ANAEROBIC BIOCONVERSION OF WASTE PAPER AND NEWSPRINT

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CHAPTER I

INTRODUCTION

Development of This Research

Waste paper has been one of the most important categories of solid waste going to sanitary landfills. It is the major contributor to solid waste by either weight or volume. Currently, approximately 40 to 50% of landfill space is occupied by waste paper (Suflita et al., 1992). Construction and operation of landfills are costly. Because of its unpopularity with the general public as a method for solid waste disposal, obtaining sites for new landfill facilities has become very difficult. The resistance to the development of new facilities has imposed a capacity limitation on the existing facilities and caused a significant increase in the cost of refuse disposal in recent years.

Historically, waste paper was one of the earliest refuse components being recycled, and the trend of recycling has been growing constantly. According to Pfeffer (1992), material and energy recovery accounted for 7.1% of the 118.3 million tons of urban wastes generated in 1970. This increased to 14.6% for the 148.1 million tons generated in 1984 and is expected to reach 30.3% for the 182.2 million tons generated in year 2000. At that time the "average" composition of the refuse generated in the United States will include 41% paper and paper products. If the predictions are valid, it is

apparent that there is a large market for competitive technologies for waste paper utilization. Currently, plant capacity for the production of recycled paper is sufficient to meet present demand, so any reduction in waste paper load to landfills in the near future must come from other alternative uses of the material.

One potential use of waste paper is for feedstock to anaerobic reactors to produce methane or other fermentation products. Implementation of such systems requires knowledge of the potential limiting factors on the process, which would have to be overcome to maximize efficiency. These include limits on the biological conversion steps of enzymatic hydrolysis, fermentation of hydrolysis products, and production of methane and other final products from fermentation intermediates. In addition to limits on biological processing of cellulose, physical and chemical factors such as particle size, presence of surface treatments or printing ink and the ratio of cellulose to lignin and other complex polymers in the paper may limit conversion rates.

Much of the advancement in understanding and applying mesophilic anaerobic bioconversion of waste cellulose results from research on the conversion of agricultural and wood residues. Studies devoted to waste paper digestion generated relatively slow conversion rates and low conversion extent on newsprint because they were either undertaken with other easily digestible cellulosic materials in a limited duration, or with no means to disrupt lignin-cellulose association in the newsprint fibers.

The basic experimental approach of this study was to use anaerobic bench-scale screening studies to quantify the rates of waste paper hydrolysis and conversion to fermentation intermediates such as organic acids, and, ultimately, methane. Thus, limits

on each bioconversion step could be defined. These findings then led to the development of a pretreatment method which might alleviate one of worst limits defined above and improve the rate and extent of the conversion processes.

Experimental variables studied included anaerobic microbial inocula from different sources, type and previous use of paper, and the initial size of untreated, shredded or ground paper loaded to the reactors. Monitoring of the systems included determination of the conversion rate of paper to methane and carbon dioxide, and the changes of pH, alkalinity, volatile solids, cellulose to lignin ratio and COD.

Three different sources, namely anaerobically digesting sewage sludge, anaerobic landfill contents and bovine rumen contents were used as starting inocula in this study. The effectiveness of converting cellulose to methane, the conversion rate, and the ability of these consortia to dissociate lignin cellulose structure were investigated. Both semicontinuous seed reactors and serum bottle tests were conducted to investigate the effectiveness of three inocula on the digestion of waste paper.

Different paper sources and different physical sizes of each paper were used as substrate in this study. Pretreatment of the paper samples by paper shredder and grinding mill enabled quantification of the effects of size reduction on paper wastes prior to anaerobic digestion. Previous use of the paper for printing may have certain effects on the anaerobic digestion. This point is of importance in determining the potential need for deinking processes for waste paper pretreatment. Assessment of whether deinking is advantageous or unnecessary prior to anaerobic treatment was accomplished by examining rates of bioconversion of inked versus unused paper.

After basic conversion conditions were established and the limits on different bioconversion steps were defined, further efforts focused on pretreatment which might improve the microbial conversion rate and extent of the high lignin content waste paper (newsprint). Alkali treatment is one of the best known chemical pretreatments which can solubilize lignin and hemicellulose from lignocellulosic materials and obtain high purity of cellulose. However, most investigations on this treatment involve washing off the soaking reagent, loss of digestible hemicelluloses, and high caustic waste stream disposal. If the alkali dosage can be minimized and neutralized instead of washing off without sacrificing the efficiency of fractionation, and if the whole treated media can be fed to an anaerobic digester directly without disposal of the waste stream, then most of the disadvantages of the process can be avoided. In addition to the alkali pretreatment, the possibility of acetic acid pretreatment was also investigated. The pretreatment portion of this study was designed to demonstrate these possibilities.

Objectives of This Research

Specific objectives of this study were to:

1. Develop semicontinuous seed reactors to acclimate lignocellulose digesting abilities of three different inocula sources under mesophilic conditions and supply seed for batch reactors;
2. Use the above semicontinuous reactors to evaluate the conversion rates and extent of newsprint by different inocula during normal feeding periods;

3. Use the semicontinuous reactors to conduct long-term tests to evaluate extent of the ultimate biological methane conversion of the newsprint;
4. Determine the limiting factor(s) associated with the semicontinuous reactor design;
5. Examine different inocula's activities and their effects on waste paper bioconversion by monitoring both semicontinuous reactors and serum bottle batch tests;
6. Examine different paper sources and their effects on paper bioconversion by conducting serum bottle batch tests;
7. Examine the physical size effects on paper bioconversion by conducting serum bottle batch tests;
8. Examine the effect of printing ink on paper bioconversion by conducting serum bottle batch tests;
9. Define the limiting step(s) in the bioconversion of newsprint paper from both semicontinuous and serum bottle reactors;
10. Determine the effects of alkali pretreatment on the high lignin content waste paper (newsprint) conversion rate and extent by using different alkali concentrations, soaking durations, soaking temperatures and neutralizing reagents.
11. Evaluate the possibility of acetic acid pretreatment on high lignin content waste paper (newsprint) conversion to enhance rate and extent.

CHAPTER II

LITERATURE REVIEW

Scope of Review

In order to understand the reactions of waste paper biodigestion processes, many interrelated factors should be understood. These include the composition and constituents of waste paper, the constituents of natural fiber materials from which paper is made, the biodegradation of cellulose and lignocellulose, the relationship between microorganisms and cellulosic material, the relationship between the enzymes excreted by microorganisms and cellulosic material, and influences of physical and chemical properties of printing ink and other additives of the paper on its biodegradation.

It is well known that enzymatic hydrolysis of cellulose is controlled by the high-order molecular packing of its crystalline regions. In addition to this rate-limiting structure of cellulose, its association with lignin and hemicellulose, as in the case of natural lignocellulosic materials, further inhibits enzymes' penetrability and represses its microbial degradation. In earlier days, shortages of conventional food/feedstuffs forced people to explore the millions of tons of unused lignocellulosic crop residues. Chemical and physical pretreatments have been explored to alter the fine structure of cellulose, disrupt or open up the lignin-cellulose association, and increase substrate surface area-to-mass ratio to enhance chemical, microbiological or enzymatic conversions. Recently,

more investigations are concentrating on the structural properties of lignin and its biological degradation.

To date, many research activities reveal that cellulose and lignocellulose can indeed be modified by various physical, chemical and biological manipulations. Physical subdivision by ball milling can thoroughly break the cellulose-lignin complex; irradiation with high energy electrons can significantly increase digestibility of lignocellulosic materials; base and ammonia soaking can swell and separate lignocellulosic structural elements; and selectively removing lignin by white rot fungi and other microorganisms may leave cellulose basically untouched. Although some of these treatments have been put aside because of economic or other limitations, others are still being investigated in a search for effective, low cost pretreatment alternatives.

Waste Paper Constituents

Waste paper is the major contributor to solid waste for disposal or recycling. In 1990, 21 million tons of waste paper were recycled according to EPA (Miller, 1994). The constituents of waste paper from different sources, for example office waste paper or waste paper from a residential recycling program, are significantly different. Estimated generation of waste printing and writing paper in offices in 1990 is listed in Table 2.1, while composition of residential mixed paper in a curbside recycling program is listed in Table 2.2.

Table 2.1 Estimated Generation of Office Paper in 1990

Paper type	Generation (thousands of tons)
Business forms	2,510
Reprographics(copy paper, ledger)	2,360
Commercial printing composite	1,750
File folders	480
Magazines	440
Stationery and tablets	260
Envelopes	230
Books	100
Other	110
Total	8,240

Source: Miller, 1994.

