WEED SEED SURVIVAL IN AN AGRICULTURALLY-BASED ANAEROBIC DIGESTER.

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(ABSTRACT)

Weed seeds contaminating cattle feed can pass unaffected through the animal's digestive tract and may germinate when manure is returned to land as fertilizer. This investigation was undertaken to determine the effects of anaerobic fermentation of raw manure on the viability of Johnsongrass and Fall Panicum seeds which may be present in the waste.

Dairy waste containing Johnsongrass and Fall Panicum seeds were subjected to batch and continuously-fed anaerobic fermentation processes in laboratory-scale digesters. The effect of influent solids concentration (4% and 6%) and retention time (15 days and 20 days) on seed viability was observed. The experiments were carried out in the mesophilic temperature range [35°C±1°C].

Fall Panicum seeds were found to be less resistant to anaerobic digestion than Johnsongrass seeds. Greater seed destruction was achieved in 20-day digesters than in the 15-day digesters. The influent solids concentrations did not have significant effect on seed viability. By applying

the Schafer-Chilcote seed population model, it was observed that the fermentation process was more effective in destroying non-dormant seeds than dormant seeds.

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INTRODUCTION

The application of animal manure to soil has been traditionally thought to increase yield and improve soil physical condition (Herran and Erhart 1965). However, in recent times the ready availability of commercial fertilizers has rendered land application of animal manure a net economic burden for many operators (Shuler, 1980). Nevertheless, when part of crop production, this practice continues to be most appropriate for the disposal of animal wastes (Loehr,1977), and it falls within legal constraints impossed on agricultural operations.

Although it is possible to control land application rates to achieve pollution free operation, it is almost impossible to eliminate the agronomic problem of weed seed transmission associated with this method of waste disposal. Early studies reveal that commercial animal feeds contain weed seeds that pass apparently uninjured through the digestive tracts of farm animals (Harmon and Keim 1934, Oswald 1908, Atkeson et al. 1934, Goss 1924, Hills and Jones 1907). These seeds are consequently returned to land with the manure. Although it can be argued that modern animal feed is of superior quality, silage and freshly cut grass, which constitute an

important part of farm animal rations, contain weed seeds which can be transmitted to cropland where they remain dormant but viable for extended periods of time (Muenscher,1980).

This study was focused on two of the most noxious weeds, Johnsongrass (Sorghum halepense), and Fall panicum (Panicum dichotomoflora), that are found in the feed of dairy cattle. Hagood[1] (1981) found that Johnsongrass was the most difficult weed to control in corn followed by Fall Panicum. In alfalfa, Johnsongrass was the fourth and Fall Panicum the fifth-most difficult weed to control. Johnsongrass in particular is a problem not only in the US but also in Central and South America, Europe, the Mediterranean, Africa, India, the far East and Australia. Therefore, if animal waste is returned to land it is extreamly important that appropriate procedures be developed to eliminate weed seeds in order to control their proporgation.

This investigation was an effort to achieve the destruction of Johnsongrass and Fall Panicum seeds by anaerobically treating dairy waste containing these seeds at mesophilic temperatures [35°C±1°C]. If this procedure is found successful, it will have the advantages of eliminating weed seeds and reducing organic pollutants while preserving the

[1] Virginia Weed Survey, Fall 1981. Dept. of Plant Pathology, VPI & SU. Unpublished data.

fertilizer value of the manure.

The specific objectives of this investigation were:

1. to build and operate bench-scale anaerobic digesters;
2. to determine the effects of anaerobic treatment on the viability of Johnsongrass and Fall Panicum seeds present in animal waste;
3. to identify the level of treatment that has the greatest influence on seed viability, and;
4. to attempt to interpret the changes in weed seed viability using the Schafer-Chilcote buried seed population model.

Chapter II

LITERATURE REVIEW

2.1 ANAEROBIC DIGESTION

The treatment of waste products so as to reduce bulk and render them less obnoxious is becoming more and more necessary in present-day society. The types of waste produced vary widely in their characteristics and pose different levels of disposal problems. Probably the largest portion of the total waste output is organic in nature. Fogg (1980), reported that about 1,800,000 farms in the United States have some type of livestock or poultry. The total amount of dry waste produced in 1978 was approximately 187 million tons (USDA estimation). This reflected an increase of nearly 25% of that produced in 1974. Table 1 gives a breakdown of the different farm wastes. Nearly 40% of the total waste is collectable and therefore readily available for treatment.

Anaerobic treatment has proved particularly effective for concentrated wastes such as those from farms, meat processing plants, and slaughter houses. The advantages of anaerobic fermentation have been enumerated by many investigators.

TABLE 1

U.S. Livestock Manure Production (Fogg 1980).

Types of livestock	Confined %	Total animal units.* 1,000's	Total manure dry wt. 1,000t/yr	Collectible manure dry wt. 1,000 t/yr
Dairy cattle	65	17,800	27,500	17,900
Beef cattle	25	63,000	92,500	23,100
Broilers	99	1,300	3,000	3,000
Other chickens	97	1,700	4,000	3,900
Swine	80	5,800	6,900	5,500
Sheep and goats	13	1,000	1,500	200
Turkeys	68	700	1,700	1,000
Ducks	78	100	100	100
Horses	29	7,300	21,100	6,100
TOTAL	38	98,600	158,200	60,700

* One animal unit = 454 Kg live weight.

McCarty (1964a), Hobson et al. (1974), and Lettinga et al. (1979) emphasized the following benefits:

1. a high degree of waste stabilization is possible at heavy organic loading rates;
2. low production of stabilized sludge;
3. low nutrient requirements;
4. no oxygen requirement, and;
5. the production of a useful end product in the form of methane.

The investigators also listed the following drawbacks of methane fermentation:

1. high temperature requirement for optimal operation;
2. not suitable for dilute wastes, and;
3. slow growth rate of methane producing bacteria.

However, McCarty (1964a) noted that, for concentrated wastes, the advantages of anaerobic digestion far outweigh the disadvantages.

2.1.1 Economic and Technical Feasibility

The fact that organic material, rotting under conditions where it is out of contact with air, will produce a flammable gas has been known for centuries. The technology of anaerobic digestion to extract energy has been developed, based on scientific experience. A variety of farm wastes,

plant residues and combinations thereof have been subjected to anaerobic treatment. The common objectives of all these investigations have been to demonstrate the technical, and economic feasibility of the process from the point of view of energy production and pollution control.

Morris et al. (1975) assessed the economic feasibility of incorporating anaerobic fermentation into the dairy farm both as an alternate energy source and as a waste management practice. Their investigation revealed that energy generated by anaerobic digestion is comparable in cost to conventional energy for a herd size of 1000 dairy cows or more. When compared with a liquid handling system for waste management, anaerobic digestion was found to be economically competitive. A similar herd size for beef feedlots was recommended by Hashimoto and Chen (1980). Their results indicated that anaerobic systems constructed and operated by the farmer would be economically feasible for feedlot sizes between 1,000 and 2,000 head as opposed to 'turn-key' systems which required a much larger herd size of 8,000 head to break even.

In a case study of a farm in central Iowa, Smith et al. (1977) reported that an economically viable system would be one in which, in addition to methane extraction, the digester sludge is recycled to take advantage of the nutrients.

Jewell et al. (1980) conducted a two-year investigation with full scale plug-flow and completely-mixed reactors based on a dairy size of 50-65 animals. They successfully developed and demonstrated a practical and economically feasible system using light, low-cost material and farm labor. The plug-flow system was found to be more economically attractive than the completely-mixed system.

Ifeadi and Brown (1975) computed the average U.S. dairy and beef facility manure production to be 0.1 t/day (dry basis) and concluded that this value was too small to achieve economic benefits. Martin and Loehr (1980) noted that the composition of biogas limits its use to space heating and generation of electricity. Since on-farm electricity demand is constant throughout the year as compared to space heating, generation of electricity was favoured as a biogas utilization alternative.

Harper and Seckler (1975) evaluated the technologies available for the use of manure as fertilizer, as fuel and as livestock feed. Their results revealed that (a) manure as a fertilizer appeals most to the small-scale farmer, (b) the most economical use of manure as a fuel is direct combustion for medium and large scale operations, and (c) utilization of waste as feed is generally more attractive than utilization as a fertilizer or fuel.

Abeles et al. (1975) concluded that although the economic feasibility of biogas digesters for an average farmer cannot be doubted, energy is just one component of a complex ecosystem comprising of both human and natural sources. Hence, a systems study that considers environmental and sociological aspects is necessary. These conclusions were based on studies conducted on three full scale and one pilot scale systems.

Technical feasibility of anaerobic digestion can be achieved by closely controlling certain environmental parameters. Jewell and Loehr (1977) investigated the potential for energy recovery from animal wastes by different methods. Among other factors, the amount of energy that can be extracted by anaerobic digestion is directly influencd by the biodegradable fraction of the waste. Dairy cattle waste is low in biodegradable organics because it has undergone partial degradation in the rumen of the animal. Chicken manure, on the other hand, has the potential to produce much more gas, indicating that a higher amount of biodegradable material is present. Table 2 details the estimated gas production from various animal wastes.

Anaerobic digestion characteristics of dairy, swine and poultry wastes were examined by Grammes et al. (1971). Their results indicated that all three wastes could easily

TABLE 2

Estimated Manure and Biogas Production per 1000 lb. Live Weight (Jewell et al. 1977).

	Dairy cattle	Beef cattle	Swine	Poultry
Manure Production (lb/day)	85	58	50	59
Total solids (lb/day)	10.6	7.4	7.2	17.4
Volatile solids (lb/day)	8.7	5.9	5.9	12.9
Digestive efficiency (% of VS)	35	50	55	65
Gas production (ft^3/lb VS added)	4.7	6.7	7.3	8.6

(lb X 0.454=kg; ft^3/lb X 0.062=m^3/kg)

be subjected to anaerobic fermentation. Dairy waste was found to be the most amenable in terms of high loading rates and low detention times. These results were substantiated by the investigations of Converse et al. (1977) who examined the fermentation of dairy manure by varying the temperature, detention time and composition of the waste. The experiment high lighted the ease with which dairy waste could be successfully subjected to anaerobic digestion.

In another study Varel et al. (1977) reported that at 60°C, the methane fermentation of cattle waste proceeded at the maximum rate. The system was observed to be stable even with variations in temperature, retention time and volatile solids loading.

In a pilot scale investigation of swine waste digestion, stable conditions were encountered at loading rates ranging from 2.4 to 2.88 kg VS/m^3. Fisher et al. (1975) conducted this experiment at mesophilic temperatures and found that the gas production rate was 0.99 m^3/kg VS destroyed. In another investigation with the same waste, Kroeker et al. (1975) obtained process stability and high rates of gas production in spite of high pH and ammonia concentrations.

The possibility of applying the concept of dry anaerobic digestion to dairy and poultry wastes was explored by Wong-Chong (1975). Results indicate that this method is well

suited for fresh dairy waste. Ammonia inhibition resulted with poultry manure. Dry anaerobic fermentation offers improved economics due to reduced reactor volume.

2.1.2 Biochemistry and Microbiology

2.1.2.1 The Two-stage Scheme

Since the 1930's methane fermentation was thought to involve only two major metabolic groups of bacteria. McCarty (1964a), Gloueke (1977), Metcalf and Eddy (1979), and Price and Cheremisnoff (1981) found the two stage process convenient to express the chemistry, microbiology and kinetics of anaerobic fermentation.

A simplified illustration of the two-stage scheme is presented in Figure 1. In the first stage, complex organics such as fats, proteins and carbohydrates are acted upon by a group of facultative anaerobic bacteria, and biologically converted to simpler products, namely organic acids. The bacteria responsible for this conversion are termed acid-forming or hydrolytic bacteria. The main functions of the hydrolytic bacteria are (a) to produce suitable enzymes to hydrolize the substrate extracellularly, and (b) to break down the hydrolyzed substrate intracellularly to simpler compounds. Chemically this step is one of hydrolysis and fermentation, in which a small portion of energy is released

for growth and a small portion of the organic waste is converted to cell mass.

The first stage merely converts organic matter to volatile fatty acids- a form suitable for the second stage of treatment. McCarty (1964a) listed as many as seven volatile acid intermediates. However, McCarty (1964a), and Toerien and Hatting (1969) have concluded that acetic and propionic acids are the most important intermediates in methanogenesis. McCarty et al. (1963) agreed that the fermentation of carbohydrates, proteins and fats produced acetic acid, while propionic acid was a product of carbohydrate and protein fermentation. Other volatile acids occurred in low concentrations only.

It is in the second stage of methane fermentation that true waste stabilization occurs. Organic acids are acted upon by a group of bacteria called methane-formers and converted to methane and carbon dioxide which are the most reduced and the most oxidized one carbon compounds.

2.1.2.2 The Three-stage Scheme

Research by Bryant et al. (1977), and McInerney et al. (1981) indicated that a three-stage scheme for anaerobic fermentation (Figure 2) best fits the current information.

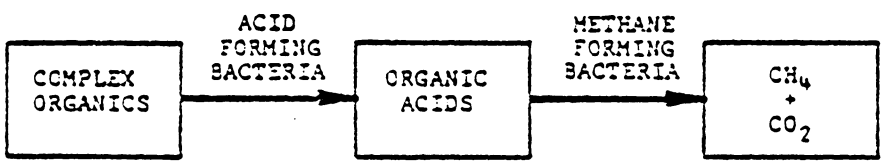

Figure 1: Two-Stage Anaerobic Fermentation Scheme.

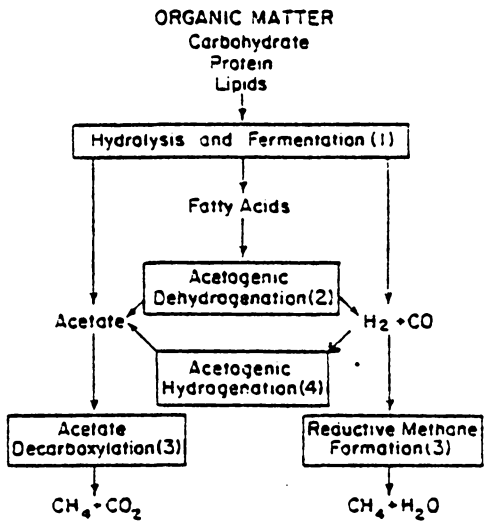

Figure 2: Three-Stage Anaerobic Fermentation Scheme (Bryant et al. 1977).

The first stage is the same as the two-stage scheme. In the second stage, a group of bacteria called obligate hydrogen- producing acetogenic bacteria, obtain energy for growth by producing acetate, hydrogen and sometimes carbon dioxide from the products of the first stage. Although the fermentative and hydrogen-producing bacteria play key roles in methane production, it is the methanogenic bacteria (the group that mediates the third stage) which has attracted much attention. This may be because (a) this group of bacteria directly produces methane, thereby stabilizing the waste (Hobson et al. 1974), (b) these microorganisms are fastidious anaerobes; hence a knowledge of their nature is of importance for succesful anaerobic fermentation, and (c) methanogenic bacteria are popularly considered to be slow growing, thus making the third stage the rate-limiting step.

The range of substrates available to methanogenic bacteria has been narrowed to hydrogen, carbon dioxide, methanol, formate and acetate (Bryant et al. 1967). Buswell and Mueller (1952) postulated that methane is probably formed by two mechanisms: the first is by direct acetic acid cleavage which can be represented chemically as:

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (1)$$

and the second is by carbon dioxide reduction:

$$CO_2 + 8H^+ \rightarrow CH_4 + 2H_2O \qquad (2)$$

This theory is also supported by McCarty (1964a).

The importance of methanogenic bacteria and acetate has been illutrated by McCarty (1964a). His scheme for the anaerobic fermentation process (Figure 3) indicates that methane-formers are responsible for most of the acetic acid formed and this acid in turn accounts for nearly 70% of the gas produced. The percentages are based on chemical oxygen demand (COD) conversion.

Mountfort and Asher (1978) concluded that 72% - 90% of the methane liberated orginated from acetate. Their work was confined to bovine waste. Jeris and McCarty (1962), Smith and Mah (1966), and Zeikus (1977) have also stressed the importance of acetate in methane fermentation.

Although acetate is an important substrate, only a few bacteria that convert acetate to methane and carbon dioxide have been isolated. Smith (1966) observed counts of acetate-utilizing bacteria 10 to 100 times less than hydrogen-utilizing bacteria. Hobson et al. (1974) while working with swine waste incubated for 4 weeks, could not account for acetate-utilizing bacteria. They concluded that prolonged incubation periods were needed to isolate acetate-utilizing cultures.

Decomposition rates of various substances were investigated in the laboratory by McCarty (1966). He reported that

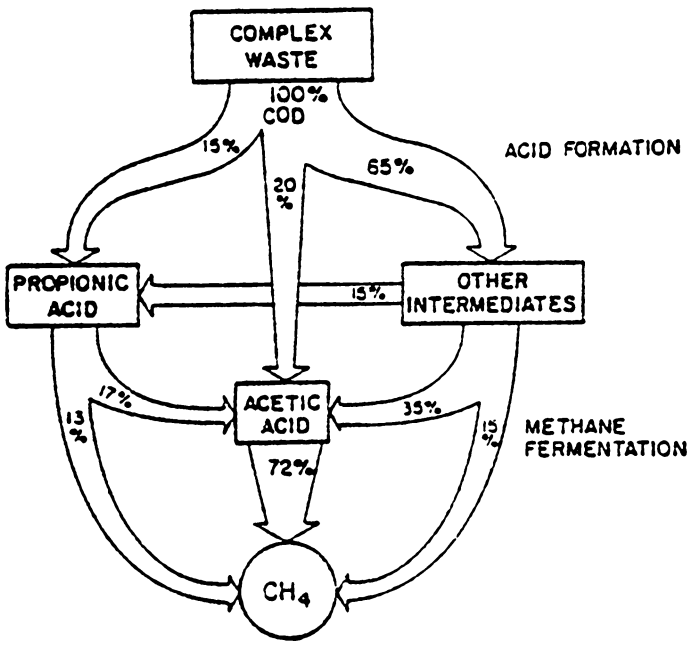

Figure 3: Pathways in Methane Fermentation (McCarty 1966).

at low solids retention time (SRT), most of the methane was produced from formate and methanol. At low SRT, washout of acetate-utilizng bacteria occurred. However, since acetate and propionate together account for approximately 85% of the methane formed, longer SRT values are required for complete breakdown of wastes. Thauer et al. (1977) state that the amount of energy released when carbon dioxide is reduced to methane is approximately the same as that liberated when formate is utilized. The amount of free-energy change associated with the reaction converting acetate to methane and carbon dioxide is very small. The slow growth rate of acetate-utilizing bacteria may be attributed to the small amount of free energy liberated (McInerney and Bryant 1981).

Ohwaki and Hungate (1977) noted that the amount of acetic acid formed was much more than could be accounted for by the reduction of organic matter; however, it was approximately what would be expected from stoichiometric considerations if hydrogen and carbon dioxide were substrates. This suggests the presence of a fourth group of bacteria (acetogenic hydrogenation bacteria) which was included in the anaerobic fermentation schemes presented by McInerney and Bryant (1980), Zeikus (1979), and Hashimoto et al. (1980a).

Very little work has been done in isolating corresponding bacteria in the thermophilic range. Hashimoto et al.

(1980b) speculated that three metabolically different groups of bacteria are again involved. Converse et al. (1977), Zeikus and Wolfe (1972), and Zinder and Mah (1979) were some of the investigators who were successful in characterizing thermophilic bacteria.

The bacterial populations in anaerobic digestion have been characterized and reported by many investigators. Table 3 summarizes work done by different individuals.

Although three distinct stages of anaerobic fermentation have been identified, these actually proceed simultaneously. Further, the microorganisms responsible for the reactions have been observed to exhibit substrate preference (Russel and Baldwin 1978). Hence, it is not surprising that more than one species of bacteria are necessary to complete a particular stage of the reaction.

2.1.2.3 Regulatory Action of Hydrogen

Toerien et al. (1969), Bryant (1979), and McInerney et al. (1981), among others, identified hydrogen as a major end product of stage-one digestion and as a regulator of methane fermentation. McInerney et al. (1981) proposed two sites during the anaerobic fermentation process at which hydrogen exercises a regulatory action.

TABLE 3

Typical Bacterial Populations in Anaerobic Digesters.

Group	Numbers	Reference
Hydrolytic bacteria	10^8 - 10^9	Kirsch (1969) Mah and Sussman (1967) Toerien and Siebert (1967)
Acetogenic bacteria	10^6	McInerney et al.(1978)
Homoacetogenic bacteria	10^5 - 10^6	Ohwaki and Hungate (1977)
Methanogenic bacteria	10^6 - 10^8	Smith (1966)

Bryant (1979), and McInerney et al. (1981) suggested that the partial pressure of hydrogen in the ecosystem controls the proportions of various products of fermentative bacteria by a phenomenon termed 'interspecies hydrogen transfer reactions'. At low partial pressures of hydrogen, pyruvate is degraded to carbon dioxide and acetate, while at high partial pressures ethanol, butyrate, propionate, hydrogen and carbon dioxide are formed. In a well operated digester, low hydrogen concentrations (and hence low hydrogen partial pressure) is maintained by the hydrogen-utilizing (methanogenic) bacteria which consume hydrogen as it is formed. High hydrogen concentrations occurs when the system is stressed.

The second site at which hydrogen demonstrates a regulatory action is in the conversion of ethanol to methane. According to Bryant et al. (1967), this fermentation is a result of a synergistic association of two species of bacteria. One of these, called the acetogenic S organism, converts ethanol to acetate and hydrogen. The hydrogen so produced inhibits the growth of S organisms. Removal of hydrogen is brought about by a methanogenic (hydrogen-utilizing) bacteria which cannot use ethanol, but which use hydrogen and carbon dioxide to produce methane. Hence, when the two organisms are combined, they are mutually dependent upon

each other. Lactate, propionate and butyrate have also been observed to be degraded by acetogenic bacteria in association with methanogenic bacteria.

McInerney and Bryant (1980) computed the partial pressures of hydrogen below which the degradation of a given substrate becomes favorable. Any increase in the partial pressure will terminate the degradation of propionate first, followed by that of butyrate and ethanol. This is confirmed by the investigations of McCarty et al. (1963), McCarty (1964b), Varel et al. (1977), and Pohland et al. (1963), which revealed that the first acid to accumulate during digester failure was propionate. Hence, it is evident that hydrogen concentration not only regulates the proportion of end products of the fermentation bacteria, but also regulates the degradation of these products by the hydrogen-producing acetogenic bacteria and hydrogen- utilizing methanogenic bacteria.

2.1.2.4 Constituents of Digester Feedstock

The waste supplied to a digester is complex in nature. The major constituents of digester feedstock and their degradation products are presented in Figure 4. Dairy cattle manure is composed mainly of cellulose and hemicellulose (Hills and Roberts 1980). Other minor sources of carbohyd-

rates are bacterial cells, gut secretion, and cells sloughed off intestinal walls. Undigested plant fibers are always present in animal feces. These fibers consist of simple sugars linked together to form polysaccharides (Hobson et al. 1981). During hydrolysis, the simple sugars are released and are utilized by the digester bacteria.

According to Hobson et al. (1981), Downing et al. (1980), Toerein and Hatting (1969), Bryant (1979), and McInerney and Bryant (1981), degradation of protein proceeds via the extracellular hydrolysis, whereby peptides are formed. These are broken down into their component amino acids before being absorbed into the bacteria. Intracellular degradation follows resulting in the production of ammonia, carbon dioxide and volatile fatty acids. Non-protein nitrogen compounds like urea, are rapidly degraded to ammonia and are used by bacteria as an energy source.

The source of lipids in animal waste is vegetable matter. However, the death of intestinal bacteria present in the feces may also contribute to the amount of lipids in the waste. Chynoweth and Mah (1971) reported that carbohyderate, protein and lipids reduction to fatty acids were 13%, 36% and 76% , respectively.

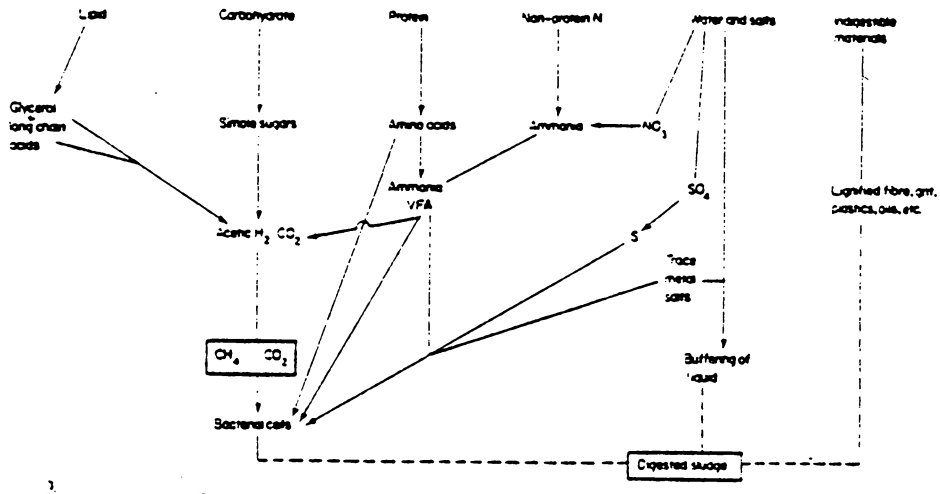

Figure 4: The Main Reactions Occuring in an Anaerobic Digester (Hobson et al. 1981).

2.1.2.5 Nutrient Requirements of Bacteria

In general, the composition of bacteria is about 90% water. The dry weight of bacterial material is composed of nitrogen (10%), carbon (50%), hydrogen (10%), and oxygen (20%). In addition, inorganic matter in the form of metallic salts, small amounts of metals, various cell structures, and sulfur also are present.

Bryant (1974) stated that most rumen organisms have very simple nutritional requirements. Cell carbon is obtained from different sources by different bacteria. Some obtain it directly from the substrate carbohydrate while others obtain cell carbon from such sources as amino acids, carbon dioxide, acetic acid or higher volatile fatty acids (Hobson et al. 1981). Ammonia or amino acids are the nitrogen sources for the bacteria (Bryant et al. 1971, Zeikus 1977, Hashimoto et al. 1980b). McInerney and Bryant (1981) included peptides, urea and nitrate as possible nitrogen sources. Sulfide or cysteine serve as sulfur sources for some bacteria. In addition, most strains need one or more B-vitamins for growth (Shuler 1980).

