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# Efficacy of Hydrolytic Enzyme Augmentation and Thermochemical Pretreatments for Increased Secondary Anaerobic Digestion of Treated Municipal Sewage Sludges

N. J. NAGLE,* C. J. RIVARD, W. S. ADNEY, AND M. E. HIMMEL

*Biotechnology Research Branch, Fuels & Chemicals Research & Engineering Division, Solar Energy Research Institute, Golden, CO 80401*

## ABSTRACT

Rising costs for landfill disposal of municipal sewage residues have prompted evaluation of alternative methods for reducing the bulk of the final waste. Representative samples of municipal sewage sludge residues were obtained from three major treatment plants in the United States, including Los Angeles (Hyperion), Denver (North Metro), and Chicago (Stickney). The majority of the treated, dewatered sewage sludge solids was found to be volatile (50–60%) and, presumably, biodegradable. Additionally, much of the volatile content was solubilized by both acid detergent fiber and neutral detergent fiber treatments, and was presumed to be proteineous microbial biomass in nature. Both low- and high-solids anaerobic digester systems, as well as the standard biochemical methane potential (BMP) assay, were utilized to evaluate the anaerobic digestibility of these sewage sludge residues. The low methane yields and, thus, the poor organic waste conversion indicated the need for treatment prior to bioconversion. The effectivenesss of various pretreatments based on assessment of increased soluble protein or organics and anaerobic digestibility as determined by the BMP assay was evaluated.

**Index Entries:** Sewage sludge; pretreatment; thermochemical; enzyme; anaerobic digestion; CSTR; high solids; BMP.

*Author to whom all correspondence and reprint requests should be addressed.

## INTRODUCTION

Modern municipal sewage water treatment plants convert most of the solids in the incoming waste water to microbial mass, carbon dioxide, water, and methane. The overall result of these plants is the conversion of a water pollution problem into a solid waste disposal problem. Recently, the solid waste volumes produced have increased dramatically as a result of increases in the organic loading of waste waters and environmental regulations that require a higher degree of waste-water treatment (*1–3*).

The ultimate disposal of sludge is generally by land application, landfilling, or combustion (*4,5*). Disposal by landfilling is becoming increasingly expensive, especially in the eastern United States. The maturing and subsequent closure of existing landfills, reduced sitting of new landfills, public concern over ground-water contamination, and safety problems associated with methane production as a result of biological activity in landfills further compound the problem. Public concern over possible hazardous emissions through combustion processes and possible heavy metal contamination from the resulting ash has also reduced acceptance of combustion as a disposal option for muncipal sewage sludges (*6*). The land application of sewage sludge is also problematic, because biological activity produces methane and residual volatile solids often result in ground-water contamination by organics.

Today, the cost of disposing of a given amount of sludge is often high and is growing higher. Furthermore, increased loads on existing treatment plants also lead to sharply higher disposal costs. Increasing environmental requirements on the quality of waste-water treatment have resulted in a more complex process (*7*), which produces greater microbial biomass for disposal. The greater organic loading of waste-water streams (*8*) has created a higher stress on the treatment processes that often reduces the organic removal efficiency. Higher levels of volatile organics in the sludge reduce its dewaterability substantially (*9*), increasing the water content and volume of the final waste. Increasing dewatering efficiency at a higher volatile solids content requires the use of organic polymers to enhance flocculation (*10–12*). Polymer usage increases both the disposal costs and the organic loading of the final waste. The net result is that the amount and cost of sludge disposal can increase disproportionately when an existing plant must deal with increased loadings and clean-up requirements.

Most recent research has focused on reducing the volume of the final waste by improving dewatering technology (*13–18*). However, the pollution potential of the sludge is unchanged, because the dewatering does not reduce the volatile solids fraction. The volatile solids content of treated sludges range from 40–75% (*1,4,19*), and the content of undigested sludge is even higher. Clearly, the potential for further reduction in sludge volume still exists. However, because sewage sludge is primarily water and the standard vessels employed in digestion are stirred tank reactors, which are large and expensive, further (secondary) anaerobic digestion is not currently cost effective.