Table 2.2 Composition of Residential Mixed Paper in a Curbside Recycling Program

Paper type	Generation (%)
Groundwood	41.7
Magazines	22.0
Newsprint	15.9
Catalog	2.4
Phone books	1.2
Other	0.2
Corrugated containers	16.3
Paper bags, sacks	4.8
Boxboard	8.9
Ledger paper	10.0
White ledger	8.0
Colored ledger	1.8
Other	0.2
Junk mail	8.7
Other	9.6

NOTE: Newspaper was collected separately from residential mixed paper in the sampled area. Newspaper in this sample was included with residential mixed paper.

Source: Miller, 1994.

As more material has been added to collection programs, paper recycling is more complex today than ever before. According to an American Forest and Paper Association (AFPA, Washington, D.C.) study, mixed paper will increase from 4 million tons in 1992

to 11 million tons in 2000 (Miller, 1994). Its growth is faster than any other grade of recovered paper, and its recycling is the key to the new recovery goal in the future.

The constituents of curbside mixed paper vary widely as the collection programs are very different from city to city. However, the biggest problem, according to Miller (1994), is that mixed paper generally has negative value. Programs collecting a truly mixed grade must expect to pay a tipping fee to a processor. This trend may make anaerobic digestion a stronger competitor above other paper recycling processes.

Natural Fiber Material Constituents

The chemical constituents of natural plant fibers include cellulose, hemicellulose, lignin, a wide variety of extraneous materials, and a small amount of inorganic matter. The amounts reported in Table 2.3 (Bartholic et al., 1986), indicate the approximate cellulose, hemicellulose and lignin contents of some agriculture residues and wood.

Cellulose is the most abundant organic material in the biosphere, and plays a central role in the global carbon cycle (Bolin, 1979). It forms the bulk of the cell wall material of all higher plants (Cowling, 1963). As the major component of wood, pulp, paper, cotton, rayon, and many plastic products, cellulose is one of the least expensive and most useful renewable natural resources.

Table 2.3 Some Agricultural and Wood Residues

Biomass	Cellulose Content, %		Hemicellulose Content, %		Lignin Content, %	
Barley	Str	41.9	E Hu	44.3-5.66 27.0-30.1	16-22	
Corn	Sta	34.4	Sta	23.7	Sta	10.5
	C	34.9	C	37.3	C	7.4
Sorghum	Sto	39.6	Str	25	Hu	13.6
Hay (loose)	A	35	Str	25	A	7.3
Oats	Str	49.3	Str	25	Str	14-22
Rye	Str	41.7	Str	24		
Soybeans	Sto	46.3	Str	25	Hu	11.4
					Hu	6.5
Wheat	St	47.0	Str	25	Str	13.9
Wood						
Hardwood		45.8		30.7		20.3
Softwood		43.8		24.5		29.5

A-All hay types combined C-Cob E-Endosperm H-Hay
Hu-Hull Sta-Stalk Sto-Stover Str-Straw
Source: Bartholic et al., 1983.

Cellulose makes up about 90% of cotton fibers but only about 45% of typical wood cell walls (Cowling, 1974). In common with other natural products, cellulose is susceptible to degradation by a wide variety of microorganisms. The cellulose in different plants is very similar in molecular structure (Cowling and Kirk, 1976). Differences in composition of different types of fibers are mainly in the nature and type of substances with which the cellulose is associated.

The cellulose in cotton is associated with only small amounts of non-cellulosic polysaccharides, whereas most agricultural and wood fibers contain significant amounts of both hemicellulose and lignin. All types of fibers contain small amounts of proteinaceous, mineral, and other extraneous materials.

Cellulose is often considered to be a repeating polymer of b-1,4-linked glucose units, although the true repeating stereochemical unit of cellulose is the disaccharide cellobiose(b-1,4-D-glucosyl-D-glucose) (Gilbert and Tsao, 1983). The degree of polymerization (DP) of individual chains is usually in the region of about 1500 to 15000, giving a molecular weight of ca. 2.5×10^5 to 2.5×10^6 (Knapp, 1985). Cellulose fibers contain both amorphous and crystalline regions. The crystalline regions are considered to be more difficult to degrade than amorphous regions (Huang, 1975).

The highly ordered crystalline structures which make cellulose extremely stable are formed by interchain hydrogen bonding among cellulose molecules (Marchessault and Sundararajan, 1983; Knapp, 1985). Within the crystalline arrays, the individual chains are packed in groups of about 30 to form a so-called elementary fibril, approximately 100 of which are in turn packed into larger units called microfibrils; these microfibrils are further packed to form the familiar cellulose fiber (Weimer, 1992). Within highly crystalline regions, the lattice is packed so tightly that even water cannot penetrate. Consequently, biological attack on the crystalline regions proceeds only along the surfaces or exposed ends of crystallites.

Unlike cellulose, hemicellulose shows variability in both structure and composition. Hemicelluloses are particularly heterogeneous polymers in that they are

composed mainly of the three hexoses, (glucose, mannose, and galactose), and two pentoses, (xylose and arabinose), together with uronic acids (Kuhad and Singh, 1993). Dunlap and Chang (1980) classified them into three, well defined groups: 1. xylans that have a basic backbone of poly b-1,4-xylan with additional side links to arabinose, glucuronic acid, and arabino glucuronic acid; 2. mannans that are composed of glucomannans and galactomannans; and 3. galactans appearing as arabinogalactans. The type and amount of hemicellulose vary widely depending on plant materials, type of tissue, growth environment and method of extraction (Kuhad and Singh, 1993). The degree of polymerization of short-chained heteropolymers of hemicelluloses is usually less than 200. They are generally much smaller than cellulose and are alkali soluble (Knapp, 1985).

As the second most abundant biopolymer in nature, lignin is an amorphous, three-dimensional aromatic polymer that associates with cellulose as lignocellulose and provides the structural rigidity to vascular plants (Kirk, 1987). Its molecules vary widely in molecular weight. Much work has been devoted to obtain its average molecular weight (MW) and the MW distribution (Meister and Richards, 1989; Himmel et al., 1989; Siochi et al., 1989; Johnson et al., 1989; Forss et al., 1989; Froment and Pla, 1989). Its number- and weight-average molecular weights are estimated as 4500 and 15000 (Froment and Pla, 1989). The number-average molecular weight is obtained by dividing the total mass of the sample by the number of molecules in it, while the weight-average molecular weight is obtained by summing up the mass of each molecule times the weight fraction of that molecule in the sample (Meister and Richards, 1989).

Structurally, lignin is composed of three types of phenyl propane units linked through seven major types of C-C or C-O-C linkages. These aromatic rings are substituted with a 0, 1, or 2 methoxyl group and the interunits lack stereo regularity (Kuhad and Singh, 1993). It contains 50% more carbon than cellulose. Lignin is found in all vascular plant cells and fills the spaces between cellulose fibrils together with hemicellulose and pectin, and thus acts as binding material between cell wall components.

In addition to cellulose, hemicellulose and lignin, the plant cell wall contains extraneous materials, including extractives and nonextractives (Kuhad and Singh, 1993). On a dry weight basis, wood contains 1 to 8% extractives and 0.2 to 0.8% of nonextractives while agricultural residues contain more extractives. The extractives consist of waxes, fats, gums, starches, alkaloids, resins, tannin, essential oils, and various other cytoplasmic constituents while the nonextractives include inorganic compounds such as silica, carbonates, oxalates, etc.

Cowling and Kirk (1976) described the structural relationships of above components of wood in relative detail. The noncellulosic materials are deposited in all regions of the cell walls from the lumen through the compound middle lamella. Cellulose is in highest concentration in the secondary wall and diminishes toward the middle lamella, while hemicelluloses and lignin are in highest concentration in the compound middle lamella and decrease toward the lumen. Extractives deposit mainly in the lumina of wood cells, while nonextractives spread in all cell wall layers. Within the various layers of wood cell walls, the hemicelluloses, lignin, extractives and nonextractives are concentrated in the spaces between microfibrils or elementary fibrils. The hemicelluloses

and lignin form a matrix surrounding the cellulose. Within a given microfibril, lignin and the hemicelluloses may penetrate the spaces between cellulose molecules in the amorphous regions.

