

Development of a Novel Laboratory Scale High Solids Reactor for Anaerobic Digestion of Processed Municipal Solid Wastes for the Production of Methane

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ABSTRACT

Economic evaluations of the capital costs for anaerobic digestion systems for gas production show that the reactor is a significant cost component. The successful application of high solids digestion of processed MSW (e.g., greater than 10% solids within the digester) would allow a decrease in reactor volume with maintenance of relatively high gas production rates. However, high solids slurries do not mix well in conventional stirred tank reactors. A horizontal shaft, hydraulically driven reactor was designed and fabricated to test the anaerobic digestion of high solids concentrations. Digester performance was evaluated as a function of experimental parameters such as nutrient requirements, feeding rates, pH control, and agitator design/rotation speed; horsepower of mixing was also evaluated for the reactor. Several startup protocols were examined to obtain a biologically stable anaerobic fermentation at high solids levels.

Index Entries: High solids; anaerobic digestion; methane; digester design; municipal solid waste.

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INTRODUCTION

Anaerobic digestion of municipal solid waste (MSW) couples the potential of producing considerable energy (methane) with the simultaneous reduction in organic-waste disposal problems by a much less energy intensive process than conventional aerobic processing methods. However, several key issues must still be overcome before the methane produced is economically competitive with conventional sources of natural gas. Since the value of the methane produced is relatively low, the anaerobic process must be rather simple in design, require little energy to operate, and maintain high gas production rates.

Economic evaluations (1) of anaerobic digestion for production of gas from MSW show that reactor capital costs are a significant economic burden owing to the large reactor volumes required in current anaerobic processes operated normally at lower solids levels (3–5%). If the reactor volume could be reduced significantly, the economics of anaerobic digestion of MSW and other organic wastes would improve. Increased solids loadings are particularly promising in this respect since available kinetic data indicate that gas production rates should increase with solids concentration in the reactor (2). Thus, if these data are substantiated, a decreased reactor volume would be possible for higher solids concentrations while maintaining the same solids loading rate and retention time. However, conventional mixers do not insure homogeneity in the reactor at solids concentrations above 5–10% by weight (3), and problems develop in providing adequate dispersion of intermediates and microorganisms.

Historically, research on high solids anaerobic fermentation has focused on the single charge (batch), nonmixed reactor concept, generally with recirculation of effluent (4–9). In these designs, gas production occurs either in a single stage or leached acids may be circulated to a second methanogenic stage. With nonmixed systems, rates of gas production are generally much slower than in liquid, mixed systems (3,7,9). The retention time required to effect a near complete digestion of the substrate is on the order of months for mesophilic temperatures and may be as short as weeks for thermophilic operation.

Other workers have investigated two approaches to mixed high solids fermentations. The "Bio-Funnel" (10) was used with 21–13% solids, while Gaddy and Clausen (3) attained high solids feeding in a modified continuous stirred tank reactor (CSTR) that approached 10% solids within the digester. Reactor designs allowing the high solids material to be well mixed increase the interaction of substrate, microorganisms, and metabolic intermediates. Mixing and operation at higher solids (solids levels of 9% or greater) have resulted in problems with material movement owing to substrate bridging, scum formation when the solids levels fall below 10%, and zones of nonmixing within standard reactor designs.

The high solids reactor designed, fabricated, and employed in this project is a significantly different concept than those used in high solids anaerobic digestion research by other investigators (3,10). This reactor, used in batch or continuous operation, provides adequate mixing of high solids materials (10–100%). It is based upon reactor designs conventionally used in the mixing of highly viscous materials in the plastics industry. In this paper, several protocols for startup to obtain a stable microbial consortium at high solids levels of MSW feedstock are reported including single charge feeding, low solids sludge concentration, and gradual solids buildup.

MATERIALS AND METHODS

High Solids Reactor Design and Construction

The reactor is a 30.48 cm diameter \times 60.96 cm cylindrical glass vessel that is positioned with a horizontal axis, as shown in Fig. 1. The cylinder is capped at each end, and a shaft is positioned horizontally along the cylinder axis between the centers of the two end caps. All digester sludge contacting materials are fabricated from 304 stainless steel or TefLon™ coated. Several ports are added to the glass vessel for feed addition, sample removal, gas removal, and liquid addition. Mixing is obtained with one of several different blade configurations attached to the 3.02 cm diameter horizontal shaft.

The high solids reactors were fabricated by International Process Research Co., Golden, CO (Fig. 2). Four such reactors were constructed and operated in a 37°C temperature controlled warm room. Shaft rotation is provided by a low speed, high torque, hydraulic motor (Staffa, Troy, MI; model HMB-010-P-10, 5 piston, 35 hp maximum). The hydraulic motor is powered by a custom fabricated hydraulic power station (Warren Fluid Power, Denver, CO; 10 hp). The Staffa hydraulic motor speed was controlled by Vickers temperature compensated flow control valves (Vickers, Troy, MI; model 50). The shaft seal was fabricated as a stuffing box type seal using 0.635 cm square TefLon rope packing (John Crane-Houdaille Inc., Morton Grove, MA; #C16065), which provided a liquid/gas tight seal even at fractional rotational speed operation of the reactor.

The glass vessel was fabricated from 30.48 cm diameter by 0.635 cm thick glass pipe (Precision Glassblowing of Colorado, Parker, CO) modified with two 1.91 cm ports for liquid introduction and gas removal. A 5 cm ball valve (Asahi/America, Medford, MA; Duo-bloc, PVC) was attached with couplings to the 5 cm glass port with beaded lip at the top end of the glass vessel and was used for addition of dry feed and sampling the contents of the reactor. Several glass receiving vessels were constructed which coupled to the 5 cm ball valve and allowed gassing of the receiver vessel

