

76. UB. I-5485 (1) Dr. 1275

Prepared for:

DOE/CS/20451-T1

U.S. Department of Energy

March 1983

Assistant Secretary, Conservation
and Renewable Energy

Office of Renewable Technology

Washington, D.C. 20585

Under Contract No. DE-AC01-78CS20451

ENERGY RECOVERY FROM THE EFFLUENT OF PLANTS
ANAEROBICALLY DIGESTING URBAN SOILD WASTE

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Dist. Category UC-95e

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ENERGY RECOVERY FROM THE EFFLUENT OF PLANTS ANAEROBICALLY DIGESTING URBAN SOLID WASTE

Prepared by:
Systems Technology Corporation
Xenia, Ohio 45385
Under Contract No. DE-AC01-78CS20451

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ABSTRACT

The program objective was to study the parameters of concentration, time, temperature, and pH to find optimum conditions for enzymatically converting unreacted cellulose in the effluent of an anaerobic digester to glucose for ultimate conversion to methane, and then to project the economics to a 100-tons-per-day plant.

The data presented illustrate the amount of cellulose hydrolysis (in percent solubilized mass) for enzyme concentrations from 5 to 1,000 C₁U/gram of substrate using either filter paper or anaerobically digested municipal solid waste (MSW) reacted over periods of time of from 0 to 72 hours. With an active bacterial culture present, the optimum temperature for the hydrolysis reaction was found to be 40°C.

The feasibility of recycling enzymes by ultrafilter capture was studied and shows that the recovered enzyme is not denatured by any of several possible enzyme loss mechanisms--chemical, physical, or biological. Although rather stable enzyme-substrate complexes seem to be formed, various techniques permit a 55 percent enzyme recovery.

Posttreatment of digested MSW by cellulase enzymes produces nearly a threefold increase in biomethanation. However, the value of the additional methane produced in the process as studied is not sufficient to support the cost of enzymes. The feasibility of enzymatic hydrolysis as a biomethanation process step requires further process optimization or an entirely different process concept.

This report is submitted in partial fulfillment of Contract Number EM-78-C-01-5300 by SYSTECH Corporation under the sponsorship of the Office of Urban Waste of the Department of Energy.

SECTION 1

INTRODUCTION

PURPOSE AND GOALS OF THE PROJECT

Anaerobic digestion has a long history of application to the treatment of sewage sludge for purposes of volume reduction, waste stabilization, and methane production. Today, with the advance of waste management philosophies that emphasize reclamation of energy and resources rather than simple disposal, the technology of anaerobic digestion for methane production is beginning to be applied to municipal solid waste (MSW), agricultural wastes, and other industrial cellulosic waste streams. This is a desirable trend, both from a disposal standpoint and an energy outlook. However, the economics and some of the technology of anaerobic digestion require improvement before this energy recovery technique becomes attractive for municipal solid waste.

One of the most serious drawbacks to the use of anaerobic digestion in energy recovery from municipal solid waste is that, even in a well run wastewater digester, only 50 percent of the volatile solids present in the waste are biologically converted to a gaseous product.¹ In addition to this large portion of the potential fuel escaping fermentation, the moisture-laden residue requires further energy for dewatering and drying in order to make it suitable for incineration or land disposal.

While the digestion of municipal solid waste is similar in theory to the anaerobic degradation of municipal wastewater sludges, there are several factors that make the process more difficult in practice and render it difficult to achieve even 50 percent destruction of volatile solids. A significant portion of municipal solid waste, 60 percent or greater, is composed of cellulosic materials. The first step in biological degradation of cellulose is the hydrolysis of these complex polymers into their constituent glucose molecules. The mix of microbes present in a digester appears to be deficient in the enzymes that are required to break down the cellulose chains. Because of this, much of the organic material remaining in the digester effluent is composed of cellulosic material.

The cellulosic material in the digester effluent is particularly amenable to attack by cellulase enzymes. Since the material probably was fairly well shredded prior to feed to the digester vessel, its exposure to high temperatures in a water slurry will have resulted in a swelling of the cellulose fibers, allowing enzyme attack of the cellulose. The enzymatic hydrolysis of cellulose as a treatment process has been studied for many years on the laboratory scale by various researchers, thus laying the groundwork for its application to digestion. Most of the approaches utilizing enzymatic

cellulose hydrolysis have considered this as a pretreatment for the cellulosic feedstock prior to anaerobic fermentation. It was the purpose of the present study to evaluate separate enzymatic hydrolysis as a posttreatment process rather than a pretreatment process.

PROCESS CONCEPT

The ultimate goals of this program were the development and evaluation of a process to treat the effluent from an anaerobic municipal solid waste digester with cellulase enzymes in order to improve the conversion of MSW to methane. Figure 1 shows a diagrammatic presentation of the cellulase enzyme posttreatment process studied. The digester vessel indicated is the municipal solid waste digester, which, strictly speaking, is not a part of the studied process but is shown here for completeness. Total effluent from the digester is piped into the enzyme treatment vessel, which is sized to accommodate the digester effluent for a specific hydraulic retention time. Effluent from the enzyme reactor vessel passes to a filter. The captured solids cake on the filter consists principally of inorganic materials, nonhydrolyzed cellulose, a portion of the enzymes, and the various nondegradable plastics. The filtrate is passed through an ultrafiltration (UF) unit with a membrane material designed to have a capture cutoff in a molecular weight range between 5,000 and 10,000. Ultrafiltration captures the enzymes, which have a high molecular weight, for recycle, while passing the glucose and organic acids. The concentrated enzyme stream is then recycled into the reactor. The permeate from the UF contains only glucose and other dissolved organic material and is highly amenable to bioconversion.

PROCESS EVALUATION

The overall process was evaluated in the laboratory as three separate unit operations: (1) enzyme treatment of cellulose, (2) enzyme recovery, and (3) energy recovery. Studies of the enzyme treatment unit operation were concerned with enzyme to substrate loadings, hydrolysis enhancement, and the effect of retention time on hydrolysis rate. Evaluation of the enzyme recovery unit operation dealt with identification of possible enzyme loss mechanisms and their cure as well as with optimization of filtration and ultrafiltration processes. Energy recovery due to the enzymatic treatment was evaluated by establishing a separate digester and operating it under the same conditions as the "feedstock" digester, except that it was fed only ultrafiltered, enzyme-treated digester effluent. Methane production from this vessel was measured to determine the additional energy produced as a result of the overall process.

SUMMARY OF RESULTS

The process described in this paper appears to be a technically feasible approach to recovering much of the energy lost from MSW digesters. Additional methane approaching 200 percent of that produced in the original digestion step can be produced. Furthermore, much of the enzyme used can be recovered and reused. The longevity and stability of the commercially available enzyme used was quite good even after ultrafiltration. The enzymes were not seriously inhibited by the presence of high concentrations of metals nor

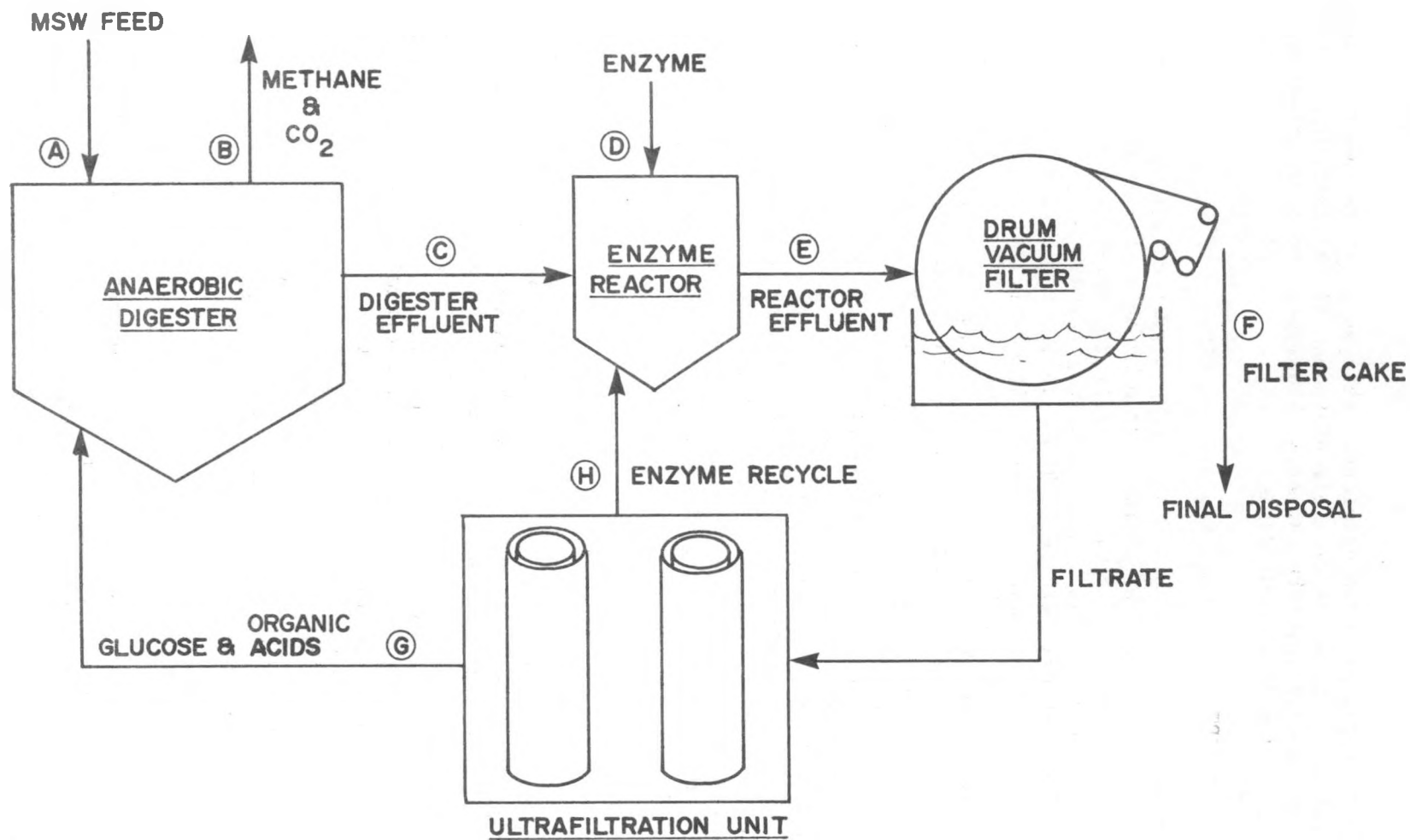


Figure 1. Process flow diagram.

degraded by bacterial proteolysis. In fact, cellulose hydrolysis rates are greatly enhanced in the presence of acetobacters due to reduction in product (glucose) inhibition effects. The only important loss of enzymes occurs during the recovery operation as losses in the filter cake solids. These losses can be minimized by various centrifugation techniques, extraction of the filter cake, or completion of cellulose destruction.

SECTION 2

UNIT OPERATIONS

In order to optimize the entire process for enhancement of energy recovery, the individual unit operations of enzyme treatment and enzyme recovery were studied individually, and each was optimized separately. These two unit operations were then combined with the final unit operation of biomethanation to allow an evaluation of the full process line.

ENZYME TREATMENT

Although cellulase enzyme products are available from several sources, the early and continued interest in this experiment shown by NOVO Laboratories, Inc., led to the use of their enzyme products throughout this study. Early comparisons of these products with those of other suppliers showed that the NOVO enzymes are at least equivalent to others, so no detriment to the experiments resulted from this choice of supplier. NOVO laboratories have determined that a pH of 4.8 and 50°C is optimum for the enzyme preparation with pure cellulose substrates in a sterile environment. In order to effect the most efficient hydrolysis of cellulose and to obtain the most methane at the least cost, the operating conditions that most greatly effect cellulose destruction in the enzyme reactor needed to be identified and then optimized. To provide some initial guidance for operation with MSW, the early enzyme loading and operating conditions were all performed on a substrate of Whatman Number 1 filter paper.

Enzyme Studies on Filter Paper in a Sterile Environment

In order to determine the most appropriate range of enzyme loading for subsequent study, a set of triplicated reactors was initiated at 1 percent, 2 percent, 4 percent, 8 percent, and 16 percent enzymes on a weight to weight enzyme-to-total-solids basis using a constant amount of Whatman No. 1 filter paper. The enzyme complex used was a nearly equal parts mixture of (1) a NOVO dry cellulase product (obtained from the fungus Trichoderma reesei), which had 400 Cellulase Units per gram as determined by NOVO on Whatman cellulose powder CC31 (written as 400 C₁U/g) and 4,772 Cellulase Units as determined on carboxymethyl cellulose (written as C_xU/g) (a soluble cellulose derivative) and (2) a liquid slurry of cellobiase (obtained from Aspergillus niger), which had 45 Cellobiase Units per gram (written as CBU/g).

A solution was prepared containing 4.0 grams of the cellulase and 4 ml of the cellobiase diluted to 100 ml with water. Appropriate proportions of this solution were added to 1 gram portions of Whatman No. 1 filter paper and diluted to 100 ml. After pH adjustment to 4.8 with an acetate buffer, these mixtures were nitrogen purged to inhibit the growth of fungus and incubated at

40°C in a dry environment. These mixtures were then allowed to react for 72 hours with little or no mechanical stirring. The solutions were filtered through preweighed glass fiber filter pads, dried, and weighed to obtain the weight loss of cellulose represented as "% Destruction" in Figure 2, and plotted versus enzyme concentration in NOVO C₁ units. These data suggested that a cellulase loading of 65 C₁U/g of Whatman No. 1 filter paper was close to optimal and that approximately 70 percent conversion of cellulose to glucose could be expected in 72 hours with an ideal cellulose substrate.

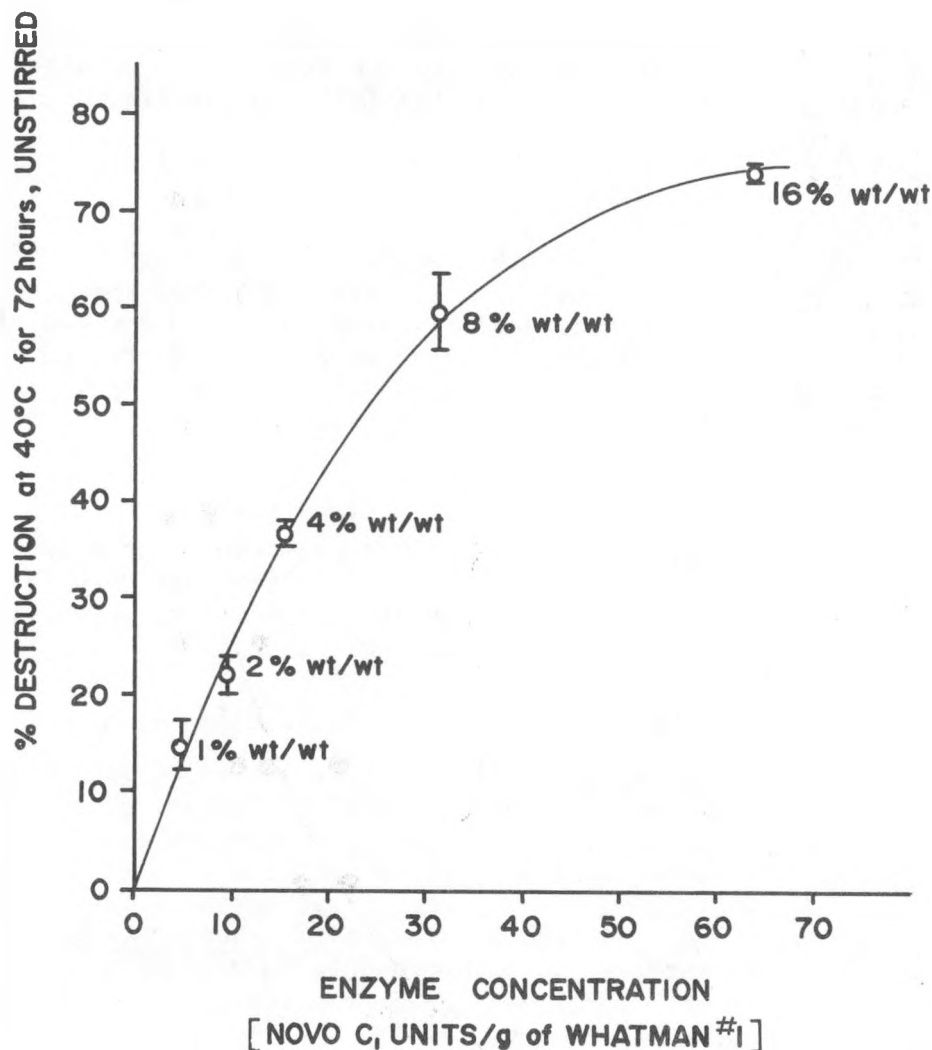


Figure 2. Destruction as a function of enzyme loading.

The shape of the filter-paper loading curve in Figure 2 suggests that some inhibitory process is taking place in the reaction. To substantiate this, a series of reactors was initiated with identical enzyme loading and identical paper substrates, but with varying amounts of glucose added to the reactors at initiation. The results of this study are plotted in Figure 3, and

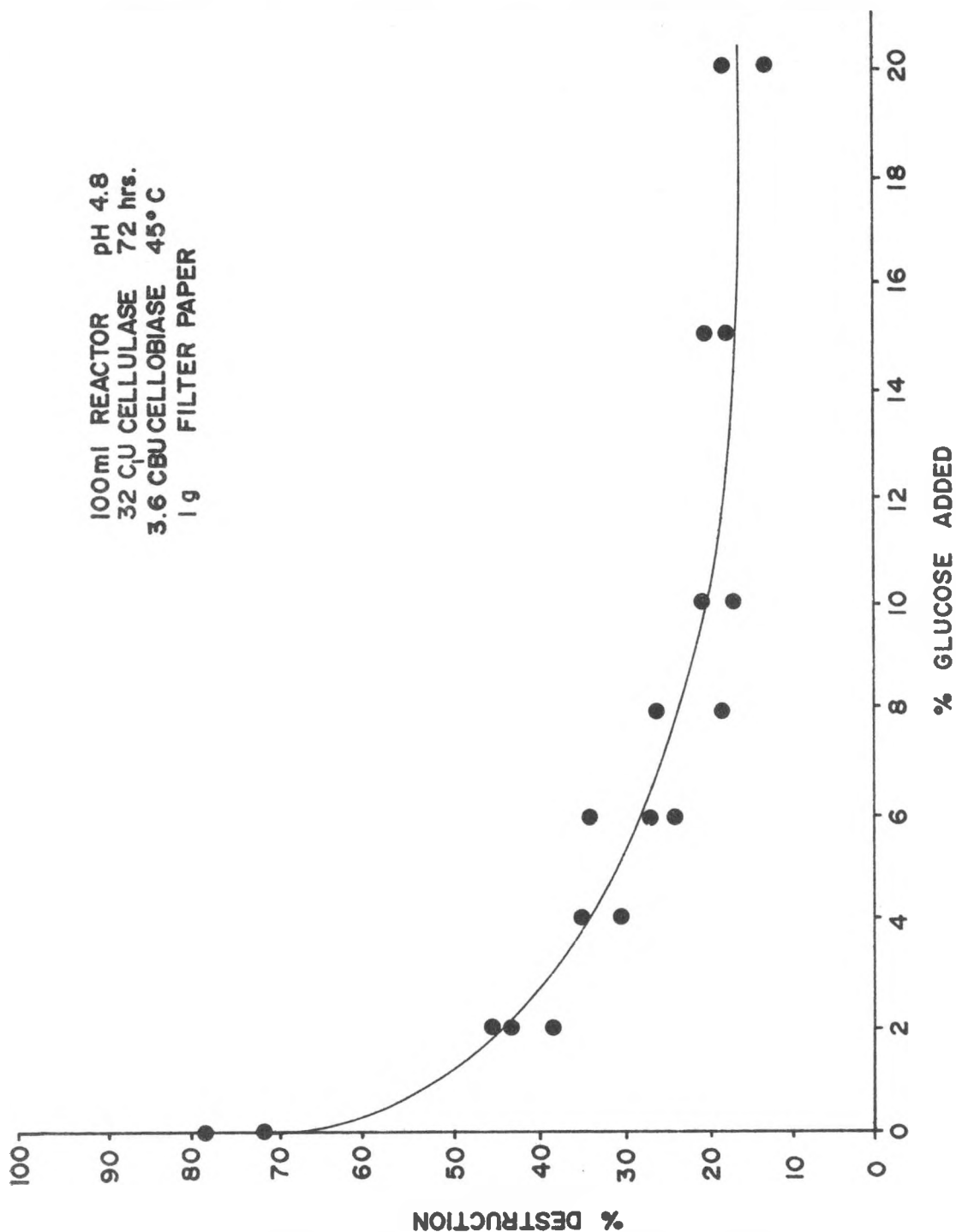


Figure 3. Glucose inhibition of cellulase activity.

indicate that a very low concentration of glucose has a very serious inhibitory effect upon the rate of cellulose conversion. It was noted that the amount of glucose contained in the dry cellulase product as received from NOVO is about 4 percent. The presence of this glucose could account for the fact that only a 75-percent destruction of filter paper was obtained even with no glucose spike.

MSW Substrate with Bacteria

Having completed these loading studies on filter paper, the next step involved repeating the experiments using municipal solid waste. In order to remove the effect of unknown variables, it was essential that accurately reproducible and identifiable "artificial" municipal solid waste be utilized in the digester test vessels. Although the actual composition of MSW is variable from site to site and day to day, a realistic version of United States MSW can be prepared from a standard mixture of organic and inorganic materials. Table 1 gives the composition of the organic fraction of the MSW based on data from several solid waste studies performed by SYSTECH Corporation.

TABLE 1. ARTIFICIAL MUNICIPAL SOLID WASTE
AS PREPARED FROM STANDARD MATERIALS^a

Item		Percent
Cellulosics	Newspaper	73
	Wood (Sawdust)	8
	Cloth	7
Proteinaceous Food Waste (Puppy Chow)		4
Oil		3
Sugar		2
Starch		2
Detergent		1

^a Composition is based on several solid waste studies performed by SYSTECH.

Since there is no cellulose assay whose results are meaningful in the presence of glucose-metabolizing bacteria (see Appendix A), some other method of assaying for hydrolysis was necessary. The effect of the enzyme on digested MSW was measured by suspended solids analysis; thus a loss in suspended solids represented the change of insoluble cellulose to soluble glucose. Since it was discovered that digested MSW could not be filtered (as called for in Standard Methods² for suspended solids analyses), a centrifugation procedure was used as per Vesilind's Treatment and Disposal of Wastewater Sludges.¹ In order to obtain accurate suspended solids analysis, the best method for sampling was to mix an amount of digester effluent in a blender for 2 seconds, take individual 50 ml aliquots for each enzyme experiment, and analyze each aliquot for suspended solids after the incubation period. For each experiment, cellulose destruction due to enzyme hydrolysis was calculated by subtracting the weights obtained for suspended solids with enzymes from the weights for blanks concurrently incubated but containing no enzymes. At the end of each incubation period, a "Stop Reagent" (0.11 molar dibasic phosphate buffer, pH 12.5) was added to effectively terminate further enzyme activity. This assay proved to be most reliable and was used throughout the enzyme studies as a measurement of the amount of insolubles destroyed.

Working with digested MSW rather than a sterile environment resulted in a need for a change of buffers. NOVO Laboratories reported that the enzyme had an optimum activity at pH between 4.6 and 5.2 in a sterile environment. This pH range was later substantiated in SYSTECH's laboratory tests for digested MSW as well. However, the acetate buffer recommended in the published procedures for sterile systems was being used as feedstock by the anaerobes from digested MSW that survived in the enzyme reactor. This resulted in the loss of buffer to the system and, therefore, a loss of pH control. A CRC Handbook of Chemistry and Physics reference to phthalate led to a series of tests with that material, which can buffer in the appropriate pH range for the enzyme reactor and, which being an aromatic, does not serve as feed for anaerobes. Laboratory tests subsequently determined that a 0.1 molar solution of potassium hydrogen phthalate (KHP) was sufficient to hold the pH at the enzyme's optimum without resorting to an initial higher ionic strength as would have been required with any "biodegradable" buffer.

The cellulose in filter paper is quite different from that found in the cellulosic component of MSW. The cellulose in MSW is cemented together with lignin, which makes the glucosidic bonds much harder to attack. In order to determine this slower rate of reaction and the efficiency of the enzyme's attack on this type of substrate, a study of the amount of cellulose destruction per unit of enzyme per weight of total solids (after digestion) was carried out using the suspended solids-cellulose destruction assay. All of the experiments were done at a mesophilic temperature of 40°C with the expectation that the viable acetobacter population could lessen the glucose inhibition of the enzyme by removing the glucose in situ. The results of these experiments are shown graphically in Figure 4 and indicate that at the asymptote a maximum of about 30 percent of the insolubles in digested MSW can be converted to soluble glucose in the 72-hour incubation period with a loading of 200 NOVO C₁U/g of total solids.

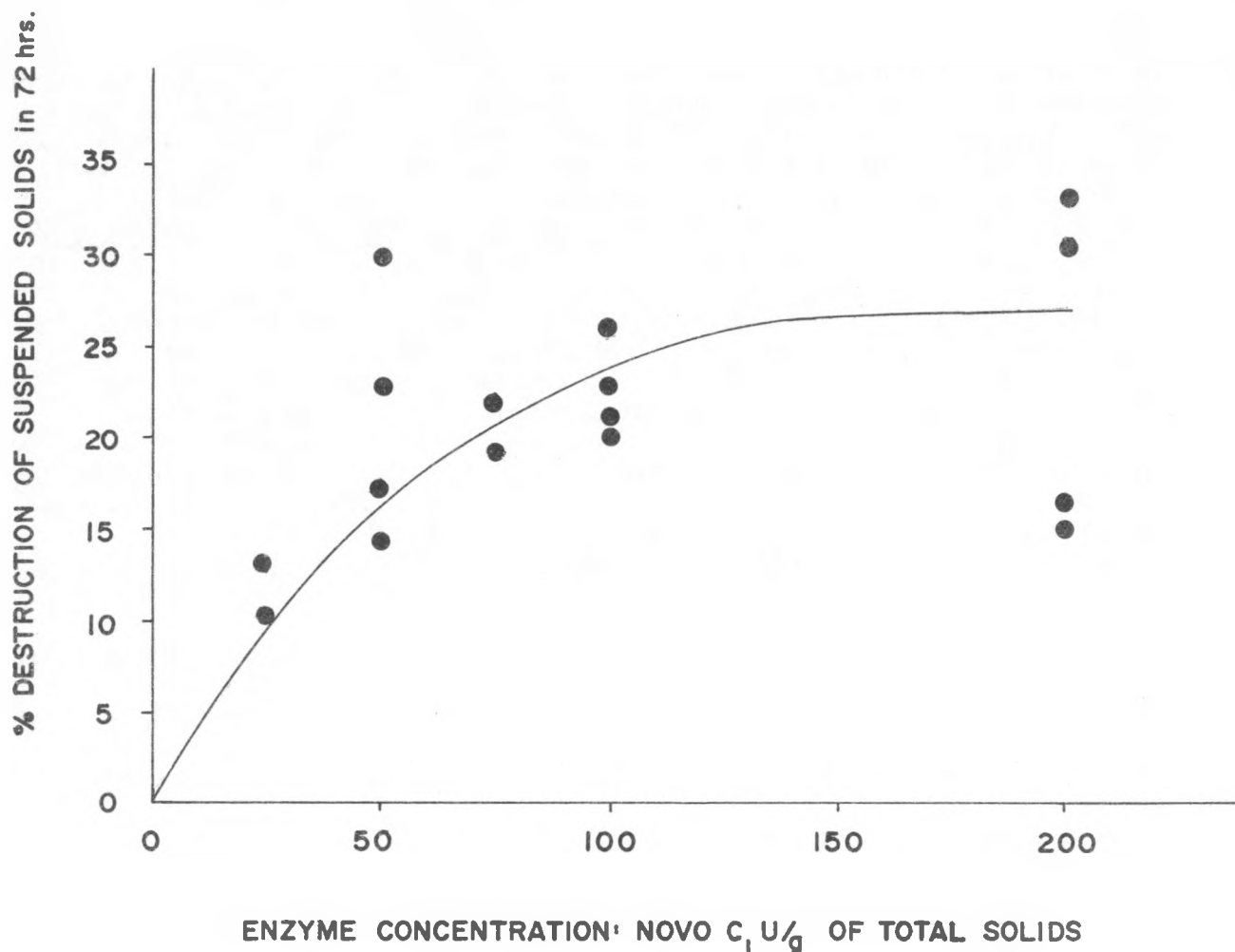


Figure 4. Enzyme-loading study on digested MSW.

The data presented in Figure 5, and all subsequent data, were generated using Celluclast® 100L, NOVO's commercial liquid preparation of cellulase. This enzyme contains 100 C₁U/g as determined by NOVO on Avicel® microcrystalline cellulose at 50°C with a 20-minute reaction time. Cellobiase 250L with 250 CBU/g was used in a 1:10 ratio by weight of Cellobiase 250L to Celluclast® 100L as recommended by NOVO.

From this same loading curve, a loading of 75 NOVO C₁U/g of total solids was chosen for a time study of suspended solids destruction for incubation periods of up to 24 hours to obtain comparison data with the 72-hour study. Figure 5 shows these results and demonstrates that the rate is nearly a straight line for about 18 hours. From these results, it would also seem that there is not much value in increasing the hydraulic retention time in the enzyme reactor beyond 72 hours.