Biodegradation of Cellulose and Lignin

Biodegradation of Cellulose

Cellulose is a linear polymer consisting of repeating units of cellobiose (Figure 2.1). It is biodegradable by hydrolytic enzyme cellulases, which convert cellulose to glucose and oligomers. Both single enzymes and enzyme complexes which can degrade cellulose are termed cellulase. The saprophytic microorganisms (including representatives of the fungi, bacteria and protozoa) are pre-eminent as cellulose degraders (Knapp, 1985). Cellulose is generally degraded by extracellular cellulases excreted by microorganisms to soluble products which can diffuse back to the cellulase-producing cells. These products can also be taken up by other microorganisms which thus depend on the cellulose degrader. Associations of cellulolytic and non-cellulolytic microorganisms are common in nature. Cellulose is often degraded more rapidly in mixed cultures due to the removal of inhibitory end-products (like cellobiose) and possibly to cross feeding (Knapp, 1985). In anaerobic conditions, cellulose degradation may be assisted by methanogens which remove inhibitory fermentation products like hydrogen (Chung, 1976). Cellulolytic microorganisms often grow in close association with their substrate (Knapp, 1985). This physical association shortens the distance of the enzymes and the degradation products must diffuse.

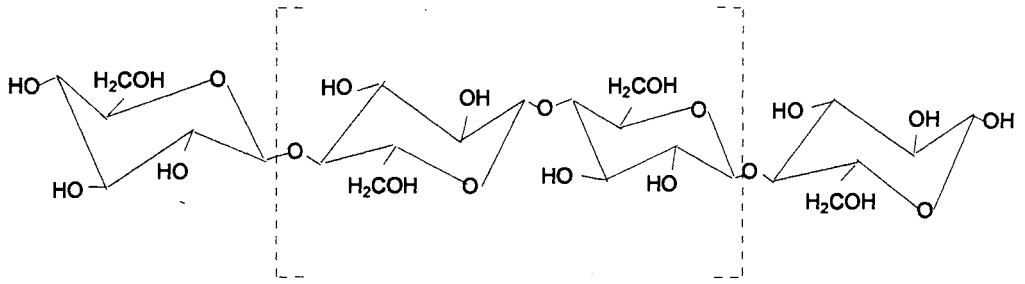

Figure 2.1 Cellulose molecular structure (From Bayer and Lamed, 1992).

The best understood cellulose degrading complexes are those of *Trichoderma reesei* and *T. koningii* (Knapp, 1985). According to Shoemaker and Brown (1978), Ladisch et al. (1983) and Wood (1985), cellulases of *T. reesei* can be divided into three major classes. They are: (1) endoglucanases or endo-1,4-b-glucanase, which initiate random attack on crystalline cellulose producing cellodextrins, cellobiose and glucose; (2) cellobiohydrolase which removes cellobiose units one by one from the non-reducing ends of cellulose chains; and (3) b-glucosidase (cellobiase) which hydrolyses cellobiose to glucose. Endoglucanases attack randomly along the cellulose fiber, resulting in a rapid decrease in the chain length of carboxymethylcellulose or H_3PO_4-swollen cellulose and yielding glucose, cellobiose, cellotriose and other higher oligomers (Ladisch et al., 1983; Wood, 1985). Cellobiohydrolyase, which is often called exoglucanase, can degrade highly crystalline cellulose, while b-glucosidase hydrolyzes cellobiose and in some cases other cello-olligosaccharides to glucose (Ladisch et al., 1983; Wood, 1985). Mixtures of endoglucanase and cellobiohydrolase account for most of the cellulase activity.

However, b-glucosidase is needed to reduce the inhibitory effects of cellobiose (Wood, 1985). When cellulose is the substrate, its hydrolysis has been shown to be rate-limiting in overall anaerobic digestion (Noike et al., 1985).

When pure celluloses (Solka Floc SW40 and Sigmacell 50) were treated by various physical and/or chemical treatments and hydrolyzed with the culture filtrate of *T. reesei,* it was found that the rate of cellulose hydrolysis primarily depends on its specific surface area (SSA) and crystallinity, but independent of treatment methods (Fan *et al.*, 1981). The rate of hydrolysis would tend to increase with an increase in SSA (m^2/g) and with a decrease in crystallinity index (CrI, %). The extent of hydrolysis after 8 hours, X_8 (g/L), can be approximately expressed as a function of both the SSA and CrI. The following empirical expression was obtained with a linear regression analysis:

$$X_8 = 0.380\ (SSA)^{0.195}\ (100 - CrI)^{1.04}$$

The large exponent for the crystallinity relative to the SSA term indicates that the rate of hydrolysis is more sensitive to CrI than to SSA.

With lignocellulosic materials, crystallinity of cellulose is less important than its association with lignin and hemicellulose and the surface area. With wheat straw as substrate, an empirical model that related relative extent of hydrolysis (REH, %) with SSA, CrI and lignin content (LC, %) was developed by Gharpuray et al. (1983):

$$REH = 2.044\ (SSA)^{0.988}(100 - CrI)^{0.257}(LC)^{-0.388}$$

In this model, the exponent for the crystallinity term, 0.257, was much lower than the 1.04 found for the pure cellulosic substrate. However, the exponent for the SSA is much higher, which has a predominant effect on the REH. The negative exponent for the LC indicates that cellulose lignin association will cause notable reduction in the REH.

Microscopic examination of cultures growing on cellulose clearly shows essentially complete microbial colonization of exposed, readily digestible tissues, and a considerably lower cell density on less readily digesting tissues and in the bulk liquid phase (Kudo et al., 1987). It was also noted that the process of cellulose digestion does not proceed if the substrate colonization is prevented or disturbed. Since the rate and extent of hydrolysis are determined by the amount of enzyme adsorbed on the specific surface area of insoluble cellulose, the kinetics of adsorption have also been explored by several investigators (Huang, 1975; Reese, 1977; Ooshima et al., 1983). Langmuir adsorption kinetics have been applied to the adsorption of cellulase to cellulose (Ooshima et al., 1983):

$$E_{ads} = \frac{K_p E_{ads,m}}{1.0 + K_p E} E$$

where E is the enzyme concentration in the supernatant in mg/mL, E_{ads} is the adsorbed enzyme in mg/mg cellulose; $E_{ads,m}$ is the maximum amount of enzyme adsorbed, mg/mg cellulose; K_p is a constant in mL/mg.

Like other models, all models of cellulose hydrolysis developed so far have their advantages and limitations. Care must be taken when applying them to predict the rate and extent of cellulose bioconversion of specific reaction systems.

In recent years, the use of cell-free enzyme extracts (commercial products) has been investigated (Broda, 1992). Pilot studies show that with cellulase products, it is possible in stirred tanks, under realistic conditions, to hydrolyze various types of cellulose to soluble sugars with substrate concentrations of 10-30% and yields of 30-60%. One could convert 1000 kg of lignocellulosic substrate (wood chips, straw, corn stover,

or fiber from urban waste) to 350 kg of glucose in 24 hours. But the economic barrier is the very large amount of enzyme complex needed to hydrolyze the cellulose (perhaps a hundred times more than the amylase needed to break down starch). In contrast, no industrial process yet exists involving biological delignification.

Biodegradation of Lignin

Lignin is highly resistant to biodegradation because of its heterogeneous bond type (Figure 2.2), which is not amenable to hydrolytic cleavage. It undergoes no significant decay in anaerobic environments like the rumen and lake sediments (Zeikus, 1980). Growing evidence indicates that it is biodegraded by a unique enzymatic "combustion," i.e. a nonspecific enzyme-catalyzed burning (Kirk and Farrell, 1987).

Due to its structural features, lignin is degraded by a narrower array of microorganisms than the other major biopolymers like starch and cellulose. Lignin-degrading enzymes must be extracellular, nonspecific, and nonhydrolytic. White rot fungi and related litter-degrading basidiomycetes were considered the only microorganisms capable of extensive lignin degradation at one time, however, the ^{14}C-assay has allowed a reappraisal of microbial lignolysis (Knapp, 1985). Besides other fungi, such as strains of *Penicillin, Fusarium and Aspergillus*, certain bacteria can also degrade lignin. It is now known that a large number of species, notably *Nocardia, Streptomyces and Bacillus, Pseudomonas, Flavobacterium, Aeromonas* and *Xanthomonas*, can cause lignin degradation. However, the rate and extent of degradation are not as great as achieved by white rot fungi.