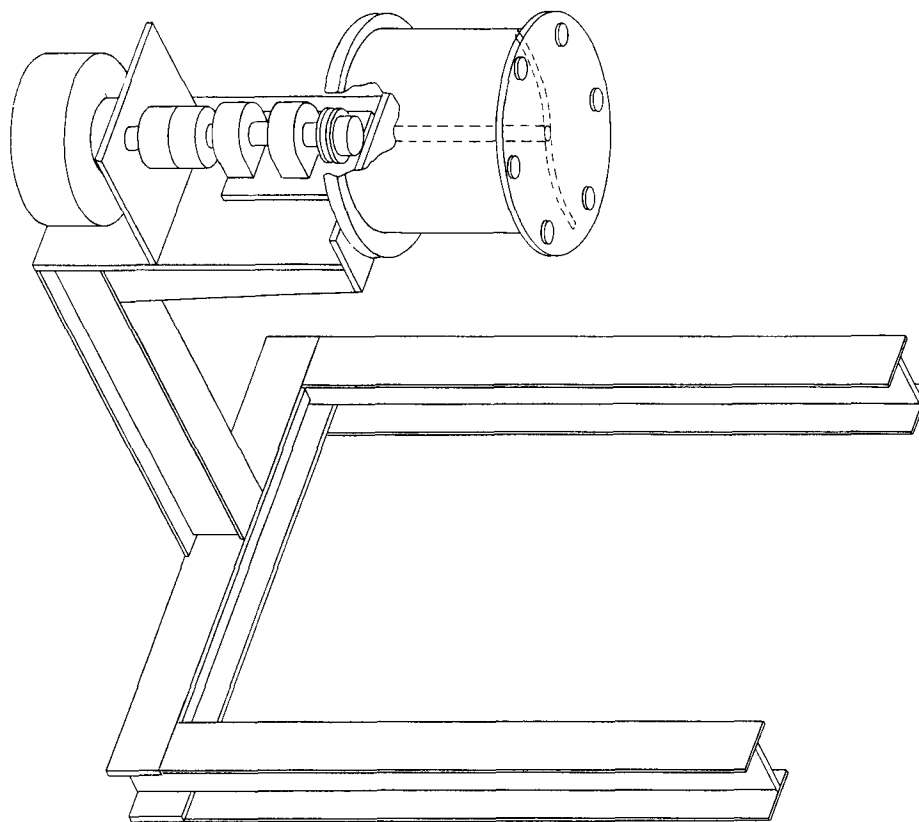


Fig. 1. A diagram of the high solids reactor system with movable stand as viewed from an aerial position. The mixing blade shown in this figure is the curved option.

with oxygen-free nitrogen gas before sampling. A plunger type addition unit was fabricated to add dry MSW feed to the reactor through the ball valve. Two gassing ports were installed on the solids feeder to allow out-gassing of the dry MSW before addition to the reactor.

Several blade configurations were designed, fabricated, and tested for mixing ability and horsepower requirements (Fig. 3). The final blade configuration chosen for fermentation studies was the 90° opposing orientation rod type agitator (Fig. 3, type E), which was TefLon coated to reduce biomass buildup and metal pitting from reducing conditions.

Horsepower Determinations

Determination of horsepower was calculated by standard methods (11) from the specific motor volumetric throughput and monitored hydraulic back-pressure ($H.P. = (GPM \times PSI) / (1714 \times E)$, where efficiency (E) is assumed to be 1.0). Horsepower requirements were determined for several blade configurations with two different milled MSW preparations.

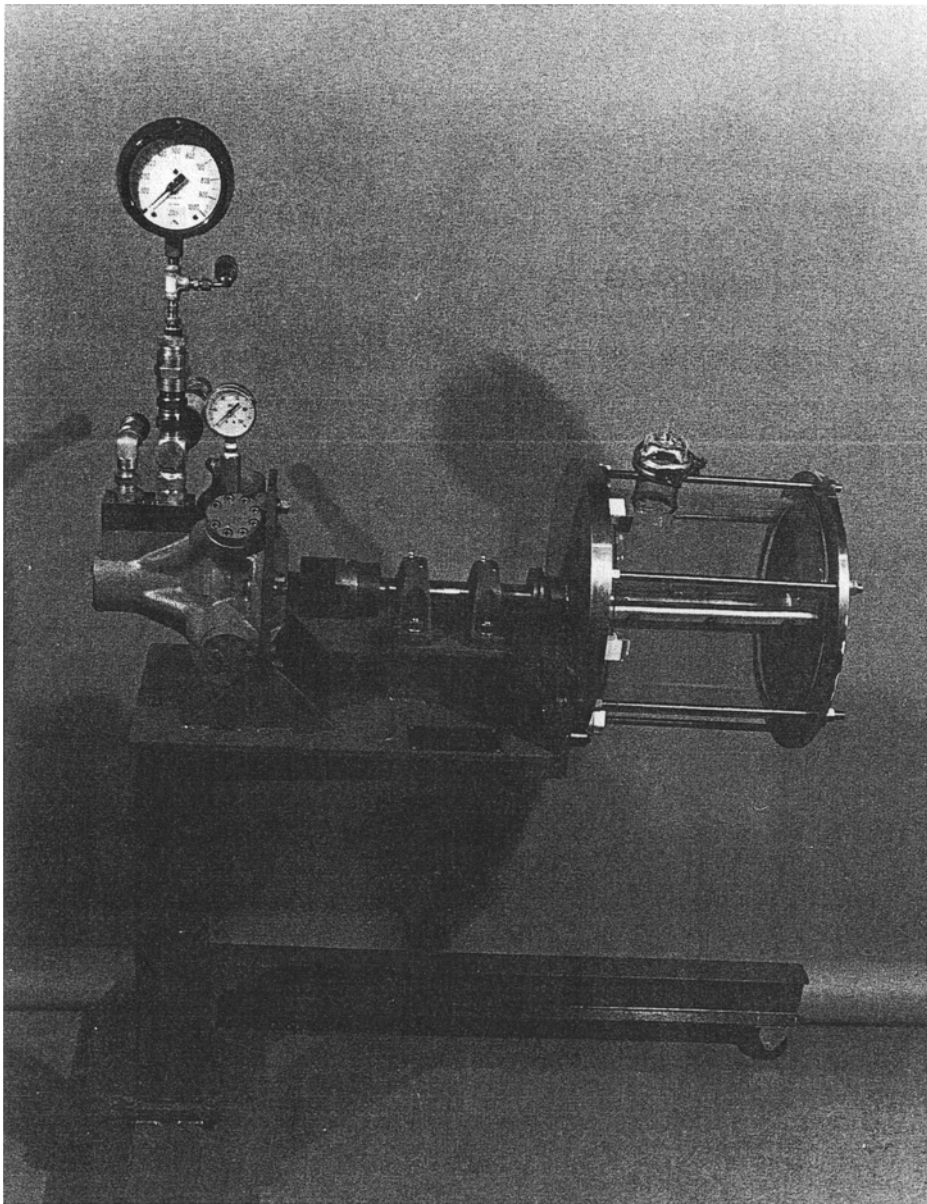


Fig. 2. The bench scale high solids digester installed in the SERI FTLB high bay area. The transparent vessel, agitator blade, and hydraulic motor with monitoring equipment are shown.