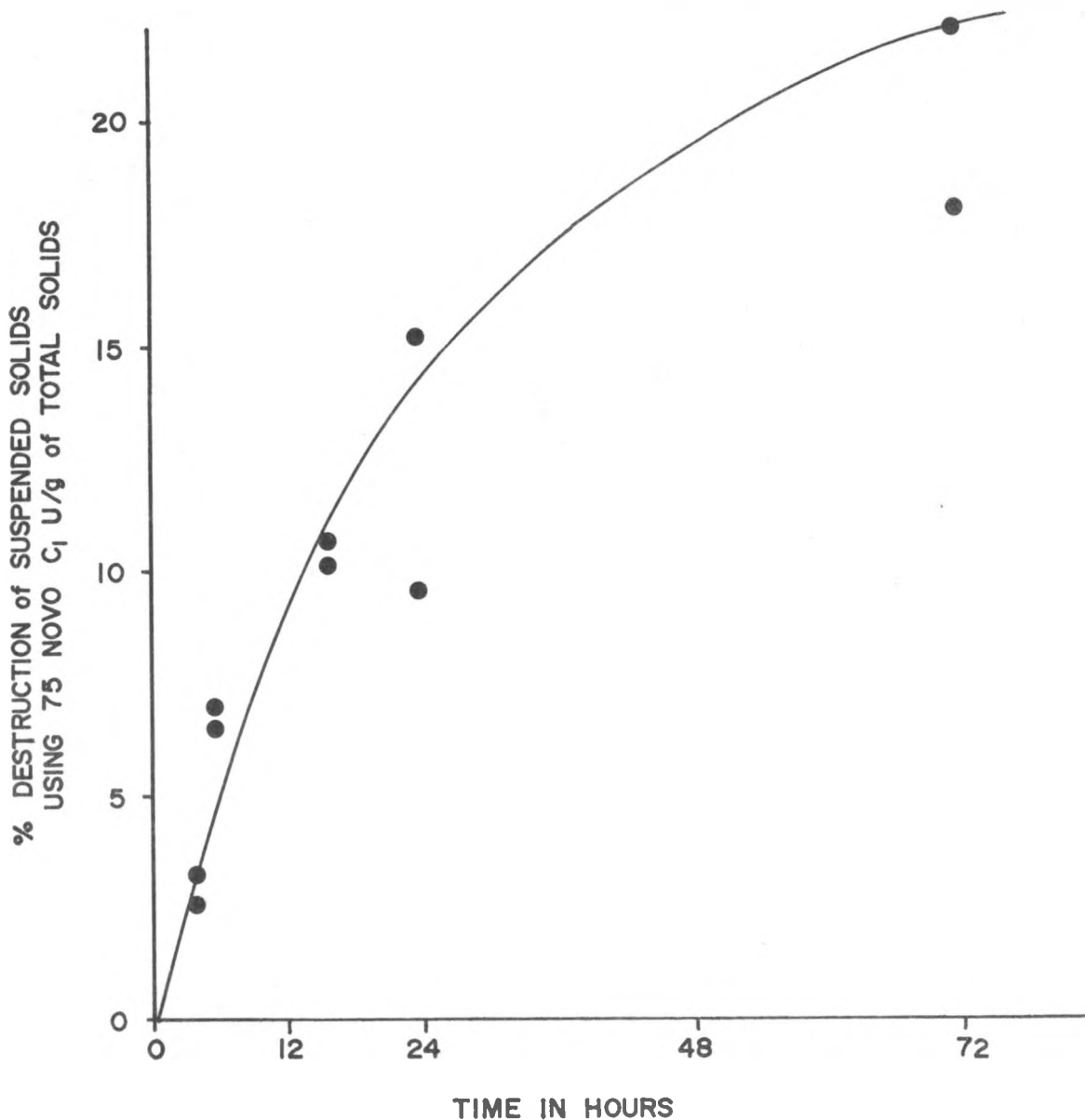


Figure 5. Enzyme time study on digested MSW.

It was suggested by NOVO Laboratories that an increase in reaction temperature to the optimum temperature as determined by the filter-paper tests might show a dramatic increase in the breakdown of cellulose with digested MSW. For comparison, two temperatures, 40°C and 50°C, respectively, were chosen as the acetobacter growth optimum and the enzyme's optimum for hydrolysis of filter paper. It was noted that 50°C would be quite low for thermophilic bacteria; however, above this limit the enzyme denatures very

quickly. The results of the temperature comparison is illustrated in Figure 6. These results show that a substantially greater destruction of suspended solids was found at the lower (40°C) temperature with two enzyme loadings. This phenomenon may be due to the lessened glucose inhibition resulting from glucose uptake by active bacteria. The lower temperature is more favorable to the proposed process as it is much less energy intensive.

Cellulose destruction could be considerably inhibited if the enzymes were affected by the attachment of certain metals to the disulfide bonds that then affect the tertiary structure (local conformation or shape) of the enzyme. Such disruption of disulfide bridges near the active (catalytic) site would distort the shape of the active site, thus inactivating it. If, however, the disulfides bridges were not close to the active site, the enzyme would still be functional.

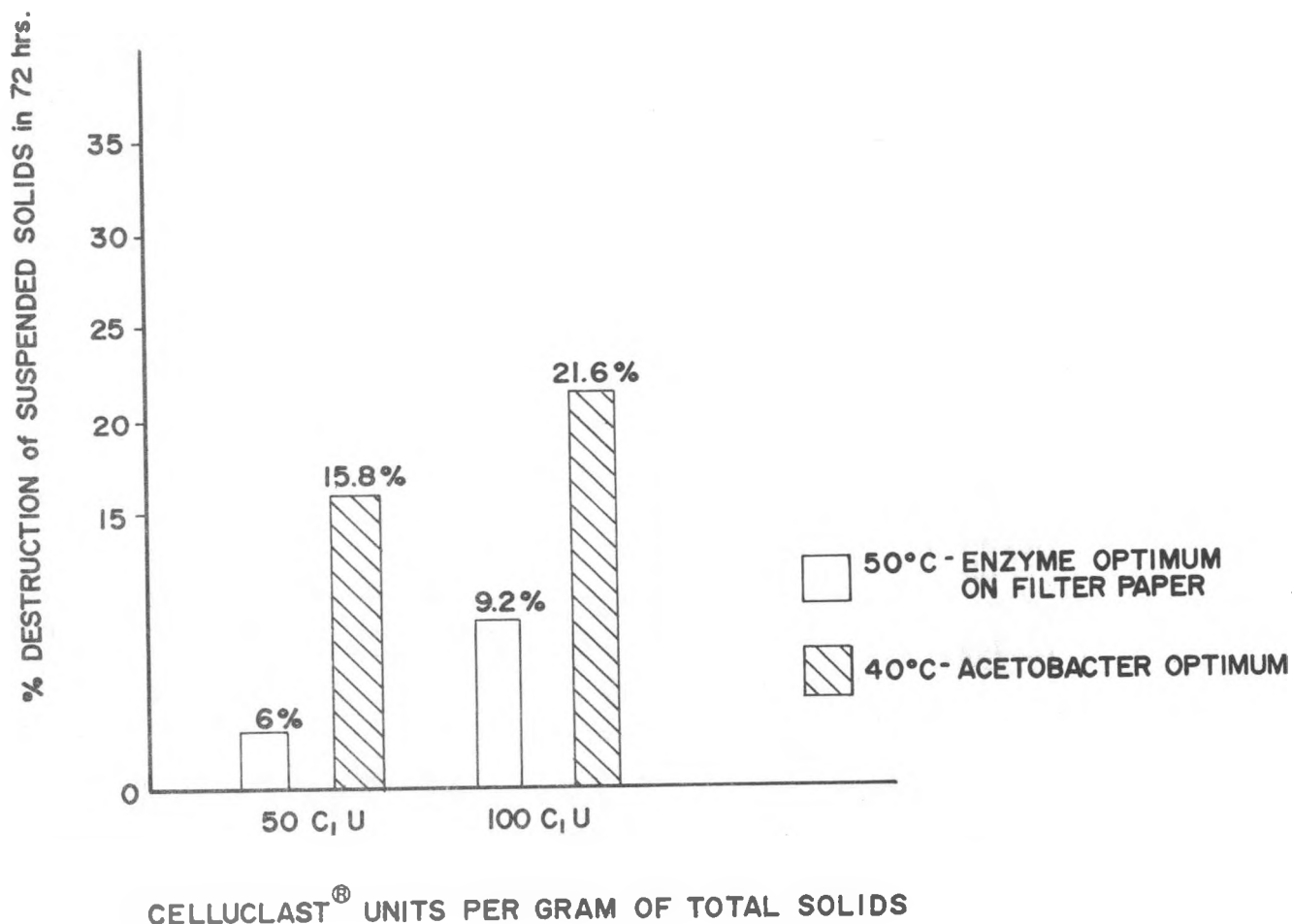


Figure 6. Temperature comparison of enzyme destruction of digested MSW.

In order to determine what the effect of metals usually found in digested MSW would be on the Celluclast® enzyme preparation, a study was completed using the concentrations shown in Table 2. This table lists the results of an atomic absorption analysis of the liquid effluent of a digester fed a 4 percent slurry of municipal solid waste. Reactions of 65 ml were set up with 2.0 grams of Whatman No. 1 filter paper as substrate, an enzyme loading of 25 NOVO C₁U/g of filter paper, and various concentrations of metals; these included no metals; the concentrations shown in Table 2; and two, five, and ten times the concentrations shown in Table 2. Blanks were also made up which had no enzyme but contained these concentrations of metals. All reaction flasks were purged with nitrogen to retard fungal growth, capped, and shaken in a Lab-Line® Orbit Environ-Shaker at 2 Hz with 2 cm displacement at 40°C for 24 hours. Since high pH would precipitate the metals from solution, no Stop Reagent was added at the end of the incubation period. Suspended solids data were then obtained using centrifugation, the results were graphed (Fig. 7). Figure 7 shows a slight depression of Celluclast® activity (about 3 percent out of 41 percent) with the 4-percent slurry concentration containing metals when compared to a slurry with no metals. Only with much higher concentrations of metals does the depression of activity become substantial. However, no digester could remain healthy at these high metal concentrations since the metal toxicity would have a severe detrimental effect on the bacterial population.³ Sufficient sulfide would have to be added to the MSW slurry to precipitate the heavy metals before they had a chance to upset the digester, and these insoluble metal salts could not inhibit the enzyme.

TABLE 2. METAL ANALYSIS OF 4 PERCENT MSW SLURRY

Metal	Concentration
Cadmium	0.05 mg/l
Lead	1.17 mg/l
Zinc	1.48 mg/l
Copper	0.23 mg/l
Nickel	0.91 mg/l
Iron	150 mg/l
Silver	0.10 mg/l
Manganese	30.1 mg/l

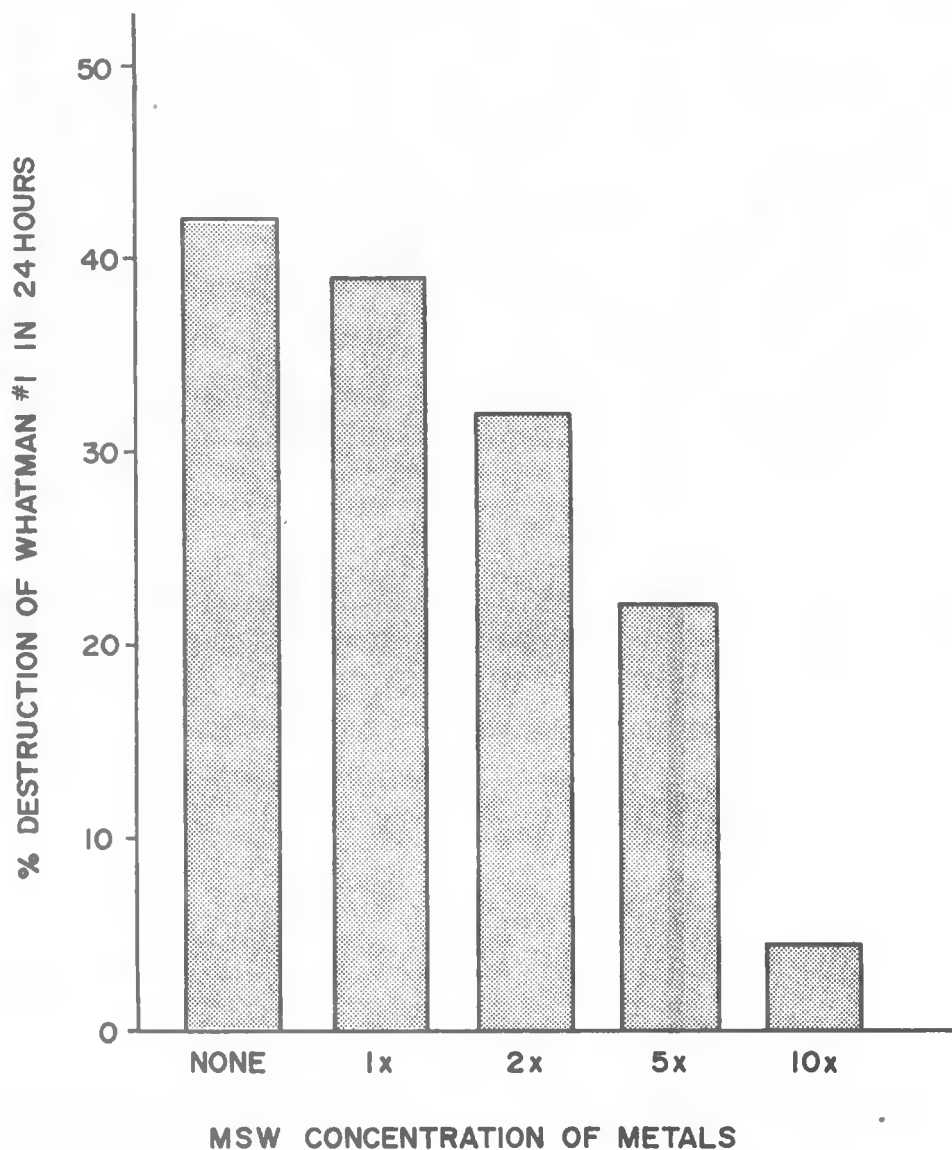


Figure 7. Effect of metals on cellulase activity.

Summary of Enzyme Treatment

Upon the completion of these enzyme treatment studies under various operating conditions, several optimum parameters were deduced. Due to product (glucose) inhibition, the enzymatic hydrolysis of cellulose is not directly proportional to enzyme concentration or incubation time, as evidenced by the curved functions obtained for the loading and time studies. At pH 5.0 using phthalate as a nonbiodegradable buffer with digested MSW, a loading of 75 NOVO C₁U/g of MSW total solids results in a maximum of 22 percent cellulose destruction in 72 hours. The substantially greater

destruction of suspended solids seen at 40°C rather than at 50°C may be due to lessened glucose inhibition resulting from glucose uptake by active mesophilic bacteria. The levels of metals normally found in MSW will not significantly affect the cellulase enzymes in the process.

ENZYME RECOVERY

Concept of Enzyme Recovery

Without the possibility of enzyme recovery, the high cost of enzymes precludes favorable economic predictions. However, the advent of ultrafiltration and reverse osmosis units as commercially available equipment allowed the consideration of the separation of the larger molecule enzymes from their hydrolysis product, the relatively small molecule sugars and sugar metabolites. Thus the captured enzymes could be reused for enzyme treatment and the sugar stream diverted to the anaerobic digester and subsequently converted to carbon dioxide (CO₂) and methane (CH₄).

Possible Enzyme Loss Mechanisms

Although ultrafiltration offered a means of recovering cellulase enzymes from an MSW slurry, there were concerns about several factors that could complicate or even preclude the effective retrieval of the enzymes. These factors follow.

1. Due to the extremely large size of enzyme molecules, shear forces generated in mixing the enzyme reactor, and especially in the ultrafiltration process, could deform and thus denature the enzymes.
2. If the enzyme possessed only a short half-life of activity, it might denature too readily to make recovery worthwhile.
3. Being themselves proteinaceous, the various enzymes in the cellulase complex might be attacked by extracellular proteolytic enzymes secreted by bacteria present in the MSW digester.
4. Preferential attachment of enzymes to the cellulose molecules would result in their loss when any unhydrolyzed cellulose was removed in the filter cake.

Shearing Effect

The possibility that shear forces would result in enzyme denaturation was examined at two points in the process line; during incubation in the enzyme reactor and during the ultrafiltration process for enzyme recycle. Experiments were conducted to examine the residual activity recoverable from buffered enzyme solutions incubated for 24 hours either in a stationary 40°C chamber or a shaking 40°C chamber agitated at 2 Hz with a 2-cm displacement. Results indicate a glucose production of 1322 ± 64 μ g glucose/ml from solutions loaded with 960 mg Celluclast® 100L and 240 mg Cellobiase 250L in 65 ml of 0.1 molar KHP buffer, pH 5.0, that were incubated in the shaker, and a glucose production of 1250 ± 38 μ g glucose/ml for solutions at the same enzyme loading incubated in the stationary incubator. Although agitation

of the sample during incubation does appear to increase the variability of the glucose production levels measured, there is no significant decrease in the activity of recovered enzyme brought about by subjecting the solution to shear forces during incubation.

The possibility of the operation of shear forces in the ultrafiltration process leading to inactivation or destruction of enzymes was also examined. Solutions containing Celluclast® and cellobiase in the concentration range covered by the standard curves (Appendix B) were prepared in 250 ml 0.1 molar KHP buffer, pH 5.0, and activity assays run on them without filtration. No significant difference was found between glucose production levels of comparable enzyme solutions, whether ultrafiltered or unfiltered. Figure 8 indicates that shear forces generated during ultrafiltration do not decrease the activity of recovered enzyme.

Short Half-Life

NOVO Laboratories reported extensive studies conducted in its laboratories on the question of the long-term stability of enzyme solutions.⁴ They found that dilute solutions of Celluclast® and cellobiase have a half life of from 10 days to 5 months at pH 5.0 and 50°C, depending on the particular activity being measured. Studies conducted by SYSTECH indicated that there is no measurable loss in activity up to 5 days at 40°C at pH 5.0.

Biodegradation

Data collected in the contractor's laboratory support the contention that the cellulase complex is not subject to bacterial degradation. Solutions containing 960 mg Celluclast® and 240 mg Cellobiase 250L, 30 ml 0.1 molar KHP buffer, pH 5.0, and either 35 ml digested MSW or 35 ml primary digested digested municipal sewage sludge were incubated for 72 hours at 40°C and then filtered through glass fiber filters. Activity assays were conducted on the filtrate of these solutions. Table 3 shows the results of these experiments as mean concentrations of glucose produced during the assay period. No significant decrease in activity was noted in enzyme incubated with sewage sludge, indicating that the microbes present in this mixtures, which are essentially the same organisms as those in the process line with digested MSW, did not inactivate or metabolize the cellulase enzymes. Additional work conducted in this study, to be detailed later, indicates that, at high loading levels, enzyme is recoverable from digested MSW at essentially 100 percent efficiency, offering further support for the absence of bacterial degradation of enzymes.

To further test for the possibility of biodegradation, an enzyme reactor was set up; it consisted of a 2-l Erlenmeyer flask having a stopper with a gas exit line and a septum for obtaining samples for gas chromatographic analysis. The reactor was fed with effluent from the 60-l laboratory anaerobic digester. Gas evolved from the enzyme reactor was collected over water allowing measurement of the displaced water. The enzyme reactor was placed on a hot-plate magnetic stirrer and kept at a constant temperature. These experiments were performed at 40°C, a temperature that is quite compatible with the mesophilic bacteria found in the digester and at which the enzyme is still active, although this temperature is substantially below the optimum temperature of 50°C. Continued emission of CO₂ was used as an indicator that the acid formers were alive and functioning.

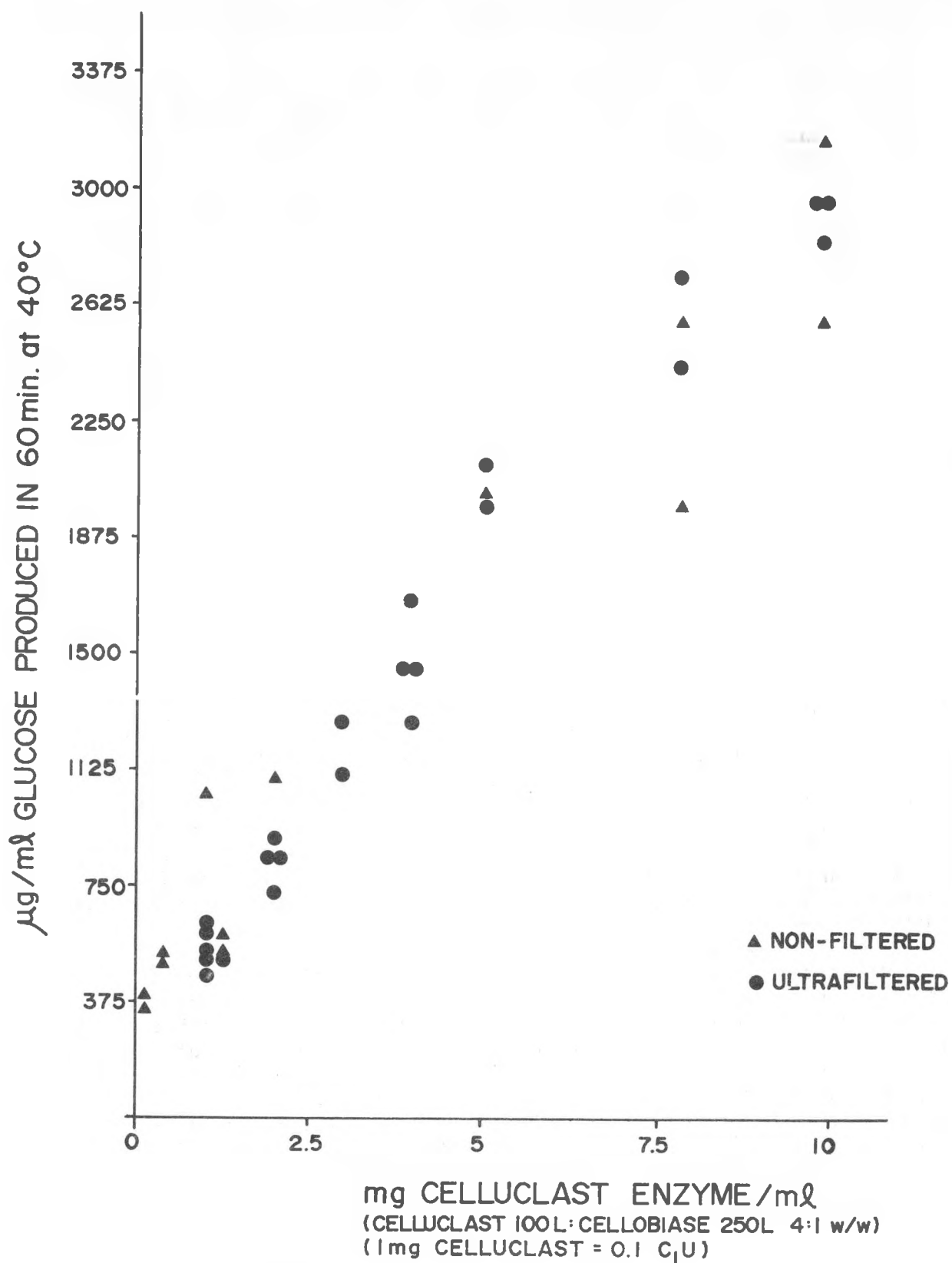


Figure 8. Shearing in ultrafilter.

TABLE 3. ENZYME ACTIVITY RECOVERED FROM
PRIMARY DIGESTED SLUDGE^a

Activity recovered	µg Glucose/ml
Theoretical (buffer)	966 ± 77
Actual (sludge)	940 ± 28

^a After incubation for 72 hours at 40°C, pH 5.0.

Using a sample of 1.0 l of digester effluent, which contained about 2.5 percent total solids, the graphs in Figure 9 show that, with 80 NOVO C₁U/g of total solids, 800 ml of water was displaced in 72 hours. (This 800 ml does not take into account the additional volume of CO₂ dissolved by the displacement water.) A gas chromatographic analysis of the flask atmosphere gave a 50 percent composition of CO₂ (and 50 percent residual nitrogen purgative), and illustrated that the acetobacters can actively produce CO₂ at the enzyme's optimum pH of 5.0. Although no methane was detected from this system at this pH, these results show that the enzyme and the bacteria culture can coexist to some extent (as evidenced by the CO₂ evolution).

Preferential Attachment and Loss in Filter Cake

In order to determine if the cellulase enzyme forms a stable enzyme substrate complex, thus precluding retrieval from unhydrolyzed cellulose in the filter cake, activity assays were conducted in conjunction with the 72-hour enzyme-loading study described in Figure 4. Assays were also performed on (1) ultrafilter concentrates from enzyme reactors at comparable enzyme loadings that had been incubated for 1 hour and (2) on concentrates from reactors that were ultrafiltered immediately after loading with no incubation. The latter test was performed to provide a basis for comparison. The enzyme reactor contents were filtered through glass fiber, rinsed, and the filtrates run through the ultrafiltration unit to concentrate the enzyme stream. The volumes of the concentrates were adjusted to provide a known concentration of enzyme to be expected if 100 percent recovery was obtained. Reducing sugar determinations made during activity assays were converted to percent recovery of enzyme values by application of the equations noted in Appendix C. The results of these analyses are shown in Figure 10, where percent recovery of enzyme is plotted against milligrams Celluclast® loaded. (It should be noted that C₁U are directly converted to milligrams of Celluclast® enzyme by multiplying by 10.) The 75 to 80 percent loss of enzyme activity after 72 hours incubation with digested MSW was originally thought to be wholly due to the preferential attachment of the enzyme to its substrate. However, these low recovery values are at least partly due to other phenomena (see Section 2.2.3).

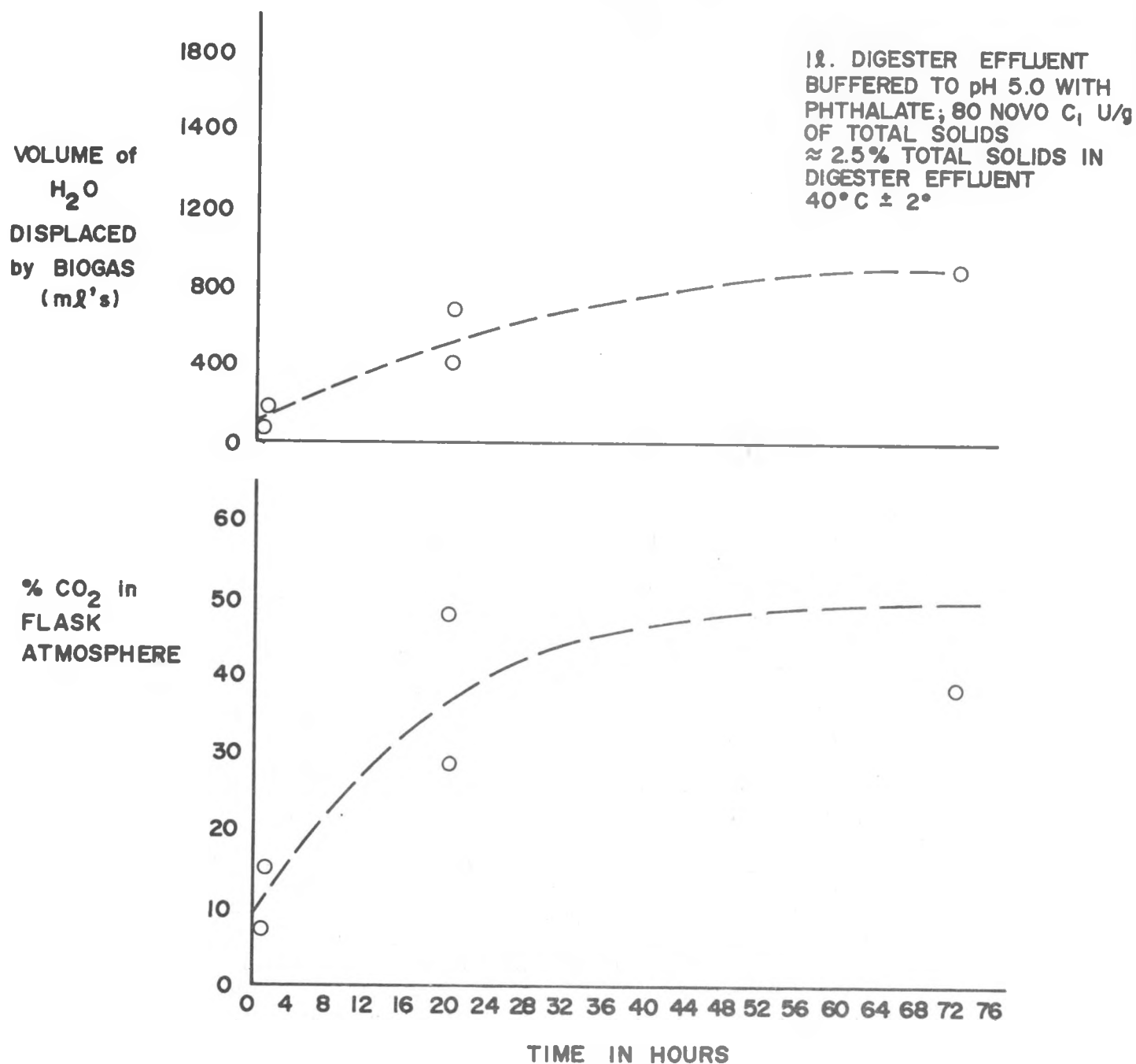


Figure 9. Carbon dioxide generation in enzyme reactor.

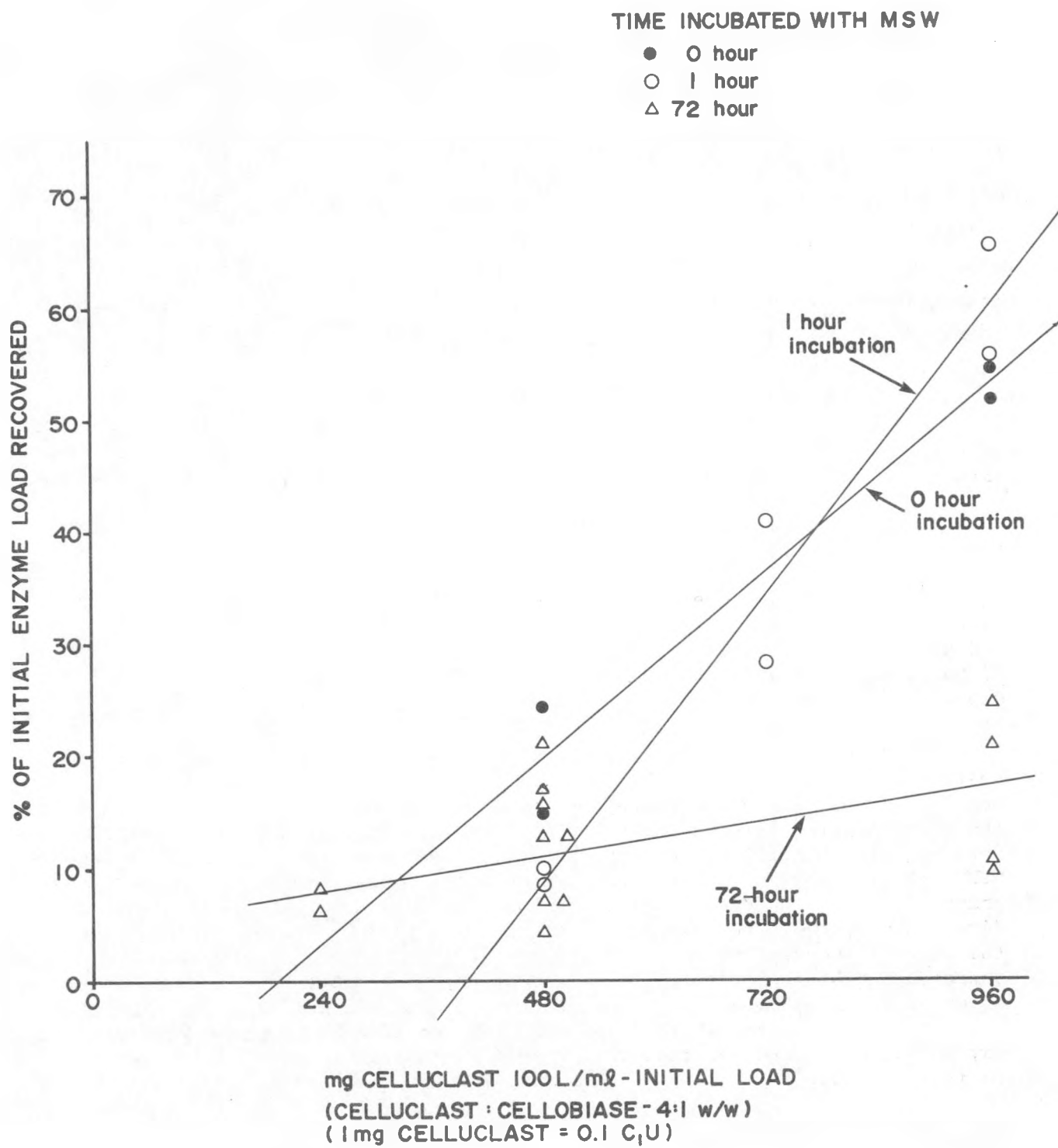


Figure 10. Recovery of enzyme from digested MSW.