2.1.3 Kinetics of Anaerobic Fermentation

Kinetic theory on the continuous steady-state growth of pure cultures of microorganisms has been well established. A single species of microorganism is assumed to be involved. Biological waste treatment processes are, however, much more complex and require many different species of microorganisms. The kinetics of pure culture growth can be used to describe that of a complex process when one step in the process is significantly slow to govern the rate of the overall process. Hence, it is possible to determine the kinetics of anaerobic treatment by evaluating the kinetics of the rate-limiting step.

According to Price and Cheremisnoff (1981), there are four potential rate-limiting steps in the anaerobic fermentation process assuming the presence of sufficient nutrients, substrate and microorganisms:

1. conversion of cellulose to soluble sugars by extracellular enzyme;
2. formation of volatile acids by acid-forming bacteria;
3. conversion of volatile acids to carbon dioxide and methane by methane bacteria; and
4. transfer of dissolved products from the liquid to the gas phase.

The investigations of Pfeffer (1968, 1978), Hobson et al. (1974), Pfeffer and Liebman (1976), Robbins et al. (1979), Dekker and Richards (1973), Grant et al. (1978), Hackett et al. (1977), and Kirk (1975) indicate that hydrolysis and fermentation of cellulose are the rate-limiting steps. Ghosh and Klass (1978) have established that the slow growing methane bacteria control the overall reaction. This fact is now widely accepted.

Heukelekian et al. (1951) have developed an empirical expression which describes the net growth of microorganisms as a function of time following mixing with organic wastes. A similar expression which can be applied to a continuous-flow, completely-mixed anaerobic treatment system is given as follows:

$$dX/dt = Y\,(dF/dt) - bX \qquad (3)$$

where,

dX/dt = net growth rate of microorganism per unit volume of digester, mass/volume/time;

dF/dt = rate of waste utilization per unit vlume of digester, mass/volume/time;

X = microorganism concentration, mass/volume;

Y = growth yield coefficient, and;

b = microorganism decay coefficient, $time^{-1}$

The rate of waste stabilization can be approximated by an expression similar to that used by Monod (McCarty 1966) to describe the relationship between the concentration of a limiting nutrient and the growth rate of microorganisms:

$$dF/dt = kXS/(K+S) \qquad (4)$$

where,

k = maximum rate of waste stabilization per unit weight of microorganism occuring at high waste concentration, $time^{-1}$;

S = waste concentration in the reactor, mass/volume; and

K = half velocity coefficient, mass/volume.

combining equations (3) and (4) yields:

$$\mu = (dX/dt)/X = [YkS/(K+S)] - b \qquad (5)$$

Where μ is defined as the specific growth rate.

In a continuously-fed system, steady state will eventually be reached when the mass of microorganisms in the total system remains constant. At this point, the specific growth rate will equal the ratio of microorganisms wasted to the microorganism in the system. For design purposes the reciprocal of the specific growth rate is more convenient to use and is called the biological solids retention time (SRT) or,

simply, retention time if solids are not recycled. The biological solids retention time is directly related to the quality of the effluent and, hence, treatment efficiency.

As the SRT is decreased, the effluent waste concentration increases. Eventually at a minimum SRT (SRT_m) value, the treatment process fails. At this point, $S=S_o$, and 'wash-out' occurs. When the influent waste concentration (S_o) is large enough to be non-growth limiting, equation (5) reduces to:

$$\theta = SRT_m = (Yk-b)^{-1} \qquad (6)$$

The minimum SRT is thus a function of the fraction of organic waste converted to biological cells, Y. The value of Y is much lower in anaerobic treatment than in aerobic treatment. For this reason, other factors being equal, SRT_m for anaerobic treatment is much larger than for aerobic treatment.

The quality of the effluent can be predicted by performing a mass balance. It is assumed that the concentration of microorganisms in the influent is low enough to be neglected and that steady state conditions prevail within the digester. The effluent microorganism and substrate concentrations are then expressed by equations (7) and (8):

$$X = [Y(S - S)]/[1+k\theta\ ] \qquad (7)$$

$$S = [K(1+b\theta)]/[\theta\ (Yk-b)^{-1}] \qquad (8)$$

These two equations demonstrate the influence of SRT on effluent quality and, therefore, the efficiency of treatment. For a specified waste, a biological community and a particular set of environmental conditions, the kinetic coefficients Y,K,k, and b can be defined. Consequently, the effluent waste concentration S, is a direct function of SRT. Similarly, for a given influent concentration, S_o, the effluent microorganism concentration, X, is also a function of SRT.

2.1.4 Prediction of Gas Production

Since gas production is a result of substrate break down, it is possible to predict the quantity of gas evolved by computing the substrate destroyed. According to Sykes (1975), the most published chemical formulae for biomass is $C_5\ H_7\ NO_2$. The oxygen demand of biomass can be computed by considering its degradation as shown in equation (9):

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + 2H_2O + NH_3 \qquad (9)$$

From equation (9), the theoretical oxygen demand of the biomass is 1.42 gm oxygen/gm cells. During anaerobic fermentation, a part of the substrate utilized is used for cell

synthesis and, therefore, is not available for methane production (Benefield and Randall 1980). At steady-state conditions, the amount of biomass produced is equal to the amount of biomass wasted each day. Hence, if dX is the biomass produced (g/day), and dS is the COD or BOD_u removed (g/day), then, the total gas production (G) is represented as:

$$G = G_o (dS - 1.42dX) \qquad (10)$$

where,

G_o = gas produced per gram of COD oxidized = 0.35 l/gm

The value for G_o is obtained by considering the oxygen equivalent of methane.

2.1.5 Inhibition and Toxicity

Inhibition has been defined as a rate reduction while toxicity is the cessation of fermentation. The onset of inhibition leading to toxicity usually proceeds by the following sequence of events:

1. reduction in methane content and the total volume of gas produced;
2. increase in volatile acid concentration;
3. depression of pH, and;
4. cessation of substrate fermentation.

Kugelman and Chin (1971) listed the important factors that determine the magnitude of toxicity in an anaerobic digester. They are concentration, antagonism, synergism, complex formation of substances and acclimation of microorganisms to toxic material.

McCarty (1964c) observed that 'toxic' and 'inhibitory' are relative terms and are strongly dependent on the concentration of the substance in question. At very low concentrations, the substance stimulates activity. As the concentration increases, the rate of biological activity decreases until inhibition sets in. At this point, the rate of biological activity is less than that achieved in the absence of the material. A further increase in the concentration brings about complete cessation of the process. The concentrations at which stimulation, inhibition and cessation occur, varies with the toxin. However, it should be recognized that microorganisms have a certain ability to adapt to inhibitory concentrations of some substances.

Antagonism is frequently defined as a reduction of the toxic effect of one substance by the presence of another. Synergism is an increase in the apparant toxicity of one substance caused by the presence of a second (Kugelman and Chin 1971).

It is obvious that only materials in solution can be toxic to biological growth. If not in solution, they cannot gain entrance to the cell. Complex formation is a method of removing a substance from effective solution. The process of precipitating a toxic material is an example of complex formation, and is used for the removal of certain heavy metals.

Acclimation is the phenomenon by which the biological population adjusts to the adverse effects of toxins. According to Kugelman and Chin (1971), in waste the toxic substances build up slowly, hence acclimation is possible. McCarty and McKinney (1961) found that Ca, Mg, Na and K were much more toxic if added on a slug-basis than when added slowly over a period of time.

2.1.5.1 Heavy Metals

Early studies of Hotchkiss (1923) established the stimulating and toxic effects of heavy metals. Kugelman and McCarty (1966) suggest that each cation may be responsible for enzyme activation in an organism. Eventually, when all the enzymes are activated, any further increase in the cation concentration will only harm the enzyme. At this point the cation becomes toxic. McCarty and McKinney (1961) concluded that on a weight basis the monovalent cations were

more toxic than the divalent cations. The investigation of Kugelman and McCarty (1965) indicated a different trend. When added on a molar basis, the divalent cations were more toxic than the monovalent cations.

The effect of heavy metals like cadmium, chromium, nickel, zinc, copper and lead on anaerobic fermentation has been reported by Hayes and Theis (1978), Matsumoto (1978), Mosey (1976), and Kugelman and Chin (1971).

Lawrence et al. (1965) maintain that it is the total concentration of heavy metal present, rather than the concentration of individual metal, that is of importance. Heavy metal toxicity results when the total concentration exceeds the equivalent concentration of sulfide present for precipitation.

In anaerobic digestion, control of heavy metals is by a complex-type reaction, which involves the precipitation of their sulfides. McCarty (1964c) states that sulfides in anaerobic treatment can result from, (a) introduction of sulfides with the raw waste, and/or, (b) biological production in the digester from the reduction of sulfates and from protein degradation.

In swine waste digesters, copper was not found to be a problem as it was almost entirely removed as copper sulfide (Hobson et al. 1981). It has been reported that the amount

of copper present in a swine waste anaerobic digester operated at an SRT of 10 days, and loaded at rate of 10% solids, would be about 85mg/L. This amount can be totally precipitated by the sulfides in the digester (Hobson and Shaw 1976).

Hao (1978) concluded sulfide is the controlling factor in the solubility of nickel only. The carbonate ion controls zinc, and possibly cadmium and lead, while the hydroxyl ion controls copper.

2.1.5.2 Volatile Acids

The effect of volatile acids produced during decomposition of complex organics is to reduce the pH. However, under balanced conditions, methane fermentation results in the destruction of volatile acids and reformation of bicarbonate buffer. Under unbalanced conditions, the acid formers outpace the methane formers, and volatile acid build up occurs (McCarty 1964a).

McCarty and McKinney (1961), Bryant et al. (1971), Kugelman and Chin (1971), and Hobson and Shaw (1976) have reported that acetic and butyric acids are comparatively non-toxic to anaerobic digestion. On the other hand, propionic acid is inhibitory. The investigations of Andrews (1969), Kugelman and Chin (1971), Hobson et al. (1976), and Cooney and

Ackerman (1975) further established the inhibitory nature of propionic acid.

Early studies resulted in more than one school of thought regarding the actual effect of high volatile acid concentration on the digestion process. Cassel and Sawyer (1959), and Kaplovsky (1951) believed that high acid concentration is the result of low activity of methane producing bacteria. They surmised that volatile acids are toxic to these organisms only in an indirect manner through the reduction of pH, a condition which can be relieved by the addition of buffering material. Shulze and Raju (1958), Mudler et al. (1959), and Andrews (1969) on the other hand concluded that it is the concentration of volatile acids rather than pH that directly inhibits methane formers. The toxic condition in this case can be relieved by reducing the organic load, or by dilution.

McCarty and McKinney (1961) presented a different theory of volatile acid toxicity. They postulatd that toxicity was mainly dependent on the type and concentration of the cation of the volatile acid salt. If volatile acid build-up exceeds the natural buffer capacity of the digester, the pH will drop due to the increase in hydrogen ion concentration. Addition of an alkaline material is then needed to replace the toxic cation. If the cation used to replace the hydro-

gen ion is also toxic, the condition will not improve, although pH would have increased. For instance, the addition of sodium hydroxide on a slug basis to neutralize the acid will result in sodium toxicity.

Any change in pH due to the change in volatile acid concentraiton is accompanied by changes in the concentraton of ionized and un-ionized forms of the acids as illustratd by the following equation:

$$CH_3COOH \rightarrow CH_3COO^- + H^+ \qquad (11)$$

As pH decreases, the equilibrium shifts to the left and the un-ionized volatile acid (UVA) concentration increases. Andrews (1969) observed that the inhibitory effect of volatile acids at high concentration is due to the un-ionized portion. Kroeker et al. (1979) reported a trend towards digester failure as the UVA concentration increases above 10 mg/L. The cell membrane is much more permeable to the un-ionized molecule, hence this form is more inhibitory. In conclusion, the concentration of the UVA is a function of both pH and total acid concentration. But, pH, being a log function, has a greater influence.

2.1.5.3 Ammonia

Ammonia, although necessary in digesters as a nitrogen source for bacteria, can be toxic when present in excess. Sievers and Brune (1978), Kroeker et al. (1979), Lapp et al. (1975), Stevens and Shulte (1979), and Converse et al. (1977) have all concluded that ammonia inhibition is a significant problem in the fermentation of ammonia-rich wastes such as those of swine and poultry.

As in the case of volatile acids, pH has an effect on the equilibrium equation for ammonia.

$$NH_4^+ \rightarrow NH_3 + H^+ \qquad (12)$$

When the hydrogen ion concentration is sufficiently high (pH of 7.2 or lower), the equilibrium is shifted to the left, so that inhibition is related to the ammonium ion concentration. At higher pH levels, the equilibrium shifts to the right, resulting in free ammonia inhibition. Although ammonia gas (free ammonia) is considered more toxic than ammonium ion (McCarty 1964c, Kroeker et al. 1979), it is the total ammonia concentration that is of interest in digester control.

Ammonia-nitrogen concentrations between 200 and 500 mg/L do not cause adverse effects on methane formation. The literature reveals conflicting results on the effect of ammoni-

a-nitrogen concentration in excess of 1500 mg/L. McCarty (1961, 1964c) stated that ammonia-nitrogen concentrations between 1500 and 3000 mg/L are inhibitory at pH levels above 7.4, whereas, ammonia-nitrogen concentrations in excess of 3000 mg/L are supposed to be toxic at all pH values. These threshold levels were confirmed by Hobson and Shaw (1976). On the other hand, many investigators reported satisfactory anaerobic digestion at ammonia-nitrogen concentrations far beyond 1500 mg/L, even in the pH range 7.5 - 8.0. Melbinger and Donnellon (1971) observed this phenomenon in the digestion of concentrated sewage sludge, while Converse et al. (1977), Fisher et al. (1977), Gramms et al. (1971), Hobson and Shaw (1976), Kroeker et al (1979), Lapp et al. (1975), Van velsen (1977), and Hart (1963) used animal wastes for their investigations. Melbinger and Donnellon (1971), and Lapp et al. (1975) attributed successful digestion at high ammonia-nitrogen concentration to acclimation and/or cation antogonism.

2.1.5.4 Hydrogen Ion Concentration (pH)

The heterogenous bacterial population in an anaerobic digester comprises of a wide variety of bacterial species. Each specific species has an optimum pH range in which its growth is best, and its metabolic processes function at op-

timum levels. In anaerobic digestion, the optimum pH range is the integral result of the contributions by the different reactions taking place. McCarty (1964b), reported that methane production proceeds quite well as long as pH is maintained between 6.6 and 7.6, with an optimum range between 7.0 and 7.2. At pH values below 6.2, toxicity is acute (Hashimoto et al. 1980b), while at pH levels greater than 7.6 the production of free ammonia at high organic loading rates causes inhibition (Jewell et al. 1976).

Halderson et al. (1973) pointed out that pH is dependent upon alkalinity and volatile acids concentrations. It changes relatively little except when the buffering capacity of the system is exceeded. Capri and Marais (1975) observed that in the optimum pH range for anaerobic digestion, the buffer capacity is almost totally dependent on the carbonic acid dissociation; volatile fatty acids and ammonia exert negligible buffer action.

McCarty (1964b) noted that under anaerobic conditions at near neutral pH, the carbon dioxide-bicarbonate system is the major chemical system that controls pH. The relationship between pH and carbonic acid is illustrated by the equation:

$$[H^+]\ [HCO_3^-] \rightarrow K\ [H_2CO_3] \qquad (13)$$

where, K is the first ionization constant for carbonic acid. Figure 5 shows the relationship between pH and the chemical species concentrations of the buffer system in anaerobic treatment.

The carbonic acid concentration is related to the percentage of CO_2 in the digester gas. According to Pohland and Bloodgood (1963), when the volatile acid concentration is very low, the bicarbonate alkalinity is approximately equivalent to the total alkalinilty for most wastes. However, when volatile acids build up, they are neutralized by the bicarbonate alkalinilty and in its place form volatile acid alkalinity. Under these conditions, the total alkalinity is composed of both bicarbonate alkalinity and volatile acid alkalinity. Hence, the bicarbonate alkalinity available can be approximated by the following formula:

$$BA = TA - 0.833\ TVA \qquad (14)$$

where,

BA=bicarbonate alkalinity, mg/L as $CaCO_3$;

TA=Total alkalinity, mg/L as $CaCO_3$;

TVA=total volatile acid concentration mg/L as acetic acid.

The factor 0.833 converts the total volatile acid concentration (mg/L as acetic acid) to alkalinity (mg/L as $CaCO_3$) contributed by volatile acid salts.

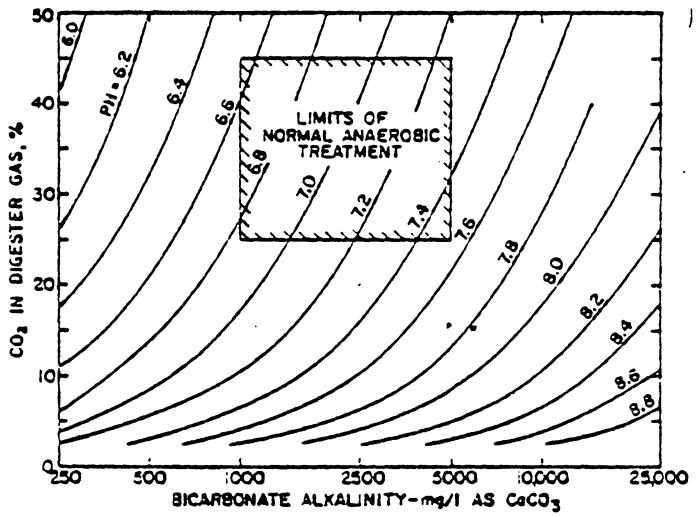

Figure 5: Relationship Between pH and Bicarbonate Concentration (McCarty 1964c).

McCarty (1964c) later added a factor of 0.85 in the TVA contribution to account for approximately 85% of volatile acid alkalinilty which is measured in titration to an end point pH of 4.0

The alkalinity consumed by the acids is essentially replaced when they are degraded by methane bacteria. Hence under steady-state conditions in a continuously-fed fermenter, no net change in buffereing capacity will result. Under unbalanced conditions, the inherent buffer capacity may not be sufficient to neutralize the acids formed. It has been demonstrated that rising alkalinity in the digester tends to increase the operating level of the volatile acids over and above those values commonly considered maximum for anaerobic fermentation process. Halderson et al. (1973) sugested that a bicarbonate alkalinity range of 2,500 to 5000 mg/L would provide good buffering capacity under unbalanced anaerobic conditions.

Sievers and Brune (1978) and Kroeker et al. (1979) reported the importance of ammonia in buffering animal waste fermentation. Digesters operating on substrates with low C/N ratios became highly buffered and more stable under varying organic loadings as compared to digesters fed with high C/N substrate. This was attributed to the action of ammonia which increased the buffering capacity and pH of the solution.

2.1.6 Temperature Effects

Temperature has a pronounced influence on the digestion process. In an early study, Fair and Moore (1934) concluded that there were four distinct temperature zones of microbial activity: thermophilic (above 42°C), intermediate (28°C - 42°C), temperate (10°C - 28°C), and cryophilic (below 10°C). In a recent work, Downing (1980) suggestd three temperature ranges: Psychrophilic (5°C - 25°C), mesophilic (25°C to 38°C), and thermophilic (50°C- 60°C). The mesophilic and thermophilic ranges are of interest in the treatment of agricultural, municipal and domestic wastes.

Temperature affects the rate of waste utilization and hence the minimun solid retention time. De Renzo (1977) reported that the rate of reduction of volatile acids was greater at 45°C than at 35°C. The influence of temperature on the period of digestion is illustrated in Figure 6.

Pfeffer (1966) found that when the temperature was reduced from 35°C to 25°C over a 10-day period, the rate of gas production dropped. In another investigation, Pfeffer and Liebman (1976) reported that the volatile solids destruction at 60°C and a 4-day SRT was greater than at 40°C and a 30-day SRT.

The dependence of gas production on temperature of the fermentation process is illustrated in Figure 7. The opti-

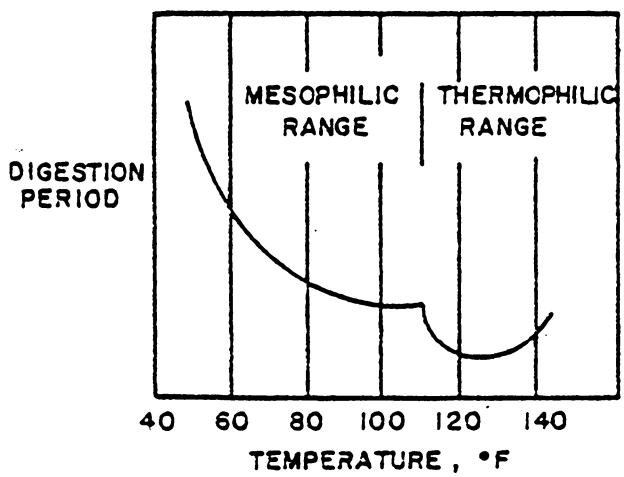

Figure 6: Influence of Temperature on Digestion Time (McCarty 1964c).

mum mesophilic and thermophilic temperatures are 40°C and 60°C, respectively (Price and Cheremisnoff 1981).

Cooney and Wise (1975) compared mesophilic and thermophilic digestion of domestic solid waste, and concluded that the gas produced was greater for the thermophilic range. Similar results were presented by Malina (1962). Garber (1977) stated that thermophilic anaerobic fermentation results in lower numbers of potentially pathogenic bacteria in the treated effluent.

The stability of the anaerobic process is closely related to temperature fluctuations. Fisher and Greene (1945) found that over a short period of time a temperature fluctuation of 3°C can affect the digester performance significantly. Golueke (1958) stated that unstable conditons due to abrupt temperature change result only if the microorganism populations in the digester are not well established.

2.1.7 Plant Tissue

2.1.7.1 Characteristics

Since the investigation presented herein concerns destruction of weed seeds, a discussion of the characteristics and biodegradability of plant material is in order.

Plant and all organic matter are composed of cells, the structure and organization of which are illustrated in Figure 8.

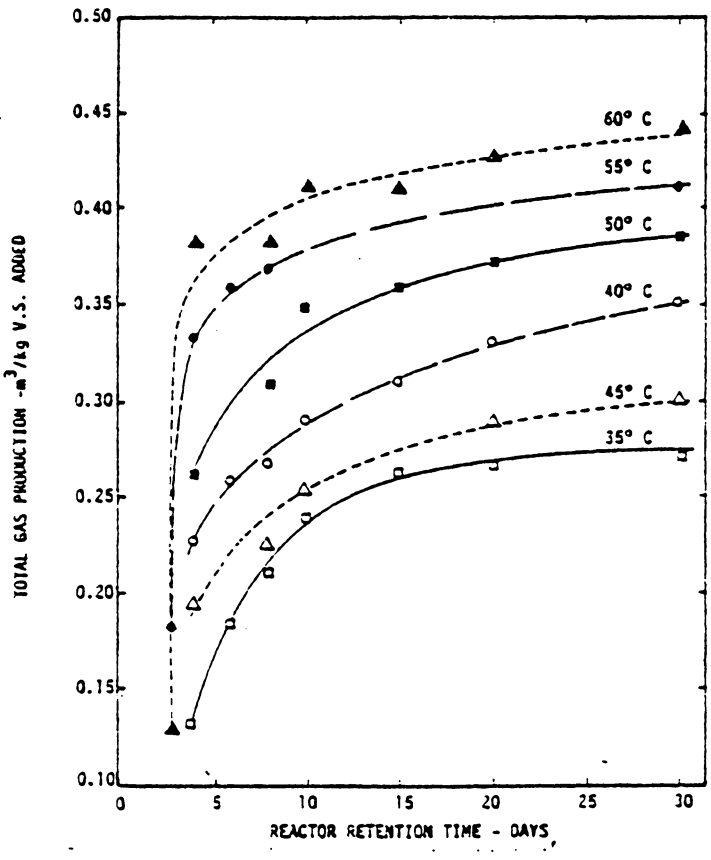

Figure 7: Influence of Temperature on Gas Production (Pfeffer et al. 1976).

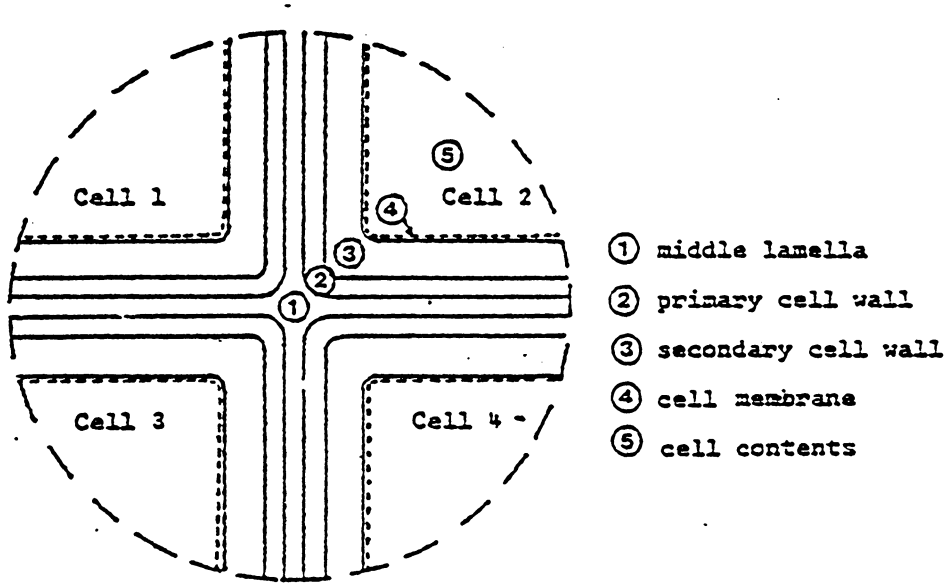

Figure 8: Organization of Plant Cells (Hagglund 1949).

The middle lamella maintains the integrity of the multicelled structure and contains pectin and lignified cells. The primary cell wall is composed of cellulose, while hemicellulose and cellulose make up the secondary wall. The three-layered cell membrane controls the flow of materials from the environment into the cell and vice versa. Cell contents consist of cytoplasm and nucleus, and are composed of starch,nucleic acids, proteins, and sugars. Pectin, cellulose, hemicellulose and starch are plant carbohydrate. In addition, plant tissue may contain cutin, silica and other minerals. Cutin and silica, in particular, may be present in seed coats.

Pectin is a polysacchraide and is closely related to hemicellulose. Hemicellulose is a mixture of polysacchride and is acid-soluble. Cellulose is the most abundant organic matter on earth (Cowling and Bromn 1969). About 20% to 40% of plant dry matter is cellulose. Lignin is a noncarbohydrate. It gives structural support to plant cell walls, and its' presence has a profound effect on organic matter degradation.