This study evaluates the use of a novel high solids reactor technology, originally developed for the anaerobic digestion of relatively dry organic materials, such as municipal solid waste (MSW), for application to the secondary anaerobic digestion of highly dewatered sewage sludge residues. Additionally, the usefulness of various pretreatment protocols in enhancing the biodegradability of the volatile solids components of sewage sludge residues was evaluated, along with the use of standard analytical techniques to predict the ultimate anaerobic biodegradability.

## MATERIALS AND METHODS

### Dewatered Municipal Sewage Sludge Residues

Dewatered sewage sludge residues were obtained from three major treatment plants across the mid and western United States. The sewage sludges were from the Stickney Water Reclamation Plant, Cicero, IL, the Metro Wastewater Reclamation District, Denver, CO, and the Hyperion Wastewater Treatment Plant, Playa Del Rey, CA. These plants treat municipal waste waters from Chicago, Denver, and Los Angeles, respectively. All treatment plants followed the basic treatment process as outlined in Fig. 1, with low solids anaerobic digestion as the final biological treatment before residue dewatering and disposal. The three treatment plants differ in the efficiency of biological treatment, the type and addition rates of bulking agents, and the type of continuous centrifuge used for the dewatering of sewage sludge. A more complete description and as a comparison of the treatment plant operation, equipment, and residue characteristics are reported elsewhere (*20*). The residues were collected in 30-gal plastic drums, and transported and stored under refrigeration at 4°C to repress microbial growth.

### High Solids Reactor Operation

The three high solids reactors utilized in this study were described previously (*21,22*). The reactor systems were 20-L vessels and were maintained at a 15-L working volume. The reactors were maintained at 37°C in a temperature-controlled warm room. The mixing rate was predetermined and remained in the range of 0.5–1.5 rpm. The reactors were batch fed daily. No additional amendments (i.e., nutrients, buffers, and so forth) were added. A total of 500 g wet wt of sewage sludge residues were added daily to each reactor. In the batch-feeding protocol, a volume of effluent equivalent to the volume of feed added was removed daily to maintain the reactor sludge volume at 15 L. The retention time of the high solids reactor system was 20 d.

