (pellet) phases. New samples were constructed according to Table 1. The pellet of each of these new samples was resuspended in and thoroughly mixed (by vortexing contents in centrifugal tubes) with its respective filtrate or distilled water. Capillary suction time was then measured at room temperature for these reconstituted samples.

Testing the role of extracellular proteins

Each of three aliquots of the filtrate from a thermophilic sample (digested at 60°C for 24 h) was treated at 37°C for 18.5 h with one of the following three protease (all are SIGMA products): T7409 (type II-S trypsin from porcine pancreas), P5147 (type XIV bacterial protease from *Streptomyces griseus*, a non-specific protease), and P6911 (a protease from *Streptomyces griseus*, typically used in nucleic acid isolation procedures). The dosage of each protease was 50 mg/L. Another two aliquots were boiled at 100°C for 20 and 60 min, respectively. After each of the treated filtrate cooled down to the room temperature of about 22°C, a pellet from the original sample was resuspended in and thoroughly mixed (by vortexing contents in centrifugal tubes) with its respective filtrate. The CST was then measured at room temperature.

Statistical analysis

The 90% confidence intervals of SCST of the feed and sludge samples that were digested for 3 h and 5 d at various temperatures were reported. Calculations of the confidence intervals ($n < 30$) incorporated standard deviations and number of measurements of both CST and TS. Results from experiments of phase partition of substances causing poor dewaterability and protease and boiling treatment were subjected to t test at 95% level of significance and were reported with 90% confidence intervals.

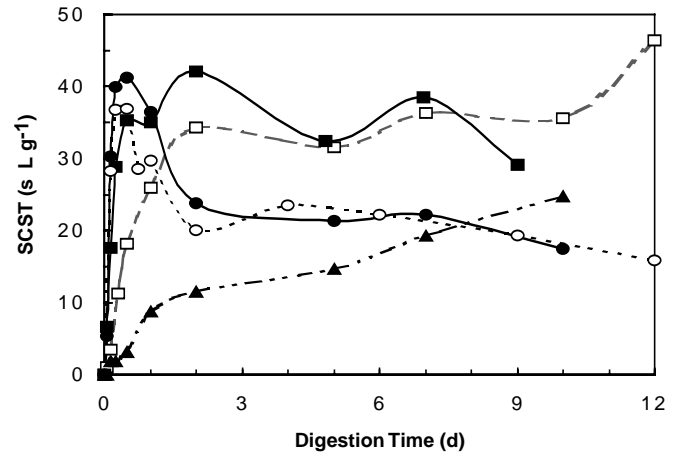
Results and discussions

Effects of temperatures

Temperature effects on the dewaterability of digested biosolids are illustrated in Fig. 1, and are summarized in Table 2. During the first few hours of digestion, higher digestion temperatures resulted in a rapid and significant deterioration in dewaterability (higher SCST). As digestion continued over the next a few days (up to 12 d), biosolids that were treated at 60°C and 70°C exhibited lower SCST than biosolids that were digested at 40°C and 50°C (Table 2). Mesophilic biosolids (22°C) showed a continued deterioration of dewaterability (gradual increase in SCST) with time.

The temperature of the feed sludge was between 15 and 20°C. Sludges in the digesters reached targeted temperatures (40°C, 50°C, 60°C, or 70°C) within 3 h, following the start-up of waterbath heating. One possible explanation of the results shown in Fig. 1 could be that extraneous substances and (or) finer particles were formed during digestion at high temperatures. The occurrence of such substances directly correlates to the supplied temperatures. For digestion at 60°C and 70°C, the SCST decreased following an initial rapid surge. Such changes suggested that prolonged treatment at higher temperatures destroyed some of the substances initially causing poor dewaterability, or changed the formation, stability, or relevant functional groups of the substances affecting SCST; as such the rate of destruction exceeded the rate of production of such substances. For digestion at 40°C and 50°C, SCST initially