2.1.7.2 Biodegradability

In order for the bacterial cell to assimilate the substrate, it must be placed in solution (Kugelman et al.

1971). This process is performed by the hydrolytic enzymes. Buswell and Halfield (1936) performed a carbon mass balance on thirteen water-soluble carbohydrates undergoing fermentation, and accounted for nearly 100% as methane, carbon dioxide and bacterial cells. Van Soest (1967) concluded that at pH 7.0, cell wall composition did not affect the extent of fermentation of organic matter.

The biodegradability of cellulose and hemicellulose is greatly affected by the presence of lignin. Lignified cells may encrust or entrap nutrients, or resist cellulolytic enzymes by the presence of lignin-carbohydrate linkages (Van Soest, 1979). Goering and Van Soest (1970) observed a strong correlation between the extent of cell wall fermentation by rumen bacteria, and cell wall lignin content. Hackett et al. (1977) using radio-labelled lignin in a variety of substrates, showed no degradation of lignin after 41 days.

Chandler and Jewell (1980) applied the analytical procedures and feedstuff digestibility model developed by Van Soest (1979) for development of a rapid, low-cost method of assessing substrate biodegradability in anaerobic digesiton. A wide variety of biomass was batch digested in laboratory fermentation at 35°C. Their results indicated a strong relationship between extent of volatile solids destruction and

substrate lignin content. A reduction in volatile solids destruction with increasing lignin content was reported.

2.2 JOHNSONGRASS AND FALL PANICUM

2.2.1 History and Distribution

Johnsongrass is believed to be native to the Mediterranean region. Closely related forms were thought to have originated in India, the Malay peninsula and the Philippines. It is generally agreed that Johnsongrass was introduced into the United States in the nineteenth century. Its distribution across the world is illustrated by Figure 9.

As can be seen from Figure 10, Johnsongrass is well established in the United States and is considered a troublesome weed throughout the southern half of the country, extending as far north as central New York, New Hampshire and Vermont in the East, and Southern Oregon in the West.

The history of Fall Panicum is not well documented. However, it has been reported in Canada, Argentina, Bangladesh, New Zealand, Brazil and Hawaii. Figure 11 illustrates its distribution in the United States. It has been reported throughout the U.S. except parts of the North Central and Northwestern States and Texas.

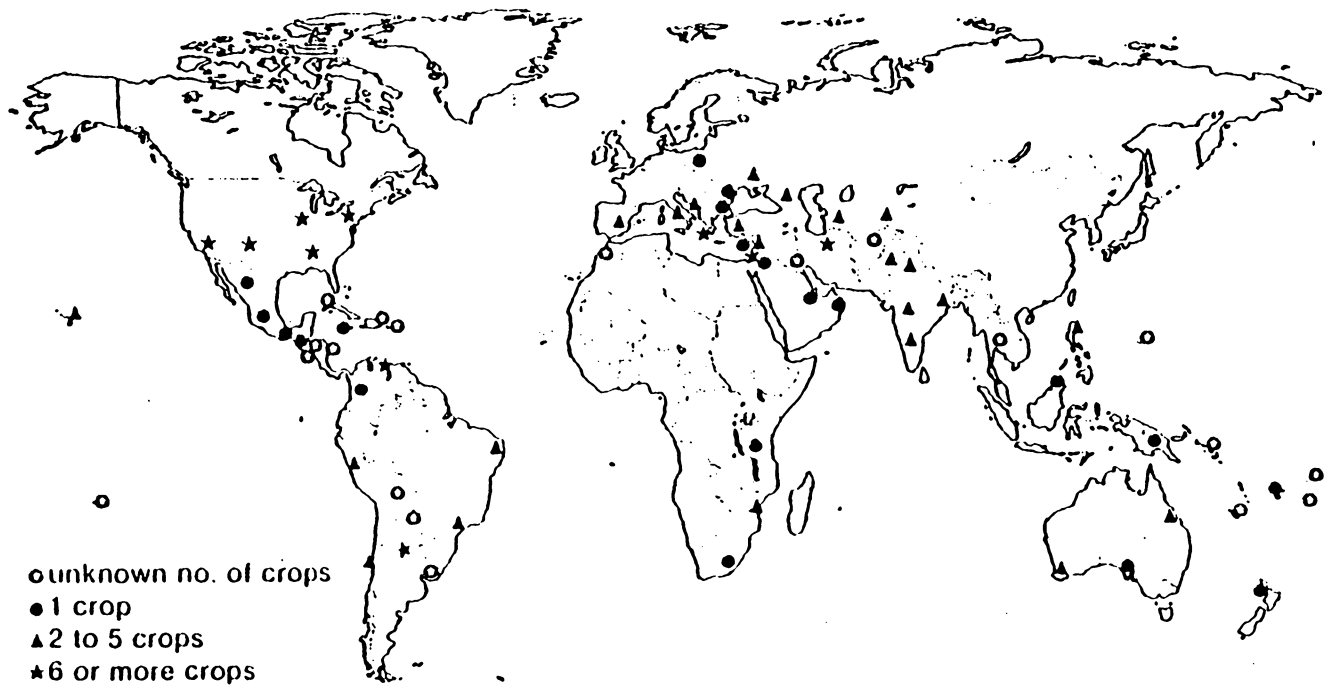

Figure 9: World Distribution of Johnsongrass (Holm et al 1977).

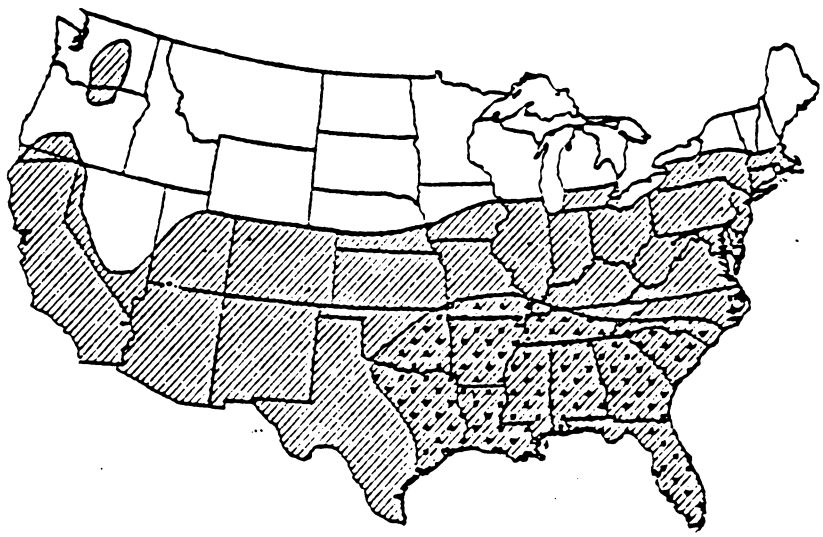

Figure 10: U.S. Distribution of Johnsongrass (ARS. USDA. 1971).

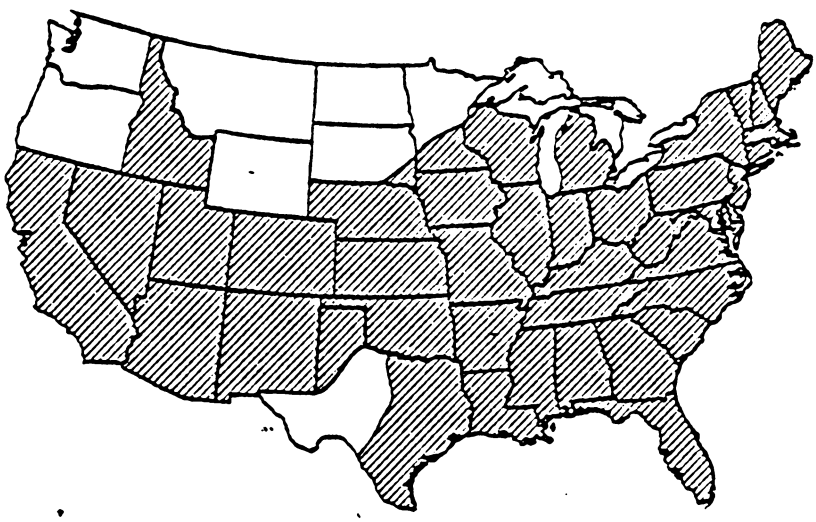

Figure 11: U.S. Distribution of Fall Panicum (ARS. USDA. 1971).

2.2.2 Damage Caused

Johnsongrass is classified as a perennial weed that is propagated by seeds and underground rhizomes. The seeds are disseminated by floods, birds, cattle and sale of unclean agricultural seeds. The rhizomes appear approximately 40 to 50 days after planting. Mattioli (1977a) observed that one Johnsongrass plant produced 40 metres of rhizomes and 700 viable seeds during one growing season. Stamper (1957) estimated that Johnsongrass produced up to 13.6 metric tons or 241 kilometers of rhizomes per acre. The presence of such an extensive network of underground rhizomes coupled with the production of numerous viable and dormant seeds has made Johnsongrass one of the ten worst weeds in the world. It is identified as a weed in 22 countries (Holm et al. 1979). In the U.S. it is considered as one of the three most serious weeds in corn and sugarcane (Holm et al. 1977), and also affects soybean and sorghum. Dale and Chandler (1979) concluded that yields of corn were inversely proportional to the number and weight of Johnsongrass plants that competed with the crop.

Iqbal and Aziz (1975) observed that Johnsongrass acts as a host to insects like grasshoppers which attack cultivated crops. It is also believed to be responsible for the spread of maize dwarf mosaic virus (Knoke et al.1981). It has been

observed that Johnsongrass leaves sometimes contain hydrocyanic acid (HCN) - a cyanide type poison. Any factor which interrupts normal growth may cause the release of HCN within plants. Rapid growth of new leaves, wilting due to drought, frost, freezing cutting or trampling are the most dangerous events. Livestock eating poisoned plants may die (Parker 1972).

Fall Panicum, though not as noxious as Johnsongrass, is an economically important weed. Its' survival ability was confirmed by Burnside et al. (1981) who found no significant loss in germination over a 10 year burial period. Fall Panicum invades corn, cotton, sorghum, soybeans, tobacco and other crops and tends to be more widespread than Johnsongrass. Ritter and Lewis (1977) reported that Fall Panicum infestations significantly lowered corn yields and corn biomass production. Vengris (1978) found that Fall Panicum together with large crabgrass represented 90% of total weed population in field trials where yields of silage corn decreased.

Losses caused by weeds exceeds losses caused by other agricultural pests, and is estimated to be 13.7 billion dollars annually in the U.S. (Burnett 1980). In Virginia, Johnsongrass and Fall Panicum are considered the most costly weeds in corn, and the most troublesome weeds in sorghum

(McCormick 1977). They are also commonly found in soybeans and cotton as well. The most recent estimate of losses due to weeds in Virginia is given in Table 4.

The data presented in Table 4 reflect the total loss due to all weeds that affect the crops shown. Of these, Johnsongrass and Fall Panicum are the most important.

2.2.3 Control Measures

Chemicals provide the most common method of controlling weeds including Johnsongrass and Fall Panicum. Timely application of the right herbicide or herbicide mixtures gives good control of Fall Pancium (Ilnicki and Michieka 1979, Parochetti 1979, Fletchall 1978, Vengris 1977).

Extensive investigations have been reported on chemical control of Johnsongrass. These studies were concerned with the effect of herbicides on the Johnsongrass plant (McWhorter 1963, Hauser and Thompson 1959, Egley and Williams 1978, Wiese et al. 1979, Veseky et al. 1980), and on its rhizomes (Millhollan 1979, Nelson 1979). Pienkowski and Kok (1976) highlighted the possibility of biological control of Johnsongrass. Researchers have reported that improved control is possible when chemical measures are combined with cultural methods (Diaz De Cespedes 1976, Jeffery and Overton 1978, Mattioli 1977b, 1978). The aim of cultural practices is to

TABLE 4

Losses Caused by Weeds in Virginia (McCormick 1977).

Item	Corn	Soybeans	Sorghum	Cotton
Cost of herbicide	3,814	1,547	162.5	27
Loss of yield	2,400	2,250	40	20
Loss of quality	100	105	2	10
Loss in extra land preparation & cultivation.	880	1,450	72	10.5
Loss in land value	120	20	2	5
Loss in increased cost of harvesting	500	300	20	4
Total Value	7,814	5,672	298.5	76.5

Values expressed in thousands of Dollers.

cut up the rhizomes and bring them to the surface where they can dry. Dale and Chandler (1979) concluded that application of herbicide combined with a maize - cotton - maize crop rotation effectively controlled Johnsongrass.

In spite of positive results of these and other investigations, Fall Panicum and Johnsongrass continue to be troublesome weeds. This is mainly due to the development of herbicide - resistant varieties. Fletchall (1978) reported that Atrazine gave excellent control of Fall Panicum when first used in Missouri, but now gives poor control. Thompson et al. (1971) observed that Fall Panicum breaks down 50% of Atrazine hence making it ineffective. Repeated application of one herbicide for many years results in herbicide-resistant varieties (Parochetti 1979). The level of herbicide tolerance of weeds may depend on past herbicide usage. Continuous use of one herbicide raises the level of its tolerance by repeatedly killing less suceptible strains, whereas more resistant strains survive.

2.3 SEED DORMANCY AND VIABILITY.

Failure of the seed to germinate when given suitable conditions for germination may be either due to dormancy or to loss of viability. Dormancy refers to a condition in a viable seed which prevents it from germinating when supplied

with factors normally considered adequate for germination. Dormancy is a temporary phenomenon which could be reversed artifically, while viability is a degenerative change which is irreversible and represents the "death" of the seed. Basically viability refers to the capability of a seed to develop into an acceptable seedling even under conditions which may not be entirely ideal, such as commonly encountered in the field (Moore 1972).

2.3.1 Types of Seed Dormancy

Harper (1957) aptly used the parapharase, "some seeds are born dormant, some achieve dormancy and some have dormancy thrust upon them", to describe the three types of dormancies: "innate", "induced" and "enforced". Alternatively, innate dormancy has been termed as primary (Crocker 1916), natural (Brenchly and Warrington 1930), inherent (Bibbey 1948), and endogeneous (Schafer and Chilcote 1969); induced dormancy is often recognized as secondary dormancy (Crocker 1916); and enforced dormancy has been described as environmental dormancy (Bibbey 1948).

The term innate describes the dormancy which is present immediately when the new embryo ceases to grow but when it is still attached to the parent plant. Such dormancy prevents the seed from germinating for some time after shed-

ding. A large variation of dormancy periods amongst seeds from a single plant is normal (Roberts 1965; Williams 1960; Karssen 1970). Innate dormancy may be due to (a) immature embryo, (b) seed coat impermeable to water and/or gases, (c) inhibitors, (d)mechanical restriction by seed coat, and (e) physiological immaturity (Maguire 1975).

Sometime after the seed has lost its innate dormancy, induced or secondary dormancy occurs as a result of seeds being supplied with water but in an environment where some other factor is unfavourable for germination. Vegis (1963), Hay et al. (1959), and Thurston (1960) have stated that high temperature and limited oxygen caused induced secondary dormancy. Kidd (1914) showed that dormancy could be induced by high carbon dioxide concentrations. Induced dormancy persists for a while after the inhibitory factor has been removed.

Enforced dormancy, like induced dormancy, is caused by some limitation in the environment. However, it is removed immediately when the inhibitory factor is withdrawn. Enforced dormancy is mostly used to describe the dormancy of buried seeds. Hence it is attributed to such factors as high carbon dioxide levels, darkness and lack of fluctuating temperatures.

Roberts and Feast (1972) concluded that survival of viable seeds depends on the nature and degree of inert dormancy, whether or not induced dormancy can develop, and the ability of the seeds to persist when dormancy is enforced. Taylorson (1970) postulated that the level of innate dormancy within species may be as important as the species in determining longevity of weed seeds in soils.

2.3.2 Mechanism of Seed Dormancy

Seed dormancy represents complex and efficient control mechanisms that regulate resumption of growth by the embryo through responses of seeds to environment which may be translated into cellular control systems. Dormancy and germination are regulated by a critical balance of inhibitor - promotor complexes. The primary promotor of germination is Gibberellic acid (GA), while Abscisic acid (ABA) may block germination (Maguire 1975).

The control of seed dormancy can be divided into four distinct developmental phases: (a) inductive, (b) maintenance, (c) trigger, and (d) germination. The inductive phase is characterized by a marked decline in hormone levels. During this stage the inhibitor-promotor balance may be shifted in favour of the inhibitor component imposing dormancy. The maintenence phase constitutes an indefinite

period of partial metabolic arrest brought about by the presence of endogenous inhibitors. The trigger phase represents a period of sensitivity to specific environmental cues. During this stage a 'triggering agent' (a factor that elicits germination, but whose continued presence is not essential), and a germination agent (a factor whose continued presence is required) together shift the relative balance of an inhibitor-promotor complex to favour the promotor. The triggering agent may be an environmental factor, while the germination agent is a hormone. The germination phase is marked by increased hormone and enzyme activity and results in germination. Figure 12 illustrates the inhibitor-promotor concept.

2.3.3 Buried Seeds

According to Lewis (1961), the most rapid depletion of seed numbers in the soil takes place during the first year of burial. This may be due to lack of soil aeration. Under certain conditions, the soil may be depleted of its seed population by in-situ germination, fungal and bacterial attack. Roberts and Feast (1973) investigated the pattern of decrease in the numbers of viable seeds remaining in cultivated and undisturbed soil. The results indicated an exponential decrease in both cases with a more rapid decrease in

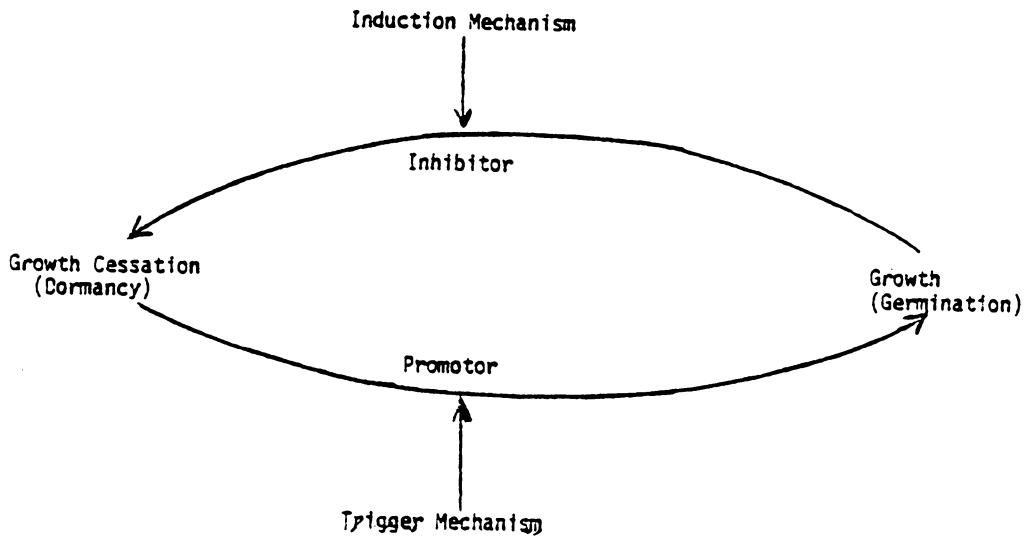

Figure 12: Mechanism of Seed Dormancy.

the cultivated soil. Harper (1957) states that the stimuli which may break dormancy are most abundant on the soil surface. Hence, any operation that buries the seeds encourages their persistance.

In general, the following observations have been reported regarding seed longevity in soil: (a) longevity increases with depth of burial (Dawson and Bruns 1975, Rampton and Ching 1966, Toole 1946), (b) acid and water-logged soil favour longevity (Champness and Morris 1948, Lewis 1961), (c) cultivation decreases longevity by promoting germination (Roberts and Feast 1973), and (d) high soil temperature favour germination and hence decreases longevity (Schafer and Chilcote 1970).

Chapter III

MATERIALS AND METHODS

3.1 PROJECT DESIGN

This study was conducted in two different phases. Phase I consisted of four continuously-fed digesters, which were loaded daily and maintained at mesophilic temperatures [35°C±1°C]. The variables selected for Phase I were influent solids concentrations of 4% and 6%, and retention times of 15 and 20 days. The different combinations of these variables allowed loading rates of 2.4 kg VS/m^3/day, 3.6 kg VS/m^3/day, 1.8 kg VS/m^3/day and 2.7 kg VS/m^3/day in digesters I, II, III and IV, respectively. Table 5 describes the different loading rates employed.

Phase II consisted of two experiments in which digesters were batch loaded and maintained at mesophilic temperatures. In Experiment 1 a feedstock of 4% total solids was digested for 15 days and 20 days, while in Experiment 2 the same retention times were maintained for a 6% total solids substrate. Four digesters were operated in each experiment to allow for two replicates. Table 6 illustrates the experimental design for Phase II.

TABLE 5

Daily Loading Rates for Phase I.

	Digester #			
	I	II	III	IV
Retention time (days)	15	15	20	20
Influent solids concentration (%)	4	6	4	6
Loading rate				
($kg\ VS/m^3$)	2.4	3.6	1.8	2.7
($lbs\ VS/ft^3$)	0.15	0.23	0.11	0.17

TABLE 6

Experimental Set Up for Phase II

Experiment	1				2			
Replicate	1		2		1		2	
Digester #	I	II	III	IV	I	II	III	IV
Influent Solids(%)	4	6	4	6	4	6	4	6
Retention Time(days)	15	15	20	20	15	15	20	20

3.2 LABORATORY-SCALE DIGESTERS.

Glass jars (3.785 L) fitted with # 14 rubber stoppers and having a working volume of 3 liters were used as digesters in both phases of the study. Two 9.52 mm (3/8 inch) holes were drilled through each stopper to allow passage of gas collection and influent-effluent tubes. Mesophilic temperatures [35°C±1°C] were maintained with a Haake Model FE circulating water bath. Warm water was circulated through 9.53 mm (3/8 inch) tygon tubing wrapped around the digesters, which were linked in series (Figure 13). This arrangement resulted in a temperature difference of 1°C between the first and last digesters in the circuit. To overcome this drawback, digesters were switched in position daily. All digesters were placed within a block of styrofoam insulation which was hollowed out to conceal the four digesters. The opening at the top was covered by a layer of styrofoam and provision was made for exit of the gas collection and influent-effluent tubes.

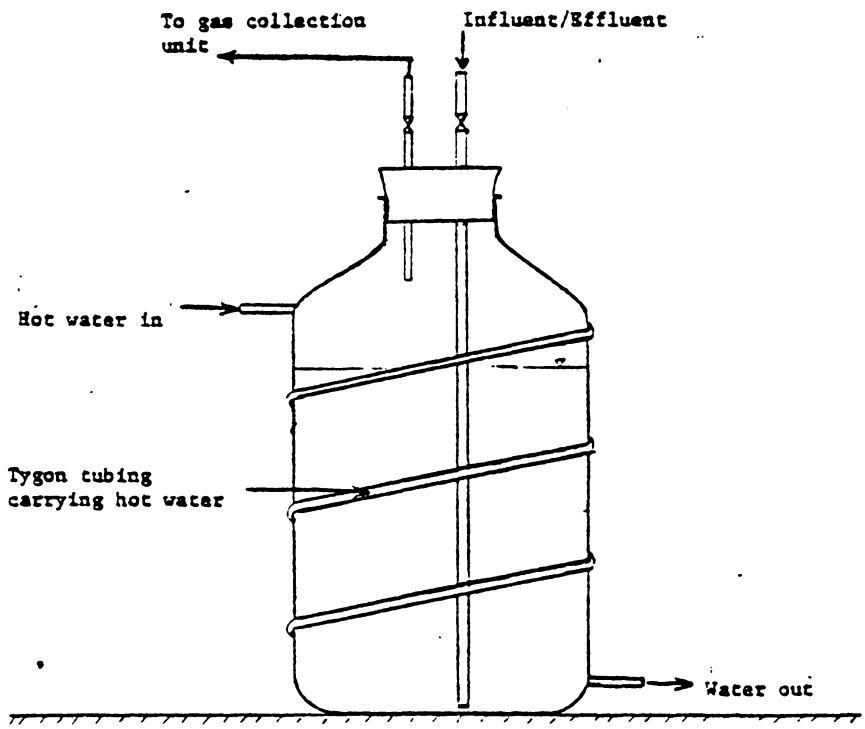

Figure 13: Digestion Apparatus.

3.3 GAS COLLECTION UNIT.

Gas was collected by displacement of a 20% solution of NaCl (Figure 14). Initially bottle A was filled with the displacement liquid. As gas pressure increased, liquid was displaced into bottle B. Gas volume in bottle A was measured at atmospheric pressure by equalizing the water levels in the bottles. The volume of head space above the digester contents and the volume of tubing connecting the digester and bottle A were then added to obtain the daily gas volume. The room temperature was also recorded. The measured gas volume was then converted to standard conditions of 760 mm pressure and 0°C.

3.4 SUBSTRATE.

Manure for this investigation was collected at one time from concrete floors in enclosed barns at the Viginia Tech Dairy Cattle Center. Care was taken to collect only fresh manure, and to avoid inclusion of sand and other inert material. Glass jars (3.785 L) were used to transport the manure to the laboratory where it was well mixed in a bucket and five random samples where taken to determine its composition. The waste was then stored in a freezer in glass jars for later use as feedstock for the digesters.

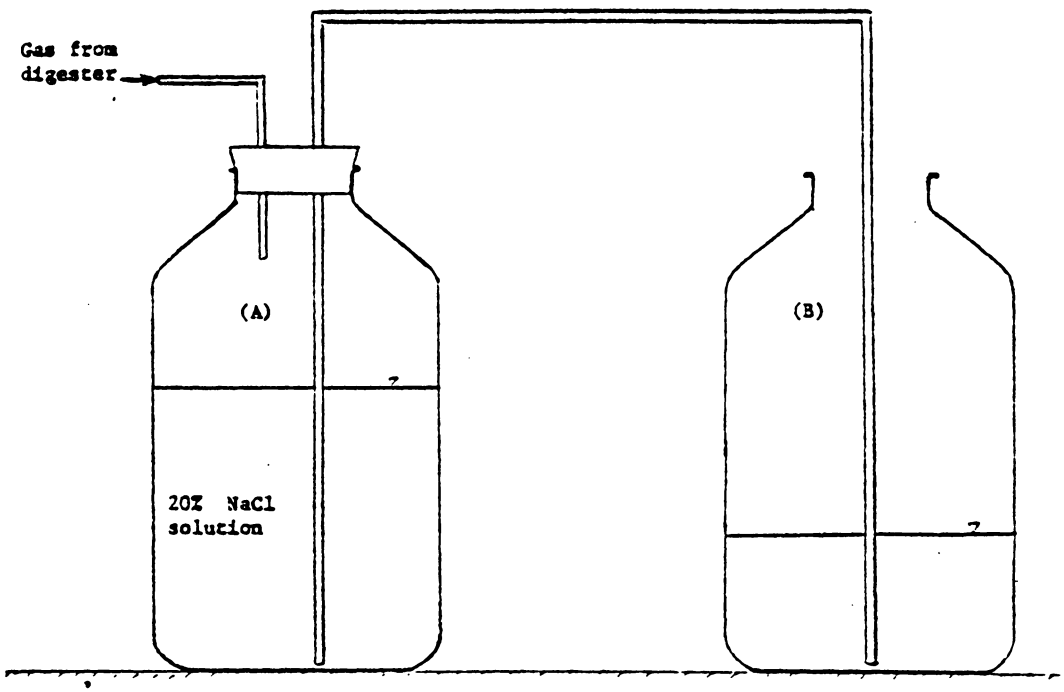

Figure 14: Gas Collection Unit.