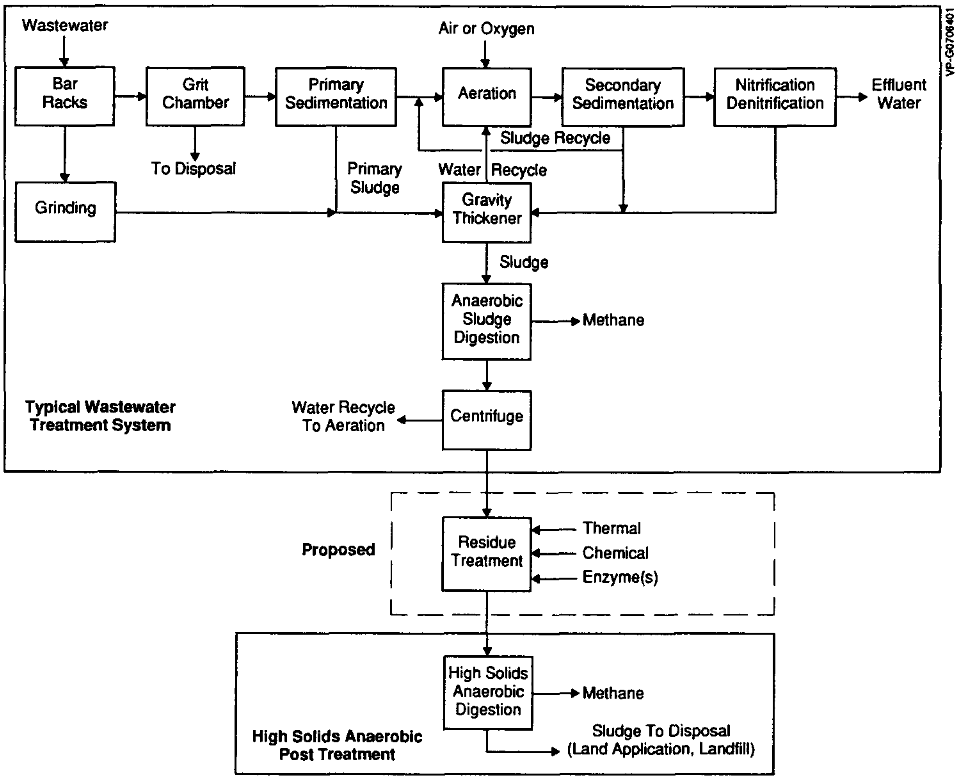


Fig. 1. Process flow diagram of conventional sewage treatment indicating the point at which the proposed pretreatment and novel high solids (secondary) anaerobic process would be intergrated.

## Low Solids Digester Operation

Four anaerobic digesters with 3.5-L working volumes and semi-continuous stirring (15 min of each 1/2 h) were constructed and operated as previously described (*23,24*). The reactors were maintained at 37°C in a temperature-controlled warm room. The anaerobic reactors were batch-fed (daily) sewage sludge residues at 3.5 g volatile solids (VS)/L reactor sludge (12.25 g VS/reactor·d). Appropriate volumes of deionized water were added to maintain a 14-d retention time. In the batch-feeding protocol, a volume of effluent equivalent to the volume of feed added was removed daily to maintain the reactor sludge volume at 3.5 L. For the operation of these reactors, the solids' retention time was equivalent to the hydraulic retention time.

## Biochemical Methane Potential (BMP) Analysis

The BMP assays were performed as previously described (*25*) to determine the ultimate methane yields for the anaerobic conversion of the feedstocks by the anaerobic consortium. Studies were conducted in 155-mL serum bottles at 37 °C and mixed using an orbital shaker. Biogas production was measured using a pressure transducer fitted to a 22-gage needle for penetration into serum bottles. After each pressure measurement cycle, the remaining overpressure was released from the serum bottles. BMP assays were conducted using whole treated samples, and incubation carried out for 60 d to ensure ultimate biodegradation.

## Digester Effluent Analysis

Digester effluent samples were either analyzed immediately upon removal from the digester system or BMP vessels, or were refrigerated at 4 °C if assays were to be performed at a later time. The solids concentrations of both representative sewage sludge feed and digester effluent samples were determined using 1.0-g aluminum weigh tins. A 20- to 30-g sludge sample was loaded into preweighed tins and dried for 48 h at 45 °C. The dried sample was then cooled to room temperature in a laboratory desiccator and weighed using a precision balance (Sartorius, model 1684MB). The solids content of the digester sludge was calculated on a wt/wt basis. The volatile solids and ash contents were determined by combustion of the dried samples at 550 °C for 3 h in a laboratory-scale furnace.

The compositions of representative sewage sludge samples were determined by acid detergent-soluble fiber analysis (*26*). This analysis permits estimation of the acid detergent solubles (e.g., microbes, fats, and protein), cellulose, lignin, and ash contents of sludge (*26,27*).

Chemical oxygen demand (COD) was determined as previously described (*28*). The COD assay employed the micro-determination method using commerically available "twist tube" assay vials (O.I. Corporation, College Station, TX).

Levels of volatile organic acids ($C_2$-$C_5$ iso and normal acids) were determined by gas-liquid chromatography (GLC). A Hewlet-Packard Model 5840A gas chromatograph equipped with a flame ionization detector, a Model 7672A autosampler, and a Model 5840A integrator (all from Hewlett-Packard) was used. The chromatograph was equipped with a glass column packed with Supelco 60/80, Carbopack C/0.3%, Carbowax 20*M* 0.1% $H_3PO_4$ for separations.

## Gas Analysis

Production of biogas from the high solids reactors was monitored daily (in conjunction with batch feeding) using calibrated wet tip gas meters. In low solids, continuous stirred tank reactors (CSTR), biogas was measured using calibrated water displacement reservoirs. In all cases, the composition of the biogas produced was determined using gas chromatography for methane and carbon dioxide. For this analysis, a Gow-Mac (Model 550) gas chromatograph equipped with a Porapak Q column and a thermal conductivity detector with integrating recorder was used.