A number of experiments were conducted to examine the mechanism of activity loss in the preliminary filtration step. Figure 11 shows the results of the 1-hour incubation study of enzyme and digested MSW reported in Figure 10, replotted as percent recovery of enzyme versus mg Celluclast® per gram of digested MSW total solids. It is apparent that this relationship is linear, and extrapolation to 0 percent recovery indicates that a quantity of 352 mg Celluclast®/g digested MSW total solids would be irretrievably lost in a 1-hour incubation study followed by filtration and ultrafiltration for enzyme recovery.

Table 4 shows the application of this value for irretrievable loss as a correction factor to a number of 1- and 72-hour incubation studies. When the recoveries of enzyme from digested MSW after 1-hour incubation are also corrected for the volume loss in filtration, a mean of 96.1 percent of the initial enzyme load is accounted for. These data indicate that a constant amount of enzyme is apparently lost, or not measured, in the loading range indicated for a 1-hour study. At 72 hours, similar correction accounts for 74.9 percent of the enzyme load. Therefore, at 72 hours, a different mechanism for enzyme loss is apparently operative.

The enzyme loss observed at 1 hour was further examined by investigating the effect of filtration parameters on recovery. Enzyme solutions were made up in 0.1 molar KHP buffer, pH 5.0, at 3.84 mg Celluclast® and 0.96 mg cellobiase/ml and filtered through three filter media: a glass fiber filter, a glass fiber filter on which digested MSW had been deposited, and a glass fiber filter on which digested MSW previously incubated for 72 hours with enzyme at the same loading as the solutions noted had been deposited.

Table 5 shows that approximately 50 percent of the enzyme activity is lost by drawing the enzyme through an MSW cake. The table also shows the results of enzyme recovery experiments (after 72 hours of incubation) in which the enzymes were filtered through different size filters and therefore different depths of filter cake deposited from the same initial weight of digested MSW. There is obviously considerable potential loss involved in passing the enzyme solution through an MSW cake, but this loss does not appear to be a direct function of the cake itself acting as a size exclusion filter since the overall recovery of enzyme does not increase as the filter-cake area increases. The fact that 30 percent more enzyme is recovered from an enzyme solution passed through digested MSW previously subjected to enzyme hydrolysis than from unhydrolyzed MSW is also significant; this increase in recovery may be attributed to the fact that enzyme is indeed preferentially adsorbed to its substrate.

Recoverability Optimization

The actual origin of the enzyme loss during recovery was found to be due to several mechanisms, some real and some due to shortcomings in the assay.

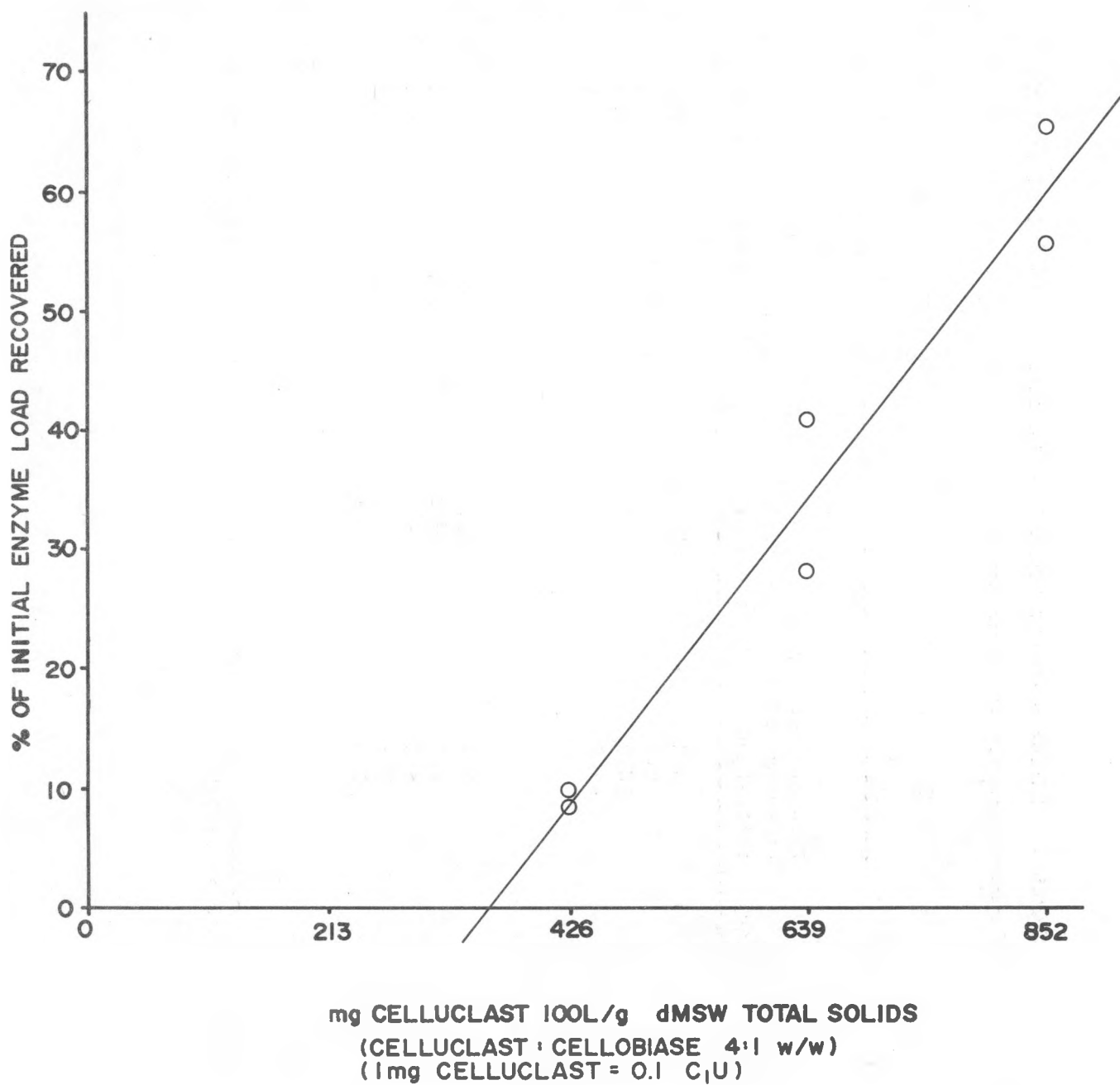


Figure 11. Recovery of enzyme from digested MSW incubated 1 hour at 40°C.

TABLE 4. ENZYME RECOVERY CORRECTED FOR IRRETRIEVABLY LOST ENZYME

	Column				
	A	B	C	D	E
Initial enzyme load (E/g MSW total solids)	Percent recovery of enzyme after filtration	A + 352 mgE/gMSW as percent recovery	Percent loss due to volume lost in filter cake	A + C	B + C
426 mg	8.5	91.0	19.0	27.5	110.0
639 mg	8.1	63.0	22.2	30.3	85.2
852 mg	18.5	59.5	20.9	39.4	80.4
1147 mg	44.0	76.0	32.6	76.6	108.6
Mean values (1-hr incubation)		$\bar{x} = 72.4 \pm 14.3$		$\bar{x} = 96.1 \pm 15.4$	
460 mg	5.3	82.0	6.7	12.0	88.7
	1.8	67.5	20.0	21.8	87.5
690 mg	8.0	59.0	6.5	14.5	65.5
920 mg	9.1	47.5	8.1	17.2	55.6
1106 mg	27.0	64.6	12.5	39.5	77.1
Mean values (72-hr incubation)		$\bar{x} = 64.1 \pm 12.6$		$\bar{x} = 74.9 \pm 14.3$	

Note: 1 mg Celluclast® = 0.1 C₁U

TABLE 5. RECOVERY OF ENZYME AFTER FILTRATION^a

Filter media	Column		A + B
	A	B	
	Percent recovery	Percent volume loss	
Glass fiber	98	0	98
Glass fiber plus dMSW	40	10	50
Glass fiber plus pre-enzyme digested MSW	52	1	53
28.3 cm ² filter glass fiber	11.2	49.0	60.2
70.9 cm ² filter glass fiber	16.8	6.5	23.3
122.7 cm ² filter glass fiber	15.1	3.0	18.1

^a All enzyme concentrations were 3.84 mg Celluclast®:0.96 mg cellobiase/ml (1 mg Celluclast® = 0.1 C₁U)

Phenomena that were found to interfere with the accuracy of the enzyme assay are discussed in Appendix B.

Notwithstanding the apparent loss of enzyme due to bacterial consumption of glucose in the assay (approximately 15 percent of the initial load; see Appendix D), there still remains an appreciable loss of enzyme, approximately 50 percent of the initial load, that can be attributed to the filtration process. This problem was addressed by an experiment designed to increase the recovery of enzyme from digested MSW through repeated extractions of the solids cake. Enzyme reactors loaded with 1097 mg Celluclast® and 274 mg cellobiase/g of MSW total solids were incubated for 1 hour at 40°C. The reactors were then filtered through glass fiber and the filtrate collected; the solids cake was resuspended in KHP buffer. The cake was extracted by shaking for 10 minutes on a shaker table and then separated by glass fiber filtration. This extraction process was repeated a total of three times.

Activity assays were conducted on each liquid fraction to determine enzyme recovery. Table 6 shows the percent recovery of enzyme at each extraction step and the cumulative amount at each step. Also reported are partition coefficients for each extraction step. It is apparent that one extraction gives a significant increase in recovery (75 percent increase) over a simple one-step filtration process. But under the conditions employed, further extractions did not yield the same distribution of enzyme between solids cake and liquid. Additional extraction studies are necessary to determine whether enzyme can be desorbed from digested MSW, as has been reported by Wilke and Mitra for newsprint.⁵

Recovery by Centrifugation Techniques

In order to circumvent the loss of activity observed by filtering enzyme through MSW, the recovery of enzyme by centrifugation techniques was examined. Enzyme reactors were loaded at 286 mg Celluclast® and 72 mg cellobiase/g MSW total solids and incubated for 1 hour in the shaking incubator at 40°C. The remaining solids were removed by centrifugation and activity assays run on the supernatants. Table 7 shows the centrifugation parameters and the percent recovery values obtained. Within the range examined, the amount of centrifugal force applied did not significantly affect the recovery of enzyme, with a mean of 23.1 percent recovery by centrifugation. By comparison, recovery assays conducted by glass fiber filtration of reactors loaded with enzyme at the same concentration gave recoveries of 15.9 percent. Assays of enzyme alone in buffer showed activities at 100 percent of theoretical when centrifuged for 1 hour at 2110 × gravity.

TABLE 6. RECOVERY OF ENZYME FROM DIGESTED MSW
BY MULTIPLE EXTRACTIONS

Extraction Number	Percent recovery of initial loading	Percent recovery of remaining loading	Σ percent recovery of initial loading	Partition coefficients
Initial filtrate	44	--	44	0.7815
1	11	20	55	0.1954
2	1	2.8	56	0.0178
3	0.3	0.6	56.3	0.0053

TABLE 7. ENZYME RECOVERY BY CENTRIFUGATION TECHNIQUES

Relative centripetal force (\times gravity)	Percent recovery of enzyme
21	24
338	20
1034	24.5
2110	24

It is unlikely that enzyme recovery optimization by extraction procedures is ultimately limited by the fact that enzyme is preferentially adsorbed to its substrate. This concept, which has been proposed by a number of workers in cellulose chemistry, offers an explanation for the low recoveries observed after 72 hours incubation if hydrolysis is not complete. For example, Huang, Wilke, and coworkers found that only 35 to 50 percent of the initial enzyme load can be found in suspension above newsprint that has been hydrolyzed to between 50 and 100 percent of completion.⁵⁻⁷ Since work to date has achieved only a 30-percent destruction of suspended solids in 72 hours at best, it is possible that the remaining nonhydrolyzed cellulose in the enzyme reactors prevents achieving complete recovery of enzyme.

In order to test this hypothesis, a series of experiments were run in which the concentration of enzyme was increased and a given amount of digested MSW maintained. Incubation was continued for 72 hours at 40°C, and activities were run on the filtrates of the reactors. Figure 12 shows the results of these experiments as percent recovery of enzyme versus enzyme concentration. From the figure, it is apparent that, at high enzyme loading levels, where theoretically all of the cellulose present should be destroyed, enzyme recovery approaches 100 percent.

At these high levels, glucose production in the enzyme reactors was examined as an indicator of cellulose destruction. Figure 13 shows the net amount of glucose remaining in the enzyme reactors at the end of 72 hours; this value approaches 0.5 percent glucose and then rapidly falls to zero as the enzyme loading level increases. From these data, it can be deduced that enzyme production of glucose is shut off when glucose reaches a level of 1 percent in the reactor, and that the bacteria present then begin to remove the glucose buildup. Since the reactors loaded at the highest level have the greatest initial concentration of glucose, they approach 1 percent glucose rapidly, and at the end of 72 hours little or no net production remains. The failure to achieve greater than 30 percent destruction of digested MSW suspended solids may be explained by glucose buildup and subsequent inhibition

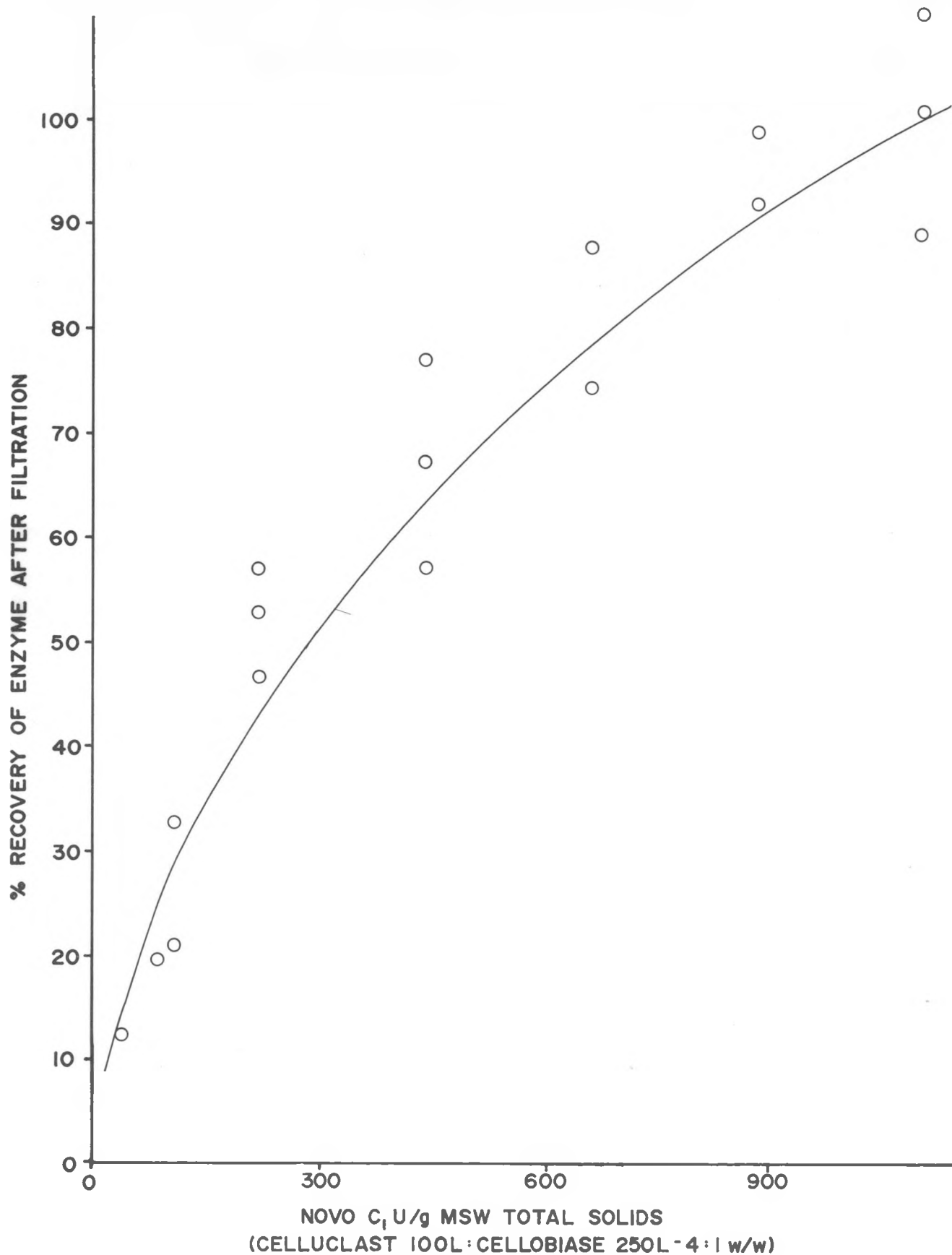


Figure 12. Enzyme recovery at high enzyme:substrate loading levels.

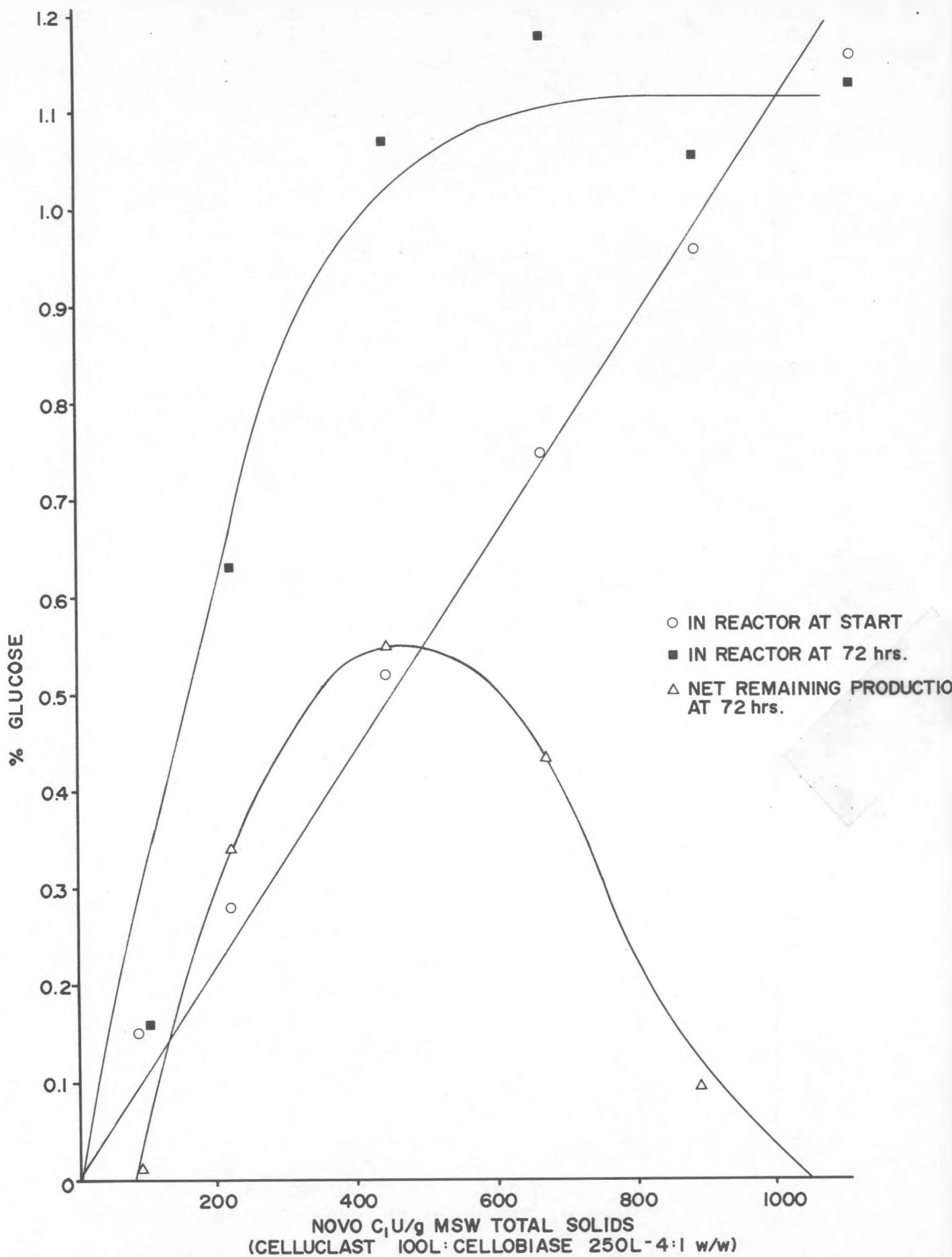


Figure 13. Glucose concentration in enzyme reactors.

of enzyme activity effectively turning off cellulose destruction before it reaches completion.

Summary of Enzyme Recoverability

Experiments conducted to date are considered promising for ultimately maximizing the amount of enzyme retrievable from digested MSW. The amount of retrievable enzyme can be increased by continued examination of three problem areas: the establishment of an assay not sensitive to bacterial uptake of glucose; maximization of cellulose destruction by glucose removal (thereby eliminating any potential adherence of enzyme to substrate; and development of more effective filtration and extraction methods for recovery of enzyme from digested MSW.

LABORATORY-SCALE ENERGY RECOVERY PROCESS

Figure 14 is a diagrammatic presentation of the laboratory-scale cellulase enzyme posttreatment process. The first digester vessel indicated was a 60- ℓ laboratory digester set up with a very controlled "artificial" diet; mixing was provided by a reciprocating agitator. Feeding and sampling were accomplished through a 3-in.-diameter pipe attached to the vessel's side, below the level of the liquid. The gas-tight lid was broached by a gas exit line, a thermocouple, and two leads for the immersion heater, all of which were sealed and gasketed. Vessel temperature was maintained in the mesophilic range appropriate for the anaerobes.

To assess the energy recovery process, a portion of the digester effluent was passed into the enzyme reactor. This consisted of a 2- ℓ stirred Erlenmeyer flask that was incubated at a constant temperature of 40°C. A gas trap was placed on top of the flask to allow any carbon dioxide to be released, while keeping the reactor oxygen-free. Enzyme load and retention time were chosen as 75 C₁U/g of dMSW total solids and 3 days for the 3-percent study and 50 C₁U/g of dMSW total solids and 3 days for the 5-percent study. These levels were selected from enzyme treatment studies previously discussed.

The reactor effluent was passed through a polypropylene filter with the captured solids or filter cake sent to disposal. The filtrate was then passed through an Amicon ultrafiltration unit with a membrane designed to have a capture cutoff of 5,000 molecular weight. In this system, the unit traps the enzyme (on the order of 45,000 to 70,000 molecular weight) and allows glucose and other nutrients to pass.

The glucose stream was then passed into a second digester vessel to measure the amount of additional methane obtained as a result of cellulose hydrolysis. (In a full-scale process, the glucose stream would be fed back into the first digester.) The second digester was a 6- ℓ polyethylene vessel kept at a constant temperature of 37°C in a circulating water bath. A feed/sample tube extending below the level of the liquid had been installed through the gas-tight lid. A sealed and gasketed gas exit line also penetrated the lid.

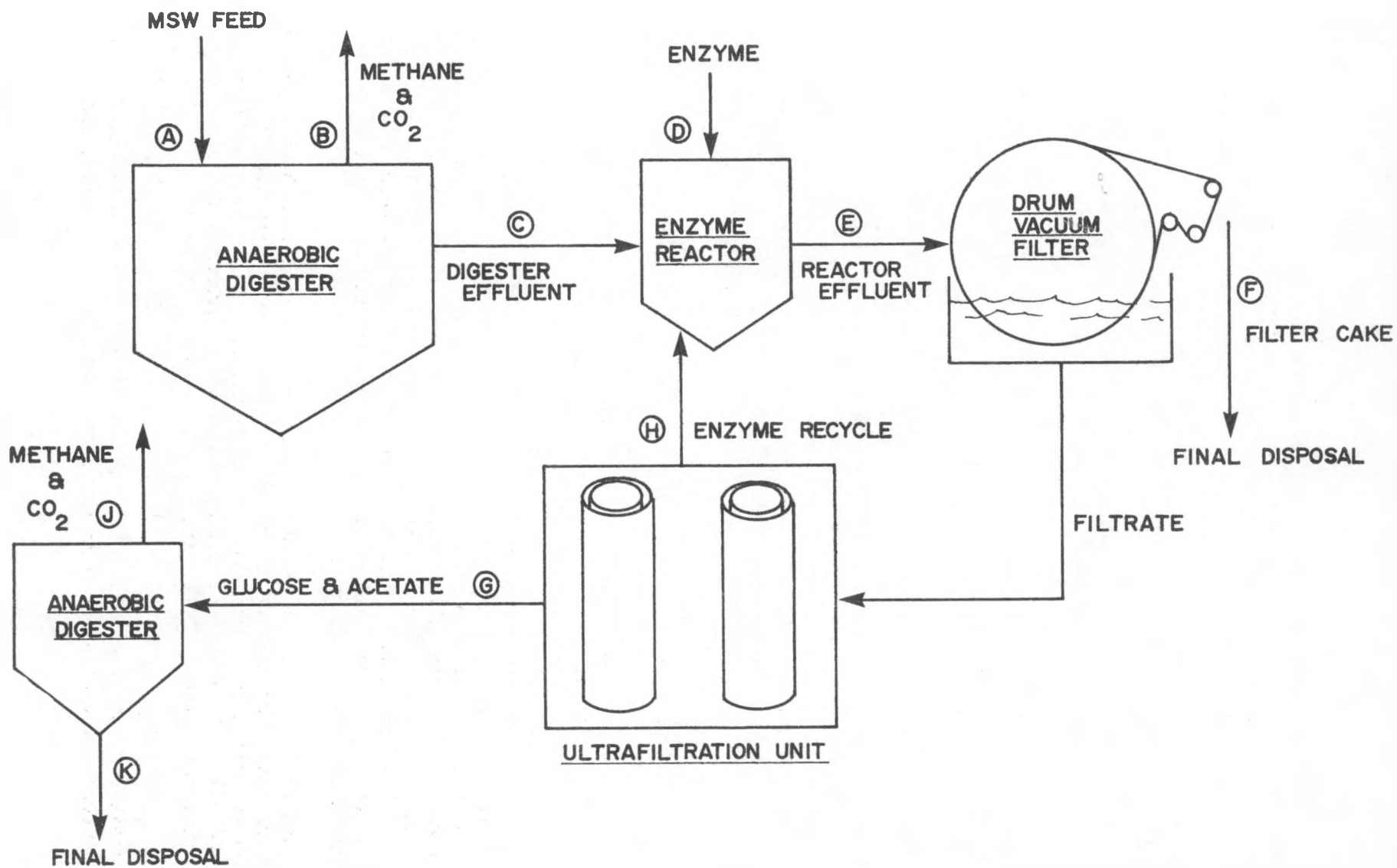


Figure 14. Laboratory-scale process flow diagram.

Two conditions were chosen for an extensive testing of the process. These test conditions are given in terms of the operating conditions of the initial MSW digester. The first condition consisted of a 3-percent total solids feed with a 15-day retention time. The second test condition chosen was a 5-percent total solids feed and 15-day retention time.

Three-Percent MSW Slurry

Following establishment of healthy digester operation, the testing program ran for 15 days, from January 30 through February 13 including weekends. On February 7, the outside temperature was -14°C . It was surmised that the heat pump at SYSTECH could not keep up with the change, causing a drop in temperature in the lab. On day 10, February 8, both digesters started on a downward trend. A temperature change of 2 or 3° can be sufficient to disturb the delicate balance between acid and methane formers.⁸

The general conditions for both digesters are shown on Figures 15 and 16. The optimum conditions shown for alkalinity and volatile acids are for sludge digesters. In a pilot-scale MSW digester study performed by SYSTECH, the measured alkalinity and volatile acids obtained for digested MSW were similar to those for sludge.⁹ However, in laboratory-scale garbage digesters the range has been found to be much lower.¹⁰ The volatile-acid-to-alkalinity ratio, which is the number generally used (along with pH) to determine the health of the digester, should be maintained below 0.5 for good digester operation.⁸

As the figures show, Digester I was in good operating condition until day 10--the day after the severe temperature drop; Digester II never achieved optimum conditions due to a buffer problem. From January 15 through January 22, sodium bicarbonate was used to buffer each day's feed to a pH of 7. The alkalinity was five times too high due to this buffering. From January 23 on, each day's feed was titrated with NaOH to pH 7.

Figure 17 shows the amount of biogas production from each digester as well as the total biogas given off. Digester II used only a portion of the effluent from Digester I, so a multiplication factor was needed to determine the actual amount of biogas that would have been obtained if all the effluent had been used. Digester I has an average biogas production of 19.0% per day and Digester II averaged 7.3 % per day. By using the enzyme reactor and second digester, an average of 38 percent more biogas production than that observed for Digester I alone was obtained.

Figure 18 indicates the methane production obtained from both digesters and the total amount of methane given off. Again, a multiplication factor was used to convert the amount obtained from Digester II. Digester I averaged methane production of 11.2 % per day and Digester II averaged 2.3 % per day. Thus, by using the enzyme reactor and second digester, an average of 21 percent more methane than that observed in Digester I alone was obtained.