Analysis of the manure indicated that it was quite high in volatile solids (Table 7). Cellulose and hemicellulose together accounted for approximately 52% of the total solids and 58% of the volatile solids. It also contained significant amounts (18%) of lignin; the pH at the time of collection was 6.9.

Substrate for the continually-fed digesters was prepared on a daily basis. Prior to preparation, the manure was thawed overnight, then mixed well. Predetermined amounts were weighed and diluted with tap water to the desired influent solids concentration (an example of the dilution calculation can be found in Appendix A). Just before feeding, contents of each digester were mixed thoroughly for 10 minutes. A predetermined volume of effluent was then drawn out immediately after mixing. Equal volumes of influent material, containing the exact number of fresh Johsongrass and Fall Panicum seeds withdrawn with the effluent, were then loaded into each digester, followed by mixing for 2 more minutes. Effluent withdrawal and influent addition were carried out with a 100 cc syringe through the tube provided for this purpose.

TABLE 7

Manure Composition

Total solids	(% wet wt.)	17.5
Volatile solids	(% TS)	89.9
Cellulose	(% TS)	27.3
Hemicellulose	(% TS)	24.8
Lignin	(% TS)	18.0
Total nitrogen	(% TS)	2.03
Crude Protein	(% TS)	12.68
Ash	(% TS)	10.1
pH		6.9

3.5 SEEDING AND MIXING OF DIGESTER CONTENTS

Digested slurry from a poultry digester was used as seed for Phase I digesters. The initial substrate consisted of approximately 50% innoculum and 50% dairy waste. Steady-state conditions were established after about two retention cycles. Steady-state was assumed when evidence of constant gas yield and gas composition were observed. The digested slurry of Phase I was used as innoculum for the Phase II study. The innoculum made up 10% of the feedstock of the Phase II digesters.

Phase I digesters were mixed for approximately 12 minutes daily - 10 minutes before feeding and 2 minutes after feeding- while Phase II digesters were each mixed for 10 minutes daily.

The mixing mechanism used in this study is illustrated in Figure 15. The digester containing the waste was placed lengthwise on two rollers, one of which was driven by a motor while the other acted as a follower supporting and stabilizing the digester.

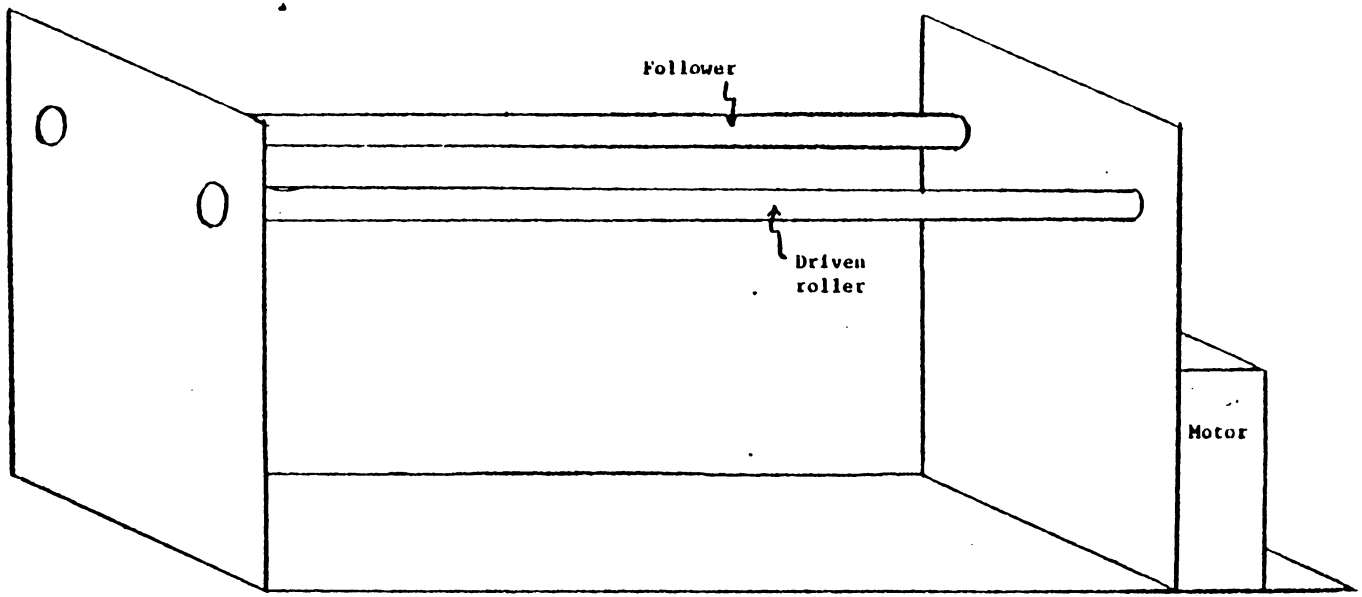

Figure 15: Mixing Mechanism.

3.6 JOHNSONGRASS AND FALL PANICUM SEEDS.

The seeds of Johnsongrass and Fall Panicum used in this investigation were purchased from Douglas W.King Seed Co., San Antonio, Texas. Prior to placing them in the digesters a random sample of 200 seeds was drawn and subjected to the standard and the tetrazolium germination tests.

3.7 SEED PREPARATION AND INTRODUCTION.

Seeds contained in animal feed pass through the intestinal tract and are subjected to the process of digestion in the rumen of the dairy cattle. The seeds in the manure are those that have survived the conditions of digestion. In order to approximate the sequence of events leading up to subsequent anaerobic fermentation of the manure, the seeds used in Phases I and II were first subjected to an in-vitro digestion process (Tilley and Terry 1963) which simulated the rumen environment of the animal in a test tube. Approximately 1 g of seed and 50 mL of rumen fluid was placed in a 100 mL tube. Carbon dioxide was directed into the space above the liquid in the tube for 15 seconds before tightly stoppering. The tube was then incubated in a constant temperature water bath at 38°C - 39°C for 48 hours, after which 20% HCl was added very slowly, followed by 5 mL of fresh aqueous pepsin. Stoppers were replaced and the tube incubat-

ed at 38°C - 39°C for another 48 hours. Seeds were recovered by centrifuging at 2500 rpm for 15 minutes. The seeds thus treated were used in all experiments described below.

In Phase I, each digester was loaded with 25 g of Johnsongrass and 25 g of Fall Panicum seeds. This was done after the digesters reached steady-state.

In Phase II the same quantity of seeds was used, however they were enclosed in bags made of nylon net. This way all seeds that were placed in the digesters were recovered at the end of the experiment.

3.8 ANALYSIS.

Determination of total and volatile solids, pH, alkalinity and total nitrogen were performed according to Standard Methods (American Public Health Association, 1975).

Table 8 outlines the frequency of sampling of the different parameters. In Phase I, sampling was initiated after the digesters reached steady-state conditions.

3.8.1 Solids

Five random samples of raw waste were placed in previously weighed crucibles and oven dried at 103° C for 24 hours to determine total solids. Dried samples were then fired at 550° C in a muffle furnace for 5 hours to determine volatile

TABLE 8

Frequency of Sampling.

	Phase I			Phase II		
Parameter	Daily	Every 2 days	Weekly	Daily	Every 5 days	Initial & final
Solids:						
total						*
volatile						*
pH	*				*	
Gas volume	*			*		
Gas composition			*		*	
Alkalinity		*				
Volatile acids		*				
Viability tests	*					*

solids. A Mettler H 80 balance was used for weighing throughout this investigation.

The method of effluent withdrawl employed in Phase I did not lend itself to accurate gravimetric measurements of effluent solids. Varel et al. (1977), Chynoweth et al. (1981), and Pfeffer (1977) have also encountered difficulties in obtaining representative digester samples with continuously-fed systems. The volatile solids reductions reported in Phase I were estimated from gas production. It was assumed that all of the carbon in the volatile solids destroyed was evolved as carbon dioxide and methane, and that volatile solids destroyed was 40% carbon. Hence, grams of VS destroyed=(moles of gas X 12 g/mol.carbon)/0.4. The carbon content of many low fat wastes, like dairy manure, containing mainly carbohydrates and protein, is near 40% (Varel et al. 1977, Singh et al. 1982).

3.8.2 pH and Alkalinity

Exactly 20.0 mL of sample was withdrawn from daily effluent for the measurement of pH and alkalinity. A magnetic stirrer kept the effluent well stirred at the time of sampling. The pH was measured using a Leeds and Northrup pH meter. The sample was constantly stirred with a magnetic stirrer and was titrated to pH 4.5 with 0.1N hydrocholoric acid to determine digester alkalinity.

3.8.3 Chemical Oxygen Demand

The sealed ampule method (micro sample digestion technique) as described by Jirka and Carter (1975), and Messenger (1981), was used to determine the chemical oxygen demand (COD). Exactly 2.5 mL of the waste was diluted to 250 mL with distilled water. 2.5 mL of the diluted sample and 1.5 mL of digestion solution were placed in a borosilicate ampule. 3.5 mL of catalyst solution (silver sulfate) was carefully added before sealing the ampule. After mixing throughly, the contents were digested at 150°C for two hours. After cooling, the ampules were washed and wiped clean and the light absorbance of the contents was measured at 600nm in a Baush and Lomb spectrophotometer. This technique measures the appearance of Cr(III). Two blanks were also prepared and analyzed in the same manner as the samples. Potassium acid phthalate was diluted to generate working standards of 25, 50, 75, 100, 250, 500, 750, and 1000 mg/L COD. The standard curve thus obtained was used to determine the COD of the samples.

3.8.4 Volatile Acid

In order to determine the volatile acid concetration, 5.0 mL of effluent sample was pipetted into a culture tube containing 1.0 mL of 25% metaphosphoric acid and 5.0 mL inter-

nal standard[2] and then frozen. After thawing, samples were centrifuged for 20 min. at 3000 rpm. Approximately 5.0 mL of sample was drawn into a 10 mL disposable syringe and forced through a membrane filter holder containing 2.0 micron metricel filter, and collected in a small culture tube. Exactly 2µL of the sample was injected into a Perkin-Elmer 881 flame ionization gas chromatograph.[3] Peaks were recorded using a Hewlett-Packard 3380 A integrator with a chart speed of 5cm/min. Areas of peaks obtained from the integrator were compared with those of the standard solution to determine the volatile acid concentration.

3.8.5 Digester Gas

A Fisher gas sampling bottle was flushed with the digester gas and immediately filled with the digester gas. bd |250 |m|L gas-tight syringe was used to withdraw 200 µL gas sample, which was analyzed in a Bendix 2300 thermal conductivity detector gas chromatograph.[4] A Linear Instruments

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[2] 2.5 mL of 4-methyl valeric in 1 L of distilled water.

[3] Nitrogen carrier gas at 40 mL/min., column at 120°C, injection port at 175°C, and detector at 210°C; 1.83 m X 3.175 mm glass column with 10% SP-1200/ 1% H PO on 80/100 mesh chromasorb WAW.

[4] Helium carrier gas at 60 mL/min., column at 45° C, inlet at 60° C, and detector at 200° C, bridge current 250 mA, 10X attenuation, and 1.829 m X 3.175 mm stainless steel column packed with Poropak N.

Corporation recorder with a 5 cm/min. chart speed was used to record the peaks. Peak heights were compared with those of a Supelco standard gas mixture to determine the relative amounts of carbon dioxide and methane.

3.8.6 Nitrogen

Total nitrogen content of the manure was determined by placing exactly 5 mL of sample in a 800 mL Kjeldahl digestion flask and diluting to 250 mL with distilled water. 10 mL concentrated sulfuric acid, 6.7 g potassium sulfate and 1.5 mL mercuric sulfate were then added and the contents boiled until the solution became clear. Digestion was continued for another 30 minutes after which the flask was allowed to cool.The contents were then diluted with 200 mL of ammonia-free water and 0.5 mL phenolphthalein indicator solution was added and mixed. Hydroxide thiosulfate reagent was carefully added so that it layered at the bottom. After mixing well, the flask was attached to the preheated distillation apparatus. 200 mL of distillate was collected in a boric acid trap and titrated with 0.1 N hydrocloric acid allowing for the calculation of total nitrogen.

3.8.7 Hemicellulose, Cellulose and Lignin

The determination of hemicellulose, cellulose and lignin were carried out as described by Goering and Van Soest (1970). To estimate hemicellulose, the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) must first be determined. NDF was measured by adding 50 mL of neutral detergent solution[5] to 0.5 of previously ground sample and refluxed for one hour, and then transfered to a tared scintered glass crucible. Vacuum was applied to remove the liquid. The sample was washed twice with hot water followed by an acetone-wash dried at 105°C overnight, cooled and weighed. The difference in weight was recorded as neutral detergent fiber (NDF).

ADF was determined by the same procedure outlined for NDF determination. The only difference was the addition of 100 mL acid detergent solution[6] in place of neural detergent solution. The difference between NDF and ADF was an estimate of hemicellulose.

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[5] 1,200 g sodium lauryl sulfate, 744.4 g disodium EDTA dihydrate, 272.4 g sodium borate decahydrate,182.4 g disodium hydrogen phosphate, and 400 g 2-ethoxyethanol in 40 L of distilled water.

[6] 1 N sulfuric acid and 20 g of cetyl trimethylammonium bromide.

Lignin was measured using the ADF portion remaining. The crucible was placed in a tray and filled with cooled 72% sulfuric acid and stirred with a glass rod to a smooth paste. The crucible was refilled with acid at hourly intervals for three hours as acid drained away. Vacuum was applied to drain off the acid, and the residue was washed with hot water. The crucible and contents were then dried at 105°C overnight, cooled and weighed. This was followed by firing in a muffle furnace at 500°C for three hours, cooling and weighing. The difference in weight, expressed as a percent of the dry matter, was the lignin content.

3.8.8 Germination Tests

The seeds in the daily effluent of Phase I digesters were separated with a tweezer after the effluent was emptied into a large shallow tray. Because of the small numbers of seeds recovered daily no effort was made to distinguish between dormant and non-dormant seeds. Only the Tetrazolium Test (TZ) was performed to determine the viable seed count for the Phase I digesters on a daily basis. In addition, 200 seeds were picked randomly from digester contents at the end of the experiment and tested. In the case of the Phase II digesters, all seeds subjected to anaerobic treatment were tested.

It was necessary to conduct two different germination tests in order to determine the percentages of dormant and non- dormant seeds. The standard germination test was first performed to measure the number of non-dormant seeds. The seeds that failed this test were then subjected to the Tetrazolium (TZ) test to differentiate between dormant and non-viable (dead) seeds (Figure 16).

3.8.8.1 Standard Germination Test

In this test, the seeds were exposed to favourable conditions for germination by placing them on a moist paper towel, and subjected to a 16-hour dark period followed by a light period of 8 hours. This cycle was repeated for 5 days. At the end of the test, the number of seeds that germinated was recorded. The seeds that did not germinate were then subjected to the TZ test.

3.8.8.2 Tetrazolium Test

The Tetrazolium (TZ) test for seed viability is widely recognized as an accurate means of testing seed viability. It was first developed in Germany by Professor. G. Lakon in 1940, and is now a standard method in many seed laboratories. It is a relatively quick method which can be completed in a few hours.

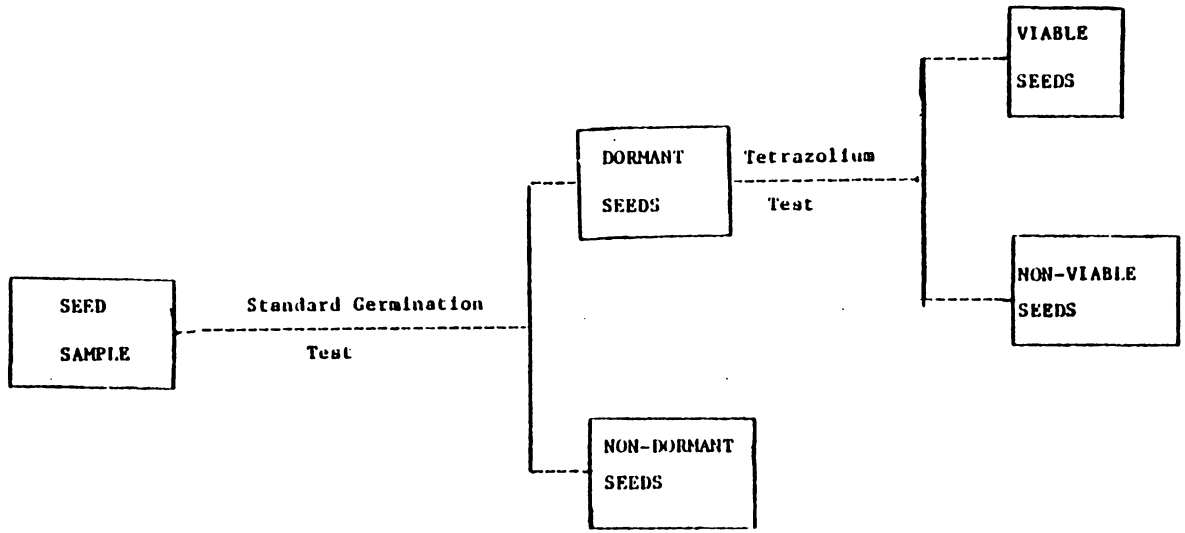
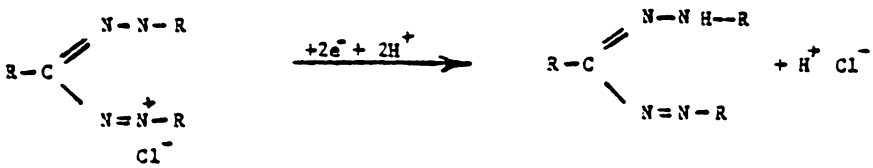


Figure 16: Interpretation of Germination Tests.

The TZ test distinguishes between viable and dead tissues of the seed embryo on the basis of their relative respiration rate in the hydrated state. Although many enzymes are active during respiration, the test is based on the activity of the dehydrogenase enzymes, which is used as an index to the respiration rate and, hence, seed viability. The highly reduced state of the dehydrogenases enables them to give off hydrogen ions to oxidized colourless tetrazolium salt (2,3,5-triphenyl tetrazolium chloride) solution which is changed into red formazan as it is reduced by hydrogen ions. Figure 17 illustrates the chemical reaction involved in the formation of formazan.

Some of the requirements for successful staining are: pre-soaking of seeds, constant temperature of about 35° C, avoidance of too short or too long periods of staining, maintenance of TZ solution pH in the range of 6.5 - 7.2, and absence of light at the time of staining (Moore, 1966, 1960; Grabe, 1970, 1959).

In this investigation, the TZ test was performed as described in The Tetrazolium Testing Handbook for Agricultural Seeds (Association of the Official Seed Analysts, 1970). Seed preparation is specific to the type of seed and is perhaps the most important step of the TZ test. In the case of Johnsongrass and Fall Panicum, the seeds had to be softened



2,3,5-triphenyl tetrazolium chloride. (colorless)

Formazan. (red)

Where, R = Penyl group =

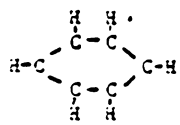


Figure 17: Chemical Reaction of TZ Test.

first to facilitate entry of tetrazolium solution into the seeds. It was found that an 8 hour soaking period was sufficient for Fall Panicum seeds, while Johnsongrass seeds had to be soaked for at least 12 hours, and then the hard seed coat pierced with a sharp needle before placing in the Tetrzolium solution.

Preliminary tests indicated that a TZ solution concentration of 1% was adequate for Johnsongrass and Fall Panicum seeds. The pH of the solution was within the recommended range of 6.5 to 7.2 and, hence, no pH adjustment was needed.

The seeds to be tested were placed between two nontoxic blotters previously soaked with TZ solution. Blotters were then wrapped with two paper towels and placed in a tray containing enough TZ solution to keep both towels and blotters saturated for the duration of test. The tray was then placed in an oven at 35° C overnight.

Seeds were bisected through the embryo under a low power microscope, and a conservative interpretation of seed viability was made based on the staining pattern of the embryo and the intensity of coloration. In this investigation, the TZ test was carried out after the standard germination test, thus enabling measurement of dormant, non-dormant and non-viable seeds. A non-germinable (non-viable) seed was defined as one in which one or more of the following were observed:

1. all embryo structures unstained;
2. lower or upper half of embryo unstained;
3. growing tip(s) unstained;
4. embryo stained very light pink;
5. embryo not stained with a uniform tone of red;
6. patches of very bright red stain;
7. embryo stained red, but with a dull purplish tinge, and;
8. central area of embryo unstained.

Chapter IV

RESULTS AND DISCUSSION

The results of this investigation will be discussed in terms of, (a) performance of Phase I and Phase II digesters, and, (b) the fate of Johnsongrass and Fall Panicum seeds subjected to the process of anaerobic fermentation.

4.1 DIGESTER PERFORMANCE (PHASES I AND II)

Performance of the digesters can be evaluated in terms of volatile fatty acids (VFA), pH, alkalinity, gas volume and composition, chemical oxygen demand (COD) reduction and volatile solids (VS) reduction. The values of these parameters are reported as means for the period of study.

4.1.1 Continuously-fed digesters (Phase I)

The results of the continually-fed digesters are presented in Table 9.

4.1.1.1 Volatile acids, pH and Alkalinity

A complex relationship exists between VFA, pH and alkalinity which exerts tremendous influence on the process of methanogenesis. Hence, it is appropriate to discuss these parameters simultaneously. Figures 18 through 21 illustrate

TABLE 9

Results of Phase I (Continuously-Fed Digesters)

Parameter	Digester I	II	III	IV
Working volume (L)	3.0	3.0	3.0	3.0
Detention time (d)	15	15	20	20
Influent TS (%)	4.0	6.0	4.0	6.0
Influent VS (%TS)	89.9	89.9	89.9	89.9
Influent Volume (L/d)	0.2	0.2	0.15	0.15
Loading rate:				
(kg VS/m^3/d)	2.4	3.6	1.8	2.7
(lbs VS/l/d)	0.15	0.23	0.11	0.17
Influent COD (1000 mg/L)	35.94	35.94	52.35	52.35
Infl.Alkal.(mg/L $CaCO_3$)	2563.0	2805.0	2410.0	2680.0
Gas production:				
(L/L/.d)	0.84	0.93	0.7	0.81
(L/g VS added)	0.35	0.25	0.39	0.3
Methane content (%)	59.0	60.0	61.5	62.0
VS reduction (%)	46.8	34.5	52.0	40.1
COD reduction (%)	43.7	45.7	61.4	58.7
Eff.Alkal.(mg/L $CaCO_3$)	3062.0	3060.0	2991.0	3205.0
Vol.acids.(mg/L Acetic)	898.0	1515.0	754.0	1354.0
pH	7.7	6.9	7.3	7.4

the variations in VFA, pH and alkalinity of Phase I digesters.

The mean VFA concentrations in digesters I, II, III and IV were 898.0, 1515.0, 754.0, and 1354.0 mg/L as acetic acid. The sudden acid build-up that occurred in digesters II and IV during the early stages resulted in high VFA values for these digesters. Acid accumulations likely resulted from process imbalance. Digesters II and IV were loaded at rates of 3.6 and 2.7 kg VS/m^3/d, respectively, which were close to the upper limit of 3.5 kg VS/m^3/d suggested by Stafford et al. (1980) for anaerobic fermentation of dairy manure. Further, when feeding was stopped for a day, the digesters recovered, indicating that the imbalance was due to high loading rates. The pH variations in the digesters were moderate, and the mean values fell within a range of 6.9 and 7.7. This compares well with the optimum range for anaerobic fermentation as given by McCarty (1964a).

Influent alkalinity is a fuction of the loading rate. The values for digesters I, II, III and IV were 2563.0, 2805.0, 2410.0 and 2680.0 mg/L as $CaCO_3$, respectively. In anaerobic digesters, alkalinity is contributed by the bicarbonate, carbonate, and ammonia components. These increase with the loading rate.