## Theoretical Methane Yield

The theoretical methane yield for the various sewage sludge residues was calculated from COD values as previously described (*25*). The ratio of actual methane yields for a given anaerobic fermentation to the theoretical methane yield calculated from COD values is a direct reflection of the organic carbon conversion of the substrate added.

## Thermal Treatment of Sewage Sludge Residues

Initially, all three sludges were thermally treated to evaluate the posssible impact of pretreatment on anaerobic digestibility. Individually, 5 kg of each sludge residue was autoclaved for 4 h at 121°C in an aluminum pressure cooker (to reduce changes in moisture content). The thermally treated sludge residues were cooled and refrigerated at 4°C until use.

## Application of Standard Analytical Techniques to Predict Anaerobic Biodegradability

Because of the lengthy incubation time involved in anaerobic digestibility analysis (BMP assay), several standard methods for rapid determination of soluble compounds (organics, protein, amino acids, and total nitrogen) were evaluated as possible predictive measures of overall biological digestibility. To address the inconsistencies that may arise in several of the analytical methods because of the level of organics or salts present, a series of four different sewage sludge treatments was evaluated and the data correlated to that obtained from BMP assays.

These pretreatments included variations in alkaline-thermal treatments, in which samples (40 g) of Denver sewage sludge residues were treated by adding 0, 0.25, and 1.4*M* sodium hydroxide (final concentration) and by heating at 121°C for 30 min in an autoclave. Following thermal pretreatment, the samples were cooled, adjusted to pH 7.0 using concentrated HCl, and diluted 10-fold with distilled water. The samples were then centrifuged at 2000 rpm for 10 min, and the supernatant was removed.

The sludge pretreatment supernatants were assayed for COD, Kjeldahl nitrogen (*29*), and several dye binding protein assays, including Lowry (*30*), Bio-Rad (Bio-Rad, Richmond, CA), and Bradford (Pierce Chemical Co., Rockford, IL). For the analytical assays in which high levels of salts are known to influence the determinations (i.e., dye binding assays), the samples were analyzed both before and after dialysis. Samples (4.5 mL) of posttreatment supernatant were subjected to dialysis to remove salts accumulated during pH adjustments. The samples were pipeted into 6000-8000 mol-wt Spectropor tubing and sealed. The samples were dialyzed against two changes of deionized water for 24 h at 4°C. Following dialysis, the sample volumes were measured and readjusted to 4.5 mL. The supernatants were also assayed for total amino acids as performed by the method of Moore and Stein (*31*). The samples were reduced and carboxyamidomethylated using the method of Fox et al. (*32*). After dialysis against distilled water and lyophilization, 50-μg samples of the reduced and alkylated enzyme were dissolved in 500-μL aliquots of 5.7*N* HCl containing 0.05% v/v β-mercaptoethanol. The samples were then placed into tubes, and evacuated, sealed, and hydrolyzed at 110°C for 24, 48, and 72 h. Hydrolyzed samples were assayed in triplicate using a Beckman model 121 CL amino acid analyzer. The final residue content was normalized for losses or apparent increases during hydrolysis.

## Data Regression Analysis

The results obtained from the various rapid analytical methods were correlated against the anaerobic biodegradation (i.e., BMP) data for the four pretreatment protocols examined using a single line regression. The $R^2$ value served as the selection for identifying the best analytical method that can be used as a predictive technique for estimating anaerobic biodegradation effects.

## Thermochemical Pretreatment of Denver Sewage Sludge Residues

The comprehensive evaluation of thermal, chemical, and enzymatic pretreatments of sewage sludge residues to enhance anaerobic biodegradability was conducted with only the Denver sewage sludge residue for expediency. Samples of Denver sewage sludge residues were adjusted to pH 0.5, 1.0, 8.5, 10, and 12 with either 50% (w/v) NaOH or concentrated HCl. The sludge samples were incubated for time periods of 5, 30, 90, and 240 min at temperatures of 22, 80, and 100°C. After pretreatment, the sludge samples were adjusted to pH 7.0 with HCl or NaOH, and aliquots (1 g) were diluted 10-fold in distilled water and subjected to low-speed centrifugation (10 min at 2000 rpm). Sample supernatants were collected and frozen until analysis.