Fig. 1. Effects of digestion temperatures on dewaterability (specific capillary suction time, SCST). Data shown represents differences in SCST between digested biosolids and their respective feeds. Digestion temperatures: □ 40°C, ■ 50°C, ○ 60°C, ● 70°C, ▲ 22°C.



increased very rapidly, then flattened during subsequent digestion of up to 10 d. Such a behaviour suggests that an equilibrium balance was achieved between production and destruction of substances (or relevant functional groups) causing poor dewaterability. The gradual increase in SCST of mesophilically digested biosolids (22°C) indicated a gradual build-up of factors affecting SCST. Destruction of these substances during mesophilic digestion did not appear to be significant.

Typically, thermophilic aerobic digestion requires a total digestion time of at least 5–6 d. Results of this study suggest that, to minimize deterioration in dewaterability of thermophilic biosolids, digestion temperatures of 60°C or higher are desirable. This would then result in a degree-day product of between 300 and 360, thus affecting digesters sizing requirements.

Locating substances affecting dewaterability

Experimental results showed that substances that affect dewaterability primarily resided in the liquid phase of digested biosolids (Fig. 2). Thermophilic biosolids (Sample A, SCST of 48 s L g⁻¹) exhibited poorer dewaterability than mesophilic biosolids (sample D, SCST of 18 s L g⁻¹). Replacing the filtrate from sample A (thermophilic biosolids) with distilled water resulted in a 64% reduction in SCST (17.4 s L g⁻¹ in sample B). Replacing the filtrate from sample A with the filtrate from sample D resulted in a 53% reduction of SCST (22.5 s L g⁻¹ in sample C). As shown in Table 3, the reduction of SCST in both cases is statistically significant at 95% confidence level. The preceding effects indicated that, with thermophilic biosolids, a substantial amount of substances causing poor dewaterability resided in the liquid phase.

Replacing the filtrate from sample D (mesophilic biosolids) with distilled water resulted in only about 22% SCST reduction (14 s L g⁻¹ in sample E), suggesting that, with mesophilic biosolids, most of substances affecting dewaterability were from the solid phase (pellet). In contrast, replacing the filtrate of

Table 2. Temperature effects on dewaterability.

| | | Digestion temp. (°C) | | | | | |
|----------------|--|----------------------|---------------|---------------|---------------|---------------|---------------|
| | | 70 | 60 | 60 | 50 | 40 | 22 |
| Feed | CST ^a (s) | 294 ± 40(6) | 215 ± 30(5) | 484 ± 22(3) | 308 ± 83(5) | 183 ± 14(6) | 294 ± 40(6) |
| | TS ^a (g/L) | 24.6 ± 0.1(3) | 24.9 ± 0.1(4) | 25.0 ± 0.2(2) | 22.9 ± 0.2(3) | 24.5 ± 0.3(3) | 24.6 ± 0.1(3) |
| | SCST ^b (s L g ⁻¹) | 12 ± 1 | 9 ± 1 | 19 ± 2 | 13 ± 4 | 7.5 ± 0.5 | 12 ± 1 |
| 3 h | CST ^a (s) | 1054 ± 81(4) | 865 ± 125(6) | | 713 ± 149(4) | 262 ± 27(4) | 341 ± 8(2) |
| | TS ^a (g/L) | 24.9 ± 0.1(4) | 24.0 ± 0.3(4) | | 23.0 ± 0.6(4) | 24.0 ± 0.3(4) | 24.7 ± 0.1(2) |
| (Net increase) | SCST ^c (s L g ⁻¹) | 30 ± 4 | 27 ± 4 | | 18 ± 9 | 3 ± 2 | 2 ± 7 |
| 5 d | CST ^a (s) | 707 ± 41(6) | | 914 ± 97(3) | 832 ± 91(7) | 685 ± 75(8) | 554 ± 23(3) |
| | TS ^a (g/L) | 21.3 ± 0.2(4) | | 21.3 ± 0.2(4) | 18.2 ± 0.1(4) | 17.6 ± 0.4(4) | 20.8 ± 0.3(2) |
| (Net increase) | SCST ^c (s L g ⁻¹) | 21 ± 2 | | 24 ± 12 | 32 ± 5 | 32 ± 3 | 15 ± 3 |

^aCapillary suction time (CST) and total solids (TS) are expressed as “average ± standard deviation (number of measurements)”.