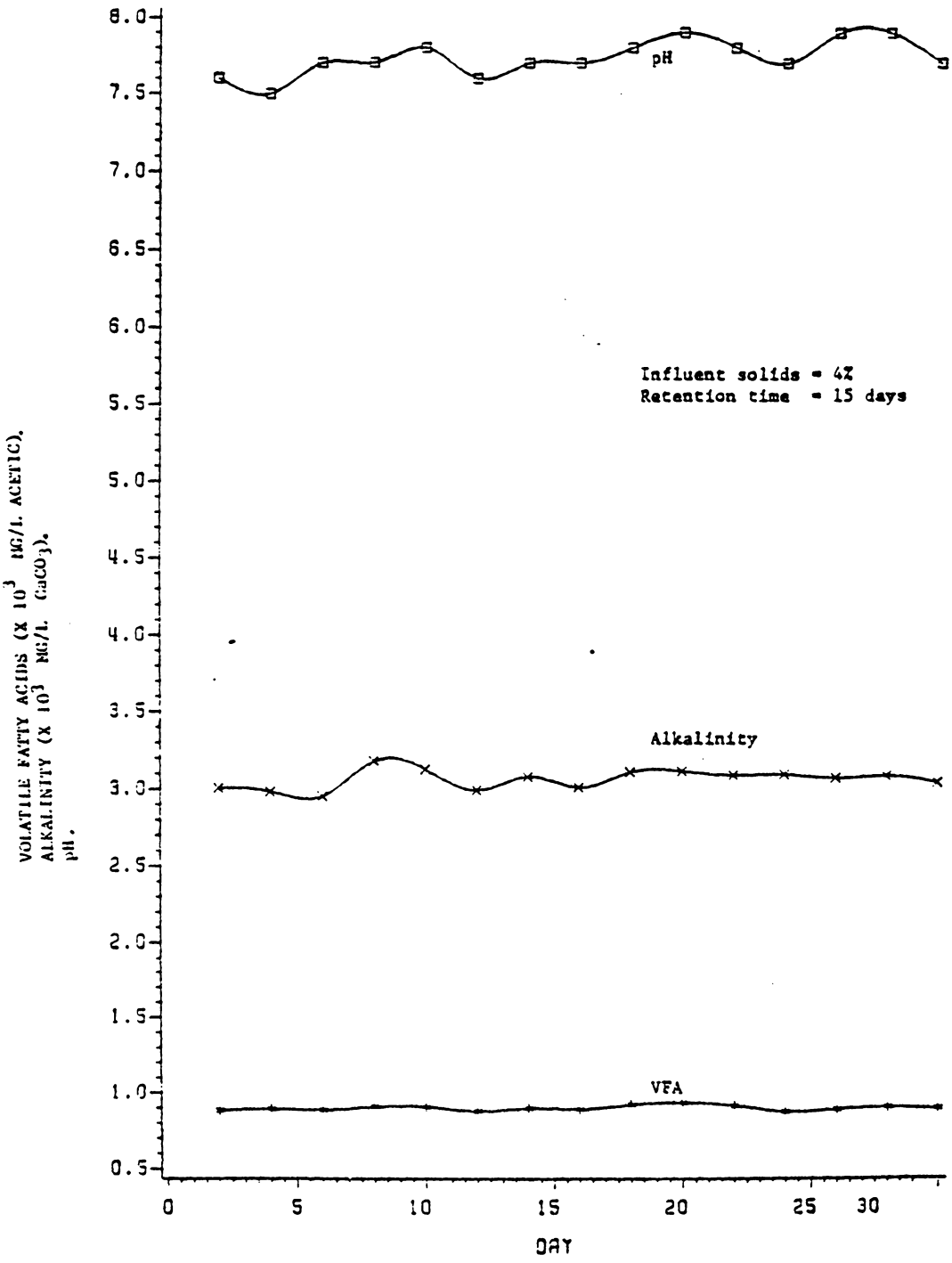


Figure 18: VFA, pH and Alkalinity Variations in Digester I (Phase I).

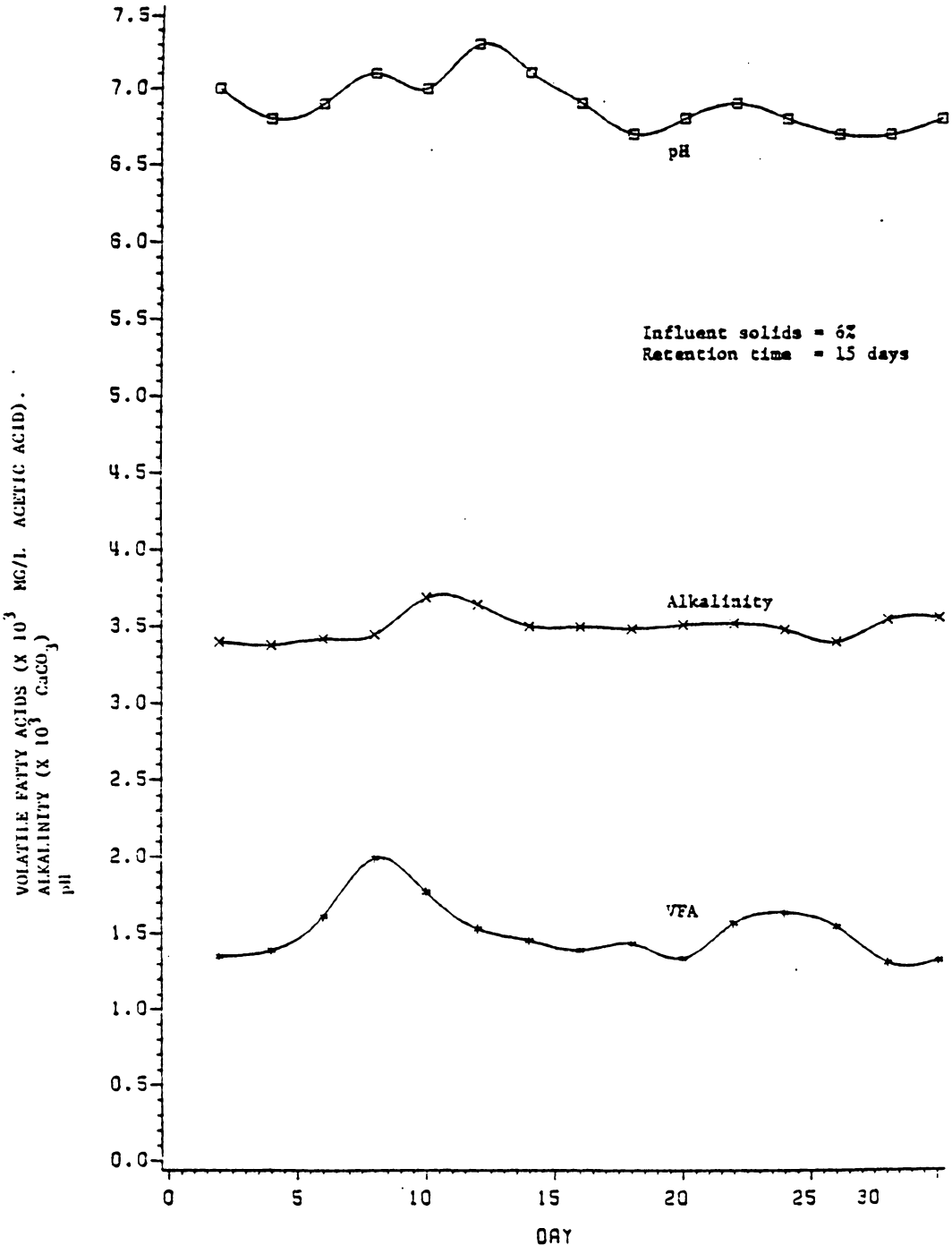


Figure 19: VFA, pH and Alkalinity Variations in Digester II (Phase I).

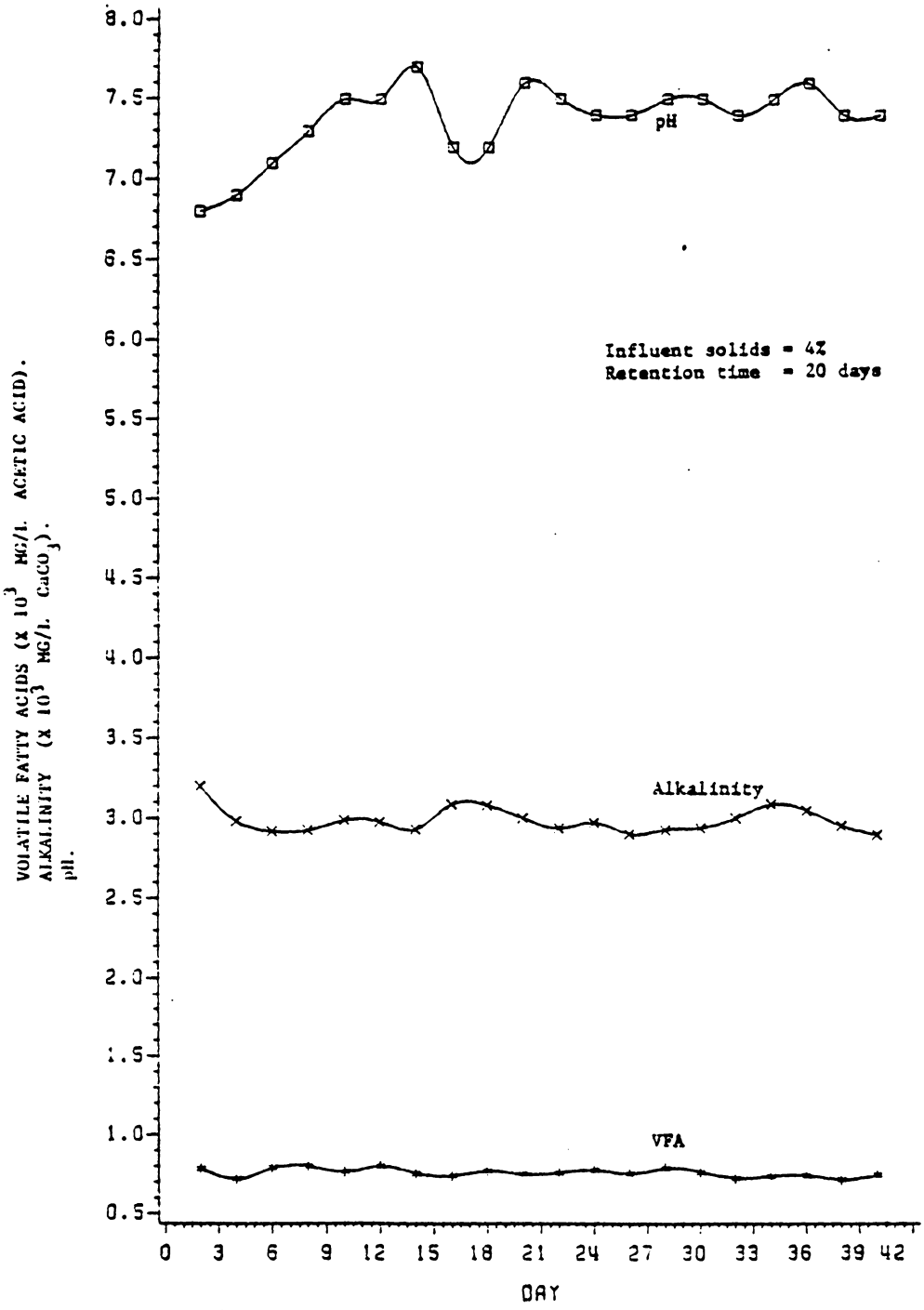


Figure 20: VFA, pH and Alkalinity Variations in Digester III (Phase I).

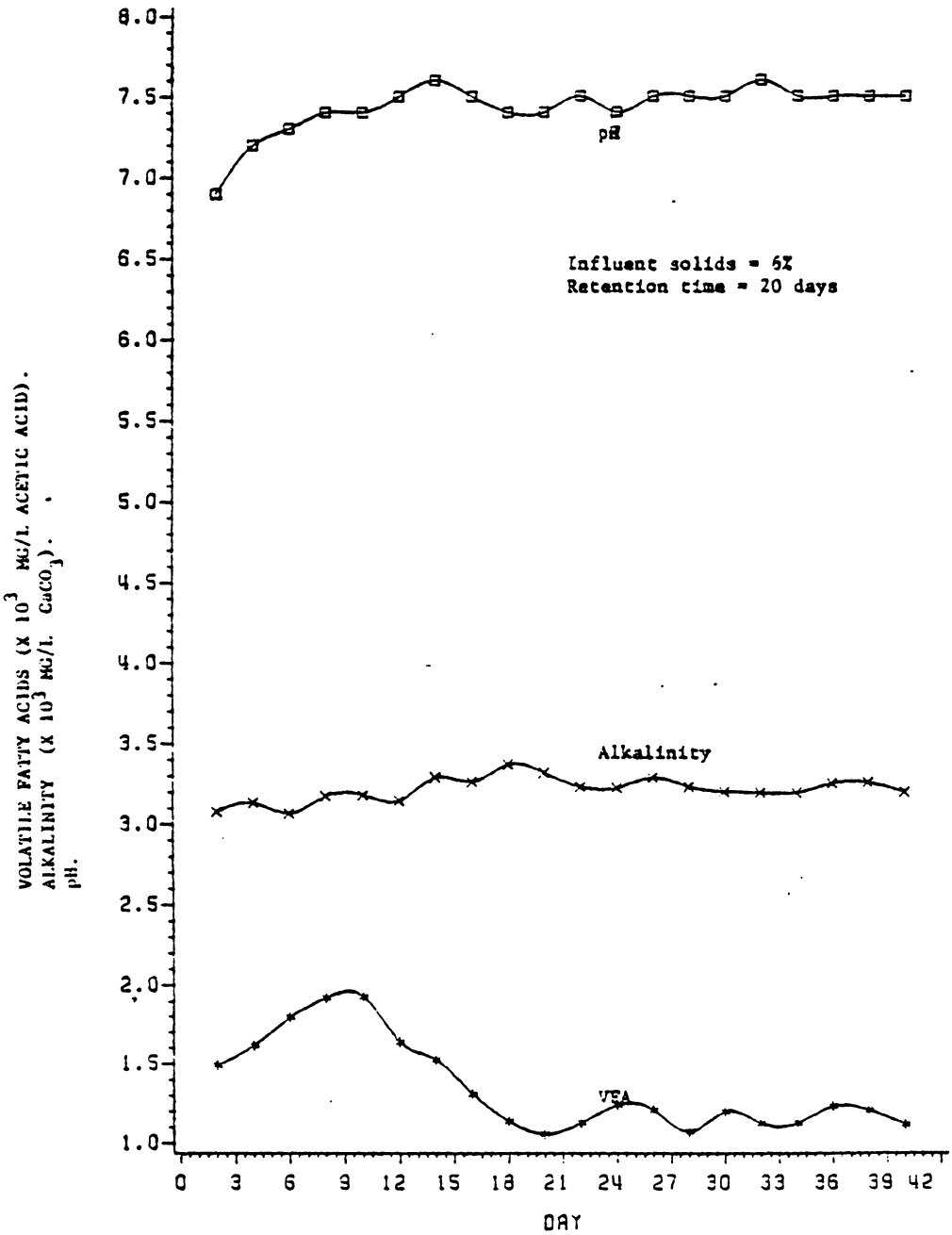


Figure 21: VFA, pH and Alkalinity Variations in Digester IV (Phase I)

The effluent alkalinity represents the alkalinity of the digester contents. The mean values for all digesters of Phase I exceeded 2900 mg/L as $CaCO_3$. The increase in volatile acids did not affect pH of the digesters. This may be attributed to the high alkalinity of the waste. According to McCarty (1964b), alkalinity in the range of 2500 to 5000 mg/L as $CaCO_3$ provides a safe buffering capacity for anaerobic treatment of waste. Acid build-up will not alter the digester pH significantly until such a high concentration is reached that the neutralizing or buffering action is overcome (Hobson et al. 1974).

4.1.1.2 Gas Production

Figures 22 through 25 illustrate the daily gas production in the digesters. The highest (0.93 L/L/day) and the lowest (0.73 L/L/day) mean values were recorded by digesters II and III, respectively, which also received the highest (3.6 kg VS/ m^3/d) and the lowest (1.8 kg VS/m^3/d) loading rates. At lower loading rates, the bacteria exhibit lower metabolic activity, hence, a smaller quantity of gas is produced.

A reverse trend was observed when gas production was expressed in terms of the volatile solids added. The mean values ranged from 0.25 L/g VS added in digester II to 0.39 L/g VS added in digester III. When expressed this way, gas

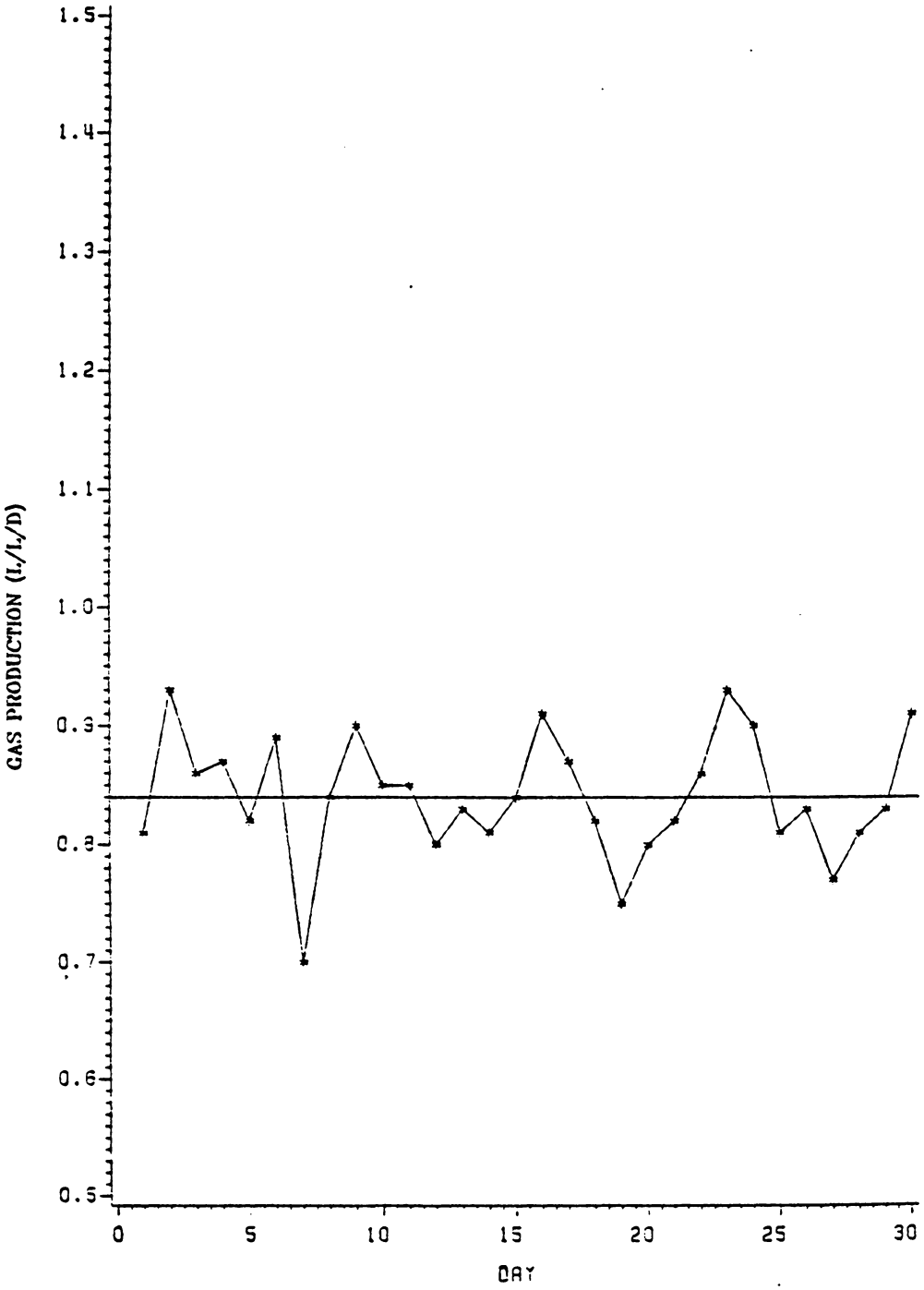


Figure 22: Daily Gas Production of Digester I (Phase I).

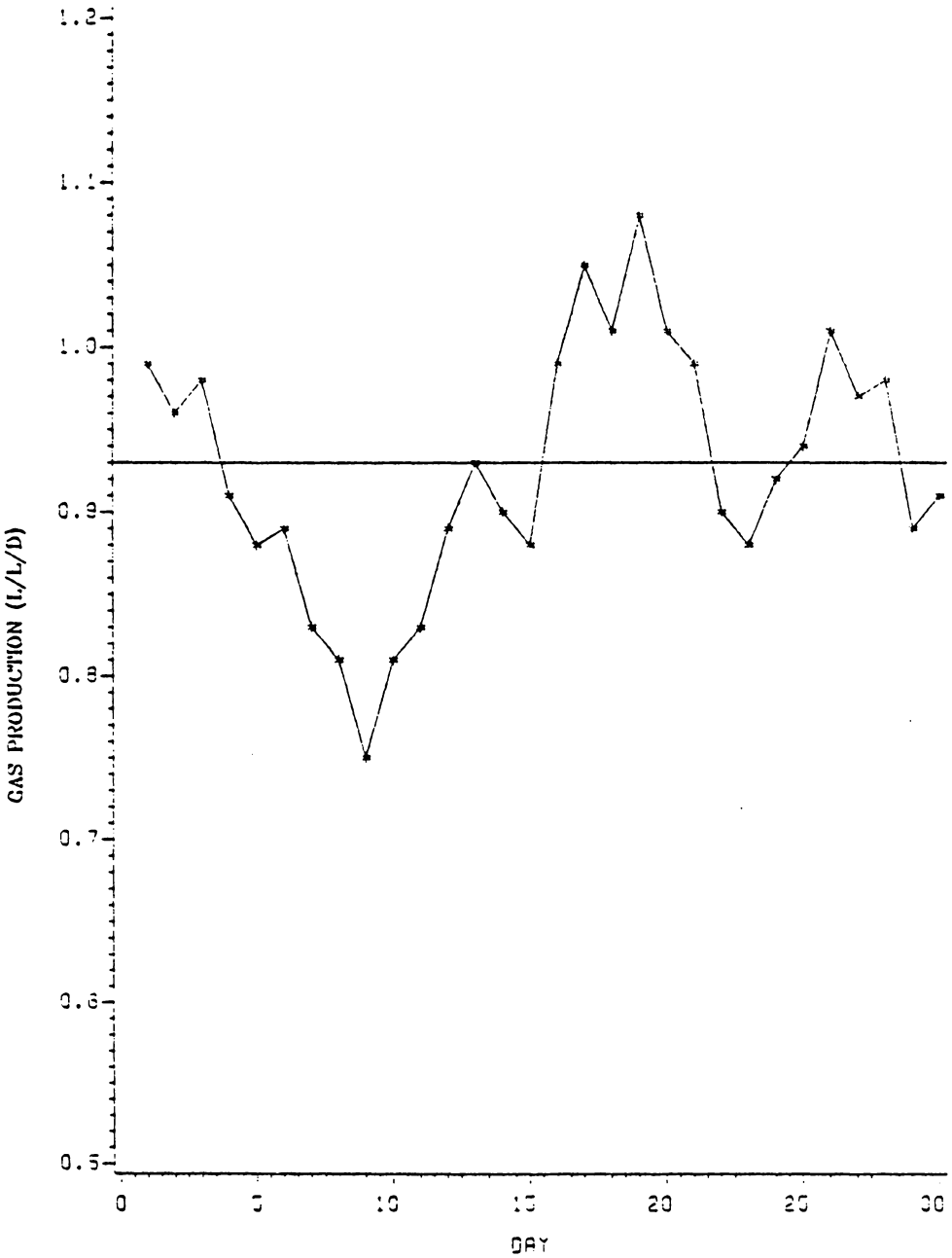


Figure 23: Daily Gas Production of Digester II (Phase I).

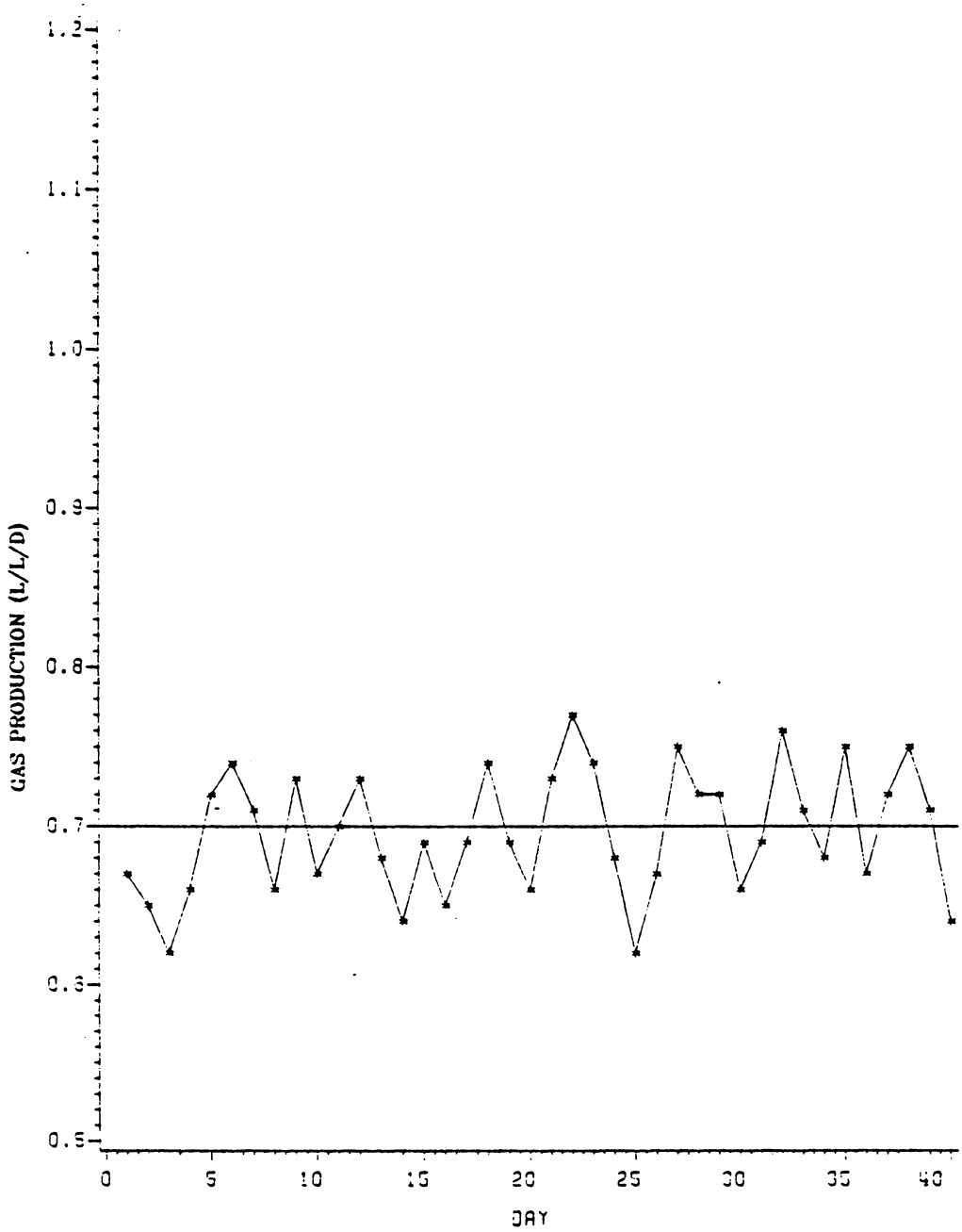


Figure 24: Daily Gas Production of Digester III (Phase I).