## Enzymatic Pretreatment of Denver Sewage Sludge Residues

Pretreatment of Denver sewage sludge residues with hydrolytic enzymes was evaluated using purified preparations of proteinase K (type XI, from *Tritirachium album*), thermolysin (type X, from *Bacillus thermoproteolyticus*), and trypsin (type XI, from bovine pancreas) obtained from Sigma Chemical Co. Lysozyme (from chicken egg white) was also obtained from Sigma. Pretreatments of both high and low enzyme activity levels were evaluated. It should be noted that, although equivalent levels of enzyme activity (9200 and 920 U/g wet sludge) were utilized for each enzyme tested, the actual activity of protease enzyme when measured on an identical substrate may be slightly different. Although roughly comparable, the units of protease activities used for the enzymes tested were deter-

Table 1
Sewage Sludge Residue Analysis (as Received)

| City | % Total solids | % Volatile solids* | Acid detergent fiber, ADF, analysis** Protein/ hemicellulose | Cellulose | Lignin/ plastic | COD*** |
|---|---|---|---|---|---|---|
| Chicago | 24.9±0.3 | 48.2±0.4 | 84.4±4.1 | 6.8±1.1 | 8.7±3.0 | 173±20 |
| Denver | 16.7±0.1 | 67.0±0.2 | 91.8±2.9 | 5.3±0.3 | 2.9±0.7 | 167±18 |
| Los Angeles | 19.6±0.1 | 56.1±0.2 | 87.1±1.5 | 5.2±0.9 | 7.7±2.2 | 176±33 |

*As a percent of the dry wt of sewage sludge residue.
**As a percent of volatile solids.
***Chemical Oxygen Demand, expressed in mg COD/g sewage sludge residue (wet wt).

mined for different substrates by Sigma. Appropriate levels of enzymes were each added individually to 40-g samples of Denver sewage sludge. Samples were incubated at 37°C, and aliquots removed to terminate activity after 0, 5, 30, 90, and 240 min. The enzyme/sludge samples (1 g) were diluted 10-fold in distilled water and centrifuged as described above. Samples were frozen until analysis.

## RESULTS

Treated, dewatered sewage sludge residues were obtained from three major treatment plants across the mid and western United States to gain a representative appraisal of general composition and efficacy of secondary anaerobic biodegradation. Analysis of the residues upon receipt (Table 1) indicates that, although the solids level of the respective sludges were different, significantly high levels of volatile solids were present. The residue solids levels are a direct result of several factors, including the level of biological degradation, the type of dewatering equipment, and the type and level of dewatering polymer utilized (*20*). For all sewage sludge residues tested, the majority of the volatile solids component was determined to be that which is solubilized by an acid detergent solution and thus composed of polymers, such as protein, hemicellulose, fats, and lipids. The source of this soluble fraction is presumed to be the microbial cells, which result from both the aerobic and anaerobic bioconversion processes of the sewage treatment plant and comprise a large portion of the residue sludge. All residues tested were similar in COD value as based on the residue wet wt. The theoretical methane yield, and thus the potential for organic bioconversion, was calculated for each sewage sludge residue tested from the COD values as previously described (*25*). The calculated yield was used to evaluate the results from actual methane yields obtained in the various digester and bioconversion assays employed.