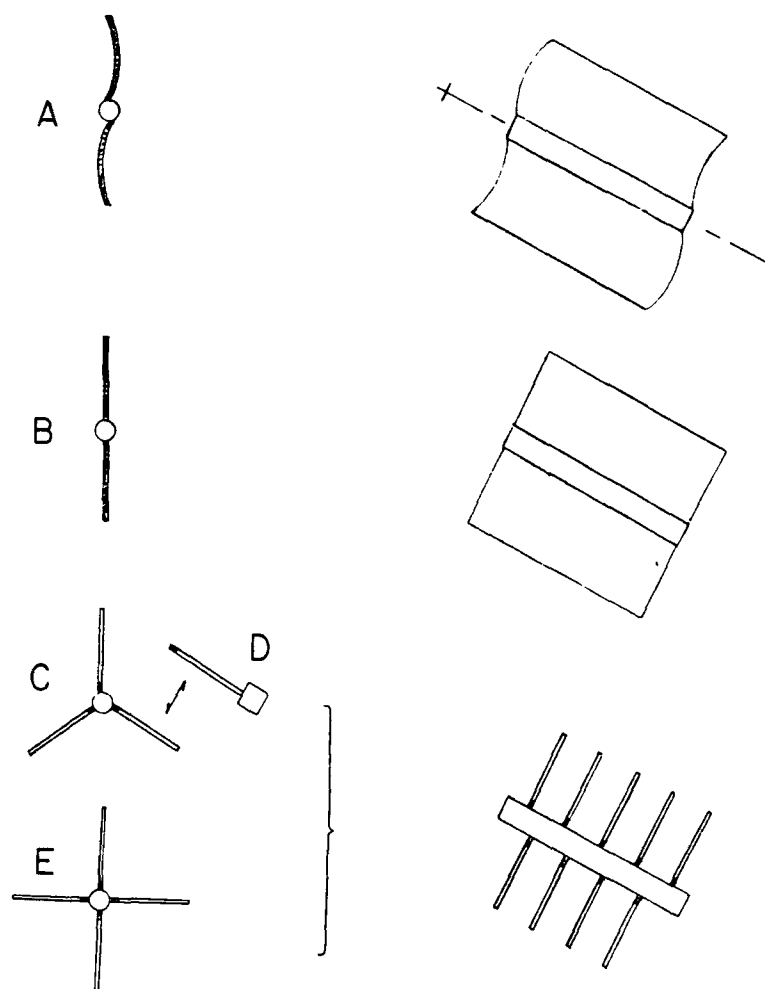


Fig. 3. Blade configurations for high solids reactor mixing: curved (gull wing) (A); straight (B); 120° rod (C); 120° rake (D); and 90° rod (E).

Feedstock

The organic feedstock for both horsepower evaluation and fermentation studies was MSW processed to remove the majority of glass, aluminum, and ferrous metals. The processed MSW was obtained from Future Fuels Inc., Thief River Falls, MN, and was shredded and densified to 2 cm pellets. Densification allows long term storage of feedstock without degradation. The pelletized MSW was milled before use employing either a small laboratory scale knife mill (Wiley Laboratory Mill, Philadelphia, PA; model 4), a pilot plant sized knife mill (All-Steel, product of Entoleter Corp., Newhaven, CT; model 8×20 , with four rotary knives and 40 hp drive), or an intermediate scale hammer mill (Pulverizing Machinery, Summit, NJ; model Mikro-2W, 15 hp drive). Milling operations utilized 1 and 3 mm round hole rejection screens.

High Solids Reactor Operation

In anaerobic fermentation studies, the 90° opposing, TefLon-coated rod blade was chosen. Several different methods of initiating the fermentation were used, and are described in the Results section. In general, the 20-L vessel was outgassed with oxygen-free nitrogen gas prior to the introduction of inoculum to remove air. The gas production was monitored using one of the ports on the glass vessel connected to calibrated water displacement reservoirs. Sludge was removed from the reactor on a daily or bidaily basis for pH determination, acid analysis, and microbial observation.

Digester Inoculum

A 100-L New Brunswick nutrient feed vessel (Edison, NJ; model NS-10) served as an anaerobic digester for supplying large volumes of low solids (approx 3–3.5%) sludge for high solids startup. The water jacketed vessel was temperature controlled at 37°C using a large capacity circulator bath (Forma Scientific, Marietta, OH; model 2067). The digester was maintained on a 30-d retention time. The reactor was fed knife-milled MSW passing through a 3 mm rejection screen. It was batch-fed daily a 5% solids milled MSW mixture containing a nutrient supplement. The nutrient supplement contained yeast extract (Difco) 8 g, K₂HPO₄ 8.71 g, 10× trace mineral solution (12) 10 mL/L of distilled water. The final pH of the nutrient solution was adjusted to 7.5.

Low-Solids Sludge Concentration

High-solids digester sludge was obtained from low-solids sludge through a large scale centrifugation-dewatering process at the Denver Municipal Sewage Treatment Plant, Denver, CO. Here, anaerobic digester sludge (3% solids) was dewatered to 21% solids through large-scale centrifugation, and the dewatered sludge was collected from a belt conveyor and stored in plastic lined 55-gal drums in a cold room (4°C) until use in the high solids reactors.

Enhanced Acetogen/Methanogen Digester

A 2-L New Brunswick Multigen fermenter (1.5-L working volume) was used for the production of an enhanced acetogen/methanogen microbial consortium. The reactor was magnetically stirred and temperature controlled at 37°C. A mixture of organic acids was used as feedstock. The addition of the organic acids was controlled by the reactor pH (set to control at pH 7.2) using a pH controller (Markson, Phoenix, AZ; model 6300) and a Masterflex pump (Cole Parmer, Chicago, IL; model 7520-30). The liquid feed contained yeast extract, 8 g/L; K₂HPO₄, 50 mM; and 10x trace mineral solution (12), 10 mL/L, as well as the following organic acids:

formic, 18 mM; acetic, 766 mM; propionic, 76mM; *n*-butyric, 102 mM; *n*-valeric, 4 mM; iso-valeric, 2 mM; and lactic, 182 mM. The final pH of the organic acid/nutrient solution was approximately 4.2. Effluent from the reactor (~500 mL) was removed weekly, and the bacterial cells were concentrated by centrifugation (5000 rpm for 30 min at 4°C, using a Beckman centrifuge, model RC-5B, and GSA rotor) under an oxygen-free gas phase in 500 mL plastic centrifuge bottles. The cell pellets were stored at 4°C until used.

High-Solids Sludge Analysis

Levels of volatile organic acids (C₁-C₅ iso- and normal-acids) were determined by gas-liquid chromatography (GLC). A model 5840A gas chromatograph (Hewlett-Packard, Palo Alto, CA), equipped with a flame ionization detector, a model 7672A autosampler, and a model 5840A integrator was used. The glass column (183 cm×2 mm) was packed with 60/80 Carbowax C/0.3% Carbowax 20M/0.1% H₃PO₄ (Supelco, Bellefonte, PA). The injection port and detector temperatures were maintained at 190°C. The oven temperature was maintained at 120°C. Nitrogen at a flowrate of 50 mL/min was used as the carrier gas. The experimental samples were calibrated against a quantitative standard (Supelco) with each run. Experimental samples were prepared for analysis by centrifugation in a microcentrifuge to remove suspended solids in 1.5 mL microcentrifuge tubes at room temperature for 15 min. The clarified samples were acidified by addition (1:1) of 1% v/v formic acid. Finally, the samples were loaded into autosampler vials, crimp caps attached, and loaded into the autosampler for analysis.

Analysis of nonvolatile organic acids in digester sludge effluent was accomplished by high performance liquid chromatography (HPLC). Experimental samples were clarified by centrifugation as described above with final removal of particulates by passage through a 0.2-micron disposable syringe filter. The samples were made to 0.01 N with sulfuric acid and loaded into autosampler vials for analysis. The HPLC system consisted of a Beckman (Palo Alto, CA) model 501 auto-sampler, Beckman model 110A pump, column temperature controller (Eldex Inc., Menlo Park, CA), Variable Wavelength Detector (Waters and Assoc., Milford, MA; detection at 210 nm), and integrating recorder (Hewlett Packard, 3390A). The column used was a HPX-87H organic acids column (Biorad, Richmond, CA). The column temperature was controlled at 45°C. The flow rate of eluent (0.01 N H₂SO₄) was maintained at 0.5 mL/min. Samples were analyzed by comparison with high purity standard mixtures of organic acids (Supelco). Analysis by HPLC identified both nonvolatile and volatile organic acids and therefore served as a backup to the gas-liquid chromatography analysis.