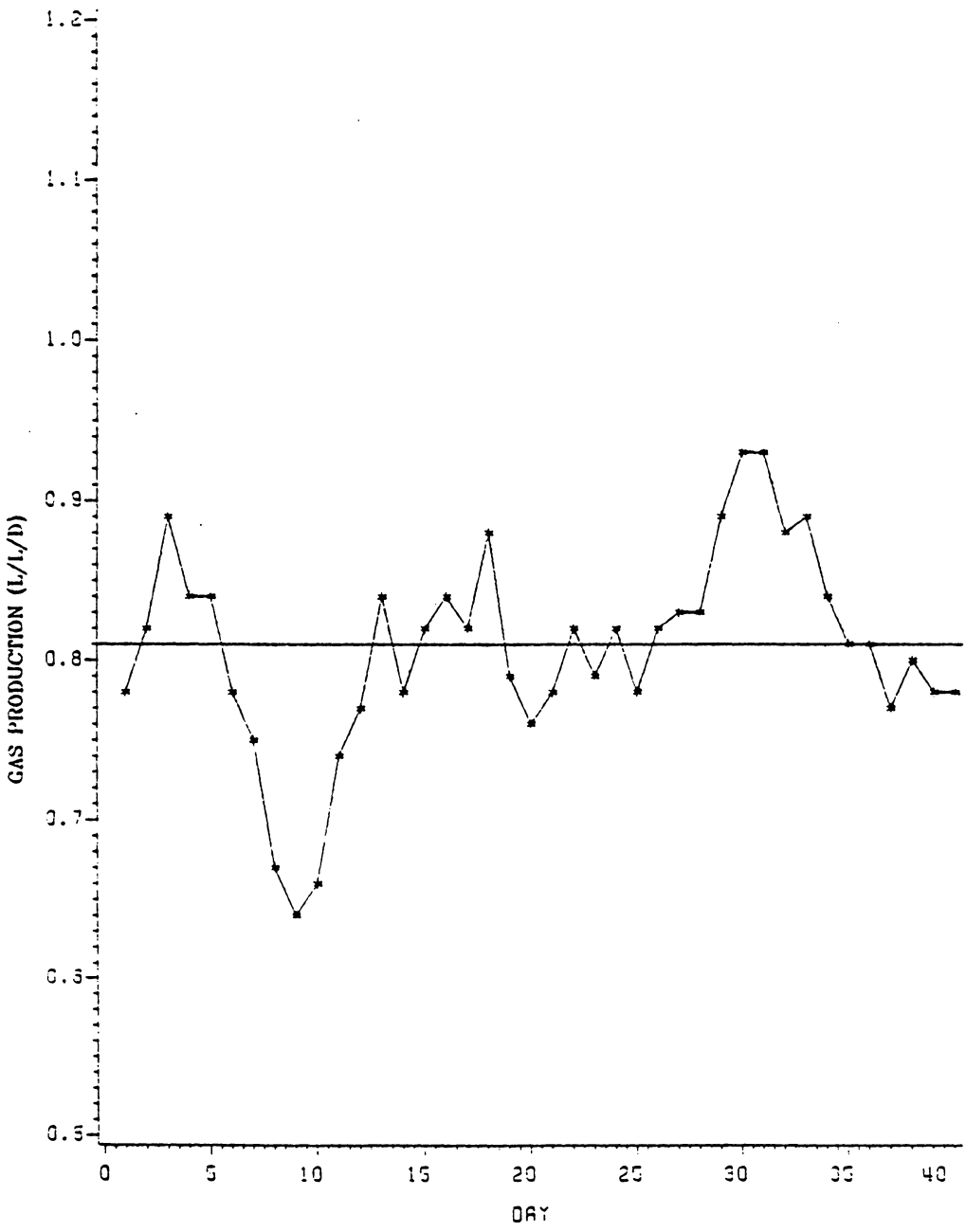


Figure 25: Daily Gas Production of Digester IV (Phase I).

production becomes a good measure of digester efficiency. It is interesting to note that although digester II produced the most amount of gas per unit digester volume, its efficiency was low in terms of the volatile solids added.

Digesters II and IV recorded a significant drop in gas production during the early stages of the study indicating process imbalance (Figures 23 and 25). The VFA concentrations in digesters II and IV were observed to approach high levels during the periods of low gas yields (Figures 19 and 21). The maximum concentrations recorded in digesters II and IV were 2005 mg/L as acetic acid, and 1931 mg/L as acetic acid, respectively. McCarty et al. (1961) and Varel et al. (1977) reported that total volatile acid concentrations above 2000 mg/L as acetic acid affected methane production. Kroeker et al. (1979) observed acute methanogenic toxicity at volatile acid concentrations between 1650 to 2600 mg/L as acetic acid.

The average composition of the gas produced by the Phase I digesters is presented in Figure 26. The methane content varied within a narrow range of 59% (I) and 62% (IV). The Roman numerals in parenthesis refer to the digesters. Literature has reported digester gas cotaining up to 65% methane (Grammes et al. 1971; Hart, 1963; and Converse et al. 1975). Based on this information, it can be stated that Phase I digesters produced good quality gas.

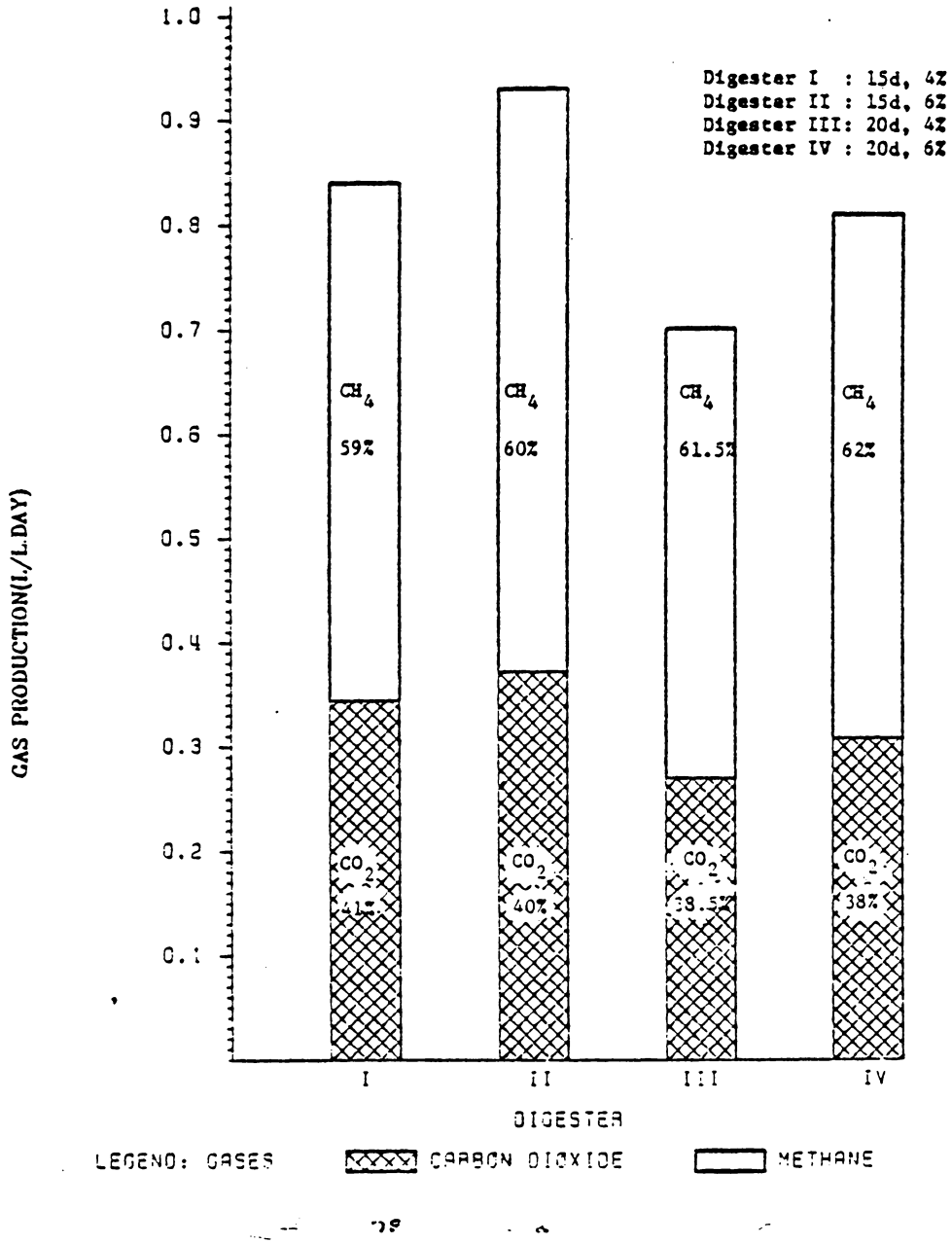


Figure 26: Gas Composition of Phase I Digesters.

4.1.1.3 Volatile Solids and COD Reductions

The volatile solids (VS) reduction determined from gas production for digesters I, II, III, and IV were 46.8% , 34.5% , 52.0% and 40.1%, respectively. The COD reduction ranged from 43.7% in digester I to 61.4% in digester III. For a given influent feed solids, greater VS and COD reductions were achieved with longer retention times. The COD reduction for the 4% influent was appproximately 40% higher in the 20-day digester (digester III) than in the 15-day digester (digester I). The corresponding increase in COD reduction for the 6% influent (digesters III and IV) was 28%. The VS reduction in the 20-day digesters (digesters III and IV) were roughly 4% and 16% higher than in the 15-day digesters (digesters I and II) for influent solids concentrations of 4% and 6%, respectively. Therefore, it can be stated that for the loading rates employed in this investigation, greater waste stabilization occured at longer retention times.

4.1.2 Batch Digestion (Phase II)

The results of Phase II (batch digestion) are presented in Table 10.

TABLE 10

Results of Phase II (Batch Digesters).

Parameter	Digester I	II	III	IV
Innoculum (L)	0.3	0.3	0.3	0.3
Substrate (L)	2.7	2.7	2.7	2.7
Retention time (days)	15	15	20	20
Influent TS (% wet weight)	4.0	6.0	4.0	6.0
Influent VS (% TS)	89.9	89.9	89.9	89.9
Inf. COD (1000 mg/L)	35.94	52.35	35.94	52.35
Digester pH	7.4	7.0	7.6	6.9
COD Reduction (%)	42.5	31.0	37.6	56.0
VS Reduction (%)	32.8	26.5	43.3	32.8
Gas production				
Total (L)	30.6	29.05	46.4	45.2
L/d	2.24	1.98	2.32	2.25
L/g VS added	0.32	0.20	0.45	0.31
L/g VS destroyed	0.96	0.75	1.07	0.95
Methane Content (%)	47.0	41.7	52.0	46.6

4.1.2.1 Gas Production and Volatile Solids Reduction

The daily gas production of Phase II digesters is presented in Figures 27 through 30. The lag period required for an active microbial population to become established in batch digestion caused low initial gas yields. Also, since methanogenic bacteria are slow-growing, most of the initial gas comes from the break down of fatty acids by the acetogenic hydrogen-producing bacteria. The end products of this decomposition are acetate, hydrogen and carbon dioxide. This resulted in low methane content of the digester gas at the initial stages (Figures 31 through 34). The two 15-day digesters registered unusually high gas yields on the 9th,10th and 11th days. This phenomenon cannot be explained.

An evaluation of the digester performance in terms of gas produced per mass of VS added, as well as per mass of VS destroyed, reveal that digester III was the most efficient while digester II was the least efficient. In batch fermentation a higher solids substrate requires longer retention time to attain maximum gas production than a lower solids feed. The gas production in digester III (Figure 29) appears to stabilize at the end of 20 days indicating that the 4% feed is at or close to maximum gas production. This probably is the reason for the high efficiency of digester III.

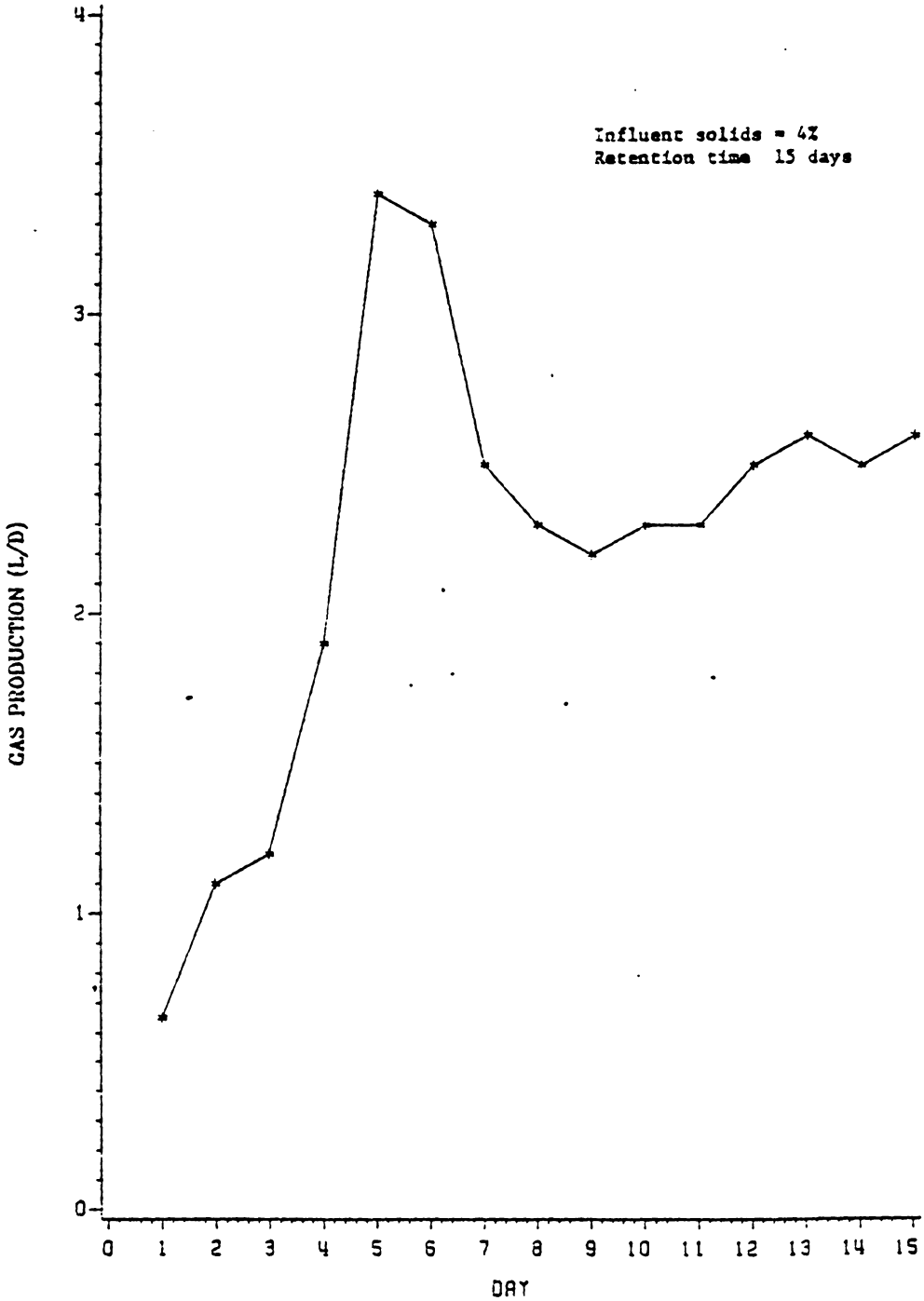


Figure 27: Gas Production of Digester I (Phase II).

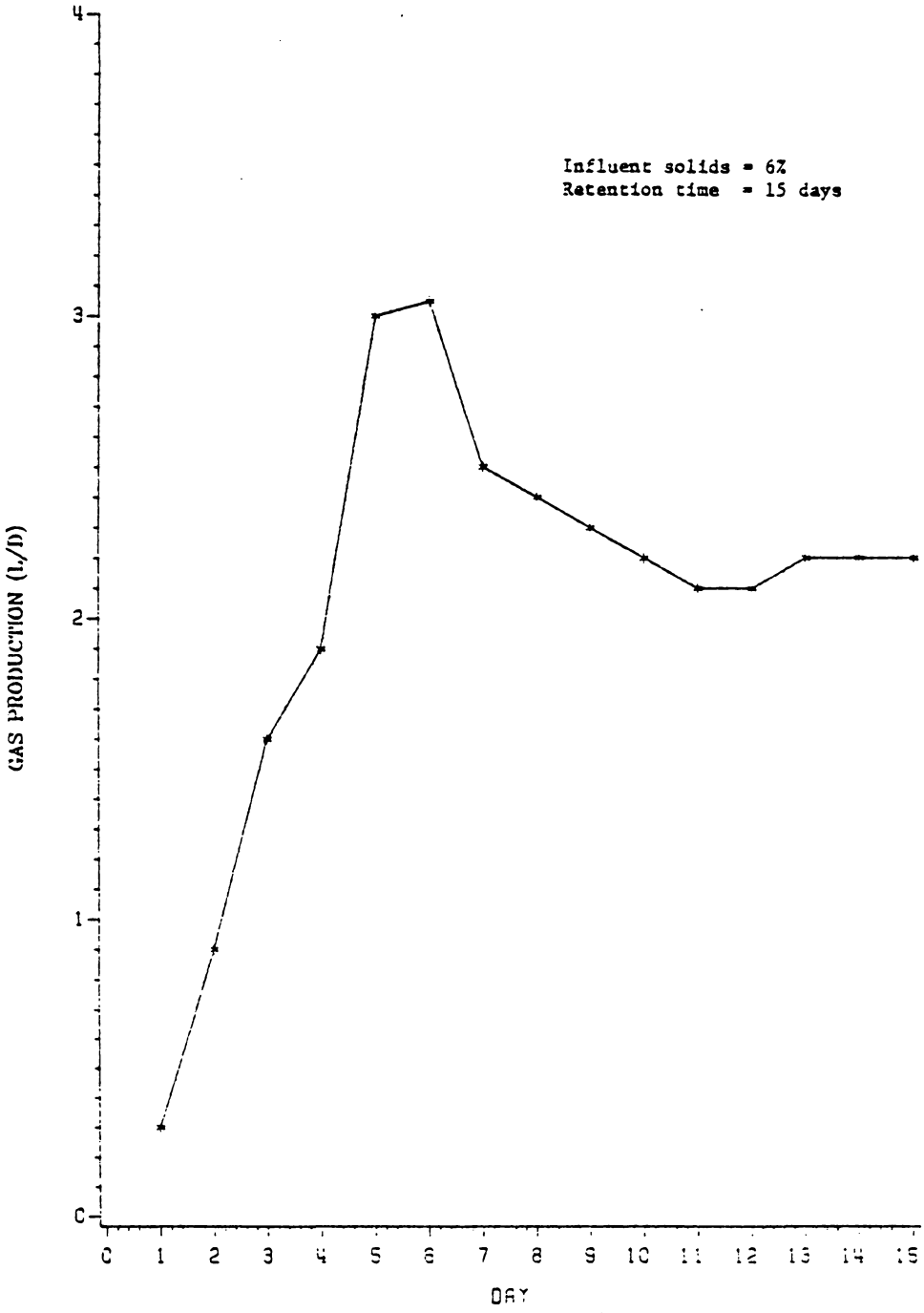


Figure 28: Gas Production of Digester II (Phase II).

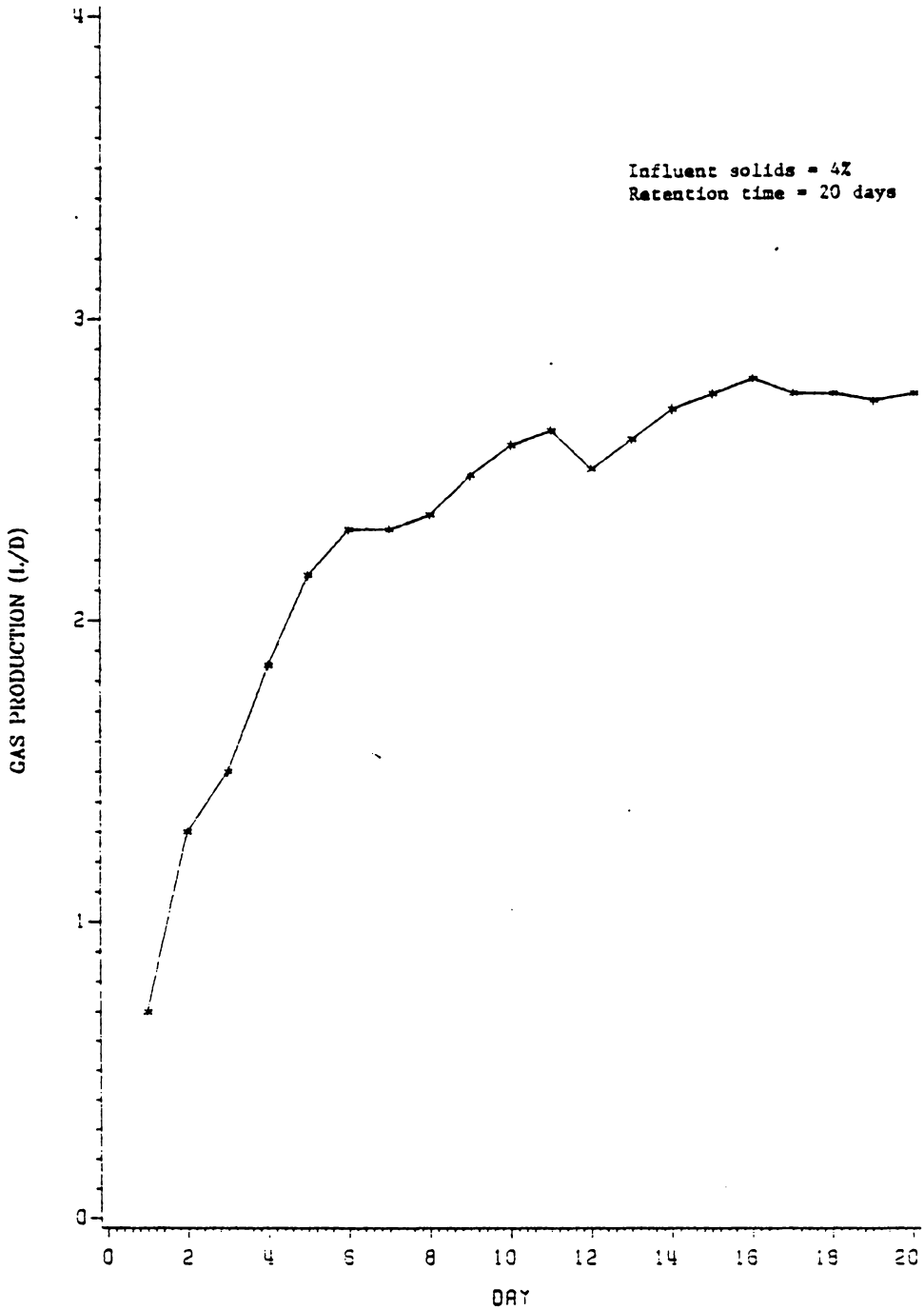


Figure 29: Gas Production of Digester III (Phase II).

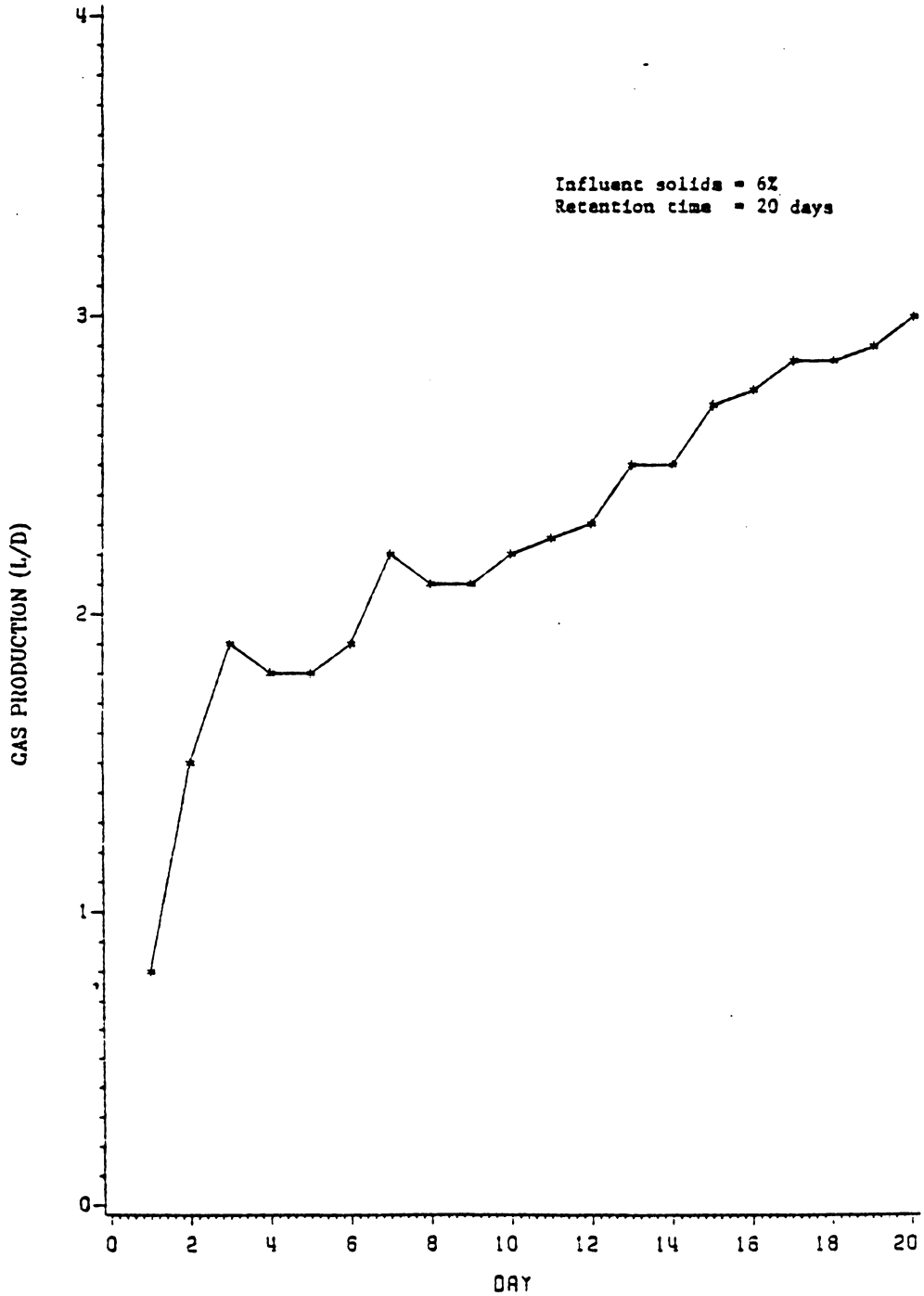


Figure 30: Gas Production of Digester IV (Phase II).