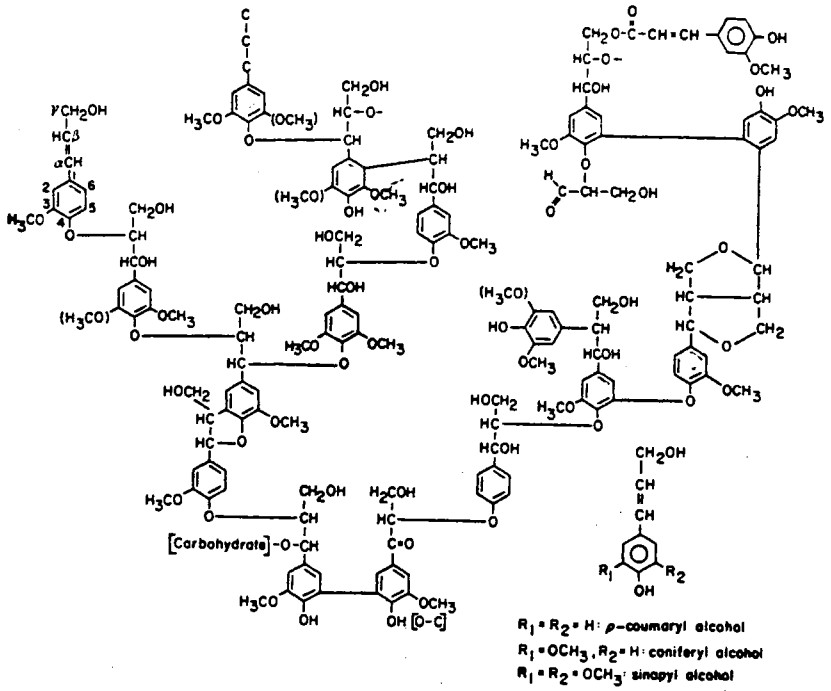

Figure 2.2. Schematic structural formula for lignin, adapted from Kirk and Farrell (1987). The structure illustrates major interunit linkages and other features described in the text; it is not a quantitatively accurate depiction of the various substructures. The three precursor alcohols are shown at the lower right: their polymerization, following one-electron oxidation, produces lignin.

Although more information is available on the white rot fungi than other organisms, it is still very incomplete. It is generally understood that lignin will require mono- and di-oxygenase enzymes to perform demethylation, hydroxylation, ring cleavage and side-chain oxidation. The nature of these enzymes is still uncertain, as is the nature of their cofactors if any are required (Knapp, 1985). *P. chrysosporium* remains the only organism for which the mineralization of lignin to CO_2 is known to be the primary process; nevertheless other organisms, notably the actinomycetes, also play important roles in lignocellulose degradation (Broda, 1992).

It is now known that *P. chrysosporium* requires O_2 for growth and high O_2 for lignolysis, which is also very pH sensitive. Oxygen tension can be crucial in determining the rate of lignin degradation by *P. chrysosporium* as well as by certain other white-rot fungi (Hatakka and Unsi-Rauva, 1983; Reid and Seifert, 1982).

The nature of the N-source is unimportant but its concentration is crucial, lignolysis being strongly inhibited by high nitrogen concentration (Knapp, 1985). Lignin degradation begins only after the cessation of linear growth, possibly in response to N-starvation.

Research has shown that *Phanerochaete chrysosporium* and *Coriolus versicolor* growth and lignin degradation cannot be supported by lignin alone and that these functions require a cosubstrate, like a carbohydrate (Kirk et al., 1976). The fact that lignolysis is proportional to the amount of cosubstrate suggests that the lignin degradation fails to produce sufficient energy to support the microbial activities. The biological rationale is that lignin is degraded as a stress response, so that the organism can obtain access to further sources of nutrients and energy previously made inaccessible by the presence of the lignin (Broda, 1992). Studies have demonstrated that lignin is not a growth substrate for white rot fungi. *P. chrysosporium* metabolizes various lignin preparations only when an alternate carbon/energy source is present (Leatham, 1986; Ulmer et al., 1983). In fact, nitrogen-limited conditions are natural for the white-rot fungi because wood is nitrogen-poor (Cowling and Merrill, 1966). Studies have confirmed that hemicelluloses and cellulose, or added carbohydrates, are always metabolized with the lignin in lignocelluloses (Agosin, 1985; Blanchette, 1984; Levonen-Munoz et al., 1983).

Due to lignin's polymeric nature, the enzymes involved in its degradation must be extracellular, and it is difficult to obtain culture filtrates with appreciable lignolytic activity. The extracellular lignin-modifying enzymes produced by white rot fungi include laccase, lignin peroxidases (LiPs) and manganese peroxidases (MnPs) (Hatakka, 1994). Ligninase production was increased by increasing either Cu or Mn (Kirk, et al., 1986). Growing evidence implies that Mn is important in lignin degradation. Not only does increased Mn lead to increased ligninase production, but this element accumulates as MnO_2 deposits during degradation of lignin in wood by several white rot fungi (Kirk and Farrell, 1987).

LiP and MnP are heme-containing glycoproteins which require hydrogen peroxide as an oxidant. LiP oxidizes non-phenolic lignin substructures by abstracting one electron and generating cation radicals which are then decomposed chemically (Eriksson et al., 1990). MnP oxidizes Mn(II) to Mn(III) which then oxidizes phenol rings to phenoxy radicals which leads to decomposition of compounds (Hatakka, 1994). Laccase is a copper-containing oxidase which utilizes molecular oxygen as oxidant and also oxidizes phenolic rings to phenoxy radicals (Eriksson et al., 1990). Another extracellular enzyme involved in lignin biodegradation is the hydrogen peroxide-producing enzyme glyoxal oxidase (Kersten, 1990).

No study has shown that lignin is mineralized rapidly or extensively by aerobic bacteria (Kirk and Farrell, 1987). As in the case of the anaerobes, a limiting factor might be the size of the lignin polymers (Kirk and Farrell, 1987). The highest reported rates of lignin degradation have been obtained by the white rot fungus *Phanerochaete chrysosporium* (Kirk and Farrell, 1987). Yang et al. (1980) reported degradation of 2.9 g

lignin per gram of fungal cell protein per day in a wood pulp, and Ulmer et al. (1983) reported rates three times higher for a lignin from wheat straw. *P. chrysosporium* degrades lignin when grown as mycelial mats. While cultivated as submerged pellets, the lignin degradation and formation of ligninase were almost completely suppressed (Faison and Kirk, 1985; Kirk et al., 1978). The agitation-induced suppression and its alleviation have not been explained (Kirk and Farrell, 1987).

Factors Affecting Waste Paper Digestion

Effects of Paper Type

Owens and Chynoweth (1993) generated estimates of ultimate methane yield for different paper samples by conducting biological methane potential (BMP) tests. Their results for estimating of ultimate methane yield for various types of paper are listed in Table 2.4. There were no significant differences among the yields of a pure cellulose control and office paper, food board and wax paper. The yield of milk carton was a little lower and the yield of corrugated cardboard was 74.3% of the cellulose control. The yields of newsprint and magazine were significantly lower.

According to Cowling and Kirk (1976), newspapers have about the same general composition as the woods from which they were derived: 40-55% cellulose, 25-40% hemicellulose, and 18-30% lignin. Waste paper prepared from chemical pulps such as corrugated fiber board and Kraft bag paper usually contain about 60-70% cellulose, 10-20% hemicellulose, and 5-10% lignin. Waste fibers from chemical pulping processes typically contain about 60-80% cellulose, 20-30% hemicellulose, and only 2-10% lignin.

The preliminary result of this research found that office Xerox paper contains about 70% cellulose and 4% lignin. The above information implies that, except for high quality office paper and waste fibers from chemical pulping processes, most potential paper related substrates for cellulose bioconversion processes are heavily lignified.

Table 2.4 Estimate of Ultimate Methane Yield for Various Types of Paper

Paper sample	Yield, m^3/kg VS (Standard Deviation)
Cellulose	0.375 (0.012)
Office	0.369 (0.014)
Corrugated	0.278 (0.012)
Newsprint (unprinted)	0.084 (0.003)
Newsprint (printed)	0.100 (0.003)
Magazine	0.203 (0.008)
Food Board (uncoated)	0.343 (0.020)
Food Board (coated)	0.334 (0.023)
Milk Carton	0.318 (0.014)
Wax Paper	0.341 (0.022)

Source: Owens and Chynoweth, 1993.