^bSpecific capillary suction time (SCST) are expressed as “calculated SCST (dividing the average of CST by the average of TS) ± 90% confidence interval”.

^cThe SCST of 3-h and 5-d digested sludge are expressed as “net increase in SCST (subtract the SCST of the feed from the SCST of the digested sludge) ± 90% confidence interval”.

Table 3. Dewaterability of the reconstituted samples described in Table 1.

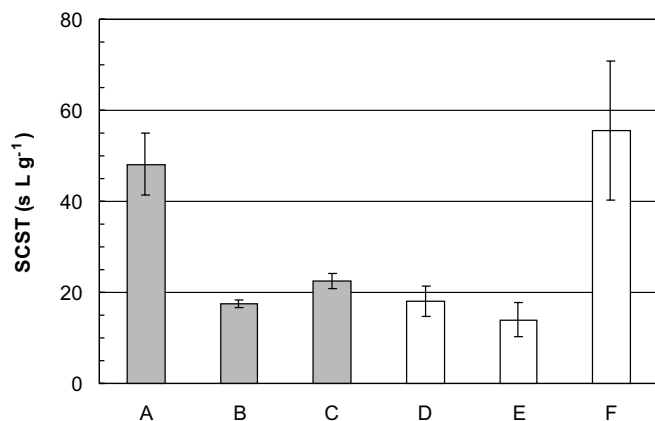
| | Sample | | | | | |
|---|---------------|---------------|---------------|---------------|---------------|---------------|
| | A | B | C | D | E | F |
| CST ^a (s) | 1125 ± 94(3) | 408 ± 12(3) | 525 ± 22(3) | 448 ± 48(3) | 349 ± 56(3) | 1383 ± 321(4) |
| TS ^a (g/L) | 23.4 ± 0.1(2) | 23.4 ± 0.1(2) | 23.4 ± 0.1(2) | 24.9 ± 0.4(2) | 24.9 ± 0.4(2) | 24.9 ± 0.4(2) |
| SCST ^b (s L g ⁻¹) | 48 ± 7 | 17.4 ± 0.9 | 22.5 ± 1.6 | 18 ± 3 | 14 ± 4 | 56 ± 15 |
| 95% critical <i>t</i> value | | 2.132 | 2.132 | | 2.132 | 2.015 |
| <i>t</i> ^c value (different from A?) | | 13.1 (Yes) | 10.7 (Yes) | | | 0.94 (No) |
| <i>t</i> ^c value (different from D?) | | | 3.6 (Yes) | | 2.3 (Yes) | 4.9 (Yes) |

^aCapillary suction time (CST) and total solids (TS) are expressed as “average ± standard deviation (number of measurements)”.

^bSpecific capillary suction time (SCST) is expressed as “calculated SCST (dividing the average of CST by the average of TS) ± 90% confidence interval.”

^c*t* test was performed using SCST.

Fig. 2. Locating substances that affect dewaterability (specific capillary suction time, SCST). Digestion temperatures were 60°C for thermophilic and 22°C for mesophilic. The bar in each column is the 90% confidence interval (see Table 1 for the key to the samples).



sample D (mesophilic) with filtrate of sample A (thermophilic) resulted in a 211% increase in SCST (56 s L g⁻¹ in sample F), which is an additional indication that the substances causing poor dewaterability in thermophilically digested biosolids are mainly from the liquid phase of thermophilic biosolids. The reduction and increase of SCST in these two cases are both statistically significant at 95% confidence level (Table 3).