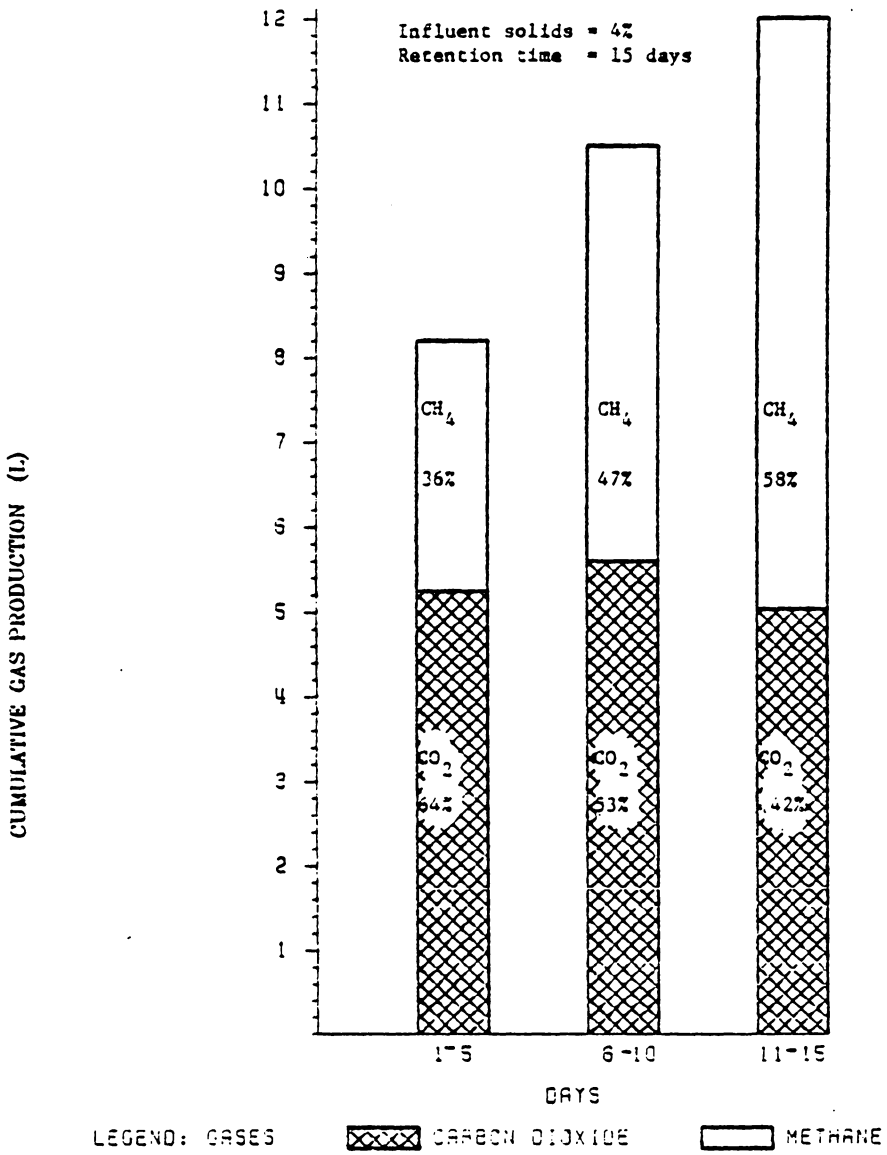


Figure 31: Gas Composition of Digester I (Phase II).

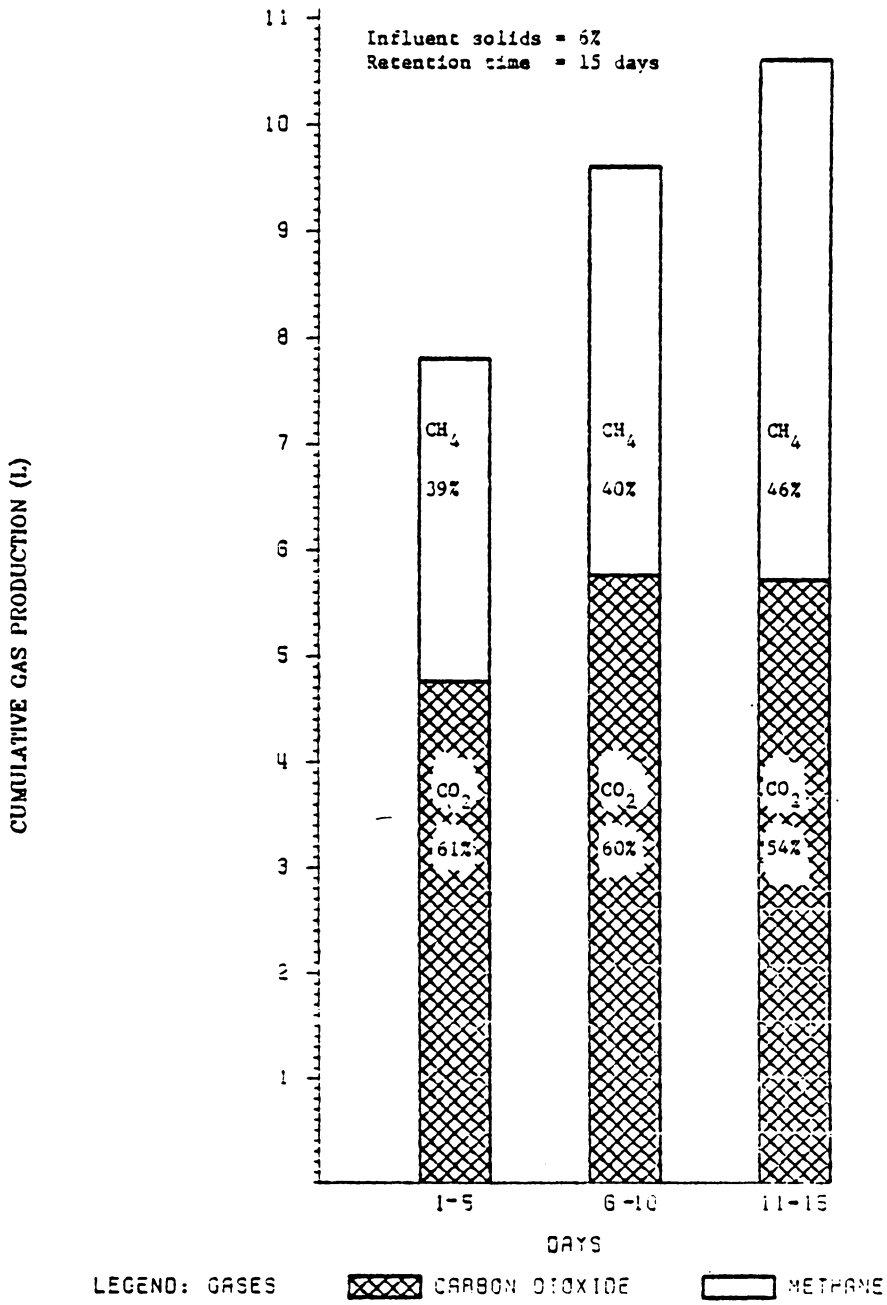


Figure 32: Gas Production of Digester II (Phase II).

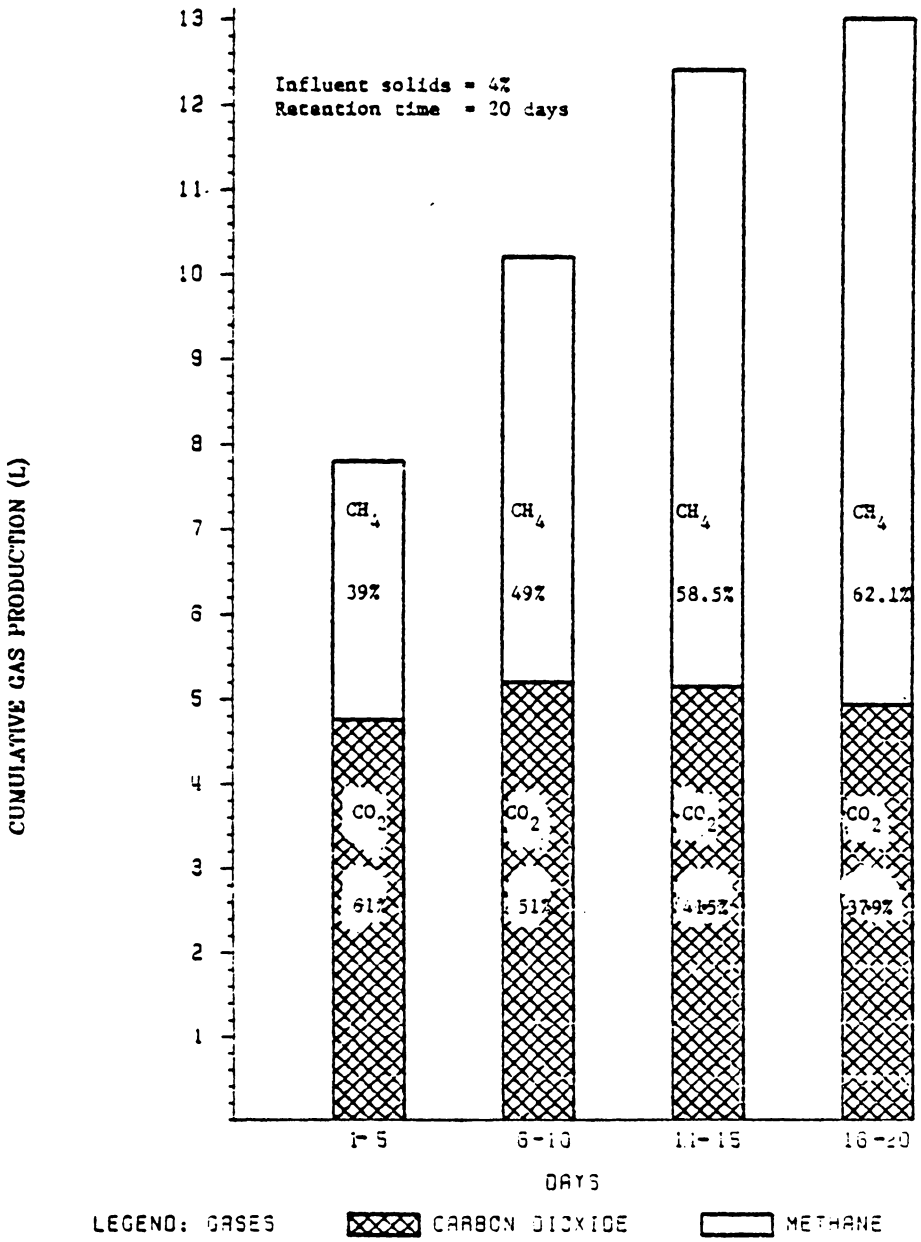


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Figure 33: Gas Composition of Digester III (Phase II).

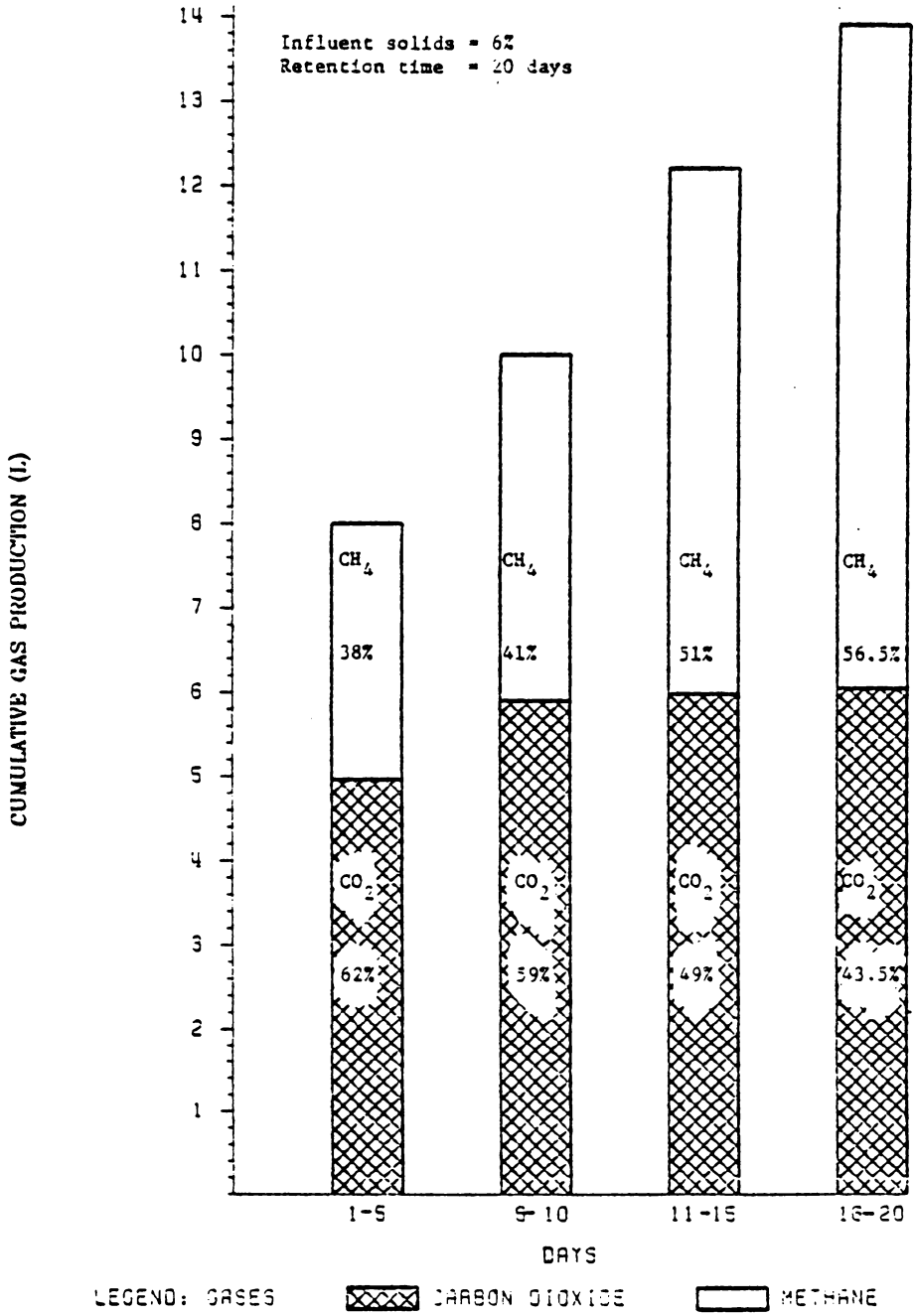

Figure 34: Gas Composition of Digester IV (Phase II).

4.2 VIABILITY OF JOHNSONGRASS AND FALL PANICUM SEEDS.

The results of the tetrazolium (TZ) and the standard germination tests performed on Johnsongrass and Fall Panicum seed at different stages during this study are summarized in Table 11.

4.2.1 Initial Germination Tests

The initial germination tests performed on seeds before placing in the digesters revealed that average viability of Johnsongrass seeds was 90.5%. Of the viable seeds, 56.9% were dormant and 43.6% were non-dormant. In the case of Fall Panicum, viability was 87% with 39.7% of the viable seeds being dormant, and 60.3% being non-dormant.

4.2.2 Viability Results of Phase I

In Phase I, the seeds withdrawn daily were seperated from the effluent and subjected to the TZ test. The daily viability percentages were recorded and plotted as five-day moving averages (Figure 35 and 36). In all treatments, a decrease in daily viability of Johnsongrass and Fall Panicum seeds was evident after two retention periods.

The data reveal that a drop in seed viability does occur over a period of time. In all cases, seed viability dropped rather steeply towards the end of the second retention cy-

TABLE 11

Results of Germination Tests.[1]

INITIAL								
Seed Type	Johnsongrass				Fall Panicum			
Viable (%)	90.5				87.0			
Dormant (% Viable)	56.9				39.7			
Nondormant (% Viable)	43.1				60.3			
FINAL								
Seed Type	Johnsongrass				Fall Panicum[3]			
Digester	I	II	III	IV	I	II	III	IV
Phase I								
Viable (%)	77.0	79.5	72.0	74.5	57.0	57.5	43.0	48.0
Non-viable[2] (%)	23.0	20.5	28.0[5]	75.5[5]	43.0	42.5	57.0[5]	52.0[5]
Dormant (% Viable)	54.1	57.3	50.1	55.3	43.1	45.0	38.1	48.7
Non-dormant (% Viable)	45.9	42.8	49.9	44.7	56.8	55.0	62.0	51.3
Phase II[2]								
Viable (%)	47.0	50.8	41.0	47.5	36.0	41.5	17.0	27.5
Non-viable[4] (%)	53.0 *	49.2	59.0[5] *	52.5[5]	64.0 *	58.5	83.0[5] *	72.5[5]
Dormant (% Viable)	92.6	93.6	98.7	90.5	62.5	63.9	82.4	70.9
Non-dormant (% Viable)	7.4	6.4	1.2	9.5	37.5	36.1	17.6	29.0

[1] Sample size = 200 seeds.
[2] Average for 2 replicates.
[3] More suceptible to treatment than Johnsongrass (significant at 0.1%).
[4] Affected by treatment (significant at 0.1% level).
[5] Significantly larger than values for digesters I and II.
* Significantly greater than values for digesters II and IV.

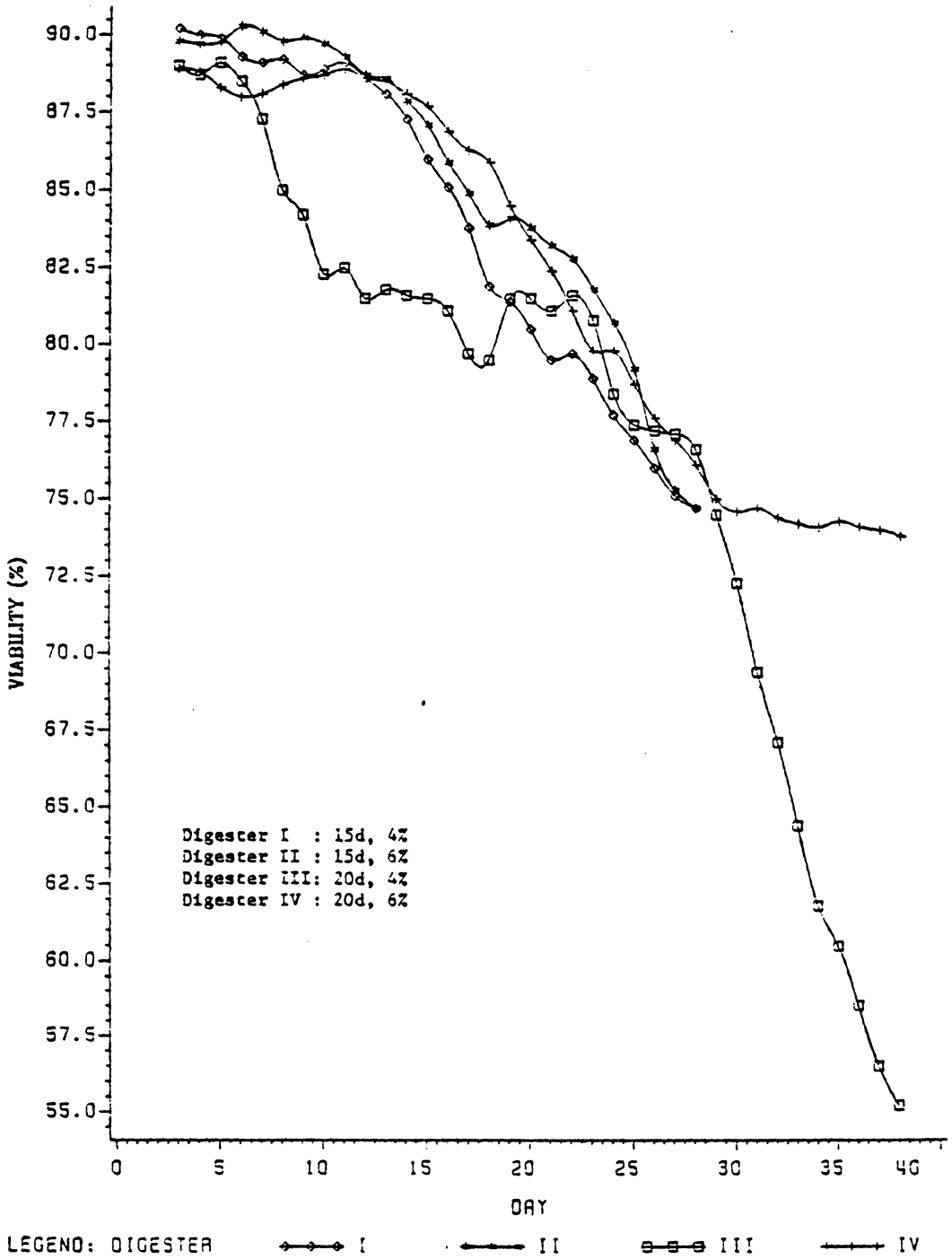

Figure 35: Viability Results of Johnsongrass Seeds (Phase I).

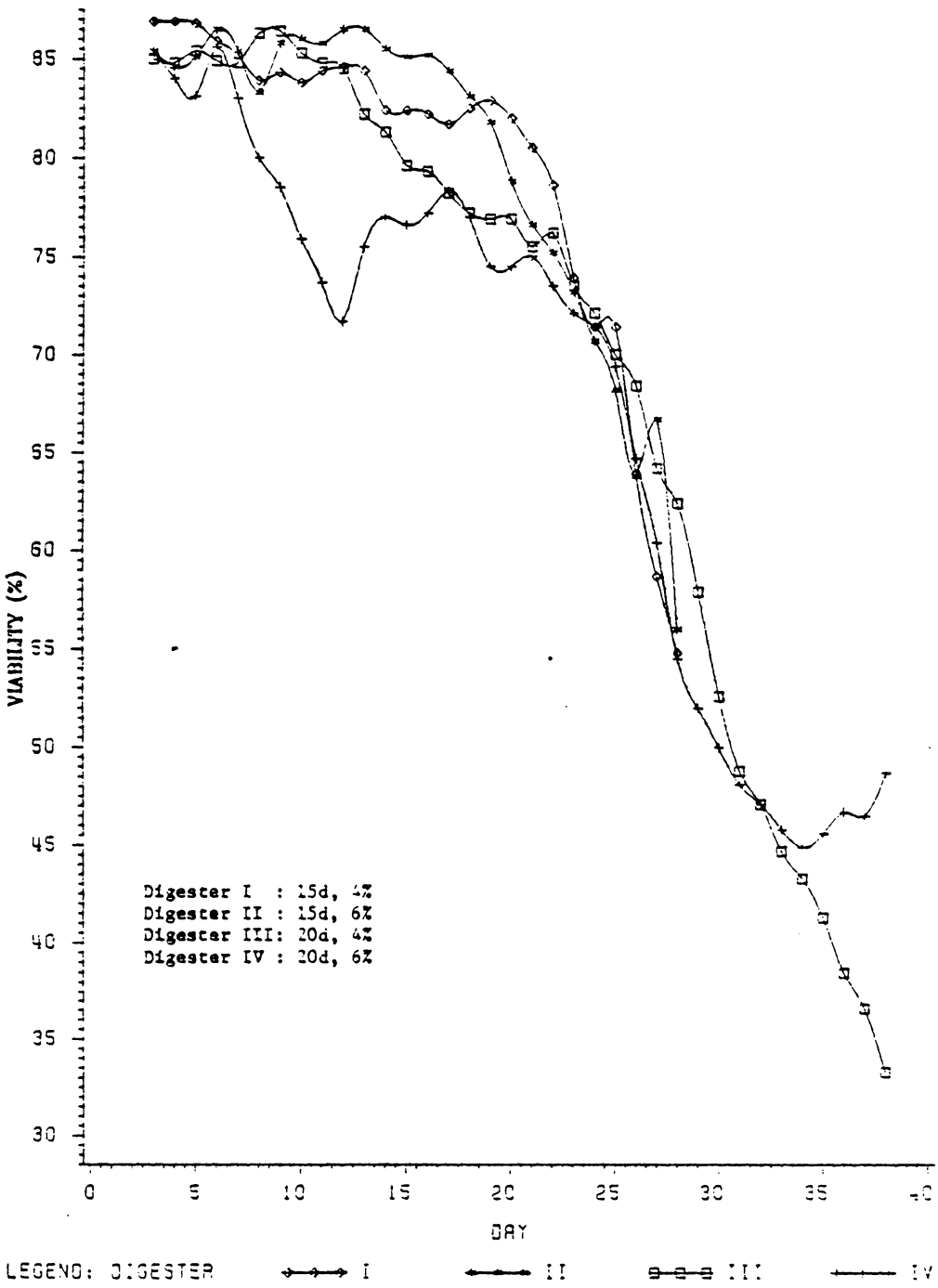

Figure 36: Viability Results of Fall Panicum Seeds (Phase I)

cle. Seeds withdrawn during the first retention cycle did not remain in the digester long enough for anaerobic fermentation to affect their viabitity. Each seed is protected by a coat which requires time for weakening by the microorganisms.

It was observed that in both Johnsongrass and Fall Panicum, greater loss in seed viability was achieved in 20-day digesters than in 15-day digesters. The greater the retention period, the longer seeds stay in the digester; hence, greater destruction occurs. Furthermore, digesters loaded at 4% solids concentration were more effective in reducing seed viability than digesters loaded at 6% solids. The difference was more pronounced at the end of the second retention cycle than at the end of the first cycle. This suggests that greater destruction of seed is in some way related to to the relative amount of solids and water in the digester. All seeds are known to imbibe water. The amount of water imbibed depends on, among other factors, the presence or absence of particulate matter in water. Thus, in the 4% substrate, more water was available for imbibition than in the 6% concentration. The greater the imbibition, the greater the number of bacteria that enter the seed, hence a greater drop in viability due to microbial attack.

It is also possible that permanent damage may have resulted due to imbibition. Early studies have confirmed soaking damage in seeds. The harmful effects of soaking have been attributed to bacterial attack (Barton 1929; Tilford et al. 1924), to loss of essential nutrients (Eyster 1940), to insufficient oxygen supply (Eyster 1940; Ross and Pollock 1971), and to direct membrane damage due to rapid water intake (Ross and Pollock 1971). During imbibition, water hydrates colloidal particles of seed tissue, causing them to increase in size as they change from solid to gel state. The increase in size leads to build up of imbibition pressure within the seed. This causes seed tissue damage. Hydration and swelling of colloids during imbibition is also accompanied by liberation of heat. Brown and Worley (1972) believe that imbibition rate is almost directly dependent on temperature, with higher temperatures accelerating the rate.