Effects of Paper Size

Some researchers (Owens and Chynoweth, 1993, Tong et al., 1990) ground the paper or solid waste before anaerobic digestion. Cummings and Stewart (1994) used shredded paper for cultivation of bacteria isolates. Tassinari and Macy (1977) reported the susceptibility of newspaper to cellulase in an enzymatic hydrolysis process had been

increased 125% with a differential speed two roll mill pretreatment (ground 8 minutes to produce a coarse product), while a ball milling of newspaper for 24 hours (to a fine particle size passing a 200 mesh sieve) gave only a 62% increase.

Research on the effects of particle size of landfill refuse to methane conversion rate gave some contradictory but interesting results. Fungaroli and Steiner (1979) milled the refuse and claimed that decreasing particle size increased methane conversion rate and yield. Ham et al. (1979) obtained the same conclusion by shredding refuse so that 90% of it by weight passed through a 7.5 cm sieve. However, Buivid et al. (1981) found that refuse with particle size 25-35 cm produced 32% more methane than that of particle size at 10-15 cm, which in turn produced 16 times more methane than that of size 1.25-2.5 cm after 90 days conversion. Barlaz et al. (1990) suggested that the reduced particle size caused too rapid a rate of hydrolysis, leading to a build-up of acidic end products and low pH, which inhibited the methanogenesis.

Effects of Lignin-Cellulose Association

The combination of lignin with partially crystalline cellulose that occurs in wood forms one of the materials most resistant to enzymatic hydrolysis (Cowling, 1963). In lignocellulosic materials, lignin forms a three-dimensional complex structure with cellulose and hemicellulose, and thus renders them less accessible to microorganisms due to the physical barrier and possibly chemical bonds connected with them (Tong et al., 1990).

A chemical bond between lignin and cellulose has been postulated for years by many investigators (Wise et al., 1952). Evidence from other past studies, however, has

suggested that the association is largely physical in nature (Cowling, 1963). Lignin apparently decreases the accessibility of wood cellulose to enzyme molecules diffusing within the fine structure of intermingled wood fibers.

Demonstration of physical break-up of the lignin-cellulose association was made by Pew (1957). When 2% of extractive-free aspen wood was ground in a vibratory ball mill to drastically reduce the particle size, up to 95% of the wood carbohydrates were solubilized by a commercial cellulase preparation. This indicated that ball milling breaks the lignocellulose matrix into such fine particles that a large amount of cellulosic surface is exposed and freed of its protective association with the lignin and thus is rendered susceptible to enzymatic dissolution. If the same is true of paper, then the effect of biodegradation on different paper types would be a function of the lignin-cellulose association, or lignin to cellulose ratio, and of the effectiveness of milling or other size reduction process to break apart this structure.

Effects of Printing Ink

Owens and Chynoweth (1993) found that when solids concentrations of media were low in BMP tests, ink of carbon black in petroleum oils did not significantly affect the methane yields in newsprint digestion. However, Cummings and Stewart (1994) found that the presence of ink containing carbon black, mineral oil, petroleum distillates and resin on heavily printed paper reduced the rate of cellulose solubilization. They considered that although the ink did not appear directly toxic to the bacteria, it might mask the surface of the paper, covering the cellulose fibers and preventing bacterial adhesion to the substrate. No reports were found on whether xerographic toner carbon,

laser print ink or ink made of other oils will affect the methane yields in office waste paper or newsprint digestion, either negatively or positively.

Effects of Inoculum Type

Microbial ecosystems obtained from municipal sewage sludges have been used extensively as inocula for paper and municipal solid waste (MSW) digestion studies (Shiralipour and Smith, 1984; Tong et al., 1990; Owens and Chynoweth, 1993). Since the experimental conditions varied, the reported methane production rates and conversion efficiencies from newsprint were quite different, but all were relatively low compared to other forms of paper.

Rumen microorganisms have been used effectively as inocula for digestion of organic waste material (Gijzen, 1987) and lignocellulosic residues, including newsprint (Camp et al., 1988). The content of a dairy cow manure slurry seed fermenter was used as inoculum for biodigestion of newsprint and other fiber materials by Chandler et al. (1980). They found an inverse linear relationship between lignin content and volatile solids (VS) destruction percentage with a correlation coefficient (r^2) of 0.94.

Five cellulolytic bacterial isolates from a methane-producing landfill were examined to determine their ability to utilize newspaper as a substrate for growth (Cummings and Stewart, 1994). Because of the high proportion of lignin, all isolates had only limited ability to degrade unprinted newspaper.

Termite soil and gut bacteria play an important role in cellulose depolymerization. According to a recent comprehensive review made by Varma et al. (1994), there are some controversies about the mechanisms involved in cellulose degradation and the

reported values of lignin degradation. The extent of cellulose digestion in wood was found to be between 59% - 99%, resulting in the production of short-chain fatty acids, mainly acetic acid, and CO_2 and H_2. The results of lignin degradation in termite guts based on analyses of termite feces were conflicting, ranging from 83% by some researchers to virtually none by others. No reports were found on comparisons of the solubilizing ability of lignocellulosic material by different consortia from different sources.

Effects of Environmental Factors

By testing anaerobic fermentation on domestic refuse at different temperatures, Pfeffer (1974) claimed that the optimized temperature for anaerobic digestion should be in either the mesophilic or thermophilic range. He found that the optimum temperature for anaerobic fermentation of domestic refuse was 41°C and the optimum temperature for thermophilic refuse decomposition was at least 60°C. The study by Hartz (1982) supported the high mesophilic temperatures for a short term refuse fermentation. However, the study by Mata-Alvarez and Martinez-Viturtia (1986) found that although the maximum conversion rate under mesophilic conditions was achieved at 42°C; the maximum accumulation of methane production was achieved at 34-38°C.

Most anaerobic biodegradation studies on cellulosic materials using anaerobic sludge as inoculum have been conducted at 35°C (Owens and Chynoweth, 1993; Tong et al., 1990). A high mesophilic temperature of 39°C was used for anaerobic degradation of solid organic waste materials by rumen microorganisms (Gijzen et al., 1987). High mesophilic temperature may also be preferred by landfill microbes. Cummings and

Stewart (1994) reported that the activities of the cellulolytic bacterial isolates from a landfill were strongly inhibited below the optimum growth temperature of 37°C.

Another factor most critical in controlling anaerobic digestion is pH. Few strictly anaerobic microorganisms grow well at low pH, and ruminal cellulolytic microbes appear to be particularly sensitive to acidic conditions (Weimer, 1992). In examining the effects of cultivating pH on growth of four predominant ruminal cellulolytic bacteria in cellobiose-fed chemostats at $D = 0.17h^{-1}$, Russell and Dombrowski (1980) found that washout occurred at pH range from 5.7 - 6.15. It is particularly important to note that the difference between washout pH and the pH of maximal observed growth yield was quite small, sometimes as low as 0.25 units (Weimer, 1992).

Optimization of alkalinity concentration for cellulose biodegradation was investigated by Khan et al. (1979). At loading rate of 1 g cellulose/L-week in a 30L semicontinuous fermenter experiment at 35°C (the fermenter maintained a COD of 3470 ± 412 mg/L), the optimum concentration of HCO_3^- for the degradation of cellulose to CH_4 was between 16 and 24 mM. At higher concentrations, HCO_3^- depressed the process. Replacement of the optimum concentration of HCO_3^- by the same concentration of CO_3^{2-} inhibited both cellulose degradation and methane formation during the first week of incubation, but this inhibition was overcome during subsequent incubation, possibly by acclimation.

Pretreatments to Enhance Microbiological Attack of Cellulosic Materials

Before efficient enzymatic hydrolysis and microbial degradation of lignocellulosic materials occur, the substrate must first be pretreated so that the relatively recalcitrant lignocellulosic matrix is more amenable to biodegradation. An ideal pretreatment would achieve reduction in lignin content, accompanying a reduction in cellulose crystallinity, and an increase in specific surface area. Many good reviews have covered this topic in detail (Millet et al., 1976; Chang et al., 1980; Puls and Dietrich, 1980; Tsao, 1984; Kirk and Farrell, 1987; Schell et al., 1991; Walker and Wilson, 1991; Kuhad and Singh, 1993), and most have classified the different types of pretreatment into physical, chemical and biological methods. Combinations of different types of treatment are also under investigation.