Glucose concentration levels in Digester I and II are shown in figure 19. The mean concentration of glucose in Digester I was $148 \pm 42 \mu\text{g/ml}$; and

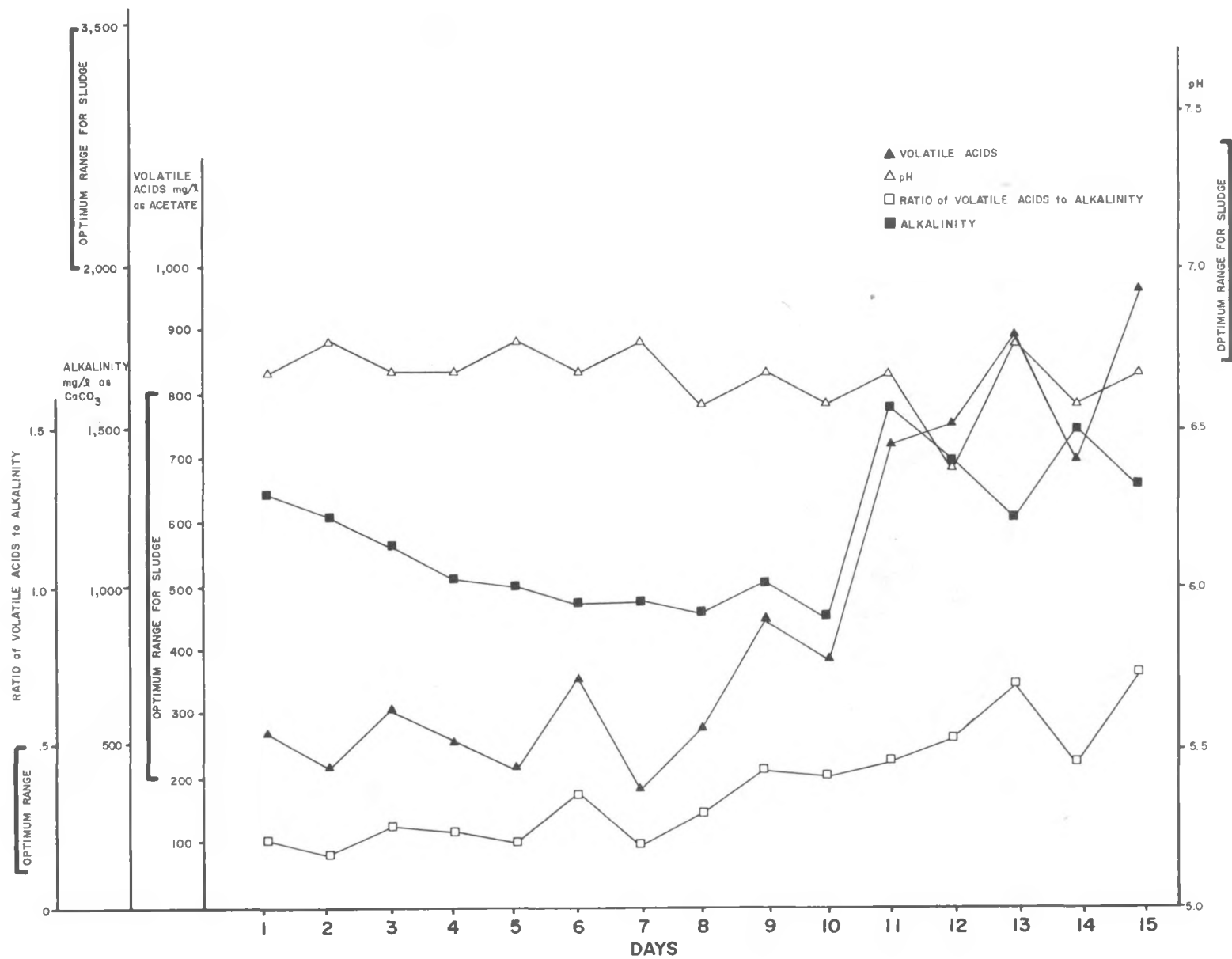


Figure 15. Digester I: general operating conditions, 3-percent solids.

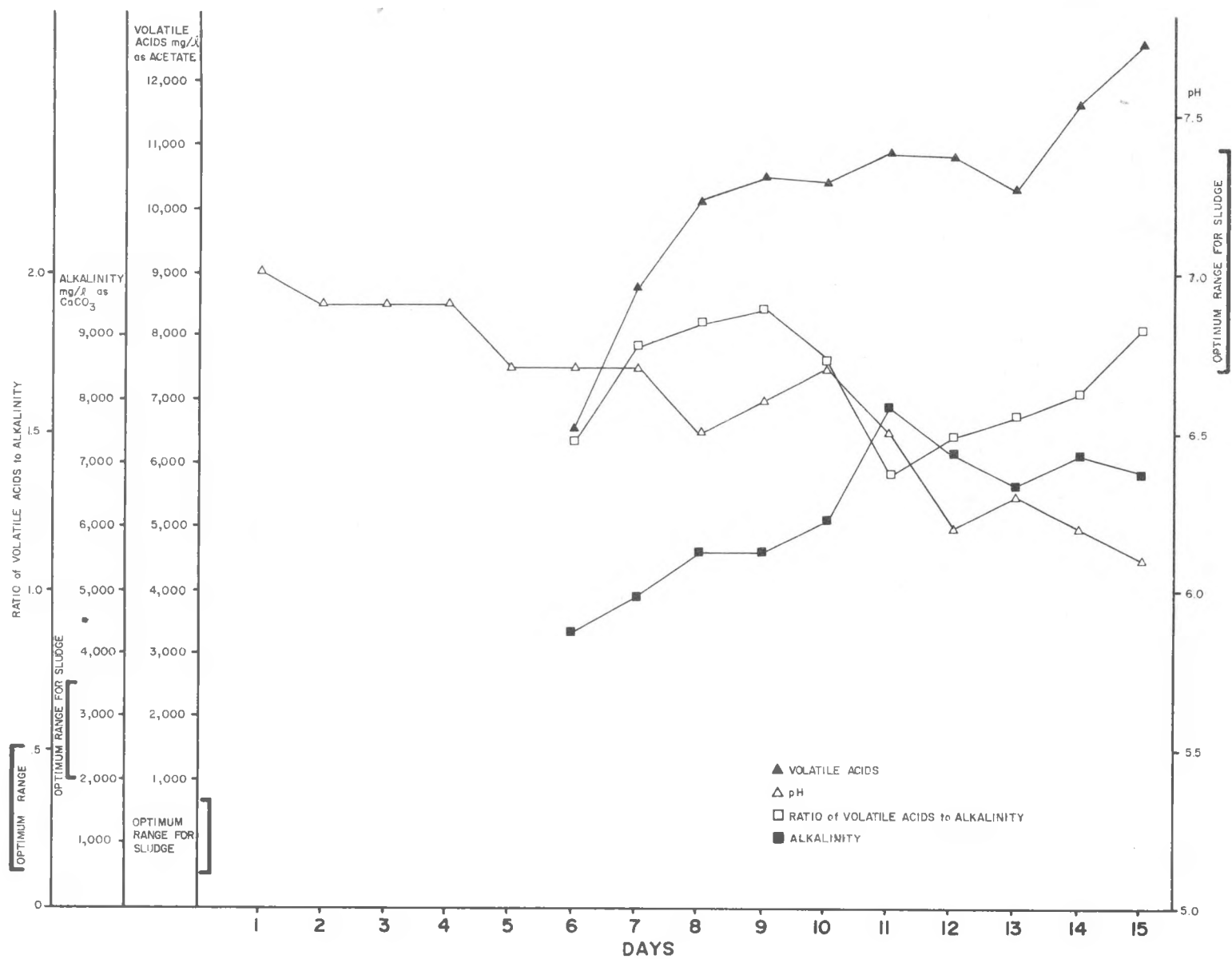


Figure 16. Digester II: general operating conditions, 3-percent solids.

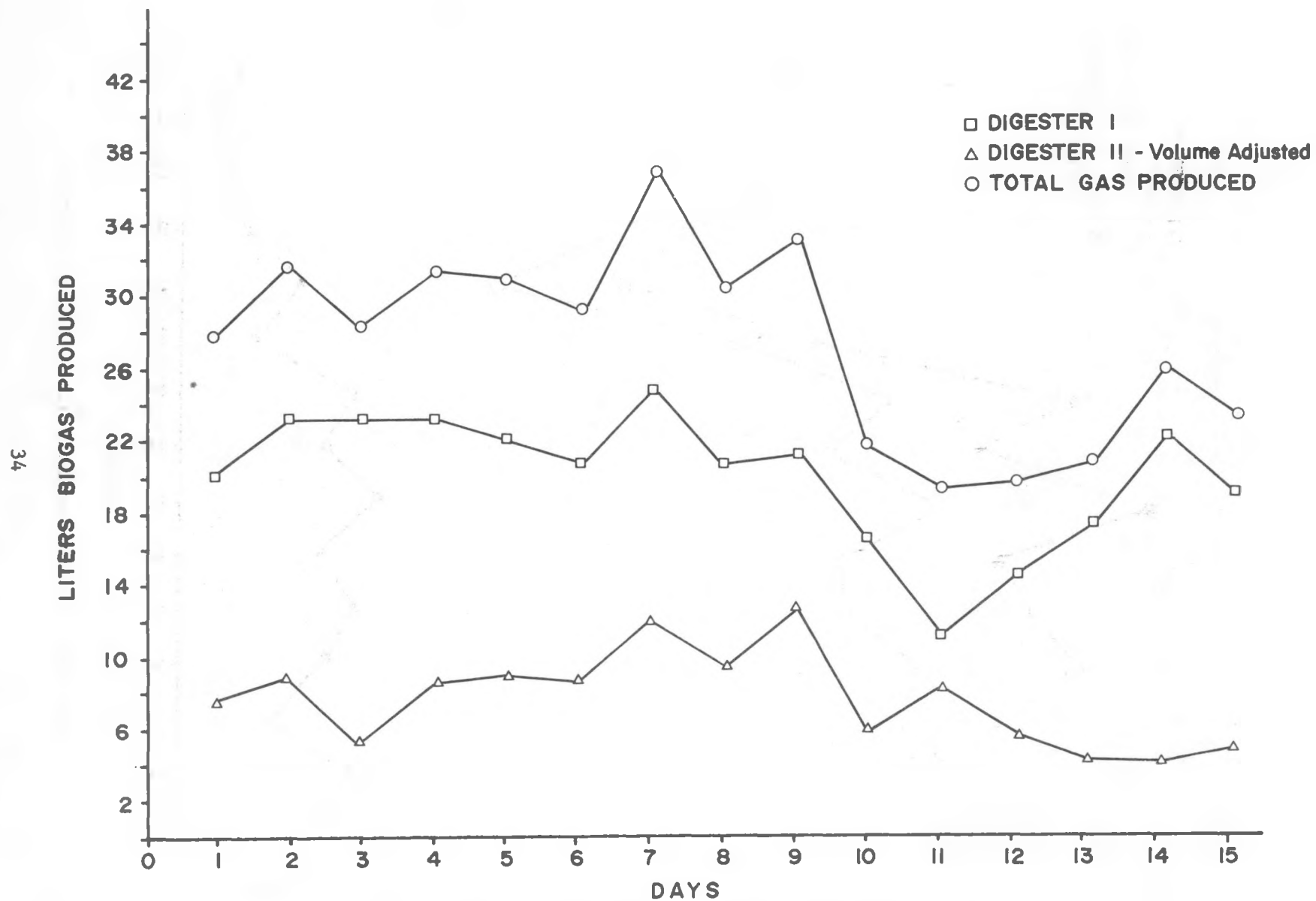


Figure 17. Biogas production: 3-percent solids.

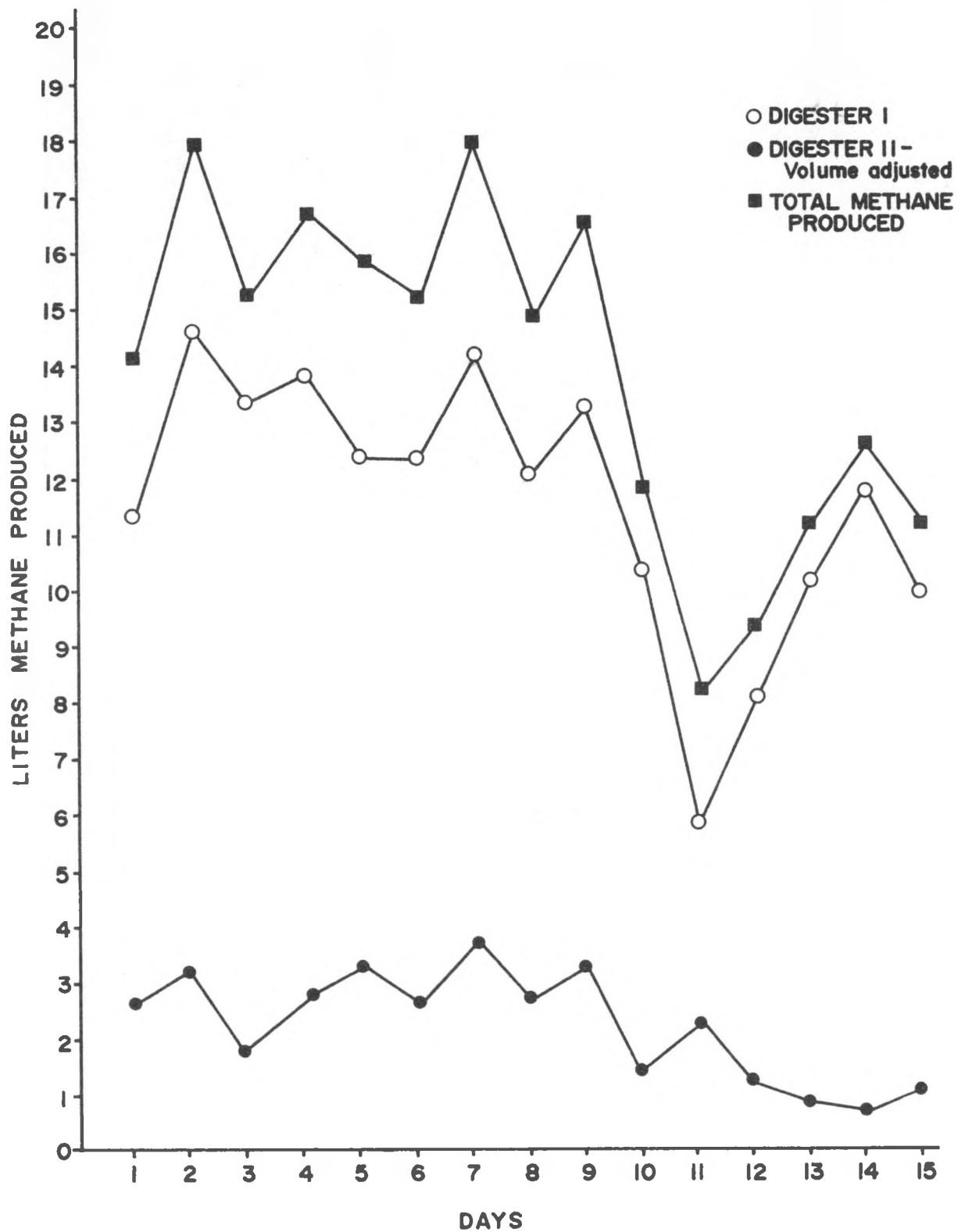


Figure 18. Methane production: 3-percent solids.

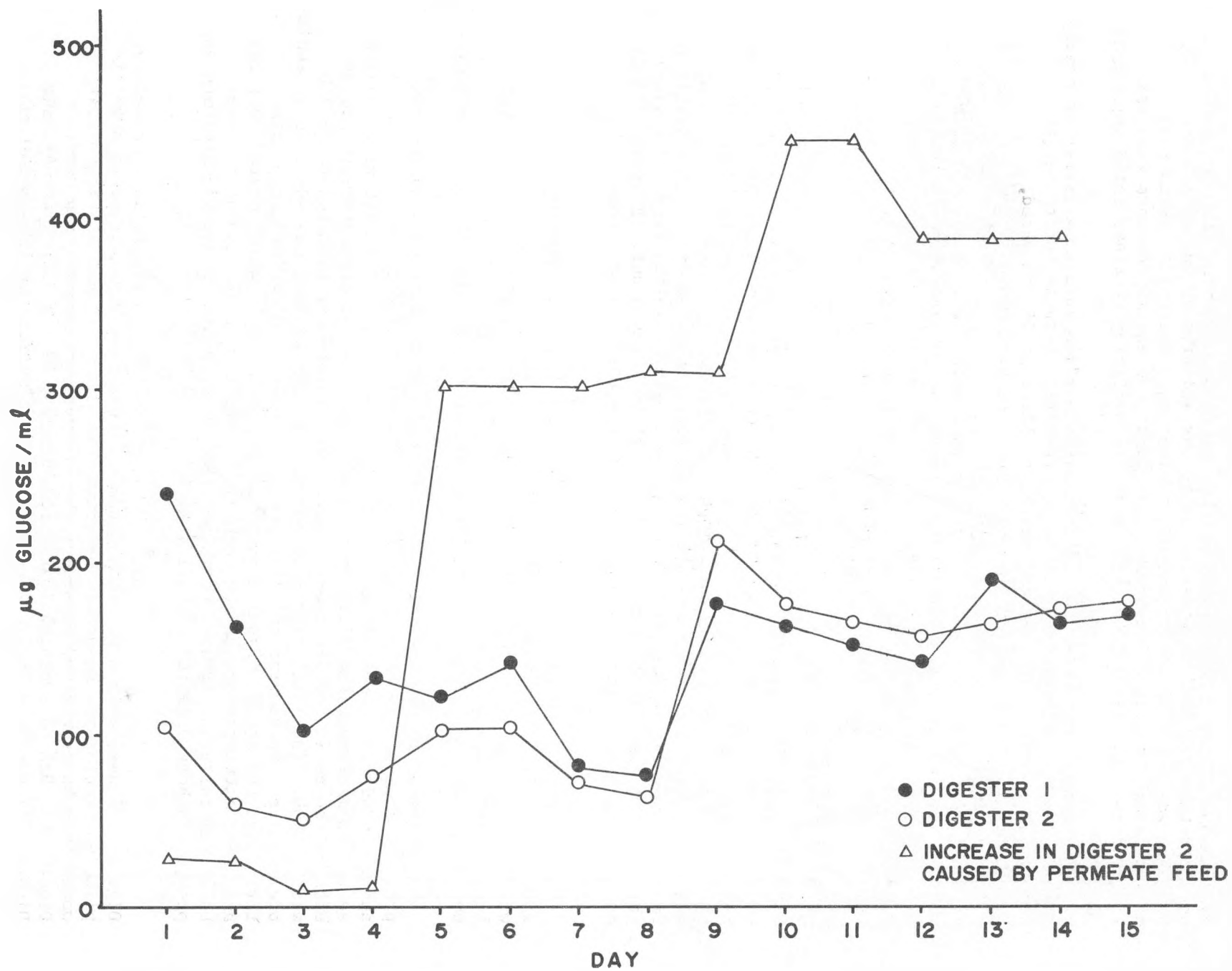


Figure 19. Glucose concentration: 3-percent solids.

in Digester II was $125 \pm 54 \mu\text{g/ml}$. The daily increment in glucose concentration produced in Digester II by the addition of enzyme reactor permeate was $377 \pm 65 \mu\text{g glucose/ml}$, indicating a bacterial consumption of approximately 1.5g glucose/day. The first two enzyme reactors were not included in this calculation because of the buffering problems noted previously.

The glucose concentration in both digesters remained essentially at steady state throughout the study, indicating a general state of health for the bacterial cultures. The overall health of Digester II, as noted, deteriorated as the study progressed. This was apparently a result of the buffering problems that made it impossible for the digester to keep up with volatile acid production and was not due to a decrease in available glucose. Glucose analyses of ultrafilter concentrates and permeates showed that glucose concentration was essentially the same in both streams. Permeate glucose levels were analyzed to assess the glucose input to Digester II from the enzyme reaction indicated in Figure 19.

Five-Percent MSW Slurry

Digester I experienced problems with solids distribution when the mixing time was 5 minutes per half hour. In order to alleviate this problem, mixing time was increased during the 5-percent study to 9 hours per weekday (full time), and the remainder of the day was left on the 5 minutes per half hour schedule. Even with the increased mixing time, sampling was more difficult for the 5-percent solids feed than for the 3-percent solids feed. The liquid phase flowed readily, while the solids had to be scraped out. Because of this problem with the solids, it was difficult to achieve uniform sampling.

Figures 20 and 21 show the general operating conditions for both digesters. Although Digester I was not as stable as in the first study, it was still considered to be healthy. The volatile-acid-to-alkalinity ratio varied between 0.4 and 0.8 instead of staying at less than 0.5. Since the pH was steady, conditions were still good even though optimum conditions were not maintained.

Digester II was healthier overall during this study than during the previous study. The ratio of volatile acids to alkalinity was high, but pH and gas production remained good throughout the study. The buffering problem seemed to have been remedied; instead of buffering the permeate with sodium bicarbonate as in the 3-percent study, the permeate was titrated to pH 7.0 with 5N KOH. The alkalinity remained high enough without the addition of sodium bicarbonate. Phosphorus and nitrogen were measured at different points throughout the study. Based upon a report by McCarty,³ which stated that the amount of nutrients present in domestic wastewater is sufficient for the bacterial cultures, it was determined that an adequate amount of nutrients was present in both digesters at all times.

Biogas production from each digester is shown in Figure 22. As before, Digester II used only a portion of the effluent from Digester I, so a multiplication factor was needed to determine the actual amount of biogas that would have been obtained if all of the effluent would have been used. Digester I had an average biogas production of $21.4 \pm 3.2 \text{ l per day}$, and Digester II averaged $24.7 \pm 5.4 \text{ l per day}$. An average of 115 percent more

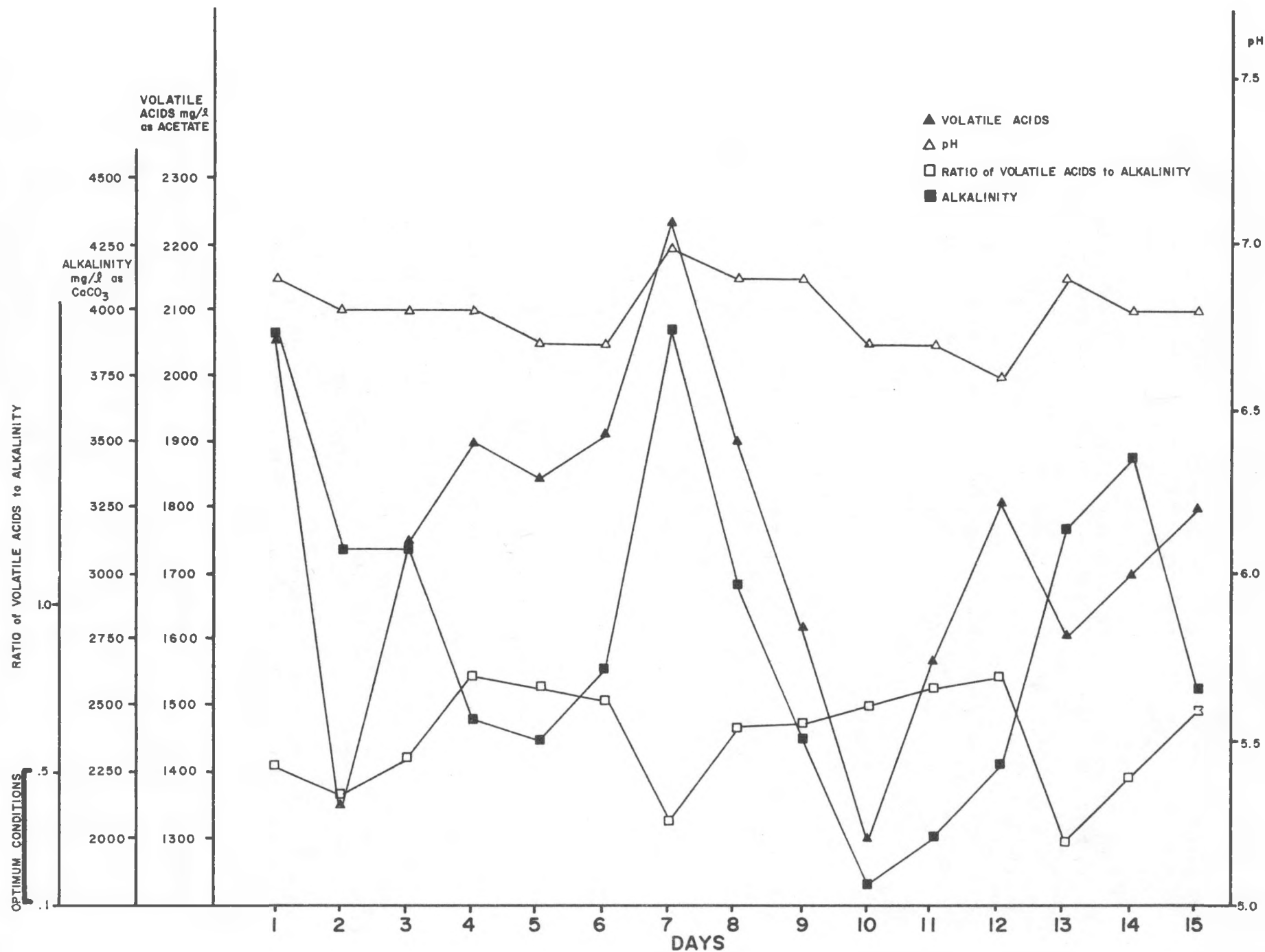


Figure 20. Digester I: general operating conditions, 5-percent solids.

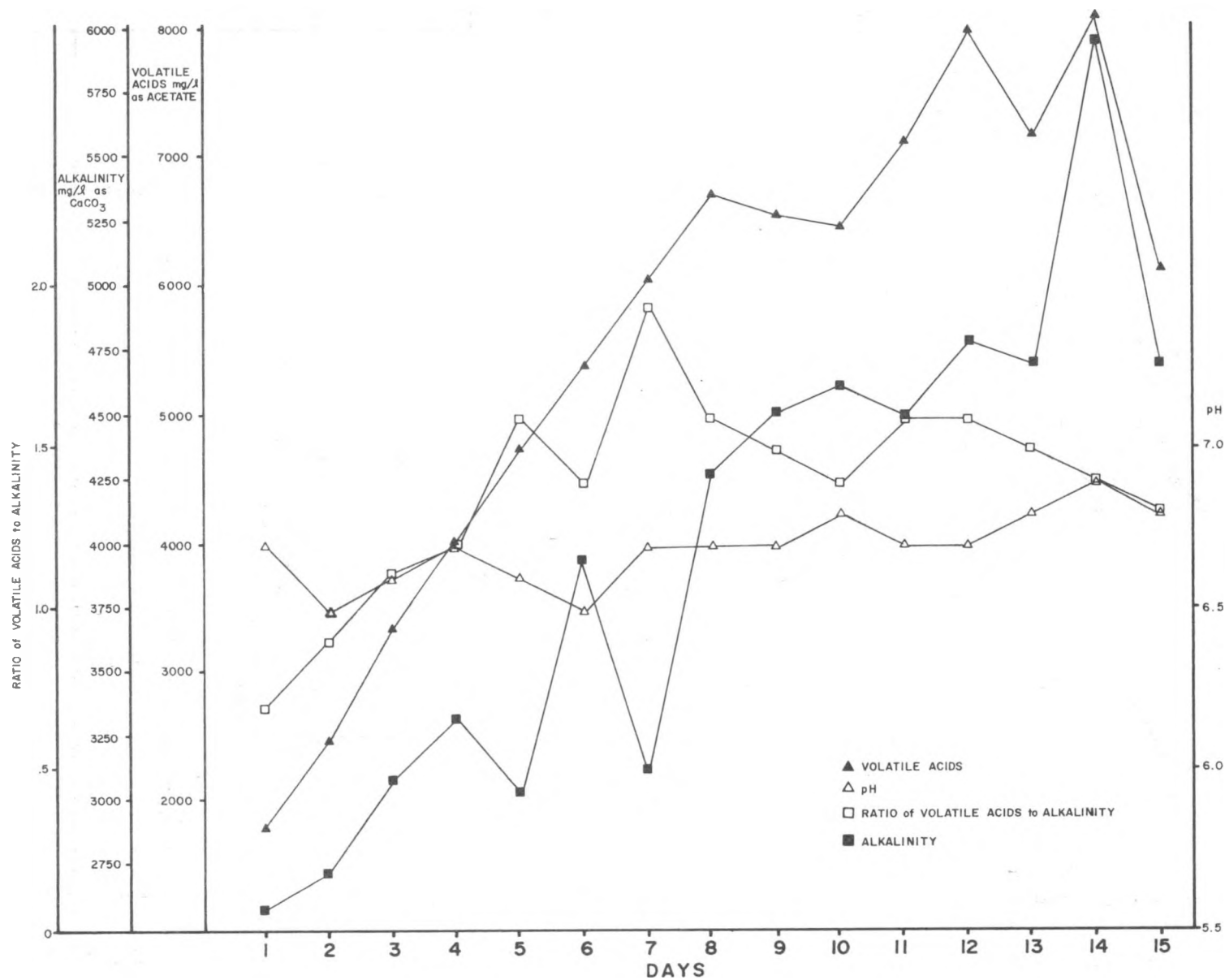


Figure 21. Digester II: general operating conditions, 5-percent solids.