It is also noted from Table 3 that the difference in SCST of sample C (thermophilic solids in mesophilic filtrate) and sample D (mesophilic solids in mesophilic filtrate) is statistically significant (95% confidence level), but sample A (thermophilic solids in thermophilic filtrate) and sample F (mesophilic solids in thermophilic filtrate) are statistically not different (95% confidence level). Such an observation provided further evidence that the substances causing poor dewaterability are mainly associated with the filtrate of the digested sludge. Findings from this work indicated that further studies on dewaterability should focus on the liquid phase of thermophilic biosolids. Characterization of filtrate constituents of thermophilic biosolids would

Fig. 3. Digestion effects on production of proteins in the liquid fraction. Digestion temperatures: □ 40°C, ■ 50°C, ○ 60°C, ● 70°C, ▲ 22°C.

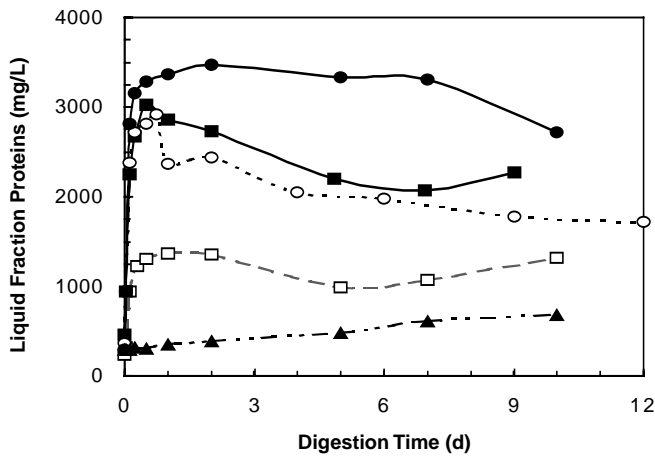
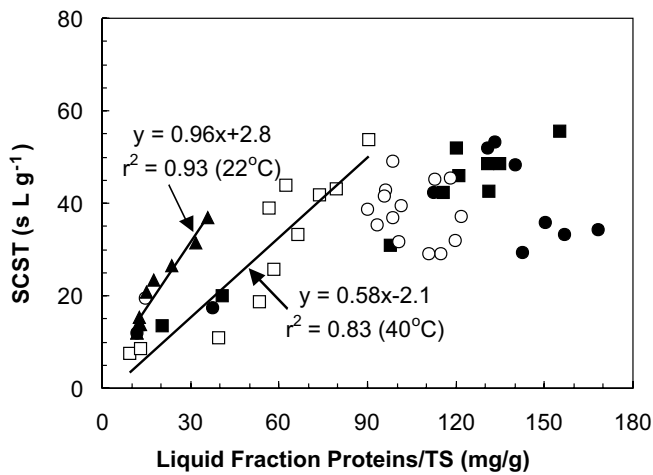


Fig. 4. Correlation between dewaterability (specific capillary suction time, SCST) and normalized protein concentrations in the liquid fraction. Digestion temperatures: □ 40°C, ■ 50°C, ○ 60°C, ● 70°C, ▲ 22°C.



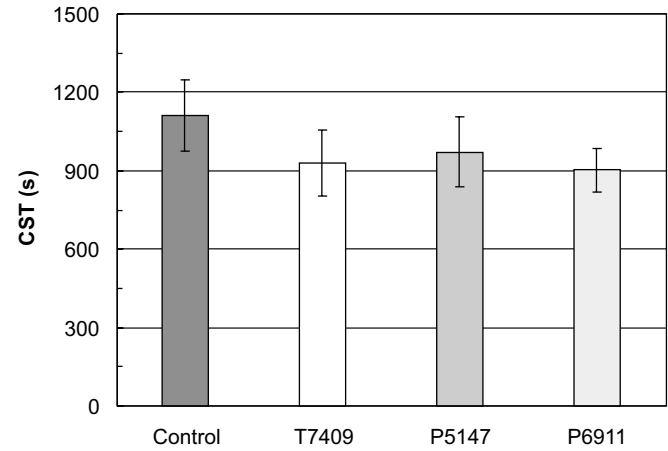
be an important step in the search for effective measures to improve dewaterability.