Chemical Pretreatments

Chemical treatments are extensively used to remove lignin from lignocellulosic materials and to disrupt cellulose crystalline structure. Chemical delignification processes in the paper industry (such as kraft or alkaline process) produce high strength, long fiber paper products. However, the processes used by the paper industry are severe and too expensive for the purposes of bioconversion or waste treatment. Numerous investigations (Millett et al, 1975; Wilson and Pigden, 1964; Han and Callihan, 1974; Chang et al., 1981; Detroy et al., 1981; Fox et al., 1989; Waiss et al., 1972; Grethlein, 1985; Gharpuray et al., 1983; Wayman, 1986) have been devoted to chemical

pretreatments with NaOH, NH_3, HCl, and H_2SO_4 as swelling or hydrolysis agents. The lignocellulosic materials were treated to increase their internal surface area, and to decrease degree of polymerization and crystallinity of cellulose.

Of the many chemical treatments to improve the digestibility of lignocellulosic materials, NaOH treatment is most prominent (Han et al., 1983). The treatment of a native cellulose with sodium hydroxide solutions of mercerizing strengths (above 20% w/w, as discovered by Mercer in 1850) causes extensive swelling and separation of structural elements (Millett et al., 1975). This treatment may partially remove lignin and hemicelluloses, and swell cellulose simultaneously. For example, Han and Callihan (1974) reported that microbial growth on rice straw and sugarcane bagasse was increased by alkali treatment. Carbohydrate utilization of treated substrates by *Cellulomonas* and *Alcaligenes* bacteria advanced from an initial 29.4% up to 73% after a 15-min digestion in 4% sodium hydroxide at 100°C. These increases were accomplished only after washing off the soaking reagent from the substrate. Fox et al. (1989) found that alkali pretreatment increased enzymatic susceptibility of sugar cane bagasse from 9.2 g/L to 37.9 g/L as the NaOH concentration in the pretreatment was increased from 0 to 10%.

The alkali treatment has also been used to upgrade the nutritive value of forage and forest residues for ruminants. When straw was soaked in about 1.5% sodium hydroxide for 24 hours, its digestibility was increased from an initial 30% up to as high as 70% (Millett et al., 1975). Considerable hemicellulose was solubilized by this process, resulting in a loss of about 20% of the straw dry matter during washing operations. These washings are both an economic cost and a disposal problem.

To overcome this problem, a 'dry' process was developed to eliminate the above drawbacks (Wilson and Pigden, 1964). In their experiment, ground wheat straw was mixed with 0 to 15 g NaOH in 30 mL H_2O per 100 g straw. The results indicated that alkali treatments up to about 9% (NaOH/substrate) caused marked increases in digestibility, but above that level no further increases were obtained.

Another long-standing approach to upgrade the feeding value of lignocellulosic materials involves treatment with aqueous or gaseous ammonia. Waiss et al. (1972) applied a variety of ammonia treatments to rice straw. Their optimum process involved a 30-day treatment of the dried straw in closed containers with 5% NH_3 and 30% water at room temperature. Product digestibility in vitro was about 62%, and it contained about 1.3% nitrogen. In vivo digestion by sheep indicated a digestibility of about 56% compared to 50% for untreated straw when both were fed at a level of 65% of the total ration. Han and Callihan (1974), using a similar treatment, showed that the utilization of rice straw by *Cellulomonas* and *Alcaligenes* bacteria could be increased from an initial 29% up to 57%, somewhat less than the improvement in digestibility for alkali treatment in the same study.

Acid hydrolysis of cellulose is a well-known phenomenon and can be carried out with concentrated or dilute acid. Concentrated acid (72% H_2SO_4 or 42% HCl) usually gives 90% or more conversion of potential glucan in biomass to glucose, but to be a viable process the large amount of acid used per unit of glucose produced must be recovered (Grethlein, 1984). By comparing the effectiveness of various acid treatments, Wayman (1986) claimed that the process based on 41% HCl has industrial potential, but the cost of recovery of the rather expensive acid is a deterrent to large scale

implementation. To avoid the recovery problem, dilute acid (1% H_2SO_4) was used to simplify the process. However, in order to get reasonable reaction rates, the temperature must be raised to above 150°C, and the glucose yield is reduced to 50 to 65% of the potential glucan with decomposed sugar compounds as byproducts (Grethlein, 1984).

Many other chemical treatments, such as using supercritical ammonia (Chou, 1986), ethylenediamine, ethylenediaminetetraacetic acid (EDTA), and dimethylsulfoxide (Detroy et al., 1980), sodium hypochlorite-hypochlorous acid (David and Fornasier, 1984), and alkali hydrogen peroxide (Gould, 1984) have also been investigated.

Generally, chemical treatments often achieve cellulose decrystallization, disrupture of lignin structures, and/or fractionation of cellulose, hemicellulose and lignin into separate streams. However, neutralization, washing or solvent recovery are always required after the treatments. All investigations found by this literature review involved some post-treatment processes or potential process waste disposal problems. No reports were found on directly feeding the whole pretreated biomass to an anaerobic digester.

Physical Pretreatments

Physical pretreatment not only decreases the crystallinity of cellulose, but also increases the surface to volume ratio of cellulosic materials, thus making cellulose more susceptible to hydrolysis. Physical pretreatments of lignocellulose include size reduction by milling (Dehority and Johnson, 1961; Stranks, 1959; Tassinari and Macy, 1977; Gharpuray et al, 1983), irradiation (Millett et al., 1970; Pritchard et al., 1962), steam explosion (Saddler et al, 1982; Grethlein, 1985) and freeze explosion (O'Connor, 1972; Puri and Mamers, 1983), among others.

Mandels et al., (1974) found that the hydrolysis rate of newsprint increased with an increase in ball milling times. They reported a high conversion ratio of 75% for 7-day ball-milled newsprint compared with 25% without milling. Three different millings were used by Gharpuray et al. (1983) to grind wheat straw. They determined the specific surface area, crystallinity and relative extent of hydrolysis of ground fibers. Table 2.5 shows that ball milling produced the highest specific surface area of the ground fiber, the lowest crystallinity and the highest extent of hydrolysis of the cellulose among the methods tested.

Table 2.5 Effect of Milling Pretreatment on the Specific Surface Area of Wheat Straw

Pretreatment	Specific Surface Area (m^2 /g)	Crystallinity Index	Relative Extent of Hydrolysis after 8 h
Untreated	0.64	69.6	1.0
Fitz-milling	0.99	65.6	1.6
Roller-milling	1.2	57.6	3.3
Ball-milling			
4 h	2.3	23.7	4.0
8 h	0.8	54.5	4.0
16 h	0.9	17.5	3.6
24 h	2.0	19.4	4.4

Gharpuray et al., 1983

In efforts to improve ruminant utilization of wood residues with vibratory ball milling, Millett et al. (1970) found that the milling response is quite species selective.

Extent of digestion ranged from about 80% to 18% for different wood species. This suggests that size reduction by milling is not a universal solution to increase the digestibility of lignocellulosic materials.

The technique of irradiating wood or straw by gamma rays or by high-velocity electrons substantially improves digestibility of these materials (Millett et al., 1970; Pritchard et al., 1962). However, a strong species specificity appears again (Table 2.6). The digestion of aspen carbohydrate was essentially quantitative after an electron dosage of 10^8 rad while spruce was only 14% digestible at this dosage. Considering the irradiation costs, the technique was deemed to have little commercial viability (Millett et al., 1976).

Table 2.6 Effect of Electron Irradiation on the In Vitro Digestion of Aspen and Spruce

Electron Dosage, rads	Digestibility	
	Aspen, %	Spruce,%
0	55	3
10^6	52	3
10^7	59	5
$5 * 10^7$	70	8
10^8	78	14

Millett et al., 1976.

Steam explosion of wood chips has received considerable attention. Cellulose was exposed to saturated steam at 3.86 MPa (260°C) for different durations and then

suddenly exposed to atmospheric pressure (Saddler et al., 1982). This explosive decompression, together with the shearing forces produced, results in increased surface area and enzymatic susceptibility of the lignocellulosic materials. During the treatment, much of the hemicellulose is hydrolyzed to free sugars and to water-soluble sugar oligomers (Saddler et al., 1993). Compared with mechanical size-reduction (hammer mills or double disk attrition mills) of poplar and aspen wood, two rapidly growing trees that are frequently considered as a raw material for a wood-to-alcohol conversion, this process saved 70% more energy than the conventional mechanical method to achieve the same size reduction and surface area increase (Holtzapple et al., 1989).