Solids analysis of digester effluents was conducted using duplicate gravimetric analysis. A 20–30 g sludge sample was loaded into the pre-

weighed tin and dried 48 h at 45–50°C. Percent solids in the digester sludge was calculated on a wt/wt basis. Ash analysis was conducted by combusting dried samples in a furnace at 550°C for 3 h in preweighed 30 mL ceramic crucibles and cooling to room temperature in a desiccator before weighing.

Gas Analysis

Production of biogas from high solids reactors was monitored on a daily basis (in conjunction with batch feeding) using calibrated water displacement reservoirs. Biogas produced in the various digesters was analyzed for methane, carbon dioxide, and nitrogen composition by gas chromatography. A Gow-Mac (model 550) gas chromatograph equipped with a thermal conductivity detector and integrating recorder was used. Separate columns (both 183 cm × 6.4 mm) installed in the chromatograph were packed with Porapak Q 80/100 mesh (Waters Assoc.) and Molecular Sieve 5A 60/80 mesh (Supelco). The injection port, oven, and detector were maintained at 100, 90, and 110°C, respectively. Helium served as carrier gas. The chromatograph was calibrated with high purity gas standards (Matheson, Chicago, IL).

Microbiological Analysis

Microbiological observations of sludge samples from high solids reactors and inocula were conducted using wet-mount slide preparations with a Nikon Labophot microscope equipped with phase contrast/epi-fluorescent illumination and 1000x power. Methane producing bacteria were detected by their unique auto-fluorescence (13) on excitation by the epifluorescent light source (narrow band width centered at 420 nm).

RESULTS

Initial testing for horsepower-mixing requirements of uninoculated MSW milled to two different sizes employed various blade configurations (see Fig. 3). The effectiveness of mixing the material was also assessed qualitatively. In general, the curved and straight solid blade designs required the most hp for mixing (Figs. 4–7), and the more coarsely milled MSW (3 mm rejection screen hole) required more horsepower than the finer, milled material (1 mm). As the distance between the blade and the glass wall of the vessel was reduced, the hp required for mixing increased; this was due to pinching of the MSW in the reactor at the blade-vessel edge. During extended operation of the reactor with the solid blades, the volume of MSW on either side of the blades became uneven owing to variations in the exact blade-vessel clearances of the two edges of the blades. This asymmetry led to large pressure variations on the hydraulic motor and translated into large hp swings.

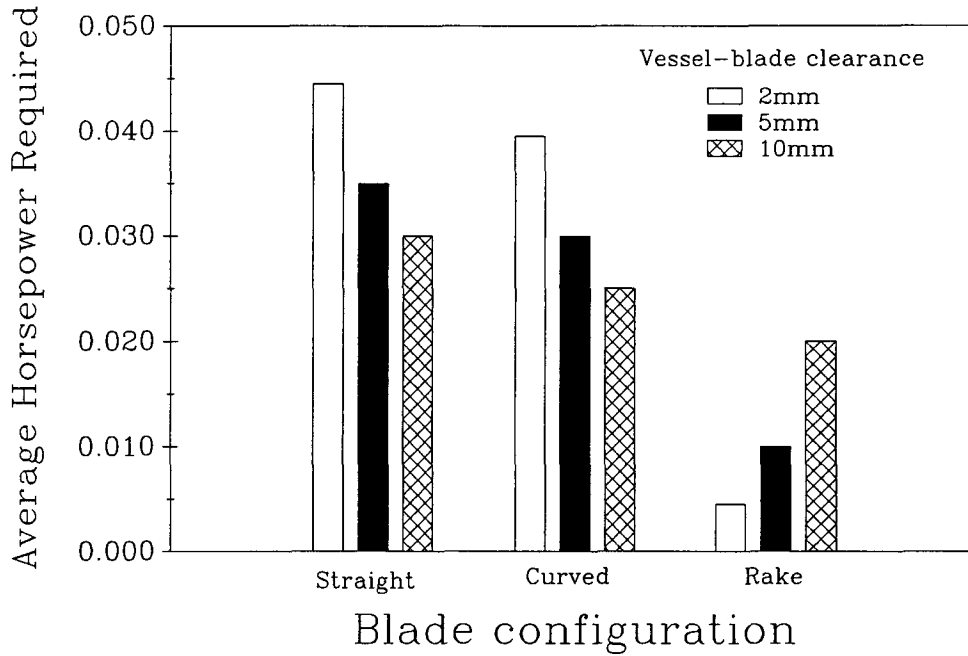


Fig. 4. Average hp required for 12 rpm mixing of uninoculated 3 mm milled MSW at 30% solids, using various blade configurations and blade-vessel clearances.

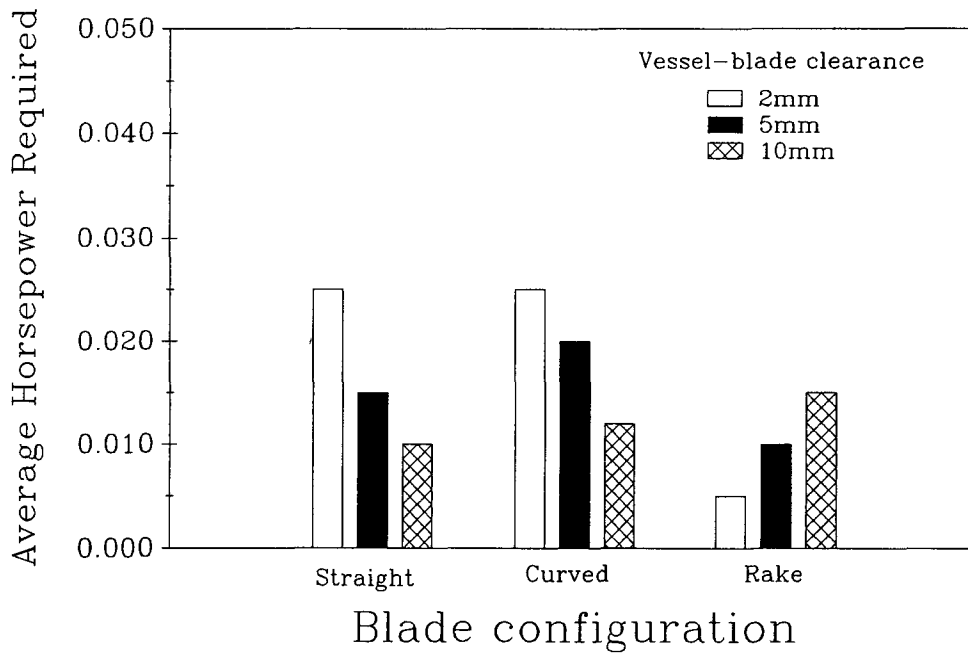


Fig. 5. Average hp required for 12 rpm mixing of 1 mm milled MSW at 30% solids, using various blade configurations and blade-vessel clearances.