Testing the role of extracellular proteins

Thermophilic digestion produced up to 10 times higher amounts of extracellular proteins than mesophilic digestion. Higher digestion temperatures resulted in the production of higher amounts of proteins. Results of measured proteins are shown in Fig. 3. The production of polysaccharides had similar profiles to that of proteins (data not shown). The SCST appeared to correlate positively with proteins in the liquid phase (Fig. 4).

The EPSs present in the feed sludge and thermophilic biosolids of this study had comparable concentrations to what were reported at some full-scale autothermal thermophilic aerobic digestion (ATAD) plants. For example, biosolids from this study

Fig. 5. Effects of protease treatment on dewaterability of the sludge liquid fraction (specific capillary suction time, SCST). The sample had been digested for 1 d before protease test. The bar in each column is the 90% confidence interval.



(digested at 60°C for 6 d) had protein and polysaccharide concentrations of 1980 and 1040 mg/L in the liquid phase, respectively. Murthy et al. (2000a) reported that biosolids from the College Station ATAD plant in Texas (similar digestion conditions) contained 2080 mg/L soluble proteins and 900 mg/L soluble polysaccharides. Samples produced in this study appeared to contain similar levels of proteins and polysaccharides concentrations to those from the full-scale plant.

The Lowry assay provides information on total amounts of proteins. However, characteristics and specifics of these proteins are still unknown. Figure 4 indicates that, likely, only a portion of measured proteins affected dewaterability. At lower digestion temperatures (e.g., 22°C and 40°C), SCST showed a linear correlation with normalized concentrations of proteins in the liquid phase (concentrations of proteins divided by TS). At higher digestion temperatures (e.g., 60°C and 70°C), after normalized concentrations of proteins in the liquid phase exceeded an apparent threshold (approximately 90 mg/g), increased proteins did not result in a further increase of SCSTs. As such, protease and boiling treatment experiments were performed to search for additional information on the proteins.

Protease treatment of the filtrate of thermophilic biosolids resulted in a statistically significant reduction of SCST (95% confidence), as shown in Table 4 and Fig. 5 (T7409: 16% reduction; P5147: 13% reduction, P6911: 19% reduction). By contrast, boiling the filtrate at 100°C, for both 20 and 60 min duration, did not result in a significant reduction in SCST (95% confidence, shown in Table 4 and Fig. 6). Boiling treatment of 60 min would denature typical proteins. Such effects are mostly due to impacts on functional structure of proteins (e.g., unfolding under boiling temperature). The ineffectiveness of boiling treatment suggested that the substances formed during thermophilic digestion were either heat stable materials, such as carbohydrates, or unusually heat stable proteins (although prolonged treatment eventually resulted in possible destruction of proteins, as observed in Fig. 1).

Table 4. Effects of protease and boiling treatment on dewaterability.

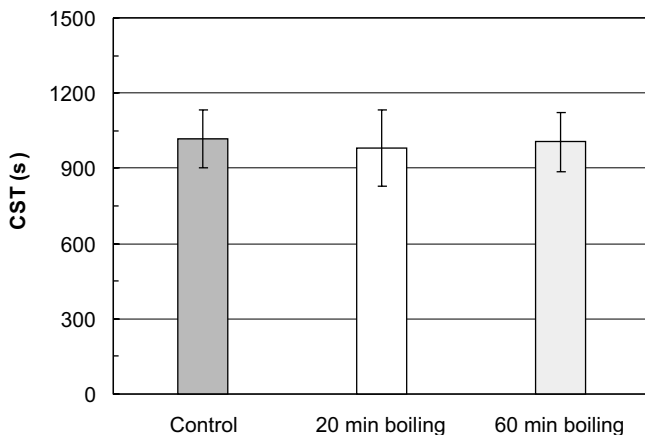
| | CST ^a (s) | 95% critical <i>t</i> | 90% critical <i>t</i> | <i>t</i> value (different from control?) |
|---------------------------------|----------------------|-----------------------|-----------------------|--|
| Control of protease test (37°C) | 1111 ± 55(3) | 2.132 | 1.533 | 1.9 ^b (Yes at 90% confidence) |
| Protease T7409 | 931 ± 51(3) | 2.132 | 1.533 | 4.4 (Yes) |
| Protease P5147 | 972 ± 54(3) | 2.132 | 1.533 | 3.2 (Yes) |
| Protease P6911 | 902 ± 34(3) | 2.132 | 1.533 | 5.7 (Yes) |
| Control of boiling test (±22°C) | 1016 ± 68(3) | 2.132 | 1.533 | |
| 20 min boiling | 979 ± 90(3) | 2.132 | 1.533 | 0.6 (No) |
| 60 min boiling | 1004 ± 71(3) | 2.132 | 1.533 | 0.2 (No) |