However, as Lipinsky (1983) pointed out, the scope of steam explosion appeared to be limited to hardwoods and grassy lignocellulose raw materials. Such softwoods as pine, spruce, and Douglas fir do not behave well in either batch steam explosion or continuous steam explosion. The limitation might be due in part to the absence of acetate groups in softwood hemicellulose. These acetate groups in hardwoods and grasses provide the acid that is conveniently located to depolymerize lignin and hemicellulose. In addition, the highly reactive aromatic rings of softwood lignin may cause rapid repolymerization of any low molecular weight lignin fractions that are formed during steam explosion of softwoods.

Steam explosion has the ability to separate wood into its three components, and the resultant cellulose gives glucose yields of 70 - 80% of theoretical conversion rate via acid hydrolysis, and may realize 100% conversion to glucose via enzymatic hydrolysis. However, the method has not yet been successfully developed for use with softwoods (Saddler et al., 1993), which are the major constituents of newsprint. At the same time,

the hemicellulose fraction suffers a large loss to degradation by the high steam temperature (Tsao, 1984).

Biological Pretreatment

If lignin can be selectively removed from lignocellulosic materials without loss of appreciable amounts of cellulose, it will be extremely attractive for biodegradation of highly lignified wastes, for pretreatment of animal feed, and for paper mill sludge treatment or biopulping processes.

Selective degradation of lignin by white rot fungi was reported recently. The influence of the growth of three higher fungi on the composition of wheat straw was investigated by Moyson and Verachtert (1991). The three selected organisms also produce fruitbodies, the so-called oyster mushrooms (*Pleurotus pulmonarius and P. sajor-caju*) and Shiitake mushrooms (*Lentinus edodes*), which are cultivated on straw in large scale industrial plants all over the world. They grew very well on lignocellulosic substrates and broke down 50% lignin of straw in 12 weeks of fungal growth. The enzymatic digestibility was doubled during that period. Together with lignin, the higher fungi consumed half of the amount of hemicellulose, leaving cellulose fairly intact.

The conditions for selective degradation of lignin by the fungus *Ganoderma austrualis* was reported by Rios and Eyzaguirre (1992). Only those wood chips that were extracted by a toluene-ethanol mixture had significant growth of fungus during the solid state cultivation. High nitrogen content and high oxygen tension stimulate the production of endoglucanase (cellulase), while the low oxygen tension and low nitrogen content stimulate Mn-peroxidase and xylanase activities.

Some actinomycete bacteria represent an alternative system for lignin solubilization in which strains differ in their spectra of activities on lignocellulose substrates (Broda, 1992). However, the actinomycete system is still at an earlier stage of study. Lignin solubilization and degradation have been more difficult to demonstrate rigorously with bacteria than with the fungal system.

Although there is still no practical lignin biodegradation process available yet, the perpetual efforts and the progress made in this area means that we can now begin to consider to select a useful organism to exploit particular lignocellulose resources. The organism that degrades lignin and hemicellulose efficiently but has minimum activity against cellulose is highly preferred. One may also exploit enzyme preparations from such an organism grown under industrial fermentation conditions. However, as Broda (1992) stated, it is still too early to predict which group of microorganisms, e.g. white rot fungi or actinomycetes, will produce the best candidate for exploitation in this manner.

CHAPTER III

MATERIALS AND METHODS

Experimental Materials

Inocula

Three different sources, namely anaerobically digesting sewage sludge, anaerobic landfill contents and bovine rumen contents, were used as inocula to establish three semicontinuous seed reactors. The contents of these seed reactors were used as inocula for succeeding experiments. The description of the initial inoculating materials is listed in Table 3.1. Among the three inoculating materials, fresh anaerobic sludge was collected, while the other inocula were obtained earlier and preserved under the conditions described in Table 3.1. Both semicontinuous seed reactor and serum bottle experiments were performed to investigate the relative effectiveness of the three microbial consortia on waste paper digestion.

Papers

In this study, two different paper sources (Table 3.2) were used as substrate to investigate their anaerobic digestibility. Previous use of the paper for printing may have certain effects on the anaerobic digestion. This point is of importance in determining the

potential need for deinking processes for waste paper pretreatment. The potential effects of these chemicals are not necessarily negative. Examining rates and extent of bioconversion of inked versus unused paper enabled assessment of whether deinking is advantageous or unnecessary prior to anaerobic treatment.

Table 3.1 Description of Inocula

Sample	Description
Anaerobic Sludge	Collected 12/28/93 from the primary activated sludge digester of Stillwater Wastewater Treatment Plant.
Landfill Contents	Supplied by Dr. Joseph M. Suflita (University of Oklahoma) from the Fresh Kills landfill, Staten Island, NY. It was sealed in a plastic bucket and preserved at room temperature.
Rumen Contents	Collected 10/22/93, from the third chamber of a killed cattle rumen, Animal Science Department, Oklahoma State University, Stillwater campus. It was stored in a 4°C refrigerator.

Table 3.2 Description of Paper

Sample	Description
Newsprint(u)	Collected 9/12/93 (for seed reactors) and 5/08/94 (for serum bottle test), from Stillwater NewsPress, supplied by Abitibi-Price, contains 20-25% recycled fiber.
Newsprint(p)	Same as above, printed with Flint ink, carbon black in 100% soy bean oil.
Office	Collected 5/08/94 from Engineering South, Annex room 204, Xerox copies of office papers.

u: unprinted p: printed

The newsprint was collected from the printing plant of the Stillwater NewsPress. For the serum bottle test, in order to investigate the effects on newsprint's digestibility caused by printing ink and/or the printing process, both printed and unprinted paper were collected from the same paper web for basic serum bottle tests.

Office paper was collected from the graduate student office, School of Civil and Environmental Engineering, Oklahoma State University. The sample used for the experiment was randomly collected from waste office paper and Xerox copies set aside for paper recycling.

Ink

The ink used for anaerobic digestibility study was also collected on 5/28/94 from the Stillwater NewsPress printing plant. This Flint ink is made of carbon black in 100% soy bean oil. The sample was preserved in a sealed glass bottle at room temperature.

Yeast Extract

Yeast extract was used to supply trace minerals and organic growth factors in most of the experimental conditions during this study. It was purchased from BBL Microbiology Systems and stored at room temperature.

Water

Tap water was used for all bioconversion tests. Milli-Q water ($\geq$ 18 MΩ·cm), which was produced by a Milli-Q purification system (Millipore Corp.) via deionization and reverse osmosis, was used for all chemical analyses.

Apparatus

Semicontinuous Seed Reactors

Semicontinuous Seed Reactors Three semicontinuous seed reactors, illustrated in Figure 3.1, were made of plastic reagent bottles. Polyethylene tubing connectors (Nalgene) had been affixed by drilling holes on the caps and side walls of the reactors. The tubing connectors placed on the caps were used as gas outlets, while those placed on the walls were used as purging gas inlets. Silicone sealant was used around the tubing and tubing connectors to prevent gas leakage. The total volume of a reactor is 4 liters, with 3 liters of effective volume. The 1 liter extra volume served as head space and buffer region in case a surge would occur.

Nalgene tubing placed over the connector on the cap was used as gas outlet tubing. A glass tube elbow was connected at the other end of the tubing, and extended into a biogas collector, the mouth of which was immersed in a water basin. The generated biogas was released from the end of the glass tube elbow upward into the biogas collector to displace water there. Norprene tubing (Cole-Parmer) was used as purging gas inlet, and extended to the bottom of the reactor. This allowed the purging gas to pass through the whole liquid depth inside the reactors, which resulted in efficient removal of air and supplied a mild mixing.