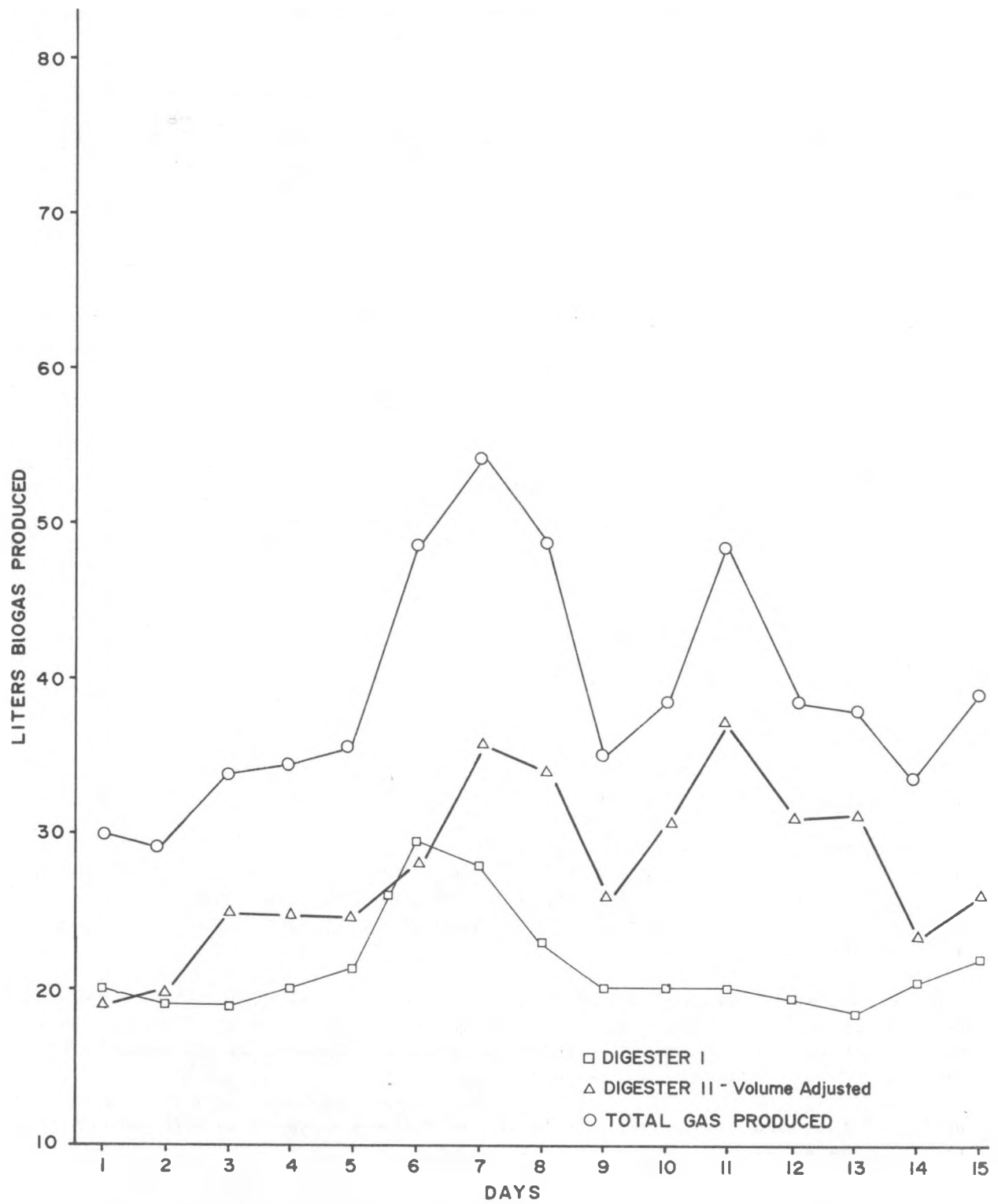


Figure 22. Biogas production: 5-percent solids.

biogas was obtained by using the enzyme reactor and second digester over that observed for Digester I alone.

Figure 23 indicates the methane production of each digester and the total amount of methane produced. Digester I produced an average of 9.5 ± 1.5 l of methane per day, and Digester II produced an average of 14.5 ± 3.6 l per day. A total production of 24.0 l per day was achieved by using the enzyme reactor and second digester. This was an increase of 153 percent of the production of Digester I.

This large improvement was partially due to the fact that methane production in Digester I averaged only 45 percent of the biogas. For comparison, the methane production averaged 59 percent in the 3-percent study. In addition, Digester II averaged 31 percent methane in the 3-percent study and 58 percent in the 5-percent study. It is surmised that the solution of the buffer problem contributed to this dramatic increase in methane production in Digester II.

Glucose production levels in Digesters I and II are shown in Figure 24. The mean concentration of glucose in Digester I was 286 ± 67 $\mu\text{g}/\text{mL}$ and 148 ± 24 $\mu\text{g}/\text{mL}$ in Digester II. The daily increment in glucose concentration produced in Digester II by the addition of enzyme reactor permeate was 340 ± 153 μg glucose/ mL , indicating a bacterial consumption rate of approximately 1.2g glucose/day. In both digesters glucose levels remained essentially at a steady state, indicating a general state of health for the bacterial cultures. Glucose analyses of ultrafilter concentrates and permeates showed that the glucose concentrations were essentially the same in both streams. Permeate glucose levels were analyzed to assess the glucose input to Digester II by the enzyme reactor (see Fig. 22).

Mass Balance

Figure 25 and Table 8 demonstrate the complete mass balance for 3 percent total solids feed calculated from the measurement of total solids destruction shown in Table 9. The total solids (TS) and total volatile solids (TVS) are shown in the figure, although only the TS are discussed in the text for simplicity.

Since biogas was collected over water in these studies, it was necessary to correct the gas production measured by water displacement for the quantity of CO_2 absorbed in the water. This quantity was calculated by Henry's Law from the water temperature and partial pressure of CO_2 in the gas. Because this quantity was not derived from direct experimental measurements, it has not been included in any calculations regarding biogas production other than the mass balances.

The first step, Digester I, had 124g TS going in and 117g TS coming out. The mass of biogas measured was 23.4g, with 8.0g CH_4 and 15.4g CO_2 . The quantity of CO_2 absorbed in the gas collection system was calculated at 13.2g. Enzyme and buffer were added to the digested MSW to give 281g TS going into the enzyme reactor and 209g TS coming out, with 72g being given off as

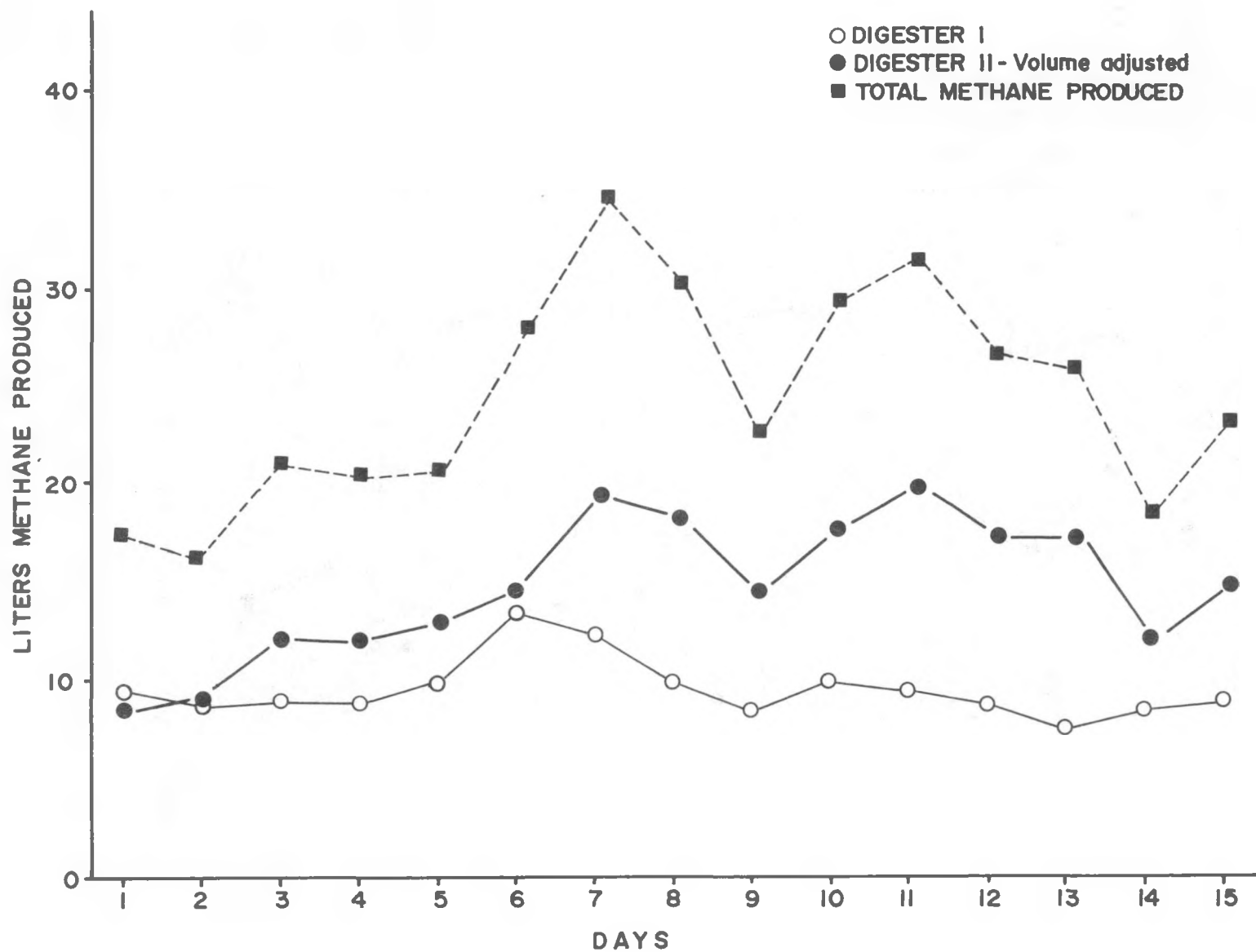


Figure 23. Methane production: 5-percent solids.

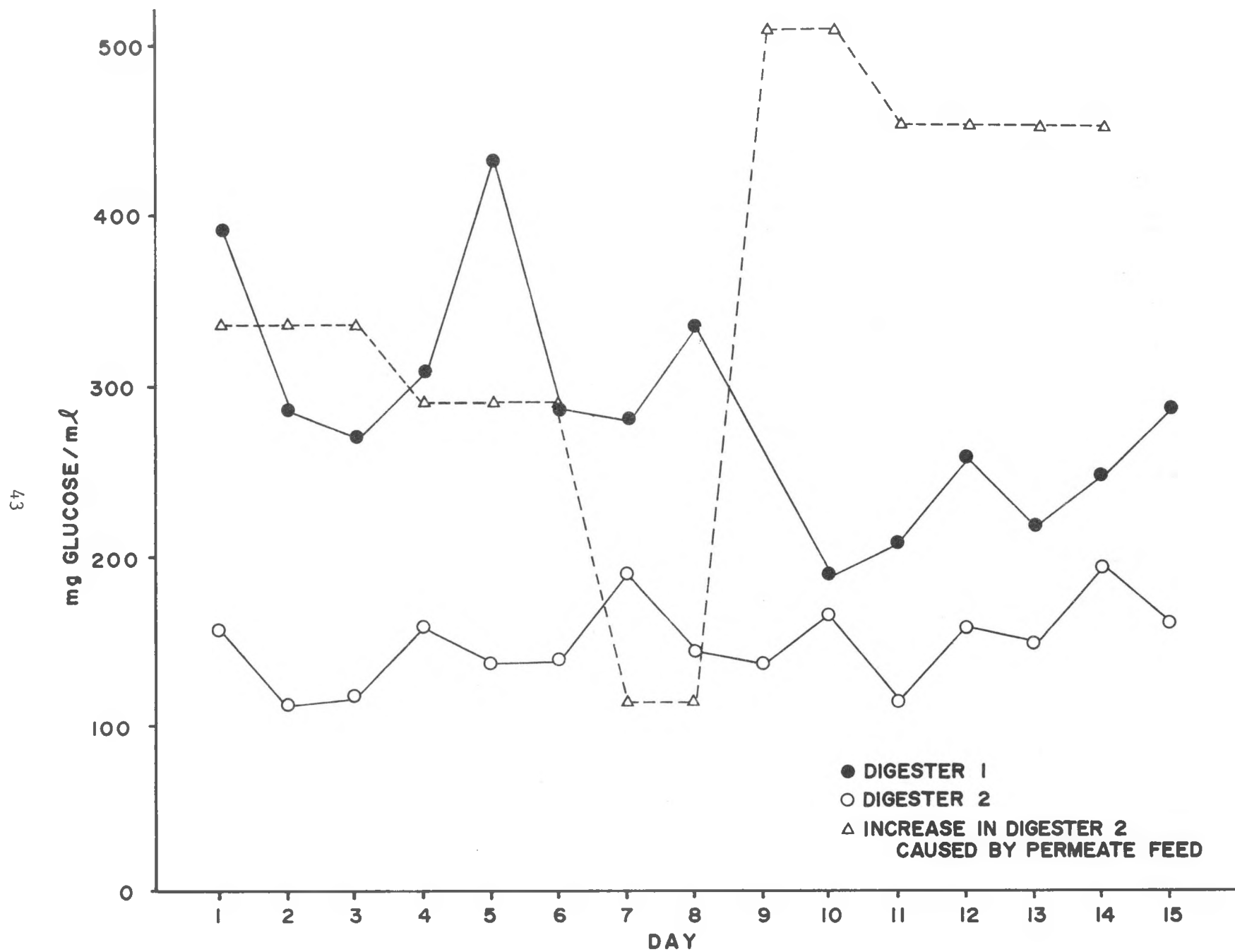


Figure 24. Glucose concentration: 5-percent solids.

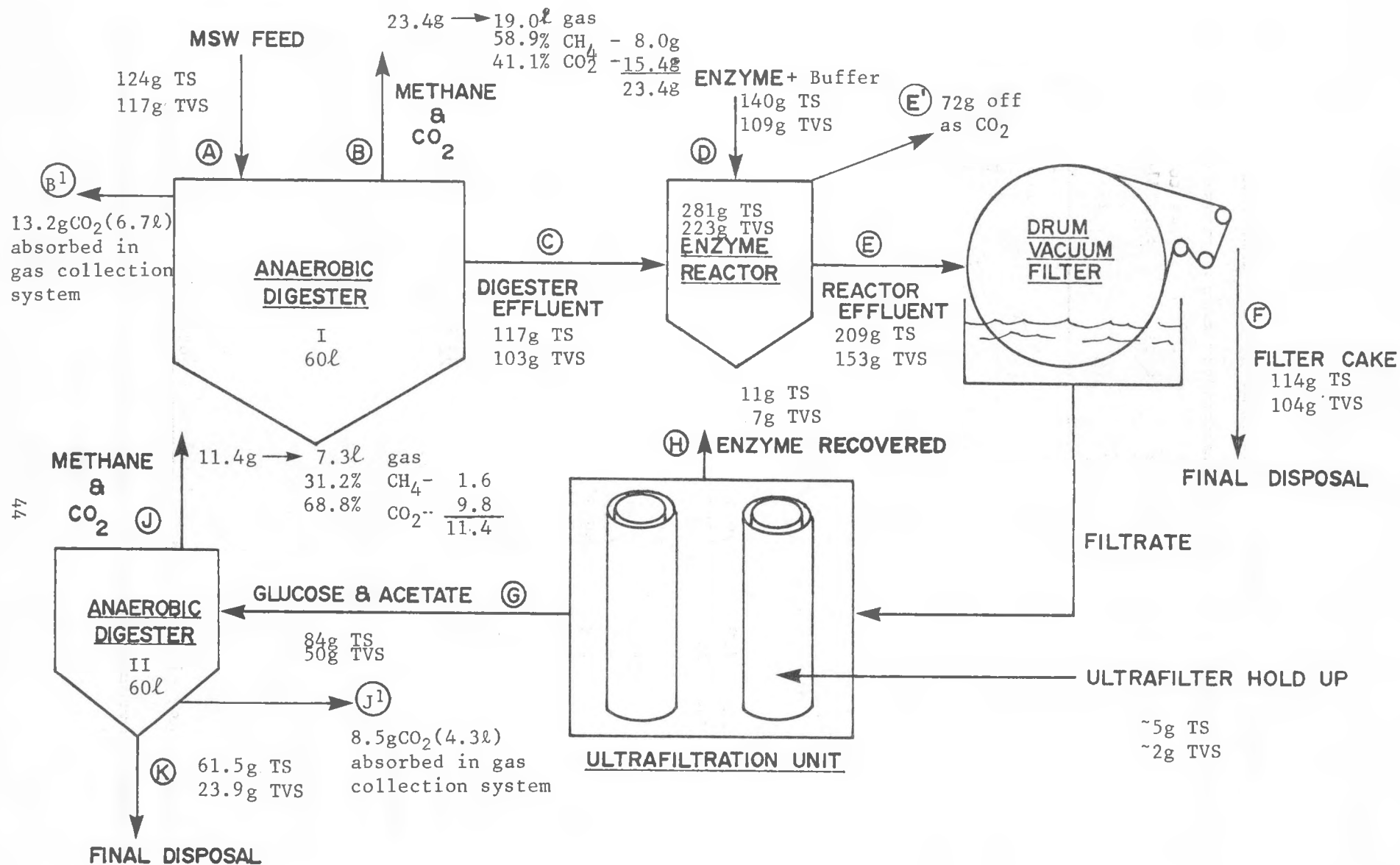


Figure 25. Mass balance per day: 3-percent total solids feed.

TABLE 8. MASS BALANCE: 3-PERCENT TOTAL SOLIDS FEED

	Total solids (%)	Total volatile solids (%)	Mass total solids (g)	Mass total volatile solids (g)	Point on Figure 25
Digester I - in	3.1	91.7	124	113.7	A
Digester I - out	2.9	88.3	<u>117</u>	<u>103.3</u>	C
Difference			7	10.4	
gas production -					B
19.0ℓ/day		{ 58.9% CH ₄ 41.1% CO ₂		8.0g CH ₄ <u>15.4g</u> CO ₂ 23.4g given off as biogas	
CO ₂ absorbed -					
6.7ℓ/day				<u>13.2g</u>	
Total				36.6g	
Enzyme Reactor - in	7.0	79.4	281	223	D
Enzyme Reactor - out	5.2	73.2	<u>209</u>	<u>153</u>	E
Difference			72	70	E ¹
Filter Cake	28.1	91.2	114	104	F
Concentrate	4.5	63.6	11	7	H
Permeate	2.8	59.5	84	50	G
Ultrafilter holdup	---	40.0	<u>~5</u>	<u>~2</u>	
Sum			214	163	
Digester II - in	2.8	60.0	83.8	50.3	G
Digester II - out	2.0	38.9	<u>61.5</u>	<u>23.9</u>	K
Difference			22.3	26.4	
gas production -					J
7.3ℓ/day		{ 31.2% CH ₄ 68.8% CO ₂		1.6g CH ₄ <u>9.8g</u> CO ₂ 11.4g given off as biogas	
CO ₂ absorbed -					
4.3ℓ/day				<u>8.5g</u>	
Total				19.9g	
Percent mass closure = 100 - $\left \frac{\text{inputs} - \text{outputs}}{\text{inputs} \times .01} \right $ = 89 percent					

TABLE 9. MASS DESTRUCTION FOR 3-PERCENT STUDY

	Digester I average	Enzyme reactor average	Digester II average
Mass in (g)	124	281 ^a	83.8
Mass out (g)	117	209	61.5
% destruction	5.7	25.6	26.6

^a Increase in mass due to addition of buffer and enzyme.

CO₂. Of the 209g TS coming out of the enzyme reactor, 114g were filter cake and discarded, 11g were recovered enzyme and available for reuse, and 84g were ultrafilter permeate and sent to Digester II. Based on the hold-up volume of the ultrafilter, it was estimated that 5g accumulated in the filter. During the final step, 83.8g TS went into and 61.5g TS came out of Digester II. Biogas production was measured at 11.4g, with 1.6g CH₄ and 9.8g CO₂. An additional 8.5g CO₂ was absorbed in the displaced water. The overall mass closure for the 3-percent study was 89 percent on a total solids basis. The major source of this discrepancy can be traced to Digester I. The measured quantity of mass converted to biogas in Digester I was 7g, whereas 36.6g of biogas were produced (including the absorbed CO₂). It is thought that this discrepancy resulted from inefficient mixing in Digester I, which prevented the obtaining of a representative sample of the digester effluent. Solids apparently settled out and accumulated near the sampling port, causing a disproportionately low estimation of the actual mass of solids converted to biogas. This explanation is supported by data obtained during the 5-percent study. A considerably better mass balance was obtained during the 5-percent study due, at least in part, to the increased frequency of mixing.

Figure 26 and Table 10 demonstrate the mass balance for the 5-percent total solids feed calculated from the measurement of total solids destruction shown in Table 11. Again, the total solids and total volatile solids are shown in the figure, while only the total solids are followed in the text.

Digester I had 188g TS going in and 139g TS coming out. The 49g of total solids converted correspond to the 51g of biogas produced, with 6.8g CH₄, 24.3g CO₂, and 19.9g CO₂ absorbed in the gas collection system. After enzyme and buffer were added to the digested MSW, there were 277g TS going into the enzyme reactor and 238g TS coming out, with 38g given off as CO₂. Of the 238g TS coming out of the enzyme reactor, 110g were filter cake and discarded, 22.8g were recovered enzyme and recycled, 12.5g were lost in the ultrafilter, and 91.3g were ultrafilter permeate and sent to Digester II. During the final step, 91.3g TS went into Digester II and 34.9g TS came out. Again, nearly all mass was accounted for with the 56.4g lost corresponding to the 43.4g of biogas produced. The overall mass closure for the 5-percent study was

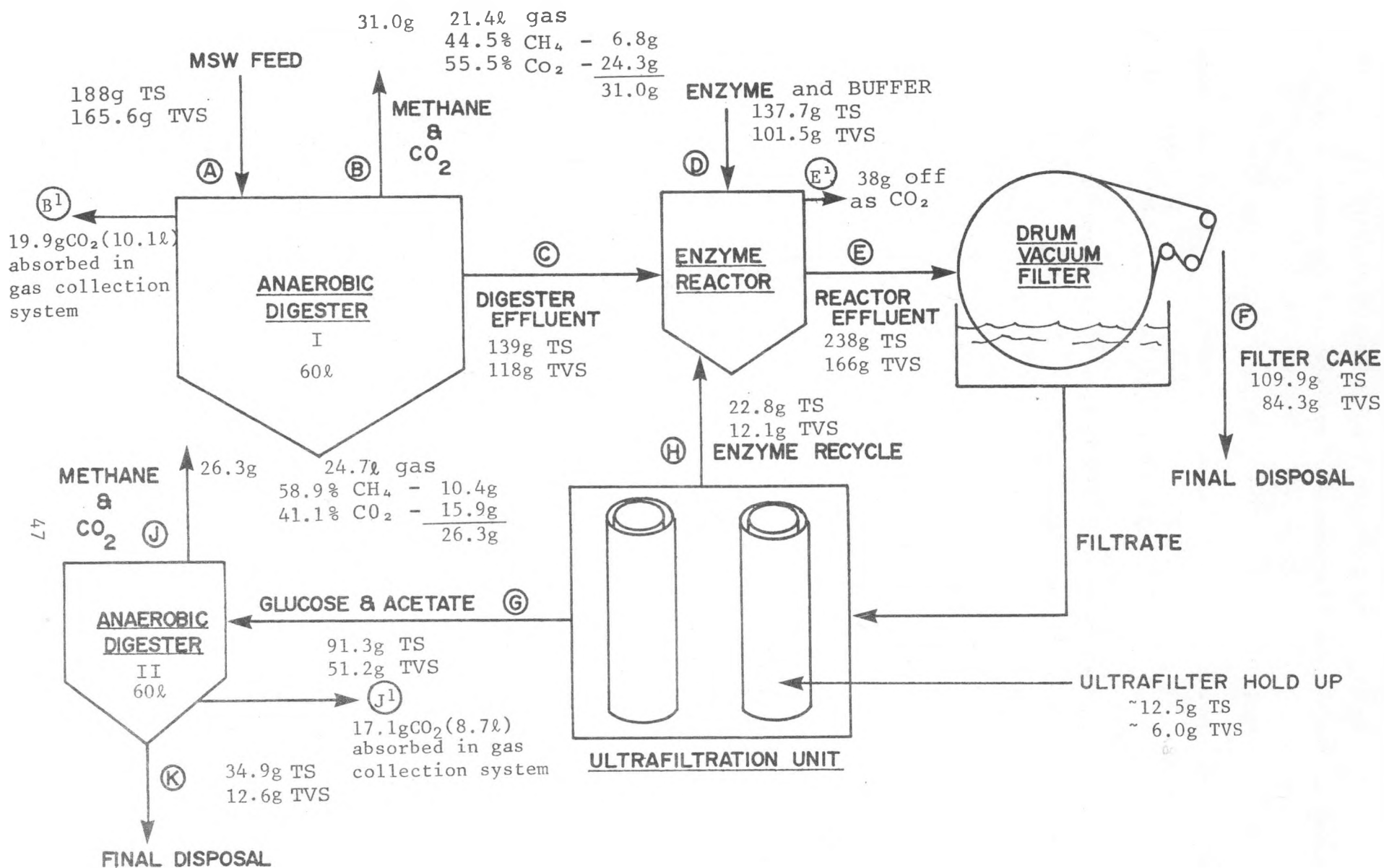


Figure 26. Mass balance per day: 5-percent total solids feed.

TABLE 10. MASS BALANCE: 5-PERCENT TOTAL SOLIDS FEED

	Total solids (%)	Total volatile solids (%)	Mass total solids (g)	Mass total volatile solids (g)	Point on Figure 26
Digester I - in	4.7	88.1	188	165.6	A
Digester I - out	3.5	84.9	<u>139</u>	<u>118.0</u>	C
Difference			49	47.6	
gas production -					B
21.4ℓ/day		{ 44.5% CH ₄ 55.5% CO ₂		6.8g CH ₄ <u>24.3g</u> CO ₂ 31.1g given off as biogas	
CO ₂ absorbed -					
10.1ℓ/day				<u>19.9g</u>	
Total				51.0g	
Enzyme Reactor - in	6.9	79.2	267.7	219.0	D
Enzyme Reactor - out	6.0	69.7	<u>238.4</u>	<u>166.2</u>	E
Difference			38.3	52.8	E ¹
Filter Cake	16.2	76.7	109.9	84.3	F
Concentrate	5.3	53.1	22.8	12.1	H
Permeate	3.4	56.1	91.3	51.2	G
Ultrafilter holdup	---	48.0	<u>~12.5</u>	<u>~6.0</u>	
Sum			236.5	153.6	
Digester II - in	3.4	56.1	91.3	51.2	G
Digester II - out	1.3	36.1	<u>34.9</u>	<u>12.6</u>	K
Difference			56.4	38.6	
gas production -					J
24.7ℓ/day		{ 58.9% CH ₄ 41.1% CO ₂		10.4g CH ₄ <u>15.9g</u> CO ₂ 26.3g given off as biogas	
CO ₂ absorbed -					
8.7ℓ/day				<u>17.1g</u>	
Total				43.4g	
Percent mass closure = 100 - $\left \frac{\text{inputs} - \text{outputs}}{\text{inputs} \times .01} \right $ = 96 percent					

TABLE 11. MASS DESTRUCTION FOR 5-PERCENT STUDY

	Digester I average	Enzyme reactor average	Digester II average
Mass in (grams)	188	277*	91.3
Mass out (grams)	139	238	34.9
Percent destruction	26.1	14.1	61.8

* Increase in mass due to addition of buffer and enzyme.

96 percent on a total solids basis. It is believed that the closure was well within the experimental and averaging errors involved. The mixing problem observed in Digester I was clearly remedied since the 49g of solids converted agreed within 4 percent with the 51g of biogas produced.

Enzyme Recovery Observed during Process Operation

Enzyme was recovered during the 3-percent solids study by vacuum filtering the enzyme reactors through a polypropylene 2/2 twill filter pad with a nominal air flow rate of $14 \text{ ft}^3/\text{min}$ per ft^2 . The solids were dried for 24 hours at 103°C , desiccated, and weighed. The filtrate was then introduced into the Amicon ultrafiltration unit, and concentrate and permeate were collected for enzyme activity assay and glucose analysis.

The results of the recovery assays conducted on ultrafilter concentrates during the 3-percent solids study are shown in Figure 27. No

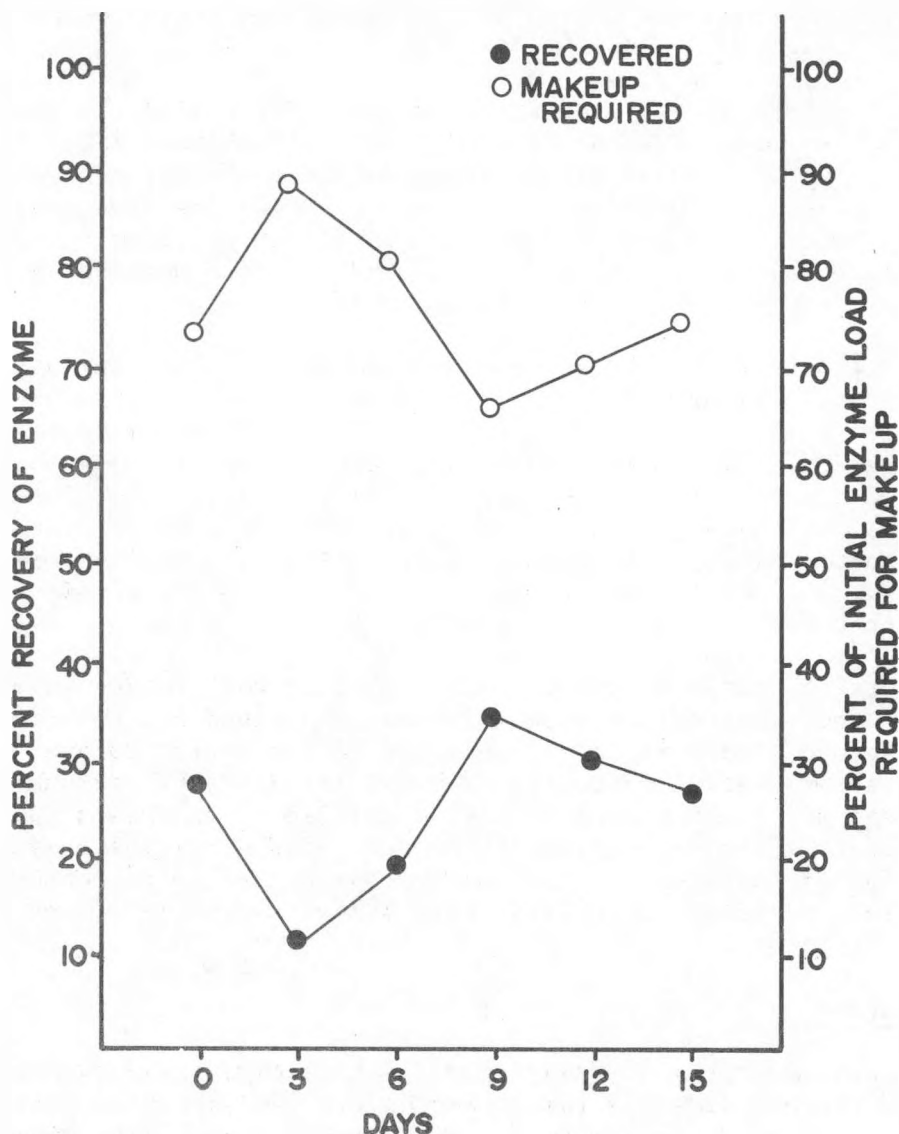


Figure 27. Enzyme recovery and makeup requirement: 3-percent solids.

activity was detected in the Digester I feed, Digester I effluent, or enzyme reactor ultrafilter permeate. Neglecting the first two reactors because of buffering problems, the mean recovery of enzyme from ultrafilter concentrates corrected for volume loss in the filter cake was 28 percent of the initial enzyme load. This value was increased to 43 percent recovered when corrected for bacterial consumption of glucose during the assay. In order to maintain the enzyme reactor at a constant loading level, this recovery data would require the addition of 57 percent of the initial enzyme load to make up for the enzyme lost in the recovery production.