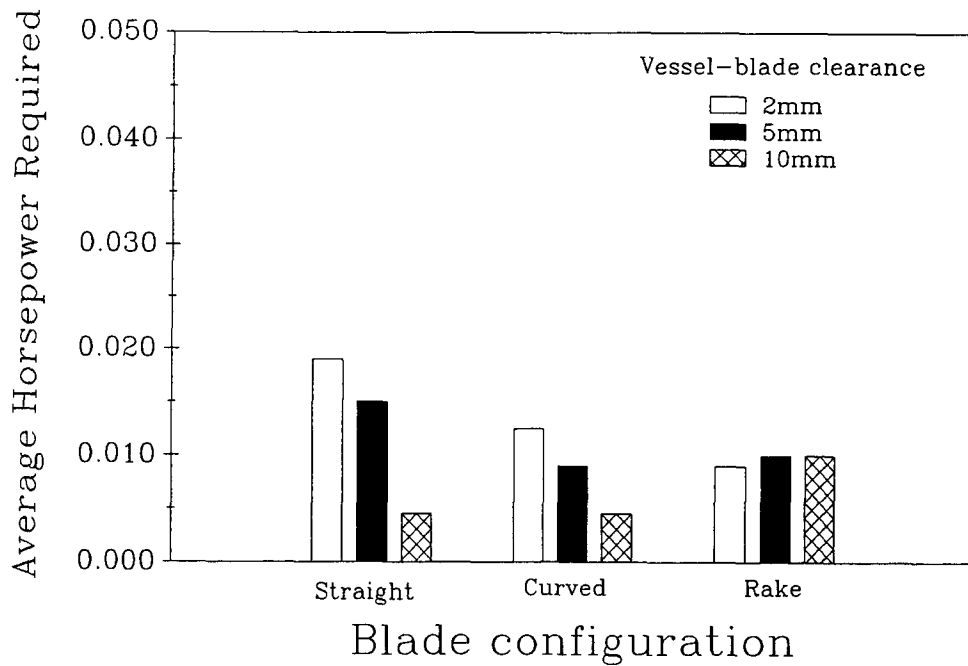


Fig. 6. Average hp required for 1.5 rpm mixing of 3 mm milled MSW at 30% solids, using various blade configurations and blade-vessel clearances.

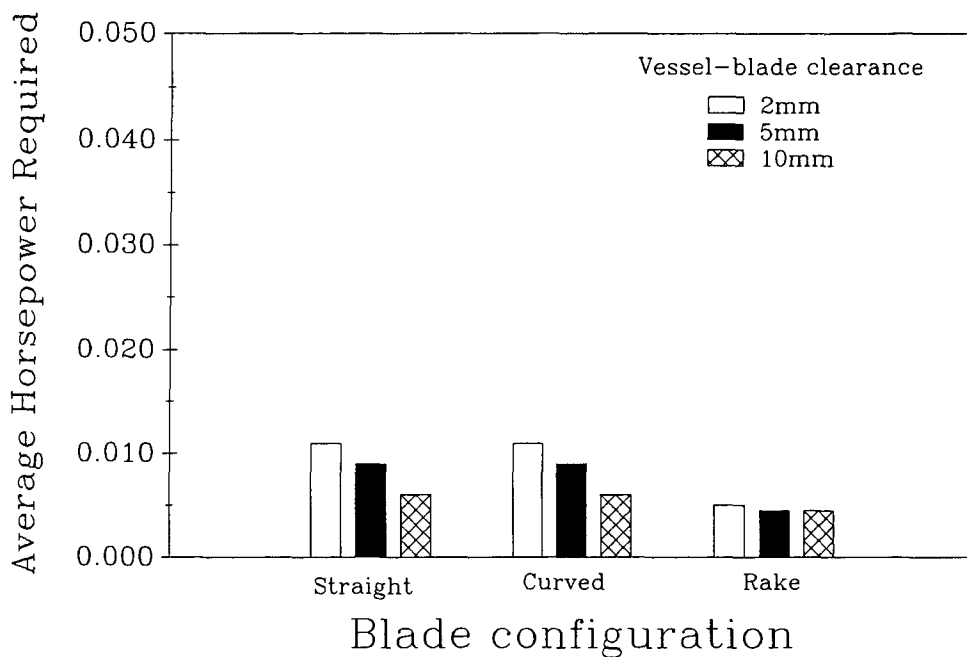


Fig. 7. Average hp required for 1.5 rpm mixing of 1 mm milled MSW at 30% solids, using various blade configurations and blade-vessel clearances.

The mixing of a 30% solids MSW material with the solid blade configuration also formed balls of material caused by tumbling of the MSW down the face of the solid blade. This clumping of material within the reactor resulted in unsatisfactory mixing performance with both the solid blade designs. The rake blade configuration (essentially a rod with end wiper) did not form clumps of material in the reactor, but in comparison to the rod blade configuration, required more hp for mixing (Figs. 8 and 9).

During the first inoculated run of the high solids reactor with the rod type blade, material adhered to the rods and hub. This problem was relieved by TefLon coating the rods and hub. The TefLon coating also resulted in a lower horsepower requirement as compared to a non-TefLon coated rod blade (Fig. 9). The angle and alignment of the rods also affected the average required hp, hp swings, and the effectiveness of mixing. In general, changing the angle from 180 to 120, and to 90° and opposing the rods rather than aligning each row, resulted in less lifting of material and more even mixing of solids. Diminished lifting of the material resulted in a lower horsepower requirement and dampened the high and low horsepower spikes. As a result, the 90° TefLon-coated, opposed-rod blade configuration was used in the digestion experiments with inoculated solids.

During preliminary single-charge MSW addition to attain high solids fermentations (data not shown), high levels of organic acids were produced resulting in the lowering of pH in reactor sludge and termination of the fermentations. This indicated a need to enhance the population of acetogens and methanogens in the digester sludge. A fermenter was initiated to produce an enhanced acetogen/methanogenic culture by controlled addition of organic acids. The type of organic acids and their relative concentrations was determined by the final acid pools in two preliminary high solids runs. The resulting microbial population consisted of many different rod and sarcina bacterial forms that auto-fluoresced under epi-fluorescent microscopy. The cells from this fermenter were harvested, and stored by refrigeration until use.