Finally, the daily-fed digesters were found to affect the viability of Johnsongrass and Fall Panicum seeds to different degrees. Johnsongrass seeds were not affected by anaerobic fermentation as much as Fall Panicum seeds. This can be attributed to the hard seed coat that protects Johnsongrass seeds. The main constituents of the seed coat are lignin, pectin and cutin which are noted for their resistance to biodegradation (Goering, 1970).

The final viability percentages of Phase I digesters were determined by performing germination tests on Johnsongrass and Fall Panicum seeds (200 each) picked randomly from the effluent remaining in the digesters at the end of two retention periods. The final viability percentages of Johnsongrass in increasing order were 72.0% (III), 74.5% (IV), 77.0% (I), and 79.5% (II). The corresponding values for Fall Panicum were 43.0% (III), 48.0% (IV), 57.0% (I), and 57.5% (II). The chi-square test of independence (Little and Hills 1978, Cochran and Cox 1957, Gomez and Gomez 1976) was used to analyze the data. The results are summarized in Table 12. Based on the statistical analysis, the following observations may be made:

1. Fall Panicum seeds were more suceptible to anaerobic fermentation than Johnsongrass seeds. The chi-square value was significant at the 0.1% probability level;
2. the viability of Fall Panicum seeds were affected by the treatment afforded, while for Johnsongrass the probability of deviation (from expected value) due to chance alone was 50%. Hence, it can be said that, although increased numbers of non-viable Johnsongrass seeds were obtained after anaerobic treatment, the statistical procedure used was not sensitive enough to attribute it to the effect of treatment;

3. the 20-day digesters caused greater loss in seed viability than 15-day digesters. The chi-square values for Johnsongrass and Fall Panicum seeds were significant at the 10% and 1% probability levels, respectively;
4. the deviations from the expected values were not significant for the 4% and 6% treatments; hence, the null hypothesis that 4% and 6% treatments affect the seeds equally, was accepted for both types of seeds.

4.2.3 Viability Results of Phase II

In batch digestion experiments actual retention times of 15 and 20 days were involved, rather than average retention time as used in the Phase I experiments. This resulted in lower viability percentages in Phase II than in Phase I. Johnsongrass seeds recovered from digesters I through IV were found to have viabilities of 47.0% , 50.8% , 41.0% , and 47.5% respectively. The corresponding figures for Fall Panicum seeds were 36.0% , 41.5% , 17.0% and 27.5%

4.2.3.1 Seed Population Model

The physiological state of the seed is of great importance to its survival. The dormant and non-dormant components of the viable seeds and the changes that occur to

TABLE 12

Summary of The Chi-square Test of Indepedence (Phase I).

Parameter Tested	Results		Inference
JG seeds vs. FP seeds	S(0.1)		FP more sucepti-ble to treatment than JG seeds.
Seed Type	JG	FP	
Treatment vs. viability	NS	S(0.1)	A relationship exsists between treatment and via-bility for FP. No such relation-ship for JG.
15 day digesters vs. 20 day digesters	S(10)	S(0.1)	20-d digesters cause greater loss in viab-ility than 15-d digesters.
4% digesters vs. 6% digesters	NS	NS	4% and 6% treat-ments affect the seeds equally.

FP = Fall Panicum; JG = Johnsongrass; S = Significant;
NS = Non-significant.
The probability levels (%) appear within parenthesis.

these components when placed in a digester can be described by a seed population model. Schafer and Chilcote (1969) proposed a model to describe the components of buried seed populations. This scheme, which is illustrated by Figure 37, is applicable to any seed population and is described by the equation:

$$S = P_{enf} + P_{end} + D_n + D_g \qquad (15)$$

Where,

S = total buried seed population of a species at a point in time;

P_{enf} = Seeds in a state of exogenous (enforced) dormancy (%);

P_{end} = Seeds in a state of endogenous (innate + induced) dormancy (%);

D_n = Seeds which have lost viability (%);

D_g = Seeds undergoing in-situ germination (%).

Of the seeds that germinate, the ones that are buried deep in the soil may not emerge (D_{gd}), while those close to the surface develop into seedlings (D_{ge}). The loss of viability of the seeds may be due to the following factors: (a)physiological aging (D_{na}); (b)the action of predators (D_{np}), or (c)events that transpired during seed development

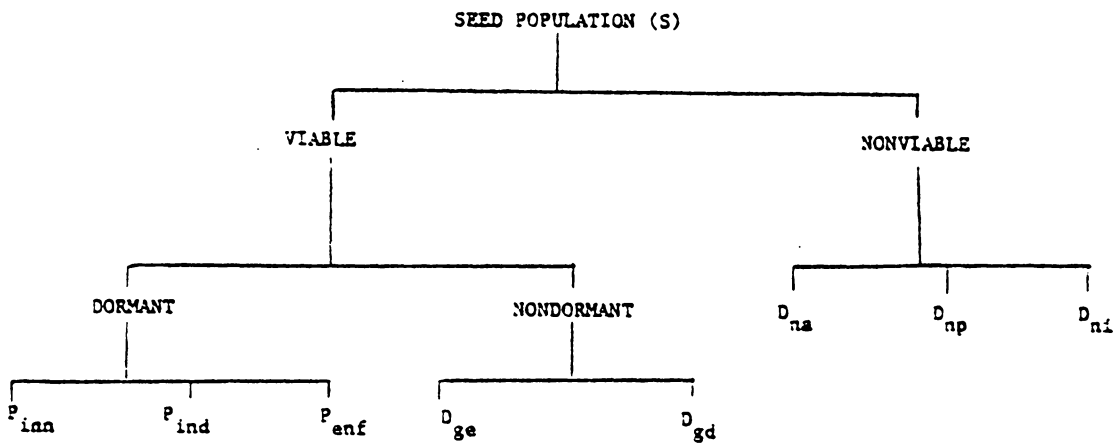

Figure 37: Seed Population Model (Schafer and Chilcote 1969).

and maturation (D_{ni}). It is also useful to distinguish between the three forms of dormancy, namely, innate (P_{inn}), induced (P_{ind}) and enforced (P_{enf}) dormancies. Hence, equation (15) can be rewritten incorporating all these components:

$$S = P_{inn}+P_{ind}+P_{enf}+D_{gd}+D_{ge}+D_{na}+D_{np}+D_{ni} \quad (16)$$

Lewis (1961) attributed rapid depletion of seed numbers in the topsoil to in-situ germination (D_{gd}). Taylorson (1970) cited in-situ germination as the major factor causing loss of seeds followed by pathogen and insect attacks. An attempt was made to apply equation (16) to various grass and clover seeds in the soil by Rampton and Ching (1970). They found it difficult to distinguish between D_g and D_n components. Investigations by Schafer and Chilcote (1970), and Courtney (1968) indicate that the most important factor influencing the depletion of any weed seed population in the soil is loss of dormancy. Taylorson (1970) observed that non-dormant weed seeds buried in soil lost viability faster than dormant seeds.

Although equation (16) was developed for seeds buried in soil, it is possible to modify it to suit this investigation, where the seeds are being subjected to anaerobic digestion. The following assumptions are necessary in order to reduce equation (16) to a desirable form:

1. seeds are from controlled storage environment and hence are not subject to adverse conditions (P_{enf} =0);
2. the D_g component refers to seeds that germinate under unfovourable conditions. These are either destroyed or give rise to defective plants. During the germination tests no seeds were observed to have germinated within the digesters (D_{gd} =0). It is assumed that all the seeds that passed the germination tests are capable of producing normal and healthy plants;
3. non-viability of the original seed sample before being placed in the digester is due entirely to physiological aging (D_{na}) and to events that transpired during seed development (D_{ni}), and;
4. the predators causing loss of viability in the digesters are bacteria.

Based on these assumptions, equations can be written for the seeds before and after they are subjected to anaerobic fermentation:

$$S = P^i + D^i_{ge} + D_{na} + D_{ni} \qquad \text{(before digestion)} \quad (17)$$

$$S = P^f + D^f_{ge} + D_{na} + D_{ni} + D_{np} \qquad \text{(after digestion)} \quad (18)$$

where the superscripts, i and f refer to situations before and after bacterial action, respectively, and $P = P_{inn}$ +

P_{ind} . From equations (17) and (18), we obtain a measure of the loss of seed viability within the digester:

$$D_{np} = (P^i - P^f) + (D^i - D^f) = dP + dD \qquad (19)$$

The dP and dD components were measured using the TZ and the standard germination tests, respectively. Figure 38 illustrates the application of equations (17) and (18) to this study, and Table 13 presents the experimental data obtained for Johnsongrass and Fall Panicum seeds.

The chi-square test of independence was used to interpret the data (Table 14). The results indicated that:

1. Johnsongrass seeds were more resistant to the anaerobic fermentation process. The high chi-square value obtained enabled the rejection of the null hypothesis (that treatments affect both types of seeds equally) at the 0.1% level;
2. there exsists a relationship between treatment and seed viability for both Johnsongrass and Fall Panicum seeds. The chi-square test was significant at the probability level of 0.1%;
3. 20-day digesters of Phase II achieved greater destruction of seed viability than 15-day digesters. The null hypothesis was rejected at the 10% level for Johnsongrass and at the 0.1% level for Fall Panicum;

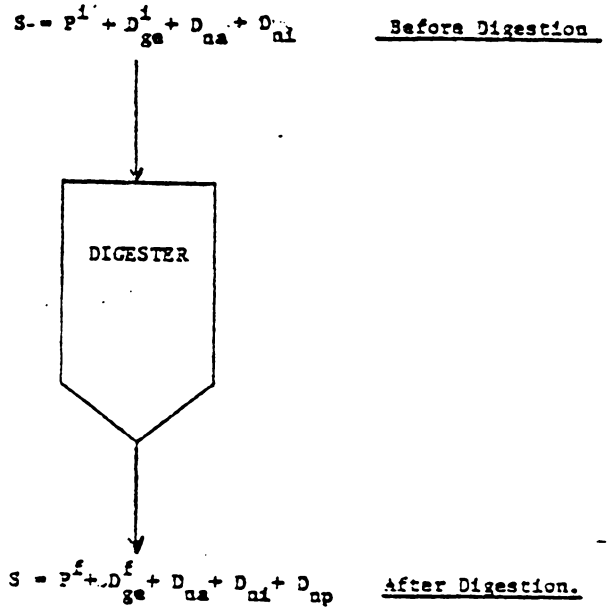

Figure 38: Application of Seed Population Model.

TABLE 13

Seed Count Before and After Treatment (Phase II).*

Component		Digesters I	II	III	IV
			JOHNSONGRASS		
Before Treatment:					
Dormant Seeds	(P_i^i)	103	103	103	103
Nondormant Seeds	(D_{ge}^i)	79	79	79	79
After Treatment:**					
Dormant Seeds	(P_f^f)	87	95	81	86
Nondormant Seeds	(D_{ge}^f)	7	6.5	1	9
$P^i - P^f$	(dP)	16	8	22	17
$D^i - D^f$	(dD)[1]	72	72.5	78	70
dP + dD	(D_{np})	88	80.5	100	87
dP/D_{np}	(%)	18.1	9.9	22.0	19.5
dD/D_{np}	(%)	81.9	90.1	78.0	80.5
			FALL PANICUM		
Before Treatment:					
Dormant Seeds	(P_i^i)	69	69	69	69
Nondormant Seeds	(D_{ge}^i)	105	105	105	105
After Treatment:**					
Dormant Seeds	(P_f^f)	45	53	28	39
Nondormant Seeds	(D_{ge}^f)	27	30	6	16
$P^i - P^f$	(dP)	24	16	41	30
$D^i - D^f$	(dD)[1]	78	75	99	89
dP + dD	(D_{np})	102	91	140	119
dP/D_{np}	(%)	23.5	17.6	29.3	25.2
dD/D_{np}	(%)	76.5	82.4	70.7	74.8

* Sample size = 200 seeds
** Average of 2 replicates
[1] Significantly larger than dP at 0.1% probabiliy level.

4. the 4% digesters were more effective in destroying seed viability than the 6% digesters. The chi-square values for Johnsongrass and Fall Panicum were found to be significant at the 10% and 5% levels, respectively;
5. the null hypothesis that the treatments affect dormant and non-dormant seeds equally was rejected at the 0.1% probability level. It was concluded that significantly more non-dormant seeds were destroyed than dormant seeds for both Johnsongrass and Fall Panicum.

The effect of treatment on dormant and non-dormant components of the total seeds destroyed is graphically illustrated by Figures 39 and 40. Dormant seeds are naturally more resistant to adverse environmental conditions. The non-dormant seeds are those which are ready to germinate and hence more susceptible to microbial attack. It was also observed that as severity of treatment decreased, the proportion of dormant seeds that were destroyed also decreased. In this investigation, severity of treatment is defined from the point of view of seed destruction. The Schafer-Chilcote model was modified to suit this investigation and allowed estimation of the total seeds destroyed by measuring the dormant (dP) and non-dormant (dD)components.

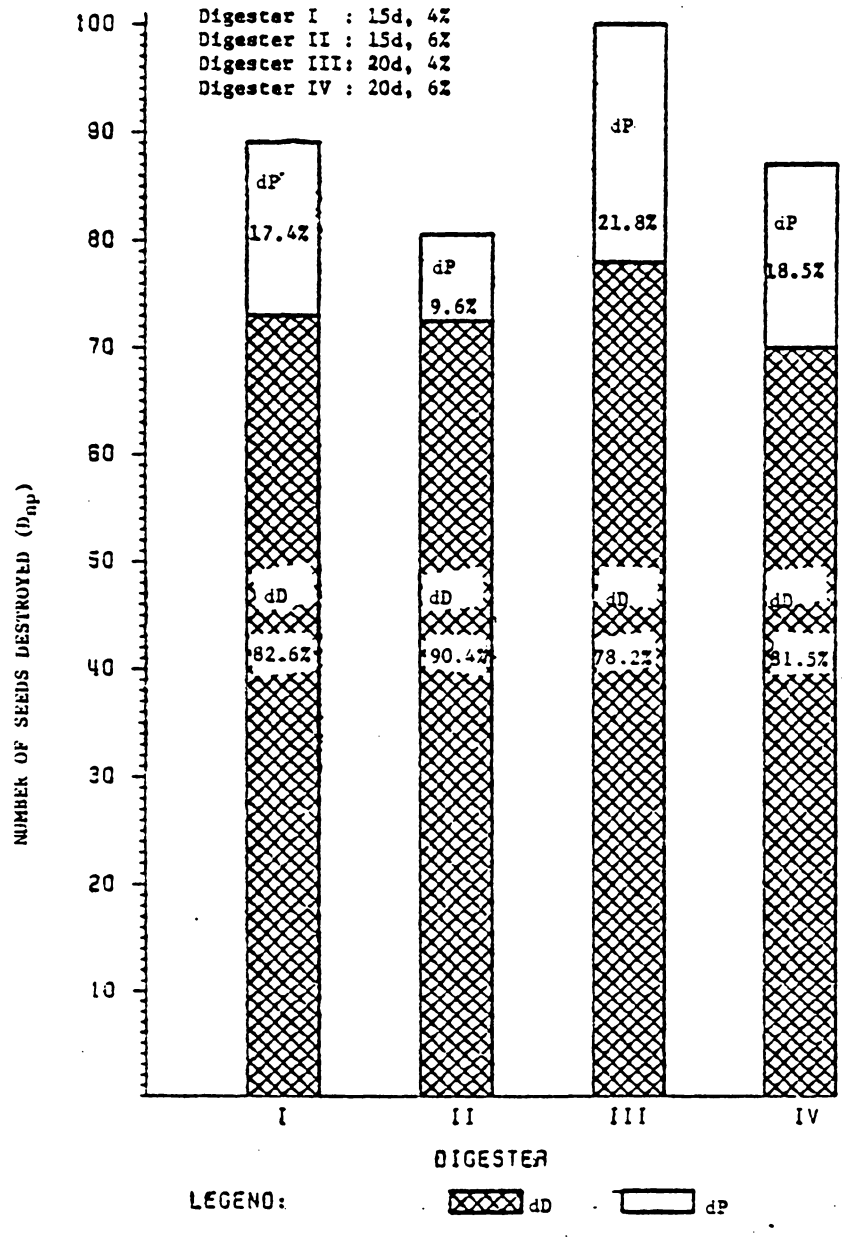

Figure 39: Effect of Treatment (Phase II) on Dormant and Non-Dormant Seeds (Johnsongrass).

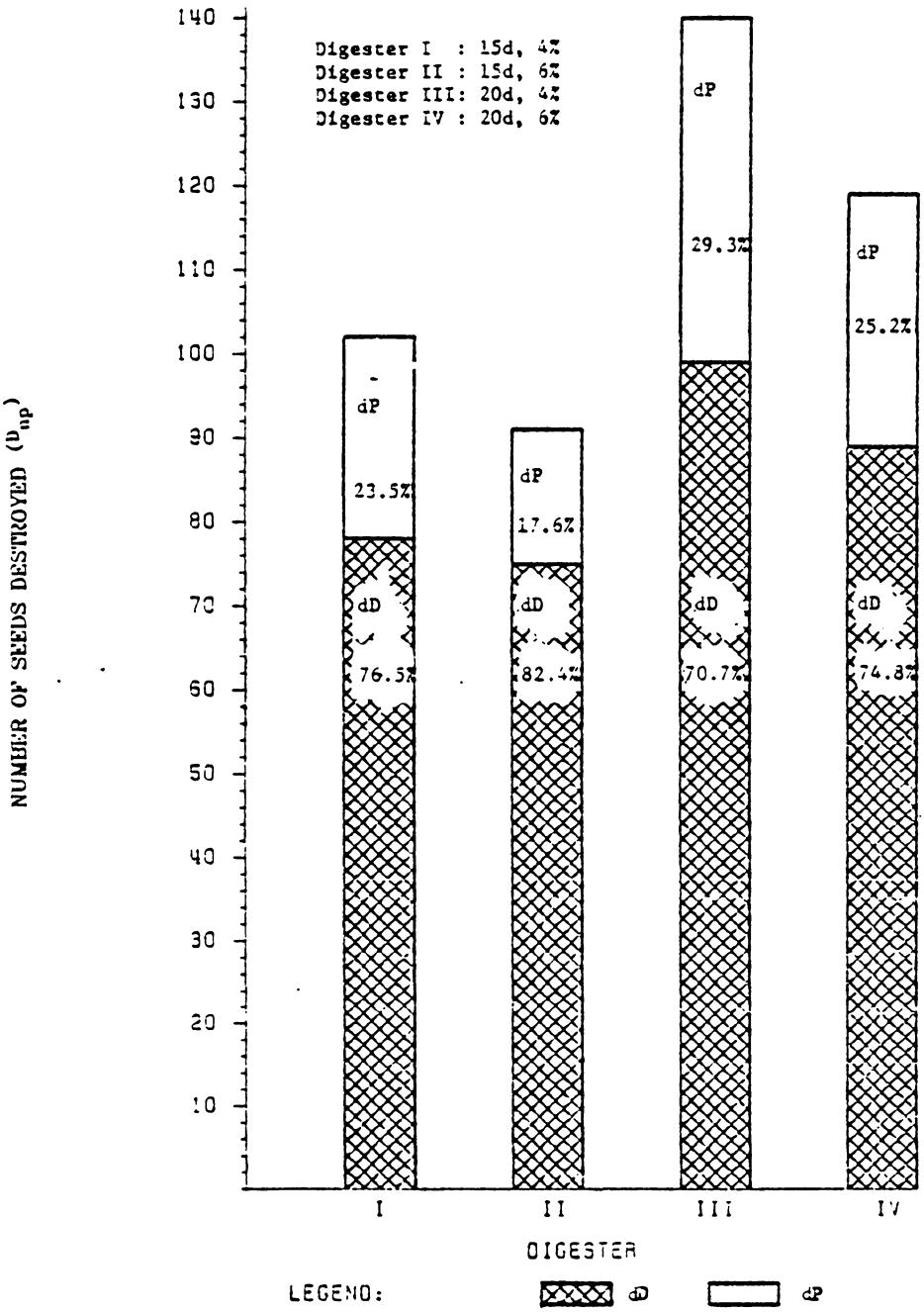

Figure 40: Effect of Treatment (Phase II) on Dormant and Non-Dormant Seeds (Fall Panicum).

TABLE 14

Summary of the Chi-square Test of Independence (Phase II).

Parameter tested	Result		Inference
JG seeds vs. Fp seeds	S(0.1)*		FP more suceptible to treatment than JG.
Seed Type	JG	FP	
Treatments vs. viability	S(0.1)*	S(0.1)*	A relationship exsists between seed viability and treatment.
15d digesters vs. 20d digesters	S(10)*	S(0.1)*	20-d digesters caused greater loss in viability than 15-d digesters.
6% digesters vs. 4% digesters	S(10)*	S(5)*	4% digesters caused greater loss in viability than 6% digesters.
dormant vs. non-dormant	S(0.1)*	S(0.1)*	more non-dormant seeds destroyed than dormant seeds

FP = Fall Panicum; JG = Johnsongrass; S = Significant.
* Probability level (%).

The contents of the seeds, determined before and after treatment, are presented in Table 15. The ash content represents inert material. In the case of seeds, this is the outter seed coat made of lignin, cutin and pectin. The seed coat encloses the easily digestible seed content (volatile solids) which includes the embryo and stored food normally used during germination. Volatile solids reduction, therefore, is a direct indication of the loss of seed content. The loss may be due to microbial attack or leaching of essential constituents, or a combination of both.

The protective characteristic of the seed coat of Johnsongrass is reflected by the high ash content of the seed. However, in spite of the tough coating, volatile solids reduction was observed. Copeland (1976) noted that in hard seeds, imbibition occurs not through the seed coat but through the microphyle which is usually plugged. Unplugging is said to be caused by impaction, action of microorganisms and passage through the digestive tract of animals. Hence, it is conceivable that during anaerobic fermentation the micropylar plug may have been degraded, thus providing a direct entry into the seed.

TABLE 15

Seed Contents Before and After Treatment

Before Treatment

Seed Type	JOHNSONGRASS	FALL PANICUM
TS (% wet wt,)	90.8	90.3
VS (% TS)	90.6	97.4
Ash (%TS)	9.4	2.6

After Treatment

Seed Type	JOHNSONGRASS				FALL PANICUM			
Digester	I	II	III	IV	I	II	III	IV
VS (%TS)	89.0	93.4	80.1	68.3	83.5	91.6	52.0	60.5
Ash (%TS)	11.0	6.6	19.9	31.7	16.5	8.4	48.0	39.5

Chapter V

SUMMARY AND CONCLUSIONS

Weeds are a menace to farmers, and their presence on cropland directly affects yields. Cattle wastes usually contain viable weed seeds which are transmitted to cropland when the waste is spread as fertilizer.

This study investigated the possibility of destroying Johnsongrass (Sorghum halepense) and Fall Panicum (Panicum dichotomoflora) seeds, which may be present in cattle manure, by anaerobic fermentation. The anaerobically stabilized sludge and supernatant can still be used as a fertilizer after energy as methane gas is removed. The main objectives of this study were:

1. to determine whether anaerobic digestion affects viability of Johnsongrass and Fall Panicum seeds;
2. to identify the level of treatment that is most effective in destroying the weed seeds; and
3. to apply Schafer and Chilcote's buried seed population model to interpret the results.

In order to achieve these objectives, the fate of seeds passing through daily-fed (Phase I), and batch (Phase II) mesophilic digesters was studied. Dairy cattle manure was used as substrate.

The following conclusions may be derived from the investigation:

1. mesophilic [35°C±1°C] anaerobic fermentation of dairy waste fed at influent solids concentrations of 4% and 6%, and maintained at retention times of 15 days and 20 days, does have an effect on the viability of Johnsongrass and Fall Panicum seeds;
2. greater destruction of Fall Panicum seeds achieved indicate that these seeds are less resistant to the digester environment than Johnsongrass seeds;,
3. at the end of two retention cycles, greater loss in viability of Johnsongrass and Fall Panicum seeds was observed in 20-day digesters than in 15-day digesters. The data also indicated that Fall Panicum seeds in the 4% feedstock were less resistant to destruction in the digester than seeds in the 6% feedstock. Apparently this is related to the phenomenon of imbibition;
4. dormant seeds of Johnsongrass and Fall Panicum are less suceptible to the destruction process than non-dormant seeds;
5. the Schafer-Chilcote buried seed population model can be modified to suit this study. The model can be used to relate the viability to the physiological state of the seed;

6. the mechanism of seed destruction in anaerobic digesters is probably one of microbial degradation or a combination of it with imbibition seed damage.

Chapter VI

RECOMMENDATIONS

1. Based on laboratory experiments, this study has established a trend in the loss of viability of Johnsongrass and Fall Panicum seeds when subjected to anaerobic fermentation. A large scale anaerobic digester study is necessary to determine treatment levels likely to achieve weed seed destruction.
2. Seed destruction by microbial action is influenced to a very large extent by the nature of the seed coat. Lignin, pectin and cutin are the most common constituents of the seed coat and have a high resistant to biodegradation. Hence, any process that weakens the seed coat, thereby making the contents of the seed vulnerable to bacterial attack, will enhance the chances of destroying seed viability. Anaerobic biodegrability of plant material has been investigated with special reference to lignin. More research is needed to determine whether similar procedures can be adopted to render seeds less resistant to microbial action.
3. The results of this investigation are confined to mesophilic conditions. Temperature has been shown to

affect the rate of imbibition by seeds. Thermophilic digestion may enhance the rate imbibition and seed damage. An appropriate investigation would provide more information on this concept.

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Appendix A

RAW WASTE DILUTION CALCULATION.

Assume:

Raw manure total solids	= 17.5% (as collected)
Influent Total Solids (TS)	= 4.5% (wet wt.)
Retention time (RT)	= 15 days
Density of slurry	= 1 g/cc
Digester working volume (V)	= 3 L

Daily influent volume = V/RT = 3000 cc/15 days = 200 cc

=3000 cc/15 days = 200 cc

= 200 g of 4% TS

Weight of influent solids = (200 g)X 0.4 = 0.8 g

Weight of influent water = (200 - 0.8) =192 g

Weight of raw waste required to provide 8 g of total solids = 8/17.5/100 = 45.7 g

45.7 g of raw manure will have (45.7 g) X (1-0.175) = 37.7 g of water associated with it.

Hence, additional water required = 192.0 - 37.7 = 154.3 g

That is, 45.7 g of raw waste of 17.5% TS is converted to 4% influent slurry by adding 154.3 g of tap water.

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