Filtration data collected during the recovery of enzyme were analyzed to provide an indication of solids removal efficiency. The filter yield observed during this process was found to be 5.9 lb/ft^2 per hr, using a 2-minute cycle of 30 s submerged, 60 s drying, and 30 s off the filter. The solids capture observed during the 3-percent solids study was 38.5 percent.

Enzyme was recovered during the 5-percent solids study by centrifugation techniques. The enzyme reactor effluents were centrifuged for 3 hours at $8000 \times$ gravity with no prior filtration. Subsequent analyses indicated that centrifuging for 1 hour is sufficient to obtain the same results. Solids removal efficiency by centrifugation was sufficient to allow the centrifugate to be introduced directly into the ultrafilter, with a mean of 46 percent of the total solids removed in the pellet.

The results of recovery assays conducted during the 5-percent solids study are shown in Figure 28. No activity was detected in the Digester I feed, Digester I effluent, or enzyme reactor ultrafilter permeate. The mean recovery of enzyme from ultrafilter concentrates corrected for volume loss and bacterial presence in assay in the centrifuge pellet was 55 percent of the initial enzyme load. In order to maintain the enzyme reactor at a constant loading level, this recovery data indicates that 45 percent of the initial enzyme load would have to be added to make up for enzyme lost in the recovery procedures.

The current status of enzyme recoverability work conducted at SYSTECH pertinent to the process line system is best described by the work conducted during the second 15-day process line study in the energy recovery process. During this study, cellulose destruction was maximized by optimum buffering conditions and efficient mixing during incubation. In this study, enzyme was recovered from the reactors at 40 percent of the initial enzyme load. If correction for the apparent loss of enzyme due to bacterial consumption of glucose during the assay is applied, this figure can be increased to 55 percent of the initial enzyme load.

Proof of Concept

For 1 week after the 5-percent total solids feed study, enzyme reactor permeate was charged directly into Digester I. Permeate was used to make up one-fourth of the water in the feed. When permeate was added without pH adjustment, the pH and alkalinity dropped sharply. Permeate was then titrated to pH 7 and added with and without sodium bicarbonate. The pH was then closer

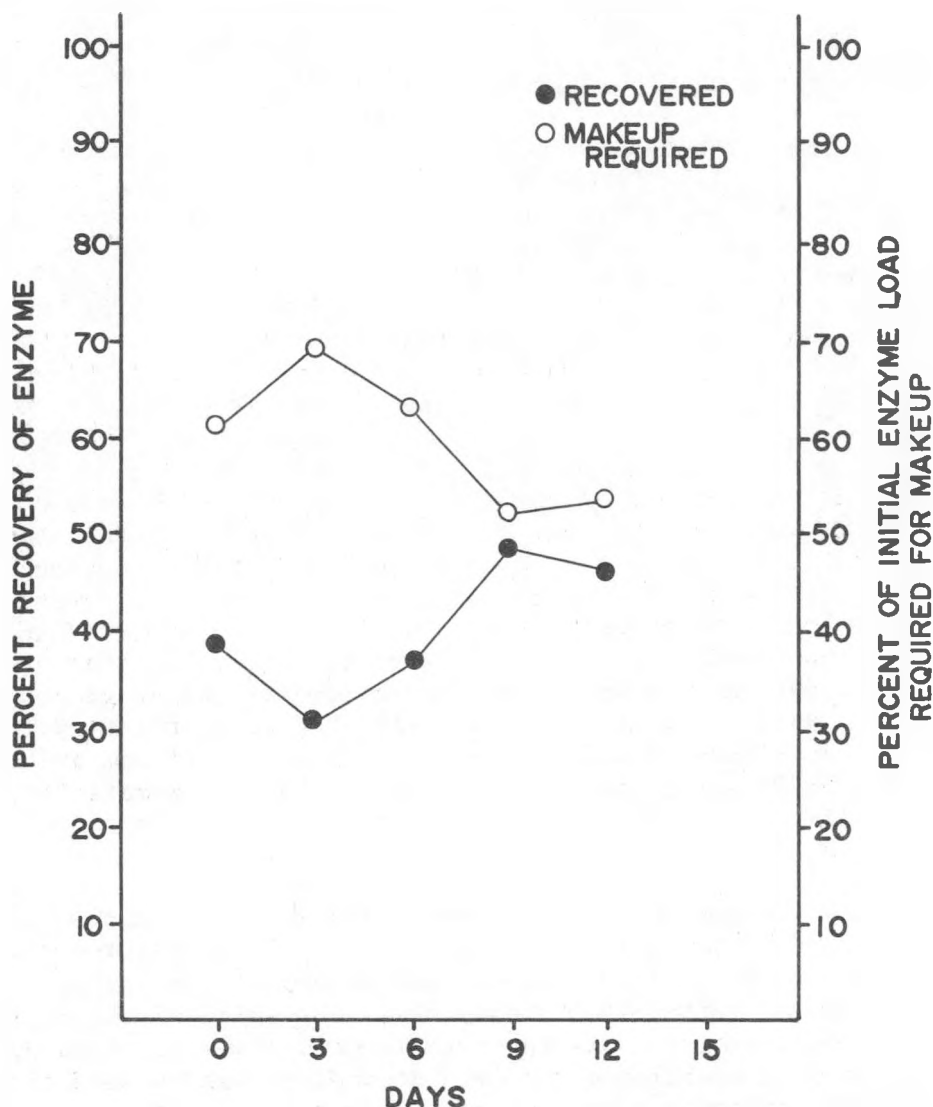


Figure 28. Enzyme recovery and makeup requirement: 5-percent solids.

to the optimum. It is difficult to determine whether the sodium bicarbonate was needed because of the permeate addition or because of the low alkalinity in the digester.

As a result of the partial permeate recycling to Digester I, the digester produced an extra 10 l of biogas per day. Although some of the biogas must be attributed to bicarbonate addition, the percentage of methane in biogas remained at 58 percent, the same level as during the 5-percent study. This amounted to an extra 5.8 l of methane due to reactor permeate in the feed. Since cycling the entire 4 l of effluent through the enzyme reactor would give 2.7 l of permeate, an extra 15.7 l of methane could be obtained.

SECTION 3

ECONOMIC EVALUATION

INTRODUCTION

The economic assessment of the system under study is based on the data obtained during the 5-percent solids loading process evaluation. The results from this evaluation have been projected to the operation of a 100 ton per day (TPD) raw MSW anerobic digester facility operated at 5-percent solids and a 15-day hydrolic retention time (HRT). The digester is followed by an enzyme reactor operated on a 3-day HRT, a vacuum filter, and an ultrafilter for return of recovered enzyme to the enzyme reactor and of glucose and acetate to the anaerobic digester. The mass balance of this 100-TPD facility is illustrated in Figure 29 and Table 12. For purposes of comparison, the mass balance of a 100-TPD simple digester facility with no enzymatic treatment is illustrated in Table 13. This system includes only locations A, B, C, H, and I from Figure 29.

In order to present this assessment in its most useful form, a number of assumptions were made regarding the operation of the digester-enzyme reactor system. First, the operational parameters of the various components as shown in Table 12 reflect the steady state operation of the entire system. That is, the view shown reflects the overall mass flows observed during each day's operation of a stabilized system. Second, it was assumed that 50 percent of the enzyme present in the enzyme reactor effluent would be captured by the ultrafilter and recycled into the reactor. This level of recovery was routinely demonstrated by experiments described earlier in this report. Third, gas production values from the system were derived by two separate methods. Gas production from the initial conversion of MSW was directly scaled up from the ratio of gas production to total volatile solids loading observed in the 5-percent laboratory-scale study. Additional gas production from the recycle of glucose and acetate into the digester was calculated from the assumption that all of this recycled feedstock was converted to gas. Data presented previously indicate that this assumption is justified. Fourth, quantities of buffer, glucose, and acetate were distributed into diverging streams based on the volumetric ratio of water diverted into these diverging streams. Finally, the glucose and acetate returned to the digester by recycling of ultrafilter permeate were not included in the calculation of the water required to maintain a 5-percent slurry. This slurry was instead defined on the basis of solids from the MSW only.

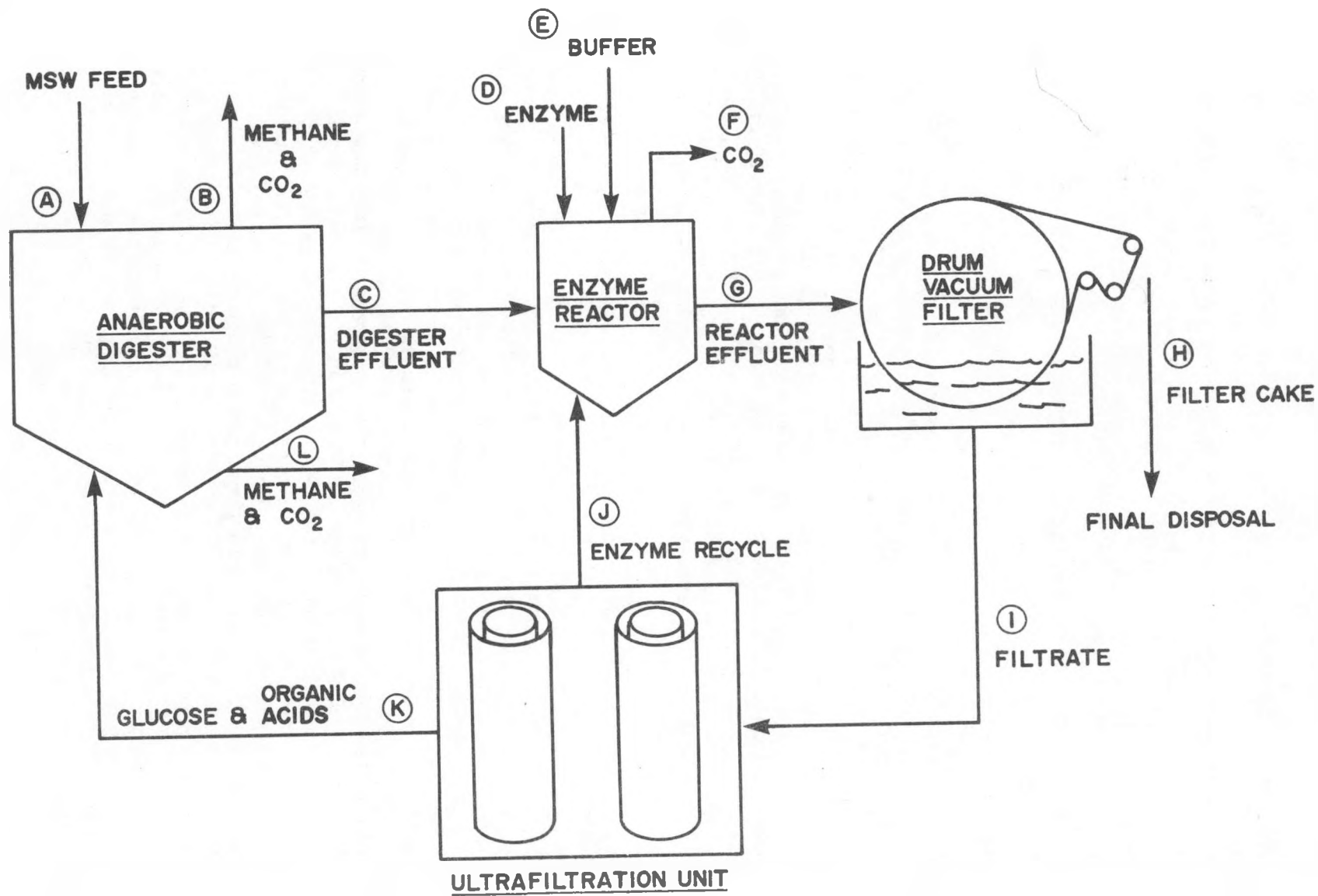


Figure 29. Process flow diagram.

TABLE 12. MASS BALANCE: 100-TPD DIGESTER-ENZYME REACTOR SYSTEM

Location on process flow diagram	Total solids (lb/day)	Total volatile solids (lb/day)	Water (lb/day)	Total gas production (lb/day)
A	1.50×10^5	1.32×10^5	2.50×10^5	
B				2.40×10^4 Total 5.42×10^3 CH ₄ 1.86×10^4 CO ₂
C	1.26×10^5	1.07×10^5	2.85×10^6	
D	3.47×10^4			
E	5.38×10^4			
F				3.45×10^4 CO ₂
G	2.16×10^5	1.50×10^5	2.91×10^6	
H	9.97×10^4	7.65×10^4	2.33×10^5	
I	1.16×10^5 3.47×10^4 enzyme 5.06×10^4 buffer 3.07×10^4 glucose plus acetate		2.68×10^6	
J	3.65×10^4 3.47×10^4 enzyme 1.09×10^3 buffer 6.62×10^2 glucose plus acetate		5.78×10^4	
K	7.95×10^4 4.95×10^4 buffer 3.00×10^4 glucose plus acetate	4.46×10^4	2.60×10^6	
L				3.00×10^4 Total 1.19×10^4 CH ₄ 1.81×10^4 CO ₂

TABLE 13. MASS BALANCE 100-TPD DIGESTER SYSTEM

Location on process flow diagram	Total solids (lb/day)	Total volatile solids (lb/day)	Water (lb/day)	Total gas production (lb/day)
A	1.50×10^5		2.85×10^6	
B				2.40×10^4 Total 5.42×10^3 CH ₄ 1.86×10^4 CO ₂
C	1.26×10^5	1.07×10^5	2.85×10^6	
H	8.79×10^4		2.05×10^5	
I	3.81×10^4		2.65×10^6	

PRESENTATION OF RESULTS

The economic assessment of this system is presented in terms of the costs and benefits associated with its operation as compared to the operation of an anaerobic digester of the same size with no enzymatic treatment components. Both systems will be examined for a 100 tons per day plant loading at 5-percent total solids and a 15-day HRT. The enzymatic system is presented with an enzyme reactor operated at a 3-day HRT. The results of this comparison are expressed in terms of the overall economic advantage or disadvantage exhibited by each system. Finally, the overall economic feasibility of the enzymatic posttreatment of digested MSW and the impact of potential improvements in the enzymatic treatment system on this feasibility are discussed.

Costs

Table 14 indicates the costs associated with the operation of both the simple anaerobic digester system and the anaerobic digester-enzyme reactor system.

Anaerobic Digester System

The anaerobic digester facility required for the disposal of 100 tons of raw refuse per day will require a daily capacity of 3.00×10^6 lb of water and total solids, or approximately 3.60×10^5 gal. Operating the digester at a 15-day HRT will require an overall capacity of 5.40×10^6 gal. Since the anaerobic digester alone and the anaerobic digester-enzyme reactor will require digester tankage of identical size, the cost of this tankage, its mixing system, and all other supporting equipment for the digester facility will not be included in this assessment.

Solids dewatering of the digester effluent to 30 percent solids may be accomplished for approximately \$880 per day. This cost is based on an

TABLE 14. DAILY COST OF DIGESTER AND DIGESTER-ENZYME REACTOR SYSTEMS

Item	Digester system (\$/day)	Digester-enzyme reactor system (\$/day)
Vacuum filter	880	648
Filter cake disposal	1,172	1,331
Subtotal (A)	<u>2,052</u>	<u>1,979</u>
Enzyme reactor		148
Ultrafilter		308
Additional labor		100
Subtotal (B)		<u>556</u>
Grand Total (A + B)	<u>2,052</u>	<u>2,535</u>

estimated cost of \$20 per ton of filterable solids to be dewatered by a conventional drum vacuum filter. Filterable solids are herein defined as being equal to the measured value of the suspended solids in the digester effluent, 8.80×10^4 lb.

At 30 percent solids the filter cake will have a weight of 2.93×10^5 lb. Landfilling this cake at a rate of \$8 per ton adds a daily cost of \$1,172. The total daily cost of the dewatering system is then \$2,052.

Not included in this cost projection are a number of items that are very difficult to estimate from the data gathered in this study. Among these items are the gas-collection and purification system and various feed conveyors and pumps required for the operation of the system. Since essentially identical components will be required for the operation of the digester-enzyme reactor system as for the simple digester, these items were not to be included in the analysis.

Anaerobic Digester-Enzyme Reactor System

Since the cost for the anaerobic digester and for supporting equipment in this system is identical to that for the simple digester system, it will not be included in this assessment.

The enzyme reactor in this system is sized to accommodate 3.16×10^6 lb of solids and water per day, or 3.79×10^5 gal. per day. This would require one tank of a 1.39×10^6 gal. capacity. The cost of this tank would amount to \$67 per day amortized at 15-percent interest over 10 years.

The digester effluent must also be mixed and heated from 37°C to 40°C in order to achieve the optimum rate of hydrolysis in the enzyme reactor. This heating will require approximately 16×10^6 Btu, for an additional daily

cost of \$40 at an estimated \$2.50 per million Btu. The total cost of heating and mixing the digester is estimated at \$80 per day.

The vacuum filter in this process line must accommodate the daily enzyme reactor effluent of 2.16×10^5 lb solids in 2.91×10^6 lb of water, or approximately 6.48×10^4 lb suspended solids. The cost of the drum vacuum filter would amount to \$648 per day, based on a rate of \$20 per ton of suspended solids filtered.

The filter cake resulting from the operation of this system will contain 9.97×10^4 lb solids and 2.33×10^5 lb of water. Landfilling this cake at a rate of \$8 per ton will add a daily cost of \$1,331.

The ultrafilter for this process line has been sized to accommodate a daily input of 2.68×10^6 lb of water and 1.16×10^5 lb of total solids. Information provided by Osmonics, Inc., indicates that the cost of an ultrafilter unit of this size sufficient for the capture of the enzymes would cost \$250,000, or \$137 per day amortized at 15-percent interest over 10 years. In addition, operating costs for this unit will amount to approximately \$0.50 per thousand gallons of permeate cycled, adding \$156 to the daily ultrafiltration cost. Approximately 300 kWh of electricity will be consumed each day to operate pumping stations required for the ultrafilter amounting to an additional daily charge of \$15 at \$0.05 per kWh. The total daily costs for the ultrafilter system will amount to approximately \$308.

Operation of the enzyme reactor and ultrafilter will also require an additional person per day at a daily rate of approximately \$100. The total additional daily cost associated with the operation of the enzyme reactor and ultrafiltration system is \$556. With the vacuum filter and filter cake disposal cost added, the cost for the daily operation of the complete digester-enzyme reactor is \$2,535. As in the simple digester system, the cost of a digester mixing system, gas-collection and purification system, and transfer conveyors and pumps required for operation were not included in this assessment.

Revenues

Every solid waste disposal facility has the potential of revenue from tipping fees. The method of calculating such fees depends on whether ownership is public or private and upon the owner's accounting methods. Thus no specific tipping fee is hypothesized herein.

Anaerobic Digester System

The daily operation of the anaerobic digester system will produce 5.42×10^3 lb CH_4 , or 1.28×10^5 ft³ CH_4 . At a cost of \$2.50 per million Btu of fuel, this daily gas production will generate \$319.

Anaerobic Digester-Enzyme Reactor System

The daily operation of the anaerobic digester-enzyme reactor system will produce a total of 1.73×10^4 lb CH_4 , or 4.08×10^5 ft³ CH_4 . Included in this figure is the additional CH_4 produced by recycling the glucose and acetate from the enzyme reactor back into the digester. At a

cost of \$2.50 per million Btu of fuel, the daily gas production from this system will generate \$1,020.

Economic Feasibility

Anaerobic Digester System

The simple anaerobic digester system would have total costs comprised of the unidentified costs for the basic digester and its peripherals plus \$2,052 per day for those items identified as being inspected by the addition of an enzyme process. The daily revenues accruing from energy recovery are estimated to be \$319. Thus, any tipping fee charged would need to be sufficient to cover the unidentified base costs plus the net identified cost of \$1,773 per day (\$2,052 - \$319).

Anaerobic Digester-Enzyme Reactor System

The basic cost of the digester vessel and peripherals, although not specifically identified, will be the same as for the simple anaerobic digester. The net costs for identified factors will be \$2,535 per day, excluding the cost of purchased enzymes. The daily revenue from energy recovery will be \$1,020. Assuming that the same tipping fee is charged as for the simple anaerobic digester system, the decreased costs and improved revenues for the enzymatic system would result in a potential daily savings (excluding enzyme costs) of \$258 over the simple anaerobic digester system.

Out of this savings must come the cost for the 3.47×10^4 lb of enzyme required each day. For the enzymatic system to break even, enzyme must be available at a cost of approximately \$0.01/lb, including shipping and handling. Given the probable high cost of producing bulk quantities of enzyme, the cost of enzyme alone may well be expected to exceed the total of all other costs, and will certainly exceed the allowable break-even cost of \$0.01/lb.

POTENTIAL FOR IMPROVEMENT

The economy of the anaerobic digester-enzyme reactor system is ultimately determined by three major factors: (1) the cost of dewatering and disposal of solids, (2) the cost of enzyme, and (3) the quantity of methane gas generation.

The cost associated with the first of these factors is determined by the overall efficiency of the vacuum filter in capturing suspended solids and by the efficiency of the digester enzyme reactor in converting raw MSW to glucose (the soluble end product of hydrolysis). The more efficient this conversion process, the lower will be the amount of filterable solids in the effluent of the enzyme reactor. As envisioned in the process under study, the efficiency of the digester in this conversion process is essentially limited by the difficulties that a feed stock high in cellulosic materials present to the biomethanation process. Improvements in the extent of enzymatic hydrolysis of cellulose to glucose are equally difficult in this

process line. This hydrolysis has been shown both by NOVO and by this study (Fig. 30) to be most aptly described by the equation¹¹

$$(\text{Product}) = a^{1/2} (\text{time} \times [\text{enzyme}])^{1/2}$$

where "a" is an empirically derived constant.* Considerable increases in either the time of enzyme treatment or concentration of enzyme used would be required to produce a reasonable increase in product formation. The drawback of this approach lies in the added cost required for the additional enzyme or for the increased enzyme reactor size to accommodate the longer detention time. It is therefore unlikely that reductions in dewatering and solids disposal costs can improve the overall economic feasibility of the enzymatic system.

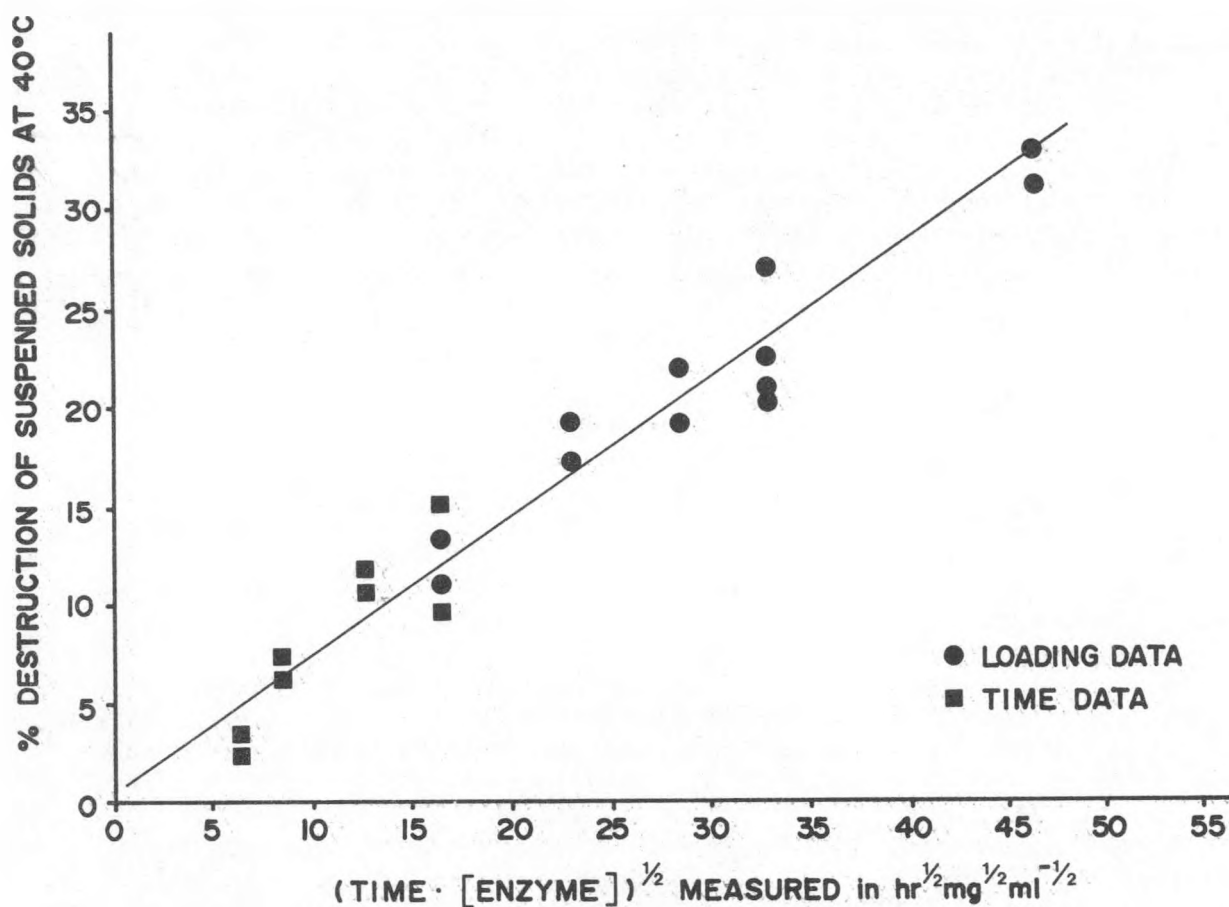


Figure 30. Composite of time and loading data on digested MSW.

* It has been found that "a" is related to the specific activity of the enzyme and to the concentration and susceptibility to hydrolysis of the substrate as well as pH, temperature, and ionic strength.

The cost of the enzyme required for this process is at present an unknown quantity, whose effect on the overall economics cannot be assessed. No matter what the cost, however, the amount of enzyme actually added on a daily basis will control the impact of this cost on the process. This amount is determined by two factors: (1) the extent of hydrolysis produced by a given enzyme loading and (2) the percentage of enzyme recovered by the filtration process. Experiments directed toward the optimization of these two factors, as described in this report, indicate that the potential is great for minimizing the quantity of enzyme added while simultaneously maintaining the same overall production of methane.

Another factor that significantly impacts the total cost of enzyme used in this system is contained in the hydrolysis equation described on page 60. Since the formation of methane is ultimately controlled by the product of time and enzyme concentration, the same amount of methane can be generated if less enzyme is used for a longer incubation period. Increasing the retention time in the enzyme reactor could therefore permit a greater expenditure per pound of enzyme used as long as the increased detention time does not exceed the useful life of the enzyme. The increase in detention time is obviously directly related to the increase in vessel size as well as to the decrease in enzyme requirements. However, since the cost of increased tankage is very slight compared to probable enzyme cost, process economics can be significantly improved by increasing the enzyme reaction time as much as possible.

One method by which the costs associated with solids removal and disposal might well be decreased would involve the relocation of the enzymatic treatment step to precede the anaerobic digestion phase. If this were done, the breakdown of the cellulosic material would begin before its entry into the digester, rendering it more susceptible to microbial digestion. If the digester were then operated under conditions favorable to enzymatic hydrolysis, considerably more glucose would be made available for the formation of methane. The effect of this process on the amount of effluent solids to be handled would lead to a considerable reduction in the costs associated with dewatering and landfilling solids, thereby improving the overall economic feasibility of the system.

The quantity of methane generated by this process line is ultimately controlled by the same factors as those controlling the quantity of effluent solids. There is no simple method by which more methane, and therefore more revenue, can be generated from the process line described in this report. On the other hand, the pretreatment of MSW with enzymes as described offers a significant possibility of increasing overall methane production. Any increase in methane production would directly improve the economic feasibility of this system.

It seems likely that the economic feasibility of the enzymatic digestion of MSW can be improved by the incorporation of a predigestion enzymatic treatment phase coupled with efforts to optimize the hydrolysis rate and recovery of the enzyme. Future increases in landfilling costs may well render enzymatic treatment a viable economic alternative to simple anaerobic digestion.

1. The first part of the report deals with the general situation of the country and the progress of the work of the Commission. It is divided into two sections: the first section deals with the general situation of the country and the second section deals with the progress of the work of the Commission.

2. The second part of the report deals with the work of the Commission in the field of the economy. It is divided into two sections: the first section deals with the work of the Commission in the field of the economy and the second section deals with the work of the Commission in the field of the economy.

3. The third part of the report deals with the work of the Commission in the field of the culture. It is divided into two sections: the first section deals with the work of the Commission in the field of the culture and the second section deals with the work of the Commission in the field of the culture.

4. The fourth part of the report deals with the work of the Commission in the field of the education. It is divided into two sections: the first section deals with the work of the Commission in the field of the education and the second section deals with the work of the Commission in the field of the education.

5. The fifth part of the report deals with the work of the Commission in the field of the health. It is divided into two sections: the first section deals with the work of the Commission in the field of the health and the second section deals with the work of the Commission in the field of the health.

6. The sixth part of the report deals with the work of the Commission in the field of the social work. It is divided into two sections: the first section deals with the work of the Commission in the field of the social work and the second section deals with the work of the Commission in the field of the social work.

7. The seventh part of the report deals with the work of the Commission in the field of the environment. It is divided into two sections: the first section deals with the work of the Commission in the field of the environment and the second section deals with the work of the Commission in the field of the environment.

8. The eighth part of the report deals with the work of the Commission in the field of the international relations. It is divided into two sections: the first section deals with the work of the Commission in the field of the international relations and the second section deals with the work of the Commission in the field of the international relations.

9. The ninth part of the report deals with the work of the Commission in the field of the science and technology. It is divided into two sections: the first section deals with the work of the Commission in the field of the science and technology and the second section deals with the work of the Commission in the field of the science and technology.

10. The tenth part of the report deals with the work of the Commission in the field of the sports. It is divided into two sections: the first section deals with the work of the Commission in the field of the sports and the second section deals with the work of the Commission in the field of the sports.

SECTION 4

CONCLUSIONS AND RECOMMENDATIONS

Work performed in the enzyme treatment unit illustrated that (1) cellulose hydrolysis is not directly proportional to either enzyme concentration or time, but rather to the square root of the product of concentration and time; (2) the cellulase complex is not significantly inhibited by metals in the concentrations normally found in MSW slurries; and (3) the reaction continues to a greater degree of hydrolysis at 40°C with viable bacteria than at 50°C, the enzyme's reputed optimum (a temperature at which the mesophilic bacteria are inactive while thermophilic bacteria are not yet active). This increase in hydrolysis in an environment where the end product is readily metabolized suggests that the cellulase system possesses a regulatory enzyme that is sensitive to feedback inhibition.