Results of single-charge MSW addition of high solids fermentation are shown in Fig. 10, which indicates that although gas production quickly ensued, the fermentation was erratic and unstable. Over a 44-d period, during which the fermentation was operated, organic acid pools increased and sludge pH decreased. Several strategies for pH adjustment were evaluated, including the addition of a concentrated acetogen/methanogen consortium, addition of the buffer morpholinopropane sulfonic acid (MOPS), urea, and outgassing the sludge with oxygen-free nitrogen to remove carbon dioxide, but they allowed only a temporary pH adjustment. Addition of the acetogen/methanogen bacterial consortium resulted in high gas production with minimal effect on sludge pH. Addition of MOPS and urea significantly affected the pH (>0.1 U) but also appeared to negatively affect gas production. Outgassing the sludge to drive off carbon dioxide had little effect on gas production and sludge pH. The fer-

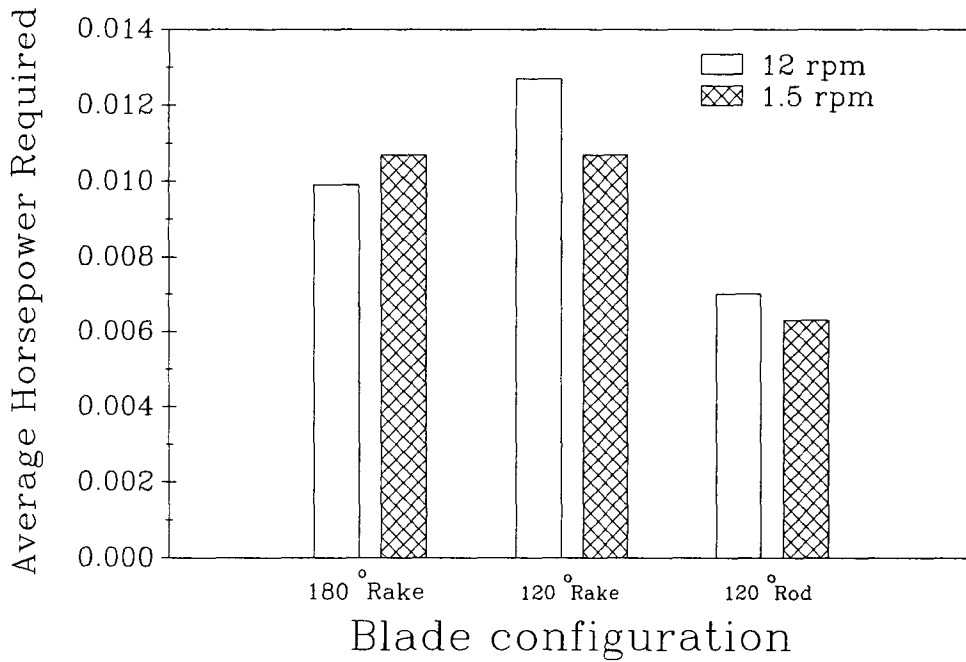


Fig. 8. Average hp required for mixing of 3 mm milled MSW at 30% solids, with 180° rake, 120° rake, and 120° rod blade configurations for 1.5 and 12 rpm.

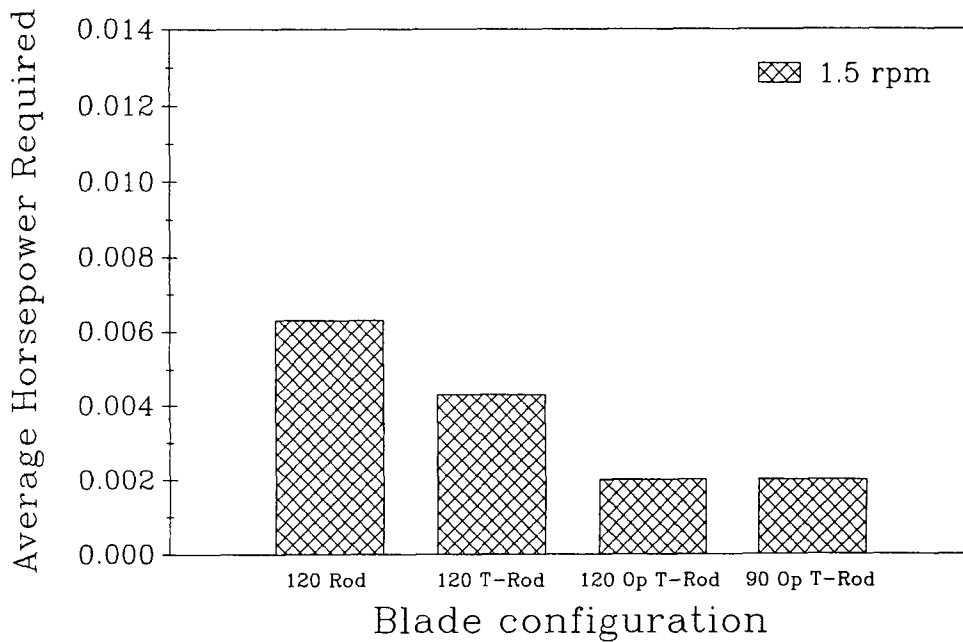


Fig. 9. Average hp required for mixing of 3 mm milled MSW at 30% solids, with 120° rod, 120° Teflon coated rod (T-Rod), 120° opposing Teflon coated rod (Op T-Rod), and 90° opposing Teflon coated rod blade configurations at 1.5 rpm.