Initial anaerobic digestion assessments of the representative sewage sludge residues using both standard low solids CSTRs and a novel high

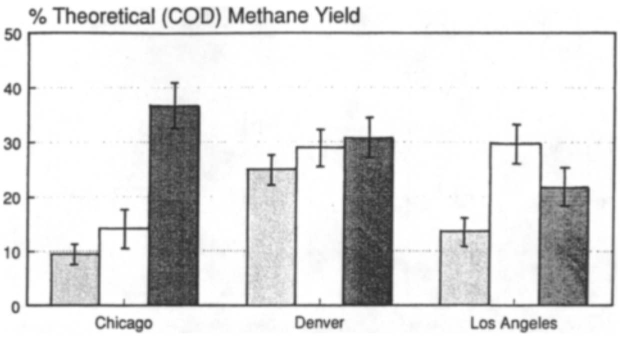


Fig. 2. Anaerobic digestion of treated dewatered sewage sludges from Chicago, Denver, and Los Angeles utilizing low solids CSTR, a novel high solids reactor system, and the classical biochemical methane potential (BMP) assay. Data for the low solids and high solids reactor systems were taken after three retention times of stable operation, and represent the average biogas and methane content for a 3-wk period. Data for BMP assays represent the average of triplicate determinations after a 60-d incubation period. Error bars represent the standard deviation of the data. ▨ Low solids system; □ high solids system; ▩ ultimate (BMP).

solids reactor system developed at the Solar Energy Research Institute resulted in stable fermentations as determined by parameters of pH, intermediate volatile organic acid pools, and daily biogas production (data not shown). However, the fermentation performance, based upon methane yields as compared to the theoretical yields determined from the respective residue COD values, was disappointing (Fig. 2; 9–30% of theoretical). For comparison, the BMP assay was used to determine the ultimate anaerobic biodegradation as determined under an extensive incubation period (60 d), which resulted in enhanced yields (22–37% of theoretical). However, although BMP yields are somewhat higher than digester systems, they are still well below the theoretical yields determined for the COD content of the residues.

The rather low level of anaerobic biodegradation exhibited in the initial fermentation studies identified a need for pretreatment of the sewage sludge residues to disrupt the organic fraction before fermentation. A preliminary thermal treatment of all three sewage sludge residues resulted in the enhancement of subsequent anaerobic biodegradation and methane yields (Fig. 3). Pretreatment of sewage sludge residues at 121°C for 240 min increased the methane yields in digester systems by an average of 49% for all sewage sludge residues tested.

To evaluate a wide range of pretreatment protocols effectively, a more rapid analytical approach to the determination of changes in anaerobic biodegradability of treated samples was necessary. The analytical methods evaluated (as a predictive measure of anaerobic biodegradability) were based on increases in soluble components released under a variety of pretreatment conditions. The assays tested examined changes in soluble pro-

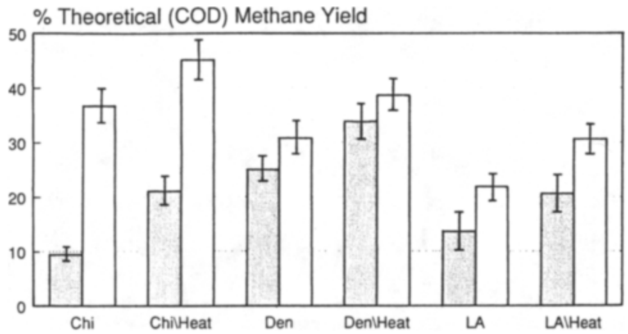


Fig. 3. Anaerobic digestion of sewage sludge residues before and after heat pretreatment (121°C for 4 h). Data for Chicago (Chi), Denver (Den), and Los Angeles (LA) digestion are as described in Fig. 2. ▨ Low solids system; □ ultimate (BMP).

Table 2
Correlation[a] Between Biological Gas Production (BMP) and Rapid Analytical Techniques for Analysis of Sewage Sludge Pretreatment Efficacy

| Method | $R^2$ |
|---|---|
| COD | 0.982 |
| Protein content | |
| Lowry | 0.944 (0.817)[b] |
| Bio-Rad | 0.752 (0.862)[b] |
| Bradford | 0.966 (0.887)[b] |
| Amino acid analysis | 0.643 |
| Kjeldahl-nitrogen | 0.974 |

[a] Single line regression.
[b] Dialyzed.