The enzyme recovery operations determined the feasibility of recycling enzymes by ultrafilter capture and showed that the recovered enzyme is not denatured by any of several possible enzyme loss mechanisms. It was discovered that (1) the shear forces generated in the ultrafilter did not denature the enzymes, (2) the enzyme was stable in solution sufficiently long enough to warrant recovery efforts, (3) cellulase enzymes are not degraded in the enzyme reactor, and (4) enzyme loss in the filter cake can be minimized to 45 percent of the initial enzyme load by using various techniques. The techniques examined in this study included recovery by centrifugation, extraction, and completion of cellulose destruction.

The energy recovery process demonstrated that with a 5-percent MSW slurry and a 15-day HRT, an increase of nearly threefold in methane production can be expected as a result of posttreatment of the effluent with cellulase and subsequent refeed of the glucose stream to the anaerobic digester. Because of a buffer problem in the second-stage digester, results of the 3-percent MSW slurry at a 15-day HRT were not as conclusive, giving only a 20-percent increase in methane production.

Posttreatment of MSW by cellulase enzymes after anaerobic digestion has been shown to greatly enhance biomethanation. However, the value of the additional methane produced is not sufficient to support the high cost of enzymes, either with or without ultrafiltration, for recapture of the enzymes. It is recommended that further research be conducted to determine if single-vessel conditions can be found under which both the cellulase and the methane-forming bacteria can remain active to achieve an economically suitable degree of additional methane production.

In addition, visual monitoring of the enzyme's action on cellulosic wastes exhibited a marked miscibility improvement even with enzyme concentrations in the parts per million range. This leads to the conclusion that mixing energies can be substantially lowered and a more homogeneous slurry established when employing cellulose treatment either previous to or simultaneous with anaerobic digestion. Viscosity studies should be conducted to determine the optimum enzyme concentration for improving slurry miscibility.

APPENDIX A

STATUS OF A CELLULOSE ASSAY

The determination of cellulose content is desired in the present study as a process control analysis to allow a determination of the rate of cellulose conversion. For the analysis to be useful, it is necessary that it be unbiased and that it be performable with a quick turnaround. This is not the case for any of the cellulose analyses presently in use.

It was found that published analytical methods for the determination of cellulose have severe drawbacks in their usefulness for analyzing municipal solid waste samples. Each of the techniques is subject to one or more of the following difficulties.

1. Prohibitively long times are required for the various steps in the analysis, most especially for the sample drying and digestion. Furthermore, such oven drying must be accomplished at low enough temperatures to prevent caramelization of sugars or pyrolysis of celluloses and yet be high enough to assure that the sample is truly moisture free.
2. Reagents and materials are hazardous and cannot be suitably accommodated in the laboratory facilities available for the present study.
3. The low-solid-content MSW slurry is by nature nonhomogeneous. The materials settle rapidly, and even large samples exhibit wide variability.
4. Sources of cellulosic materials in municipal solid waste are obviously very broad, and this results in a wide variation in the structural matrices exhibited by the cellulose present.
5. Each assay procedure depends upon hydrolysis of the cellulose to a reducing sugar for the final analysis. Various reducing sugars are already present in the municipal solid waste and these interfere with the analysis.

All the analytical procedures examined call for sample sizes in the range of 0.2 to 1.0g. Even a 2-l sample of the material typically present in our digestion studies can have varying amounts of total solids, and these total solids can be composed of different amounts of wood, paper, and cloth from day to day or even from sample to sample. Such small sample sizes, therefore, made it very difficult to obtain a representative sample. Taking a

larger sample adds additional time to the overall analytical procedure because the larger sample requires an even greater amount of filtration or centrifugation and a concomitant increase in the amount of drying time.

Because cellulose comes in many forms, each dependent upon a different structural matrix, a unique problem is found in the MSW sample that is not found in the paper-industry samples or in the analyses of forage fiber and compost. MSW has the complexities of manufactured paper and cloth as well as those of natural plant products varying from relatively soft fibers to wood. Thus an analytical procedure designed to give reproducible values on compost, for example, may give unreconcilable values with a composite of different cellulose depending on the susceptibility of the glucose polymer matrix to any specific procedure's hydrolysis steps.

Three different types of procedures and various modifications were examined since the start of the project. Lossin's Anthrone Colorimetric Method for determining cellulose in compost was employed first.¹² After running daily standard curves with the anthrone color reagent, it was noted that the reagent became rapidly unstable; thus this procedure was replaced by the more stable potassium ferricyanide method. This method for determining reduction of sugars was found to have good linearity of the standards, to require less expensive reagents, and to have a less critical reagent incubation time, as well as a higher specificity.

In order to check accuracy, some preliminary experiments were performed to determine what values of cellulose Lossin's procedure would give on filter paper. One-gram samples of Whatman No. 1 filter paper, which is known to be about 99 percent cellulose, gave values of only 0.57 and 0.60 g by Lossin's method. During the experiment, it was noted that upon subjecting the samples to 72-percent sulfuric acid for hydrolysis, some charring occurred; for this reason, the procedure gave lower cellulose values than expected. It may be that such strong hydrolysis is necessary for compost, but this treatment does not give reliable results for paper.

It was observed that the cellulase enzyme complex is the most efficient agent for breakdown of cellulose to glucose that can be accurately measured. It has also been documented in the literature that the less crosslinking the cellulose possesses the more efficient the breakdown process of that cellulose by the enzymes. A method published by the Institute of Paper Chemistry¹³ was examined; according to this method, the cellulose was dissolved by converting it to a soluble derivative in a nonaqueous solvent and then removing the insoluble noncellulose by centrifugation. Decanting into water would cause the regeneration of the parent compound, which would then have only small amounts of crosslinking, and the cellulose content could be accurately measured by glucose determination after enzyme hydrolysis. In practice it proved difficult to scale this much above the original 1.0 g of cellulose called for in the original procedure. Part of the problem seemed to be associated with the low efficiency of conversion of the cellulose to its soluble derivative and also with the considerable filtering time required for larger samples. Excess reagents for the reaction were also found to be difficult to remove as required to purify the product for subsequent enzyme hydrolysis.

APPENDIX B

ENZYME ACTIVITY ASSAY

Techniques employed to quantify the amount of enzyme present in solution continued to be improved throughout this study. Initially, the amount of enzyme present was indirectly measured by the weight loss of a given mass of cellulosic material experienced during a defined incubation period. Enzyme solutions at 100 ml volume buffered to pH 5.0 with potassium hydrogen phthalate (KHP) were incubated with 1.0g Whatman No. 1 paper at 50°C for 40 minutes. The solutions were then filtered through a glass fiber filter, and the cake was washed and dried at 105°C for 1 hour, desiccated, and weighed. The amounts of Whatman paper destroyed by the enzyme solution were then taken as an indirect measure of the amount of enzyme present.

Subsequent methods of enzyme quantification were based on measuring the amount of reducing sugars produced during the incubation of enzyme with a cellulosic substrate. Enzyme solutions are currently analyzed by incubation for 1 hour with a known amount of substrate at 40°C in 13 × 100 mm screw-capped test tubes. The tubes are placed in a preheated water vessel and agitated at 2 Hz with a 2-cm displacement in a heated Lab-Line orbital shaker. Reducing sugars produced from the substrate are then determined colorimetrically by the ferricyanide reaction.

Three potential substrates were examined at the same enzyme loading (128 mg Celluclast® 100L and 32 mg Cellobiase 250L/g substrate) for use in the assay: Whatman CF-1 cellulose powder, Avicel® Ph 105 microcrystalline cellulose (FMC Corporation), and Whatman No. 1 filter paper shredded and suspended in KHP buffer. Shredded Whatman paper was determined to be the most appropriate substrate for enzyme quantification, staying in suspension during the assay and producing an average of 390 µg/ml reducing sugar, as glucose, as compared to less than 200 µg/ml for the other substrates.

Standard curves were generated at two concentration ranges for the production of glucose by enzyme-containing solutions (Figs. B.1 and B.2). Solutions were prepared for assay by making up Celluclast® and cellobiase suspensions in 65 ml of 0.1 molar KHP buffer pH 5.0, which were then introduced to an Amicon DC-2 hollow fiber ultrafiltration unit with a 200 micron prefilter and a 5000 molecular weight cutoff membrane filter. A volume of 750 ml of 0.1 molar KHP buffer, pH 5.0, was added and the unit run with concentrate recirculation until approximately 50 ml remained in the unit, at which time 75 ml KHP buffer was added and the concentrate collected. The

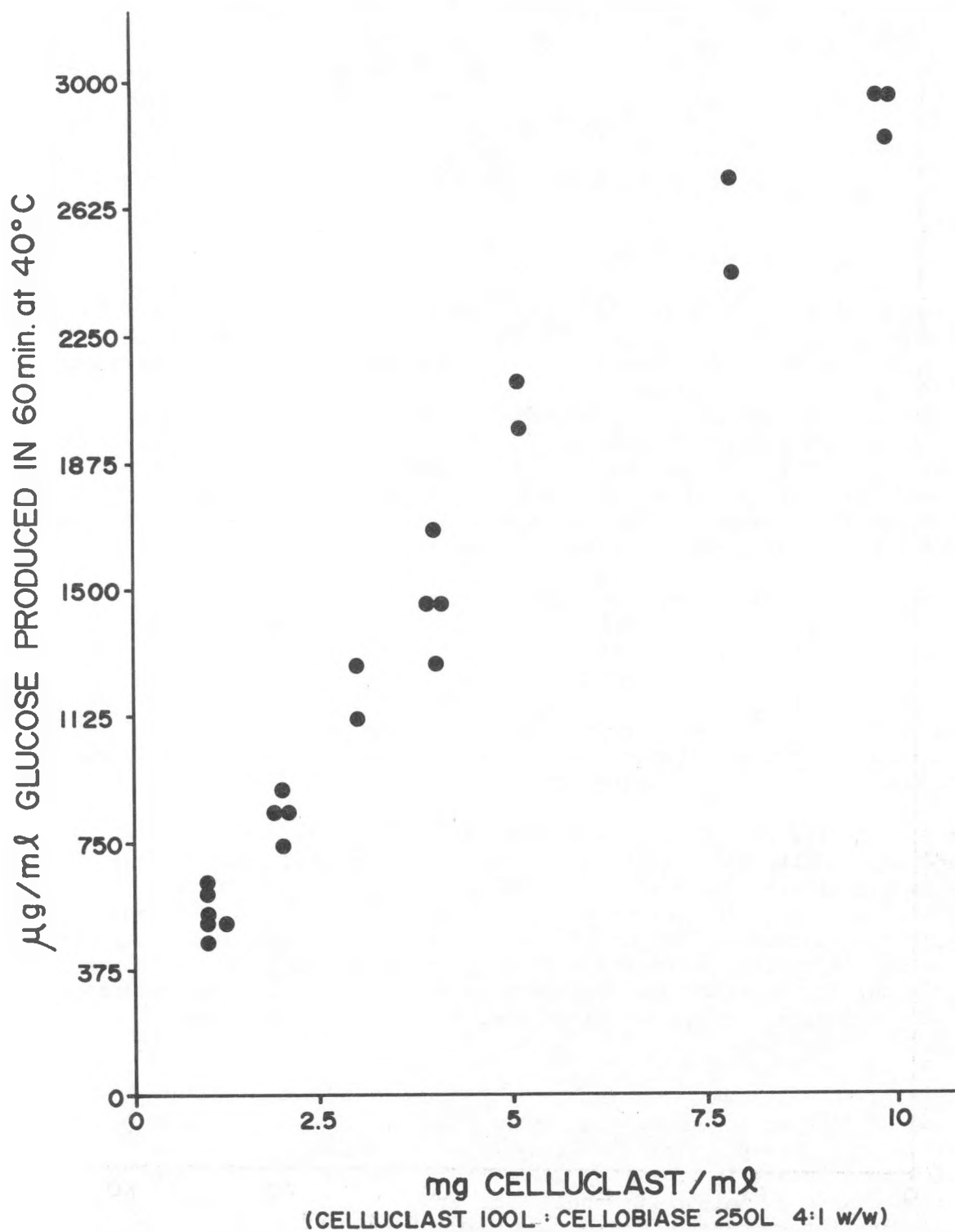


Figure B.1. Enzyme assay standard curve, low concentration.

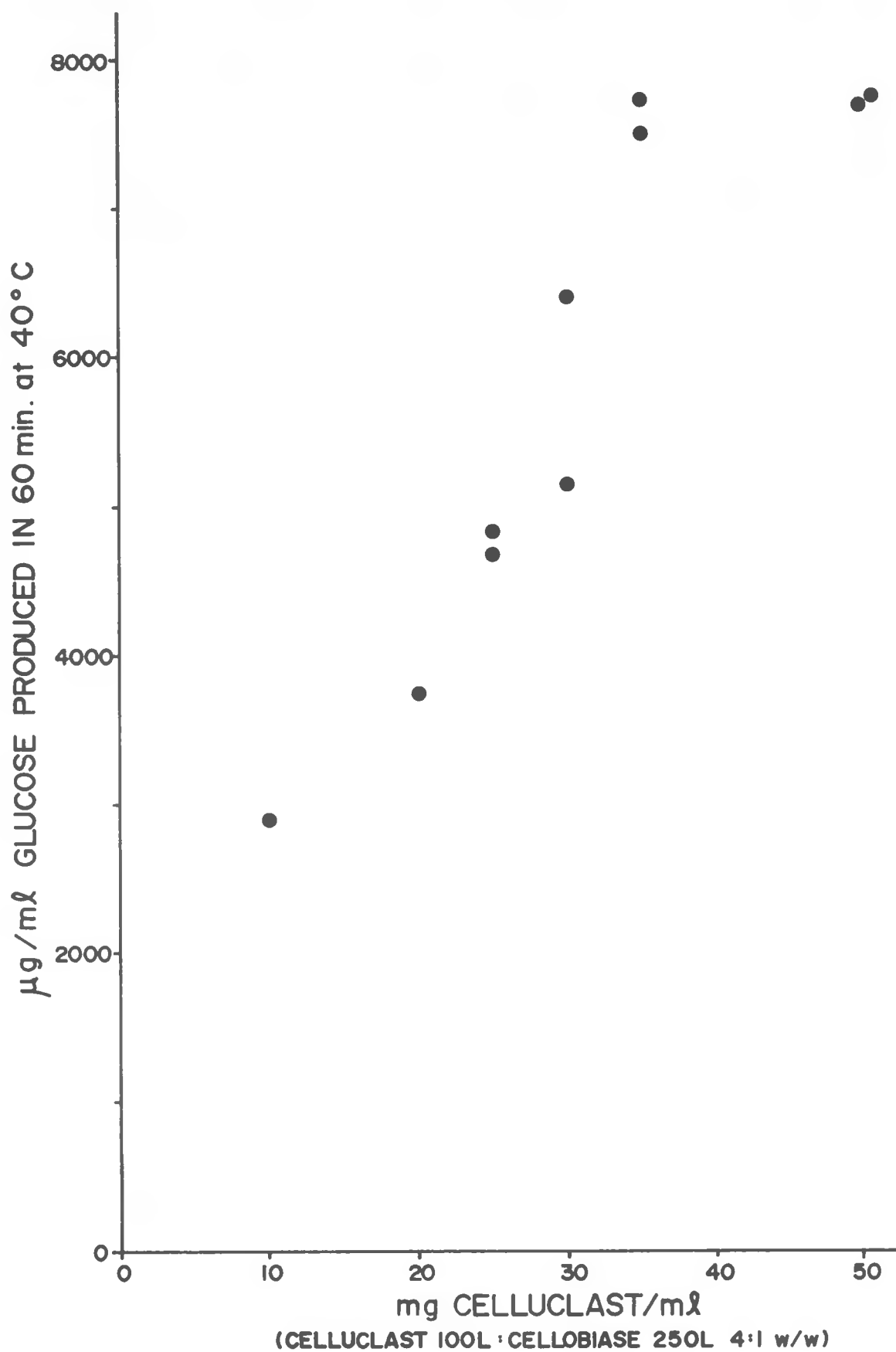


Figure B.2. Enzyme assay standard curve, high concentration.

unit was then rinsed with the second 75 ml portion and brought to a final volume of 250 ml. This basic procedure was followed throughout for ultrafiltration runs.

These data were analyzed by a least squares linear regression fit to the logarithmic form of a power curve, and two equations were generated to describe the relationship of enzyme loading to reducing sugar production. At loadings of enzyme up to 10 mg Celluclast® and 2.5 mg Cellobiase/ml, this relationship is described by the equation

$$\ln (\mu\text{g glucose/ ml produced}) = 0.5385 \ln (\text{mg Celluclast®/ml}) + 6.6161,$$

with

$$r, \text{ the correlation coefficient} = 0.9629.$$

In the range of 10 mg Celluclast® and 2.5 mg Cellobiase/ml to 50 mg Celluclast® and 12.5 mg Cellobiase/ml, the relationship is best described by the equation

$$\ln (\mu\text{g glucose/ml produced}) = 0.6864 \ln (\text{mg Celluclast®/ml}) + 6.3276$$

with

$$r = 0.9383.$$

The amount of enzyme present in a solution was then determined by the application of these equations to the amount of glucose produced from a known amount of cellulosic substrate during the assay period.

ACTIVITY ASSAY PROCEDURE

1. Suspend 1.000g Whatman No. 1 paper (shredded dry in blender) in 100 ml 0.1 molar KHP pH 5.0. Stir for 1 hour before assay.
2. For each enzyme solution to be tested, set up replicate groups of two 13 x 100 mm screw-top tubes.

To each tube add: 1.50 ml of Whatman suspension (15 mg substrate)

To one tube (sample blank) add: 1.00 ml Stop Reagent (0.11 molar Na_2HPO_4 , pH 12.5)

To each tube add: 0.50 ml enzyme containing solution

3. Place tubes in preheated 40°C water bath in shaker and incubate 60 minutes with constant shaking at 2 Hz, 2-cm displacement.
4. At end of incubation, add 1 ml Stop Reagent to each of the tubes not previously given Stop Reagent, thereby stopping enzyme reaction.

FERRICYANIDE METHOD FOR DETERMINATION OF REDUCING SUGARS

Prepare glucose standards of 0, 20, 40, 60, 80, and 100 $\mu\text{g}/\text{mL}$ and carry them through the following color procedures.

5. Remove an aliquot from tubes at Step 4 and dilute to 2.0 mL with demineralized H_2O such that glucose concentration is 20 to 100 $\mu\text{g}/\text{mL}$. Add to this 1 mL Stop Reagent.
6. Add 1.5 mL $\text{K}_3\text{Fe}(\text{CN})_6$ to this 3-mL volume in a 13 x 100 mm test tube.
7. Boil tubes for 5 minutes at 100°C . Then hold for 10 minutes at 25°C .
8. Record absorbance at 420 nm.

CALCULATIONS

Apparent concentration ($\mu\text{g}/\text{mL}$) = value from standard curve for
absorbance sample blank - absorbance sample

Actual concentration ($\mu\text{g}/\text{mL}$) = apparent concentration x
 $\frac{3.0}{0.5} \times (\text{dilution at Step 5}).$

GLUCOSE INHIBITION IN ASSAY

The possibility that glucose inhibition might cause a solution of enzyme to be measured at an artificially low value was examined by SYSTECH. Levels of glucose introduced into the assay tubes were routinely measured at less than 0.01 percent. Figure B.3 shows a study of the effect of glucose inhibition of cellulase activity at levels considerably lower than those shown in Figure B.2. It is apparent that at a level of 0.01 percent glucose no significant inhibition of activity is to be expected. Enzyme activity assays are run at such a high dilution that glucose carry-over levels become insignificant.

GLUCOSE UPTAKE BY BACTERIA DURING ASSAY

Bacterial consumption of glucose during the assay period appears to be a significant problem inherent to reducing sugar assays conducted on liquid recovered from dMSW. Examination of the data obtained in this program indicates that a mean recovery of 15 percent of initial load value should be added to the percent recovery actually observed to correct for this loss factor (see Appendix C for further discussion of this topic).

Preliminary work was conducted by SYSTECH to eliminate this error factor from the assay procedure by adding toluene to the assay tubes at the beginning of the assay period. Results of this work have been unsuccessful to date, however, perhaps due to the fact that toluene does not completely

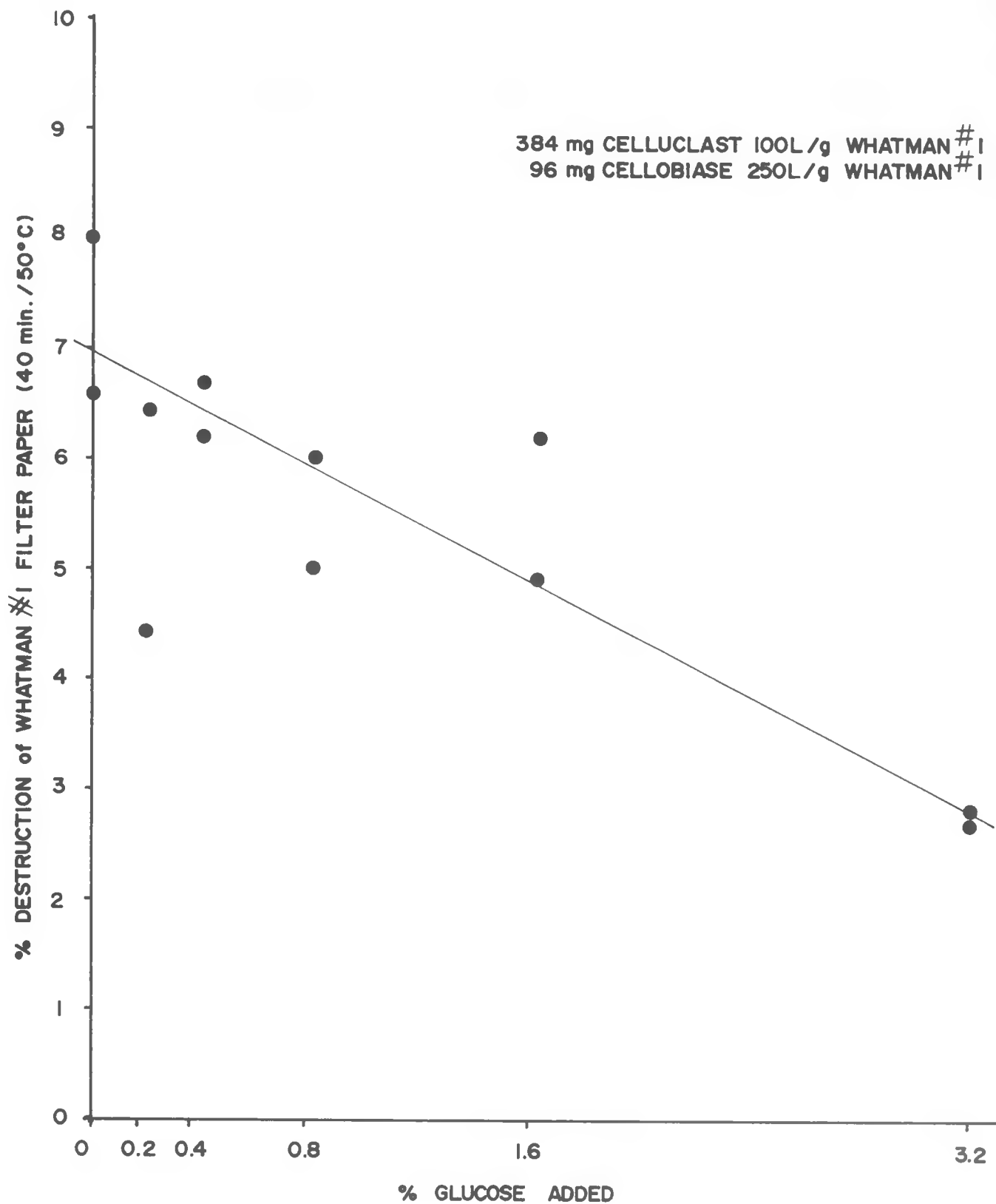


Figure B.3. Effect of low level glucose addition on cellulase activity.

eliminate the potential for removing glucose from solution. Further work is needed to clarify this problem. One approach would involve adding an excess of ATP-ase to the assay tubes, thereby preventing phosphorylation of the glucose and subsequent assimilation.

APPENDIX C

ENZYME RECOVERY TECHNIQUES

FILTRATION PROCESS

Methods used for the recovery of enzyme employed one of two filter media prior to introduction to the ultrafilter: Gelman Type A Glass fiber filter or Polypropylene 2/2 twill filter media with an airflow rate of 14 ft³/min per ft² (Eimco Envirotech). The glass fiber media was found to have an excellent solids removal efficiency, but because it is fragile and easily blinded by enzyme hydrolyzed MSW, it does not appear to be suitable for large-scale application. The polypropylene filter has a decreased solids recovery ability (measured at 38.5 percent during one study), but it is considerably less prone to blinding. Filter yield with this media has been measured at 5.9 lb/ft² per hr, and it provides a filtrate, which, although relatively high in solids, is still suitable for direct introduction to the 200 micron prefilter and then the ultrafilter.

Collection of solids was accomplished by filtering the material through a Buchner funnel under a vacuum of 10 in. of mercury, washing the cake, drying the solids at 105°C, desiccating, and weighing. The filtrate was then introduced directly into the ultrafilter for enzyme recovery. Analyses of concentrates and permeates were performed periodically to verify that the enzymes are confined to the concentrate stream.

STERILIZATION PROCESS

Bacterial uptake of glucose during the assay period is a potentially significant source of an apparent loss of enzyme recoverability. The microbial population introduced into an enzyme reactor by digested MSW is undoubtedly large and can be expected to grow even larger during an incubation period in which reducing sugar feedstock is being liberated by enzyme. This material is then further concentrated by ultrafiltration, leading to a potentially large number of bacteria being introduced into the assay tubes which might then take up glucose as it is being produced, leading to an artificial low measure of enzyme recovery. This problem was addressed at SYSTECH by comparing the measurable glucose production from enzyme solutions containing digested MSW and digested MSW that had previously been sterilized to remove bacterial contaminants.

Two methods of sterilization were applied to remove bacteria before enzyme recovery. One group of digested MSW samples was autoclaved for 1 hour at 15 psi and 121°C before incubation; another group was treated after incubation by shaking the digested MSW with 0.5 ml toluene per 65-ml

reactor for 15 minutes at 40°C to cause cell lysis and effective removal of active bacteria. Both groups of sterilized digested MSW and a nonsterilized group were incubated for 72 hours at 40°C with 960 mg Celluclast® and 240 mg Cellobiase. Enzyme activities were run on the supernatants of each group and on a solution of enzyme alone treated with toluene; the results were computed as percent of recovery of enzyme (Table C.1).

Sterilization techniques lead to a 50 to 100 percent increase of enzyme activity recoverable from supernatants of enzyme reactors. After filtration of the materials, however, there is only a 20 percent increase in recoverable enzyme gained by sterilizing the digested MSW.

ENZYME RECOVERY CLOSURE

An examination of the losses in enzyme activity recovery incurred in the sterilization experiments and size exclusion filter experiments noted elsewhere were conducted to account more closely for the amount of enzyme either not present or not measurable at 1 and 72 hours. Two possible loss mechanisms were hypothesized, and their contribution to low-enzyme recovery values were assessed. One mechanism can be described as the amount not recovered due to losses incurred in filtration. The other mechanism can be attributed to the amount not observed during the assay because of bacterial assimilation of glucose as it is produced.

Values for the amount of glucose production not observed during assays as a result of the operation of these two loss mechanisms were computed and applied as correction factors to the results of assays conducted on enzyme reactors at three enzyme loading levels under varying conditions (Fig. C.1.). Production not observed because of bacterial glucose consumption

TABLE C.1. RECOVERY OF ENZYME FROM STERILIZED DIGESTED MSW

Sample	Percent recovery of enzyme	
	Before filtration	After filtration
Digested MSW control	45	21
Digested MSW-- autoclave sterilized	91	25
Digested MSW control	48	--
Toluene treated MSW	70	--
Toluene treated enzyme solution	100	--

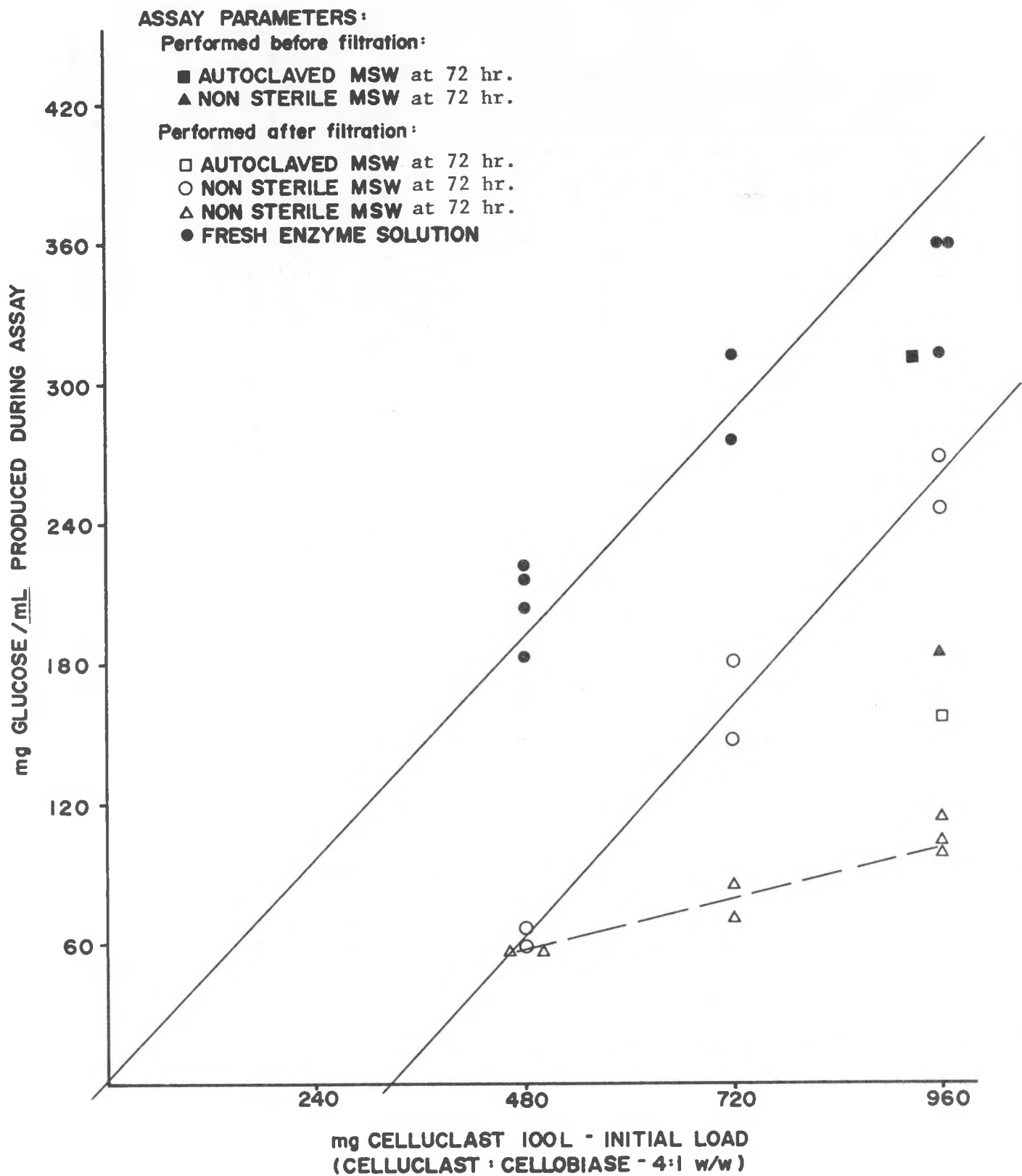


Figure C.1. Glucose production during assay.

can be computed as the difference between glucose production after filtration at an initial loading of 960 mg Celluclast® and 240 mg cellobiase between sterile and nonsterile digested MSW, or 47.0 µg glucose/ml. Production not observed because of filtration loss can be computed as the difference between the amount of glucose produced in assay of the enzyme in buffer solution and the point at which a 1-hour incubation curve predicts zero recovery, 212.5 µg glucose/ml. It is interesting to note that two other data points, the filtration of fresh enzyme through digested MSW and enzyme-treated digested MSW, give values in very close agreement for this parameter: 229.38 and 256.9 µg/ml, respectively.