Note: The 90% confidence intervals are shown in Figs. 5 and 6.

^aCapillary suction time (CST) is expressed as “average ± standard deviation (number of measurements)”.

^bControl of protease test was compared to control of boiling test.

Fig. 6. Effects of heat treatment on dewaterability of the sludge liquid fraction (specific capillary suction time, SCST). The sample had been digested for 1 d before boiling test. The bar in each column is the 90% confidence interval.



The 13–19% reduction in SCST, because of protease treatment, indicated that proteins in the liquid phase play a role in overall interactions affecting dewaterability of thermophilically digested biosolids. Such a reduction in SCST, although small from the perspective of full-scale applications, suggested that (1) the nature of extracellular proteins effect on dewaterability is complex. Thermophilic digestion may produce many different types of proteins. These proteins may all affect dewatering properties in one way or another. Therefore, denaturing proteins, by using only one type of protease each time, would not be adequate to achieve a substantial reduction of SCST. (2) Current knowledge on which proteins, or which functional groups of proteins, affect dewaterability is limited. The three types of protease used in this study may not be the most effective ones. (3) Extracellular proteins residing in the solid phases (entrapped in pellet) of biosolids may also contribute to the observed effect on dewaterability. (4) Possible compounding effects of proteins and polysaccharides are yet to be investigated.

Summary and conclusions

Based on the research work completed to date, the following comments are offered

- (1) Digestion temperatures affected dewaterability of digested biosolids differently. During the first few hours of digestion, higher temperatures resulted in more rapid and more significant deterioration in dewaterability than lower temperatures. During continued digestion of up to 12 days, dewaterability was either improved (60°C, 70°C), did not change significantly (40°C, 50°C), or experienced gradual deterioration (22°C).
- (2) The substances that affected dewaterability were primarily located in the liquid phase for thermophilically digested biosolids, and resided mainly in the solid phase for mesophilically digested biosolids. Such effects are statistically significant at 95% confidence level.
- (3) Digestion at each tested temperature (40°C to 70°C) all produced substantially higher amounts of extracellular proteins than mesophilic digestion (22°C). Dewaterability, as measured by CST, appeared to correlate directly with the concentrations of extracellular proteins in the liquid phase. Boiling treatment of liquid phase of thermophilic biosolids did not result in significant changes in dewaterability. Protease treatment of the liquid phase of thermophilic biosolids improved dewaterability by 13–19%. Such an improvement confirmed a role of proteins in affecting dewaterability.

Acknowledgements

This study was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Science Council of British Columbia (GREAT program), Dayton & Knight Ltd. of Canada, and Canadian Council of Professional Engineers (Manulife Financial Scholarship program). Laboratory work received assistance from Ms. Paula Parkinson and Ms. Susan Harper of Environmental Engineering Laboratory, Department of Civil Engineering, University of British Columbia. Preliminary results from this study were presented at the CSCE/ASCE-EWRI Environmental Engineering Conference, July 21–24, 2002, Niagara Falls, Ontario, Canada.

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