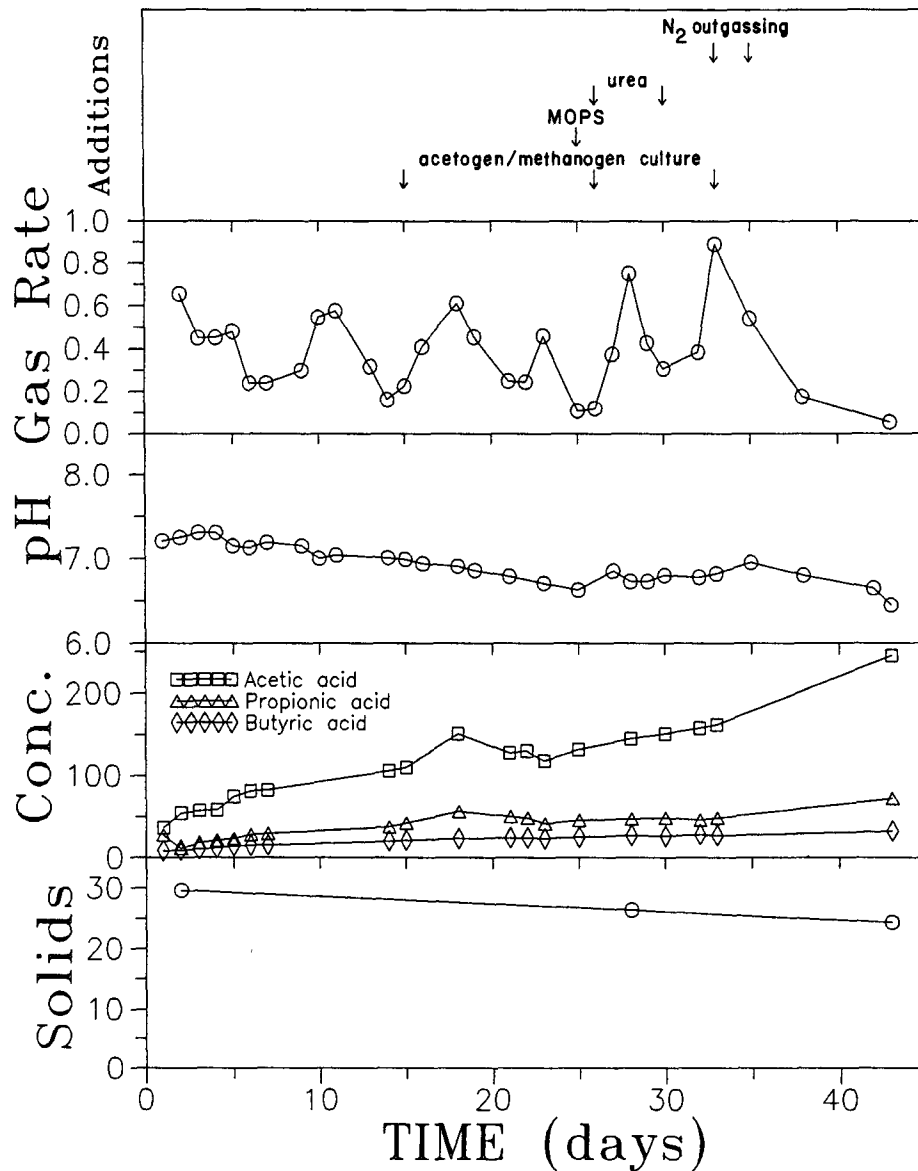


Fig. 10. The anaerobic fermentation performance for single-charge feedstock addition to initiate high solids startup. The reactor was loaded with 3 Kg of dry 3.18 mm milled MSW and outgassed for 3 h with oxygen-free N_2 gas (1.5 L/min) before introduction of 7 L of low solids digester sludge. Addition of the acetogen/methanogen culture was in 75 mL aliquots; 2 M MOPS buffer was 50 mL; urea was 30 g each addition; and N_2 outgassing of the reactor headspace was for 15 min. Total gas production is in volumes gas produced/volume reactor/d, concentration of organic acids is in millimolar, and solids were determined on a wt/wt basis.

mentation was terminated after d 44 because of the high levels of organic acid and low methane production.

Dewatering low-solids anaerobic digester sludge to high-solids levels was evaluated as a method to achieve high solids fermentation with results shown in Fig. 11. Significant gas production occurred during the first 7 d, after which it declined. Owing to the low rate of gas production and continued high levels of organic acids, feed addition to the reactors was discontinued on d 13. The appearance of the dewatered sludge was black indicating possibly high levels of reduced sulfide/metal complexes. A decline in fluorescing methanogenic bacteria in the digester solids indicated a probable inhibitory condition, and addition of low solids sludge to lower the solids concentration and supply new microbes only transiently improved the fermentation performance. The fermentation was terminated on d 50.

Fermentation performance with gradual solids buildup to attain high solids digestion levels is shown in Fig. 12. Rates of addition of dry MSW feed were slowly increased during the fermentation. Fermentation performance has remained stable with low levels of organic acids produced, stable pH, constant percent ash in the solids (data not shown, indicating no buildup of volatile solids), and high total gas production rates. At the time of this writing, the solids level for these fermentations had reached 13.8%.

DISCUSSION

A novel high-solids digestion reactor was designed, equipped, and fabricated at SERI. This reactor has achieved flexible operation (even at low rpm) as well as ease of gas collection, sludge removal, and feed introduction. The single TefLon-stuffing-box seals provided a rugged, effective gas tight seal. Several blade configurations for stirring the feedstock in the reactor were evaluated on the basis of average hp requirements and qualitative mixing effectiveness, using two different milled MSW substrates. The most effective mixing was accomplished by the agitator with rods spaced at 90° angles around the shaft. This design produced no clumping of high solids materials, the lowest hp-mixing, and the least high/low hp spikes. A problem with solids adhering to the rods and hub (and not being effectively mixed) was solved by coating the rods and hub with TefLon.

Initiating the fermentation at high solids levels by the single-charge feed addition method gives high levels of undigested substrate in the reactor, which results in severely unstable anaerobic fermentations. This problem is primarily a result of the rapid buildup of organic acids at high substrate concentrations because of the inability of the acetogen/methanogen populations to process the acid pool fast enough to prevent its buildup. Addition of a concentrated acetogen/methanogen bacterial consortium

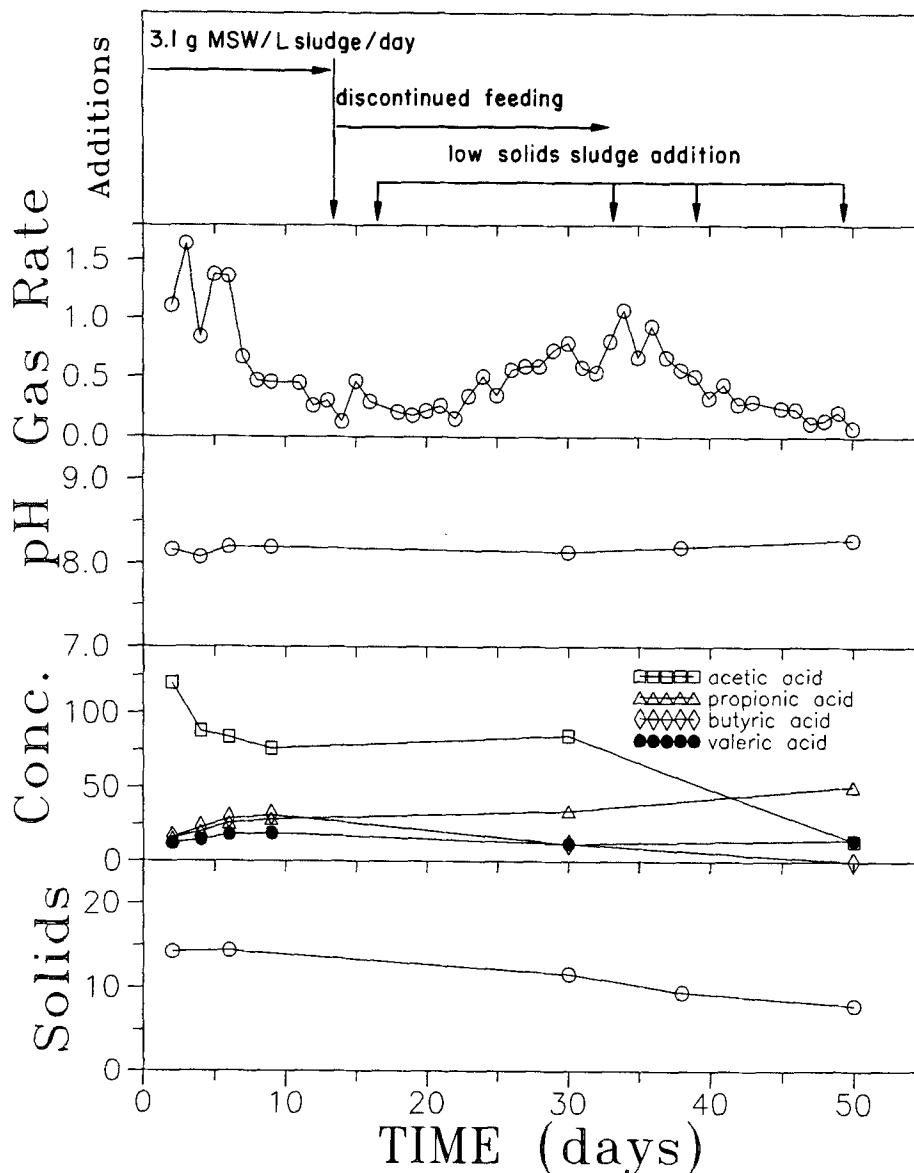


Fig. 11. Anaerobic fermentation performance for dewatered, low-solids sludge to high-solids levels startup. The dewatered digester solids (7L, ~15% solids) were from the Denver Municipal Sewage Treatment Plant as described and were loaded into each of four reactors. After addition of solids, the reactors were outgassed with N_2 (1.5 L/min) for 1 h. Dry 3 mm milled MSW feed was introduced on a daily basis at the rate indicated. A concentrated nutrient solution (30 mL) containing 60 g/L yeast extract, 500 mM K_2HPO_4 , 10x trace vitamin/minerals solution (12), and at a pH of 8.3 was added bidaily. Low solids sludge (~3% solids) was added at the rate of 500 mL/reactor/addition. Data represent the average of four reactors.