Table C.2 shows the approach to enzyme recovery closure obtainable by application of these two correction factors. These data show that, at 1 hour, correcting for filtration loss of enzyme accounts for essentially all of the enzyme loaded. Data obtained for 72-hour incubations of digested MSW and enzyme indicate that after filtration essentially all of the enzyme loaded is accounted for by correcting for filtration and bacterial losses. Application of both of these factors simultaneously to 1-hour incubations tends to overcorrect the recoveries, apparently due to the fact that there is some overlap between the correction factors. The filter loss factor undoubtedly contains some correction for bacterial loss also since there are bacteria present in the 1-hour incubation study reactors. Data obtained for 72-hour incubations of sterile MSW indicates that essentially all of the enzyme is accounted for by correcting for filtration loss of enzyme.

TABLE C.2. ENZYME RECOVERABILITY CORRECTED FOR BACTERIAL CONSUMPTION OF GLUCOSE IN ASSAY AND FILTRATION LOSS

Assay conditions			Percent recovery of enzyme	Percent recovery of enzyme corrected for bacterial consumption of glucose	Percent recovery of enzyme corrected for filtration loss	Percent recovery of enzyme corrected for both loss mechanisms
Material assayed	Incubation time (hr)	Enzyme loading mg celluclast/ml: mg cellobiase/ml				
Sterilized dMSW:						
Before						
filtration:	72	3.84:0.96	84	--	--	--
After						
filtration:	72	3.84:0.96	43	--	98	--
Nonsterile dMSW:						
Before						
filtration:	72	3.84:0.96	49	61	--	--
After						
filtration:	72	3.84:0.96	30	43	86	98
	72	2.88:0.96	24	39	89	104
	72	1.92:0.48	22	40	102	121
	1	3.84:0.96	66	79	122	134
	1	2.88:0.96	46	61	111	126
	1	1.92:0.48	21	39	102	120

APPENDIX D

ACHIEVING DIGESTER TEST CONDITIONS

Artificial MSW was anaerobically digested in a 60-l laboratory digester to provide typical digester effluent for this study. This digester was operated within stable operating conditions for several months. During this time it had a stable pH and good gas production with 4-percent solids feed and a 30-day HRT. The conditions required for the first study were 3-percent total solids and a 15-day HRT. In order to avoid shocking the system, a period of gradual change to this HRT was needed. Figure D.1 shows the slow increase in pH, volume of biogas, and percent of methane in the biogas during the adjustment period. When the pH dropped below 6.3, anaerobically digested secondary sewage sludge and sodium bicarbonate were added to increase the methanobacter count and to raise the pH. The digester was run at stable conditions for 6 days before the beginning of the 15-day study.

The second anaerobic digester was initiated with digested secondary sewage sludge. For one complete HRT of 15 days prior to as well as during the 5 percent solids study, this digester was fed glucose permeate from the enzyme reactor; Figure D.2 shows the general conditions of the digester during this start-up period. For 1 week of this period, January 15 through January 22, sodium bicarbonate was used to buffer the daily feed to pH 7. The alkalinity was five times the desired value due to this buffering. The testing program was run from January 30 through February 13, although a buffer problem existed throughout the study, which eventually led to the souring of the second digester about a week after the end of the first testing program.

In preparation for the second study, the feed for Digester I was adjusted to 5-percent total solids on February 14, the day after the first 15-day study was completed. Figure D.3 shows the general conditions during the preparation period. Secondary digested sewage sludge and sodium bicarbonate were added to stabilize the methanobacter culture and to raise the pH during this time. The motor on the digester was inoperable for 3 days, from February 20 through February 22. After the motor was replaced, it was noted that solids were collecting on the bottom of the digester, causing uneven sampling. As a partial remedy, commencing on March 13, the day before the second study was to begin, the digester mixing time was increased from 5 minutes per half hour to 8 hours per day.

Digester II was restarted with digested secondary sewage sludge on February 22. Figure D.4 shows the conditions for this digester after the start-up. Each day's feed was titrated to pH 7 with 5N KOH. The alkalinity held steady so no additional sodium bicarbonate was added.

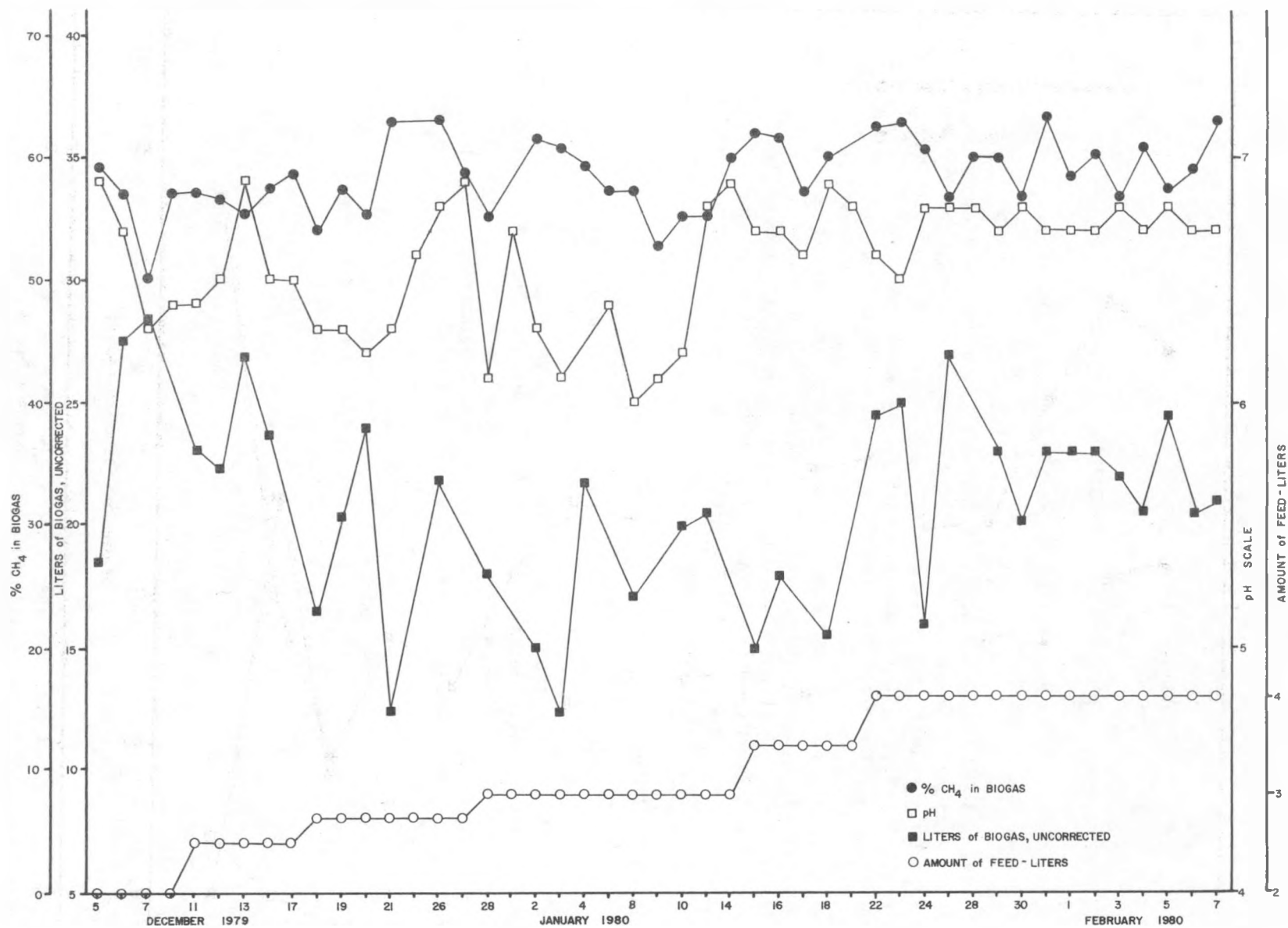


Figure D.1. Achieving Digester I test conditions: 3-percent solids.

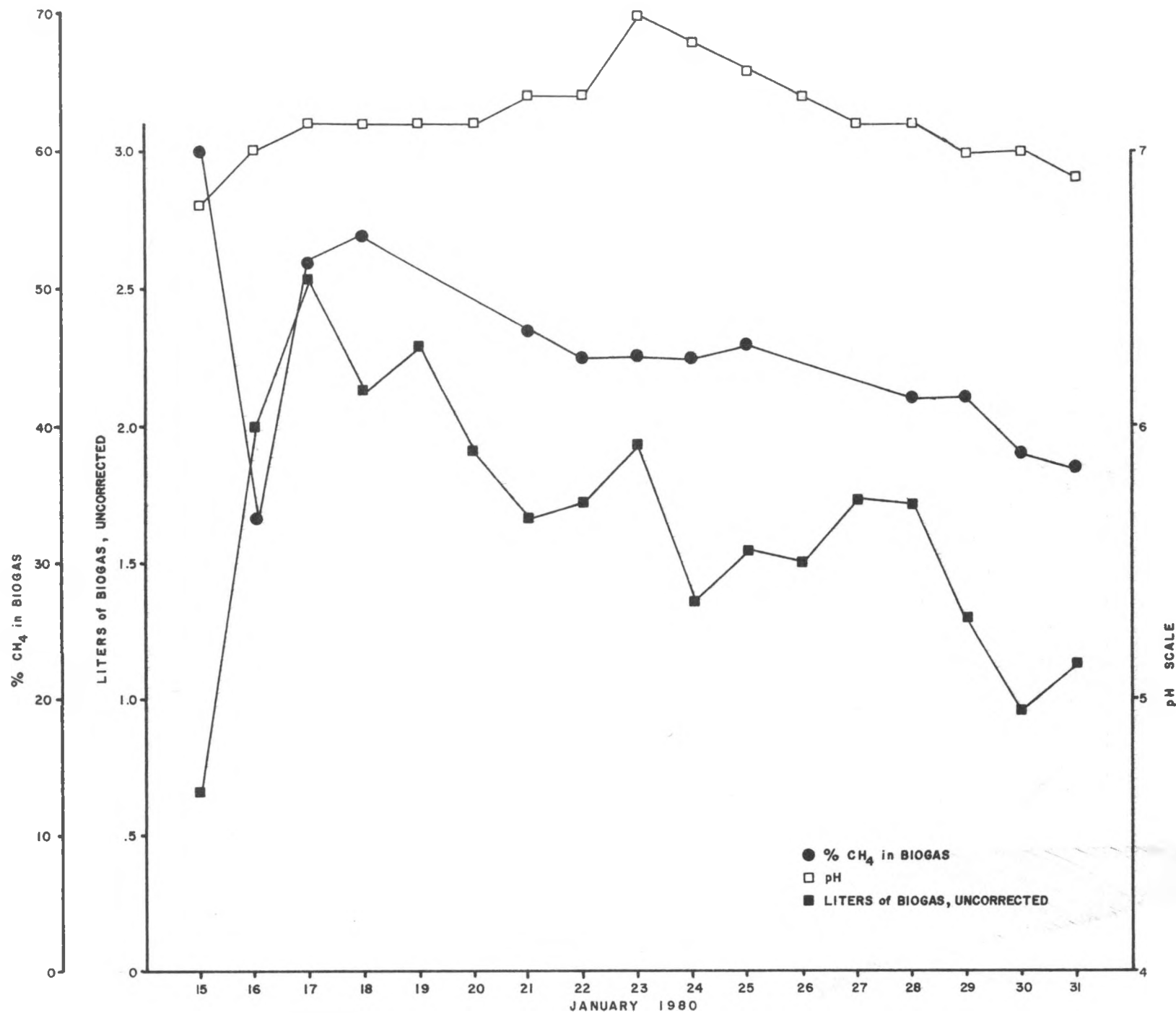


Figure D.2. Achieving Digester II test conditions: 3-percent solids.

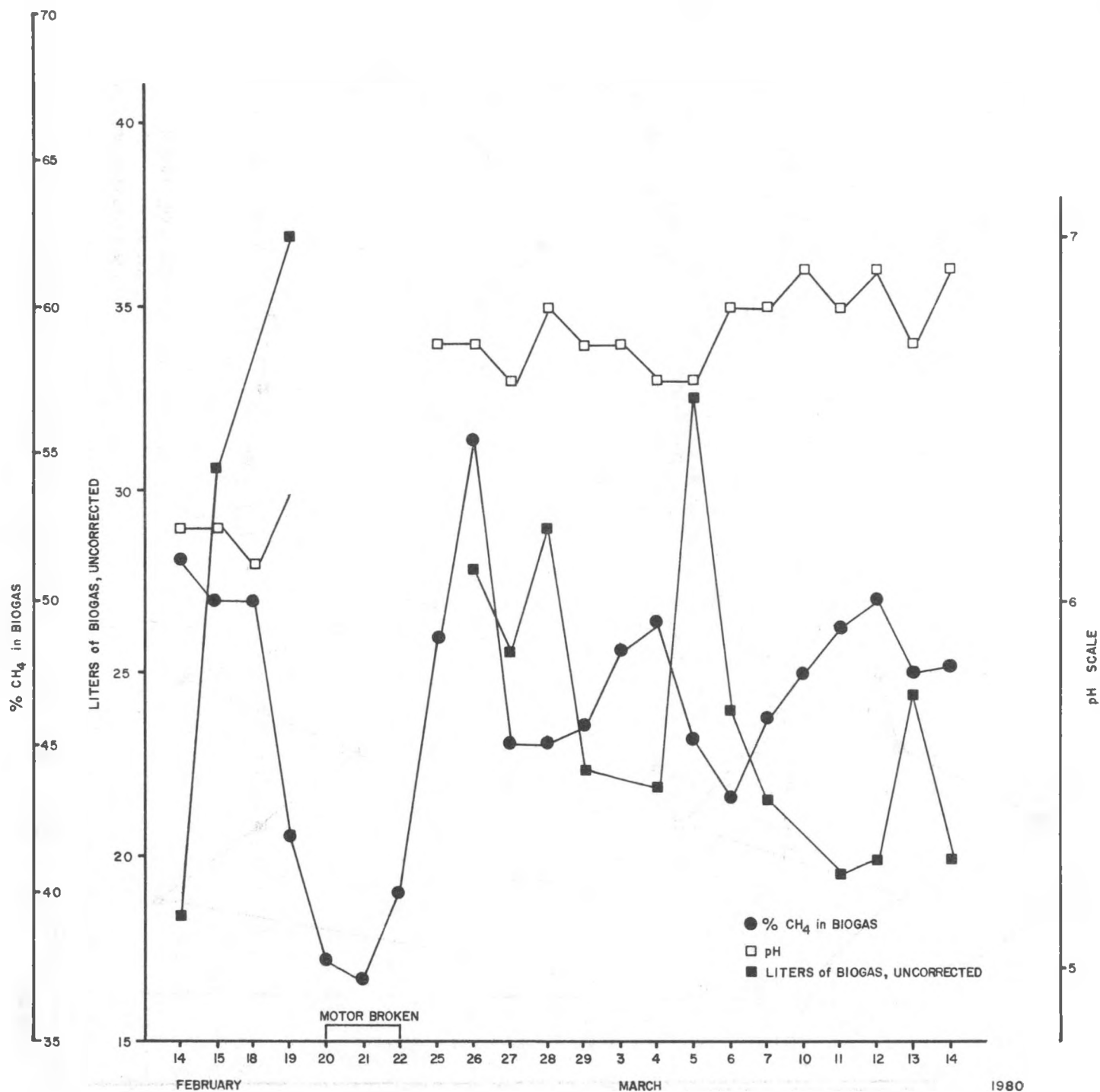


Figure D.3. Achieving Digester I test condition; 5-percent solids.

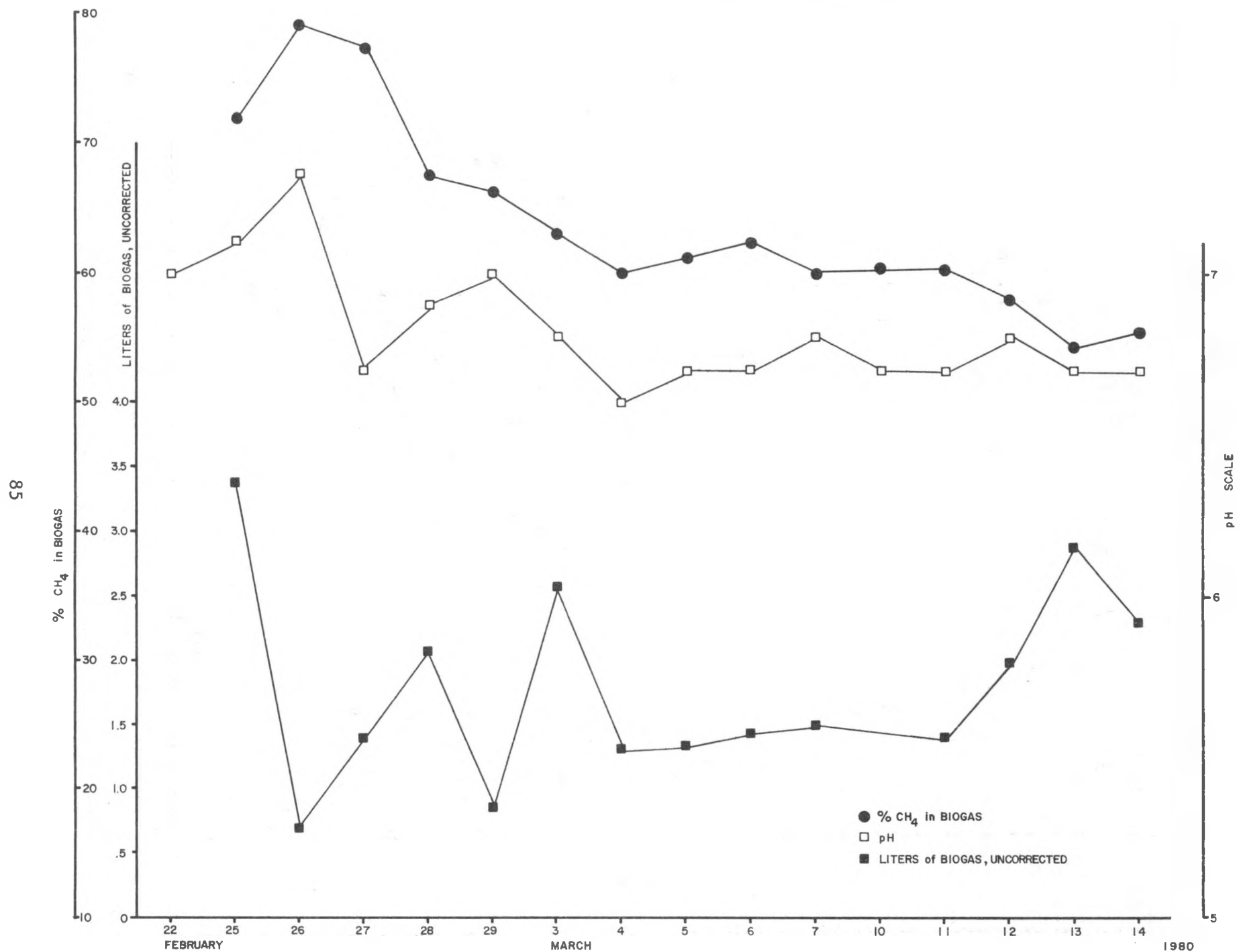


Figure D.4. Achieving Digester II test conditions: 5-percent solids.

APPENDIX E

ENERGY RECOVERY TEST PROCEDURES

Table E.1 and the laboratory process-flow diagram (Fig. E.1) show which analyses were performed at what test points and how often. The letters on the chart correspond to the letters at points on the flow diagram.

TABLE E.1. ANALYSES PERFORMED AT EACH TEST POINT

Type of test	Test points									
	A	B	C	D	E	F	G	H	J	K
Volume	✓		✓	✓	✓		✓	✓		✓
Total Solids	X		✓		✓	✓	✓	✓		✓
Volatile Solids	X		✓		✓	✓	✓	✓		✓
Glucose	X		✓		✓		✓	✓		✓
Suspended Solids	X		✓		✓					
Biogas Composition		✓							✓	
Volatile Acids			✓		✓					✓
Alkalinity			✓		✓					✓
Enzyme Activity	X		✓		✓					
Weight				✓		✓				
Filter Yield						X				
Solids Captured						X				
pH			✓		✓					✓
Gas Volume		✓							✓	
Heat Content	X		X			X				
Nutrients	X		3X				3X			3X

NOTE:

✓=Every Day

X=Once

A=MSW Feed

B=Digester I Gas

C=Digester I Effluent

D=Enzyme

E=Enzyme Reactor Effluent

F=Filter Cake

G=Ultrafilter Effluent Permeate

H=Enzyme Recycle Concentrate

J=Digester II Gas

K=Digester II Effluent

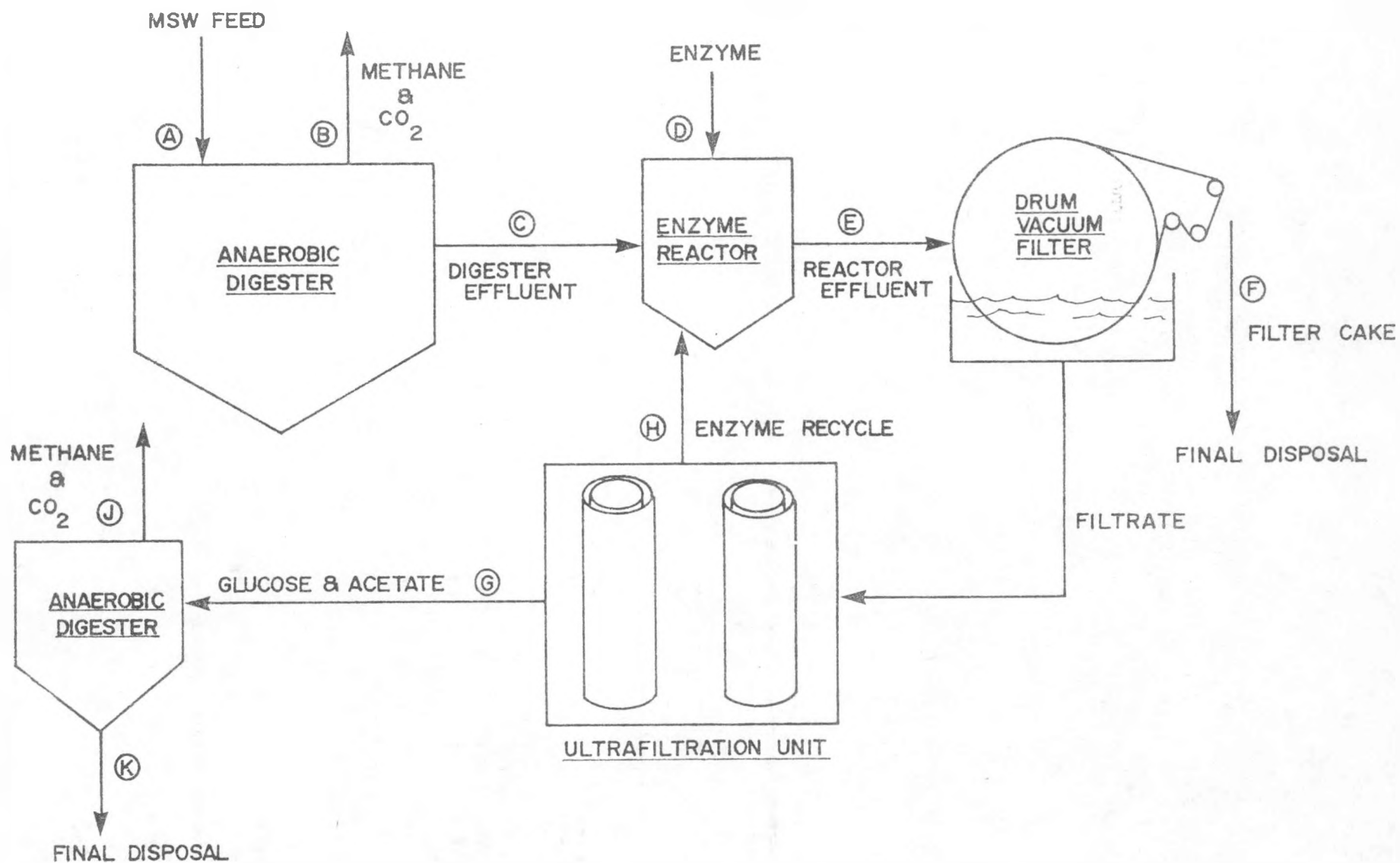
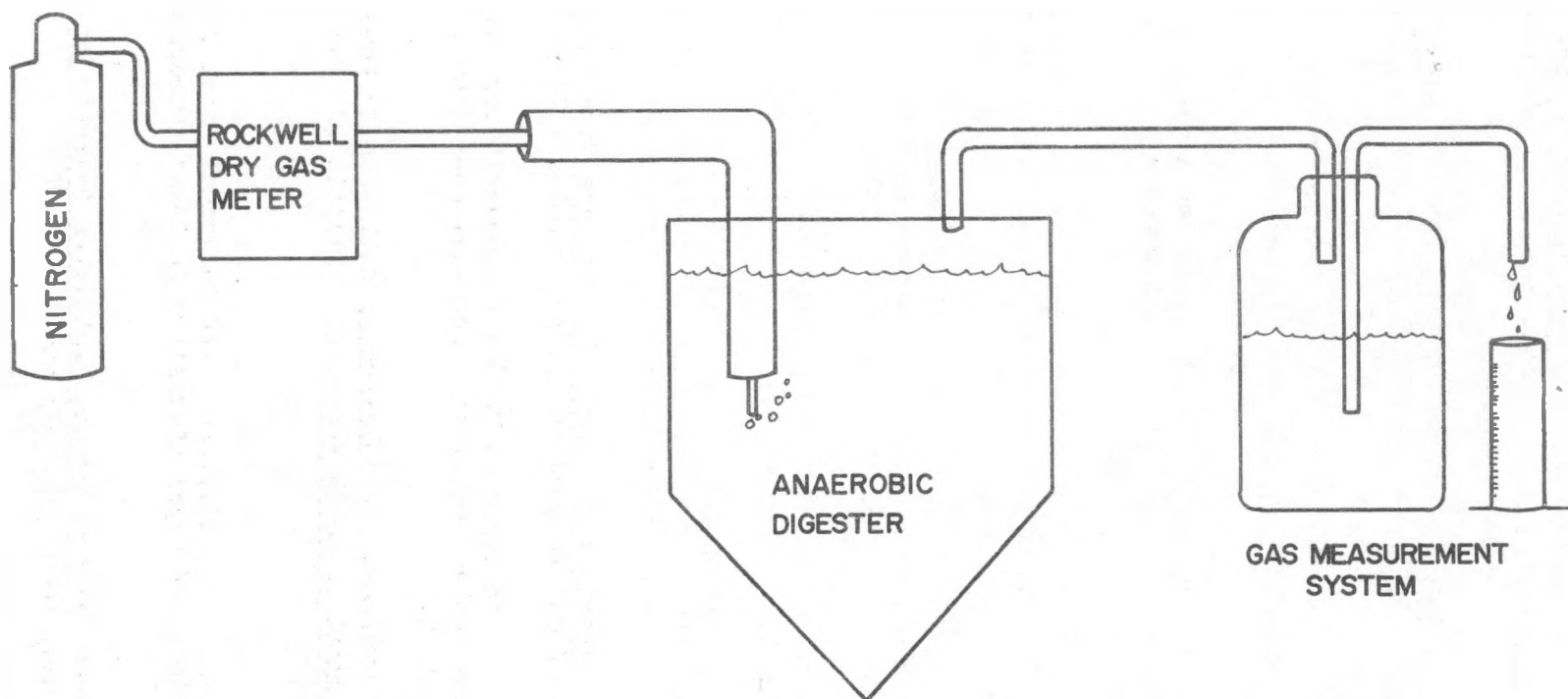


Figure E.1. Laboratory-scale process flow diagram.

The following test procedures were obtained from Standard Methods²: total solids, volatile solids, volatile acids, alkalinity, and total phosphorus. Test procedures obtained from Treatment and Disposal of Wastewater Sludges¹ were: suspended solids, filter yield, and solids capture. Biogas composition was measured on a Varian 1420 gas chromatograph using a Porapak Q column at room temperature connected to a thermoconductivity detector. Ammonia-nitrogen for nutrients was measured by a Hach kit using the procedure in the Hach Company's manual.¹⁴ Glucose and enzyme activity were measured by assays developed at SYSTECH (see Appendices B and C).

The entire digester system, including the gas measuring apparatus, was tested for leaks before and after each testing program by the procedure shown in Figure E.2. Nitrogen, measured by a Rockwell Dry Gas Meter, was pumped into the digester through the feed/sample tube. The gas collection subsystem was designed to maintain an internal pressure very close (± 1 in. of water) to the ambient barometric pressure. The measured nitrogen introduced was, therefore, expected to cause a displacement of an equal volume of water from the gas collection apparatus. Each time the system was tested, the volume of water displaced was equal to the volume of nitrogen introduced plus the volume of biogas normally produced by the digester in the 20-minute test period, thus demonstrating the integrity of the gas measurement system.



AMOUNT GAS IN = AMOUNT WATER OUT

Figure E.2. Diagram of apparatus involved with testing for leaks in system.

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