tein (Lowry, Bio-Rad, and Bradford), amino acids, total nitrogen (Kjeldahl), or soluble organic carbon (COD). Table 2 serves to compare the correlation of the rapid analytical techniques tested with that of true anaerobic biodegradation as determined by the BMP assay for replicates of sewage sludge residues under a series of different thermochemical treatments. The COD assay provided the best correlation to values obtained from BMP results for all treatment protocols. Correlations of Kjeldahl nitrogen (total nitrogen) and various protein dye binding assays were also comparable, although less accurate. Significant variations in the correlations were observed when the protein dye binding assays were performed on dialyzed supernatants designed to reduce the presence of interfering ions. The general trend here was a diminished correlation with BMP values when pretreated sludge samples were dialyzed, perhaps indicating that low-mol-wt, dialyzable components had a substantial effect.

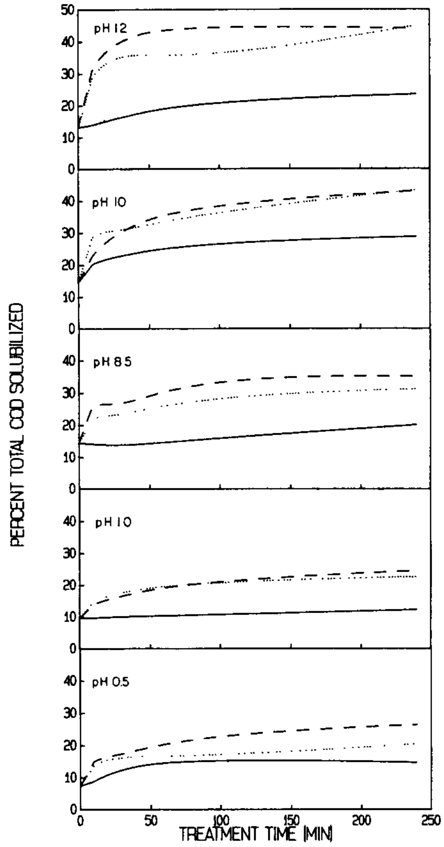


Fig. 4. Effects of initial sewage sludge pH, pretreatment temperature, and pretreatment time on the solubilization of the total COD. Data represent the average of duplicate determinations. — 22°C; ···· 80°C; —— 100°C.

To evaluate the effects of more extensive thermochemical pretreatments on Denver sewage sludge residues, changes in the soluble COD of the samples were utilized as an indicator of enhanced anaerobic biodegradability. The results, as shown in Fig. 4, indicate substantial increases in COD solubilization with increasing pH. The maximum COD solubilization (45%) occurred with sludge residue treatment at pH 12 and 100°C for 90 min; however, 44.3% of the total COD was released at the same pH and time period at 80°C. In general, increases in the treatment temperature over room temperature (22°C) significantly affected COD solubilization with less of an effect from 80 to 100°C. The effect of treatment time was most profound during the first 50 min, with less impact from 50–240 min.

Because the majority of volatile organics in the sewage sludge residues were acid detergent soluble and thus composed of protein, hemicellulose, fats, and lipids (and presumed to be microbial cells), approaches to