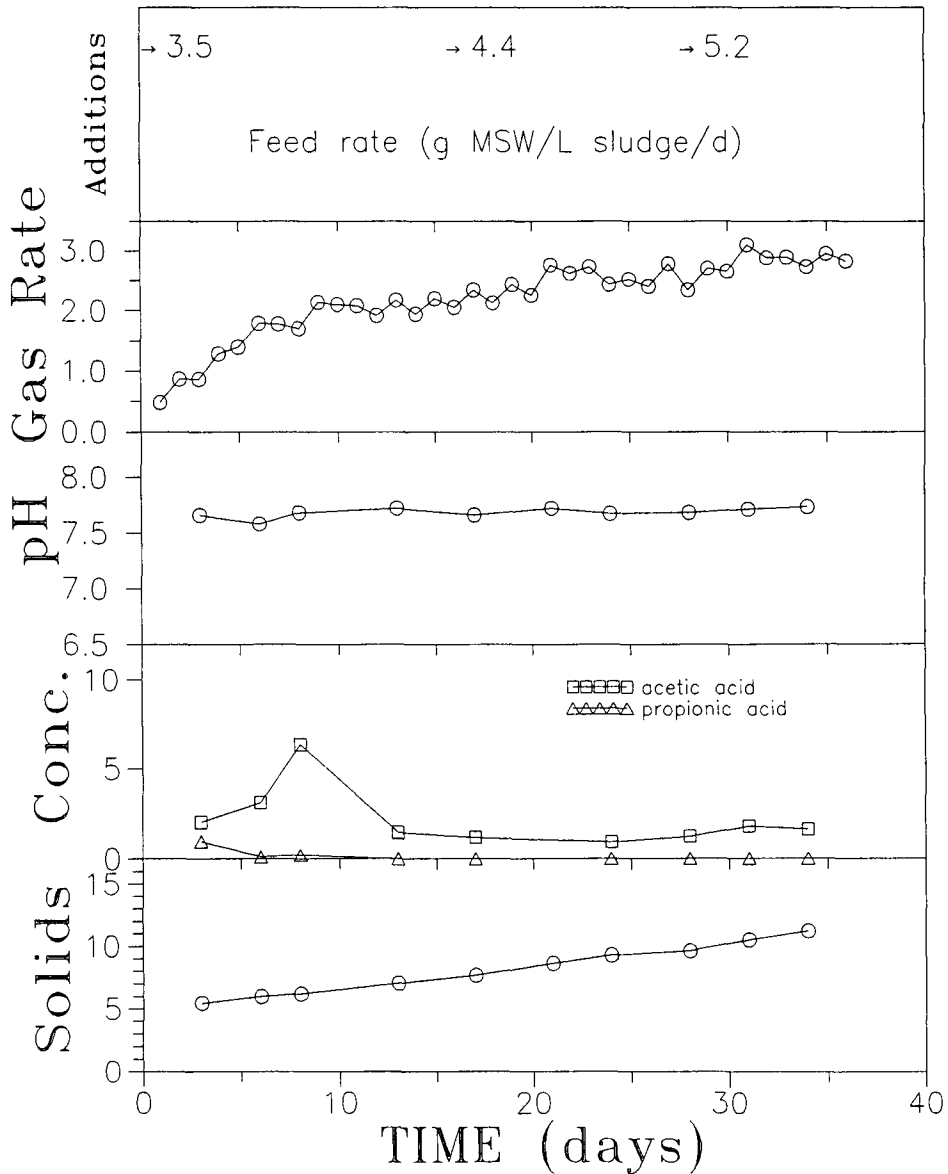


Fig. 12. Anaerobic fermentation performance for gradual solids buildup for startup of high-solids digestion. Reactors were outgassed with N₂ for 2 h followed by the introduction of 5 L of low solids digester sludge. Dry MSW feed and nutrient additions were as described in Fig. 11. Data represent the average of four reactors.

tailored to the specific acids and acid levels allows only temporary relief of the problem of acid buildup. Initiation of high solids fermentations by dewatering low solids digester sludges results in adequate fermentation startup; however, performance soon decreases, either because of the increase in solids or possibly concentration of inhibitory compounds that adversely affect the overall viability of the microbial populations in the digester.

Currently, we are assessing startup of the high solids fermenter by gradual solids buildup from low solids sludge to allow slow adaptation of the microbial consortium to higher solids levels. This protocol appears to be the most effective way of developing a stable and active anaerobic digestion consortium of bacteria.

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REFERENCES

1. Goodman, B., Lowenstein, M. Z., Neenan, B., and Ashworth, J. (1985), *SERI/SP-231-2624*.
2. Srivastava, V. J., Fannin, K. F., Biljetina, R., Chynoweth, D. P., and Hayes T. D. (1987), *Aquatic Plants for Water Treatment and Resource Recovery*, Reedy, K. R. and Smith, W. H. eds., Magnolia, Orlando, FL, p. 713.
3. Gaddy, J. L. and Clausen, E. C. (1985), *SERI/PR-231-2691*.
4. Ghosh, S. (1984), Eleventh American Society of Mechanical Engineers: National Waste Processing Conference, Orlando, FL.
5. Goebel, R. P. (1983), U.S. DOE Technical Report #DE-FG03-81 SF-11613.
6. Lin, Y.-M. PhD Thesis, Michigan State University, 1983.
7. SEG (Snell Environmental Group) (1983), U.S. DOE Technical Report #DE-FG02-81R510329.
8. Jewell, W. J. (1980), Volume II U.S. DOE Project #DE-AC02-76ET20051.
9. Wujcik, W. J. and Jewell, W. J. (1979), 2nd Symposium on Biotechnology in Energy Production and Conservation, Gatlinburg, TN.
10. Goldberg, T., Bjergbaek, B., Djernaes, E., and Mouritzen, A. (1981), International Gas Research Conference, 1981.
11. Vickers Industrial Hydraulic Manual (1984), 1.
12. Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R., and Wolfe, R. S. (1979), *Microbiol. Rev.* **43**, 260.
13. Cheeseman, P., Toms-Wood, A., and Wolfe, R. S. (1972), *J. Bacteriol.* **112**, 527.