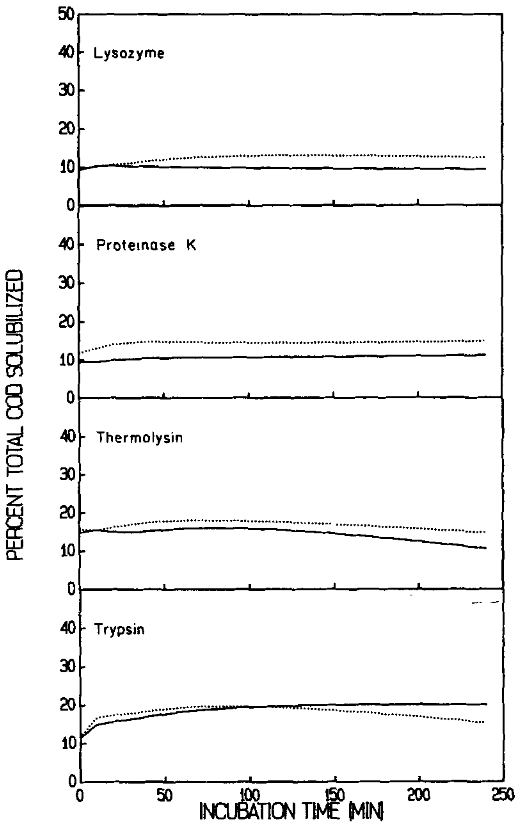


Fig. 5. Effects of lysozyme and various proteinase pretreatments at both high and low enzyme loadings and incubation times on the solubilization of the total COD. Data represent the average of duplicate determinations. Enzyme loading: — 920 U/g wet sludge; ···· 9200 U/g wet sludge.

hydrolyze the protein and cell walls of the samples enzymatically were evaluated using commercial enzyme preparations. Proteinases and lysozyme enzymes, respectively, were used for this purpose. The results, shown in Fig. 5, indicate that in general the enzymatic treatments had very little effect on the level of soluble COD of the sewage sludge residue even after an extensive incubation period. Enzymatic treatment with the proteinase, Trypsin, had the greatest effect with release of 22.1% of the total COD. No substantial differences were noted between the use of high or low levels of enzyme addition.

## DISCUSSION

Clearly, treated municipal sewage sludge residues contain substantial levels of volatile solids that can be further degraded to reduce waste volume and pollution potential, increase dewatering efficiency, and ultimately reduce disposal costs and produce additional energy (methane). The three sewage sludge residues tested were found to be similar in many respects, including polymer composition of the volatile solids fraction and COD values (based on sample wet wt). Although the sewage sludge residues tested resulted in stable anaerobic fermentations, methane yields in both low and high solids reactor systems for all sewage sludge residues were relatively low and, thus, indicated poor biodegradation of the volatile solids. Preliminary thermal treatments designed to enhance the biodegradation of the volatile solids portion of the sewage sludge residues were effective, resulting in the enhancement of anaerobic biodegradation of 23–122%.

Although the BMP assay is extremely effective in determining the ultimate methane yields for a sample, the extensive incubation period limits the number of evaluations that may be accomplished. To allow a more comprehensive evaluation of thermochemical treatment effects of sewage sludge residues on the anaerobic biodegradation potential, a rapid analytical method that effectively predicts anaerobic biodegradation was necessary. Of the various rapid analytical protocols examined, the soluble COD assessment was found to result in the best correlation with BMP results over a broad range of treatment protocols. This applicability of COD as a predictive measure of anaerobic biodegradability is not surprising, since the COD assay serves as a measure of all oxidizable organics and not just nitrogen-containing compounds, such as proteins, amino acids, or total nitrogen. Additionally, although the correlation of BMP with Kjeldahl nitrogen and protein assays was very good, these assays may only be appropriate for samples that contain high levels of proteineous materials, such as microbial cells (as in sewage sludge residues), and thus, the correlation for other types of samples may be considerably less.

Of the treatments evaluated, alkaline conditions had the greatest impact on solubilizing COD and, thus, enhancing anaerobic biodegradation, even at shorter pretreatment times and lower temperatures. The use of hydrolytic enzymes, such as general proteinases and lysozyme, was largely ineffective in increasing soluble COD even at high levels of enzyme addition and extensive incubation periods.

Clearly, the representative sewage sludge residues tested do not undergo substantial further anaerobic digestion as tested in standard low solids CSTR systems or in high solids systems. The lack of biological degradation of the volatile organic fraction of these residue materials may be owing to the inaccessible form of the organics, such as intact microbial cells. Thermochemical pretreatments designed to disrupt organics in general were shown to increase the level of soluble COD and, thus, anaerobic biodegradability of the residues. The use of commercially available hydrolytic enzymes was less effective than thermochemical treatments. Further treatment evaluation for the enhancement of anaerobic biodegradability of sewage sludge residues is necessary before an accurate asment as to the application of secondary anaerobic treatment may be made.

## ACKNOWLEDGMENTS

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