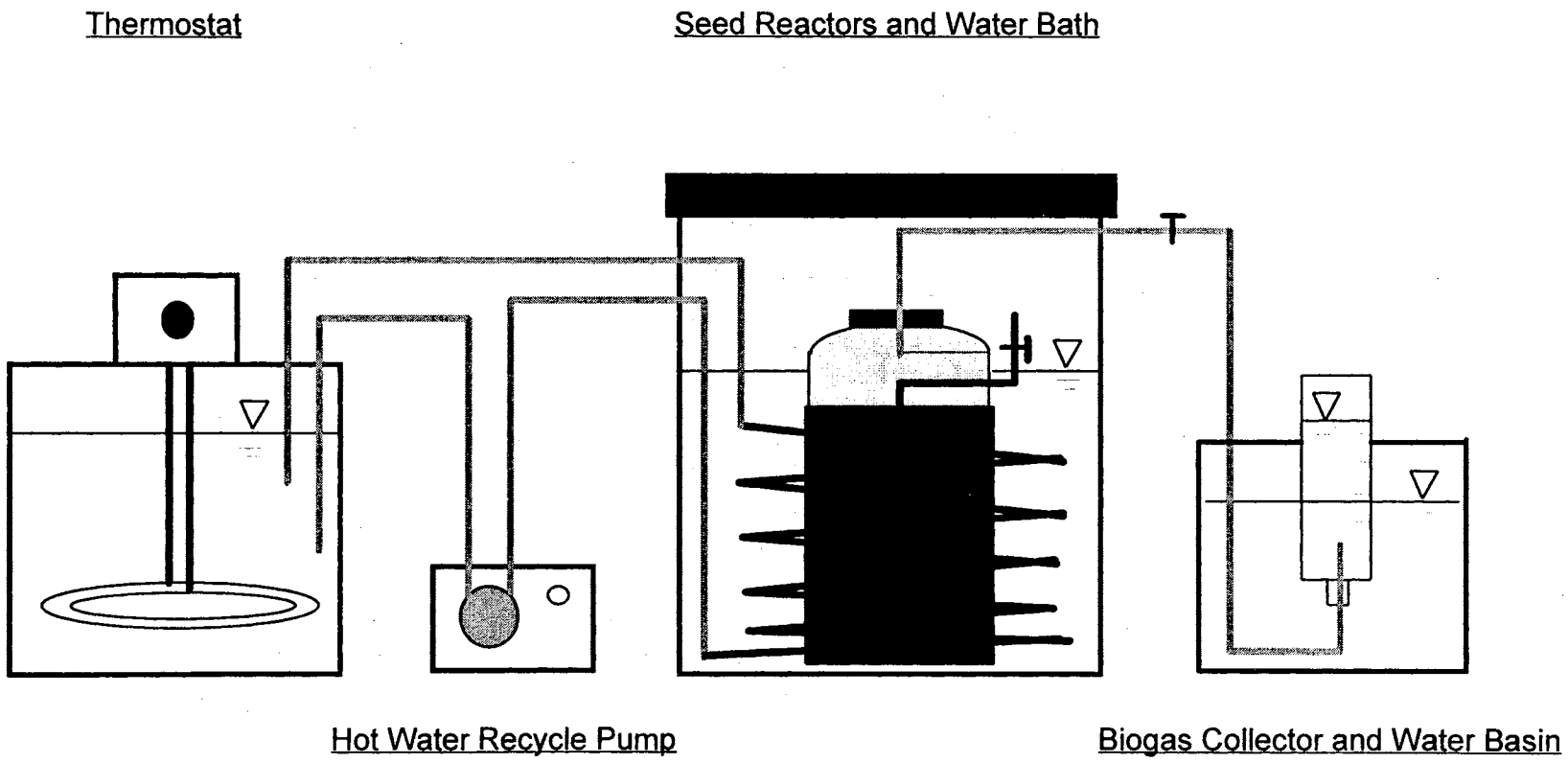

Figure 3.1 Schematic diagram of seed reactor system.

Incubator and Temperature Controls A 35°C water bath, in which the reactors were placed, served as an incubator (Figure 3.1). The temperature was maintained by circulating 60°C water from a second thermostat - controlled water bath via a positive displacement pump (7553-50, Cole-Parmer) through a Nalgene tubing coil laid inside the first water bath. The pump was fitted with a model 7015-20 pump head (Cole-Parmer). The 35°C temperature was adjusted by altering the pumping speed of high-temperature water.

Biogas Collection and Measurement The biogas collectors were made of long body, narrow mouth 500 mL glass bottles which were scaled, filled with water, and immersed in a water basin. Biogas was collected by replacing the water inside the collectors (Figure 3.1). The collected biogas was measured and analyzed periodically for methane and carbon dioxide with a gas chromatograph (GC), Gow-Mac model 350, equipped with thermal conductivity detector (TCD). Some early samples were assayed for methane only, using a HP 5890II GC with flame ionization detector (FID).

Serum Bottle Reactors

Serum Bottles 160 mL Wheaton "400" Brand serum bottles were used as batch anaerobic reactors. To each bottle, 100 mL medium was fed, and the remaining 60 mL volume served as head space. The bottles were stoppered with black butyl rubber septum-type stoppers (GeoMicrobial Technology), which were air impermeable even after repeated sampling with syringes. After being stoppered, the bottles were further sealed with 20 mm aluminum crimp seals (Wheaton).

Incubator and Temperature Controls The serum bottles were incubated in a Thelco Model 4 incubator (Precision Scientific, ambient - 60°C temperature control range), which has 3 equal space layers. Triplicate samples could be placed on three different layers to avoid the effects of uneven temperature distribution inside the incubator. The temperature was controlled at 35 ± 1 °C.

Biogas Collection and Measurement To avoid temperature fluctuation inside the serum bottles, the biogas was collected at 35°C. A 30 mL glass syringe (B-D YALE V 7871) was used to release the biogas accumulated inside the serum bottles periodically. The collected gas was analyzed for methane and carbon dioxide with GC immediately upon sampling.

Experimental Methods

Semicontinuous Reactor Experiment

Start-up For the seed reactor test, in order to acclimate the microbial consortia to attack the cellulose-lignin structure of paper, only unprinted newsprint (low cellulose-lignin ratio) was used as substrate. The paper was homogenized with a 2-speed stainless steel Waring blender (Fisher Scientific) with water for 5 minutes at 20,000 rpm. Each time, about 8 grams of torn paper were blended with about 500 mL water, resulting in homogenized paper pulp. Then the pulp was boiled to remove air and cooled to room temperature under nitrogen atmosphere before being fed into the reactors.

Total solids (TS) and volatile solids (VS) of the three inocula were analyzed before the reactors' start-up. At the same time, the TS, VS, cellulose, acid insoluble

lignin (AIL) contents and chemical oxygen demand (COD) of the newsprint were also analyzed. The concentrations of the inocula and feed materials were decided according to these analyses.

Speece and McCarty (1962) found that digestion of acetic acid alone could not proceed at high rates if only the normal inorganic salts were present. They found thiamine, proline, calycine and benzimidazole could increase acetate utilization rates significantly. Since yeast extract contains comprehensive trace minerals and organic growth factors, 1 g/L yeast extract was added with inorganic nutrients.

The formula of the nutrient solution was similar to that of Owen et al. (1979), with some modification (Table 3.3). The yeast extract and the inorganics (except sodium sulfide and sodium bicarbonate) were dissolved separately and boiled to remove dissolved air, cooled in a nitrogen atmosphere to room temperature, and mixed. Then the sodium bicarbonate (solid reagent) was added to the mixed nutrient solution. The sodium sulfide was dissolved separately and boiled to remove dissolved air, then mixed with the nutrient solution when it was fed into the reactors.

The amounts of the three inoculating materials added to the three reactors at start-up were based on equal amounts of volatile solids content for each reactor at a loading of 500 mg VS/L. Inocula were added only once and gradually digested or replaced by new feeding materials (paper pulp and nutrient solution). The inocula additions to the reactors are listed in Table 3.4.

Table 3.3 Composition of Nutrient Media

Constituent	Conc., mg/L	Constituent	Conc., mg/L
$NaHCO_3$	6.0 g	$CoCL_2 \cdot 6H_2O$	0.5 mg
NH_4Cl	1.2 g	$CuCL_2 \cdot 2H_2O$	0.5 mg
$(K)_2HPO_4$	0.5 g	$ZnSO_4 \cdot 7H_2O$	0.5 mg
$MgSO_4 \cdot 7H_2O$	0.3 g	Na_2SeO_3	0.5 mg
$Na_2S \cdot 9H_2O$	0.2 g	$AlCL_3 \cdot 6H_2O$	0.5 mg
$CaCl_2 \cdot 2H_2O$	0.1 g	$MnCL_2 \cdot 4H_2O$	0.5 mg
$FeCl_2 \cdot 4H_2O$	40 mg	H_3BO_3	0.5 mg
KI	2 mg	$NiCl_2$	0.5 mg
		NH_4VO_3	0.5 mg
Yeast Extract	1.0 g	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.5 mg

Table 3.4 Inocula Addition (Same Amount of Volatile Solids)

Reactor Name	Inocula Type	Total Amount	VS Content	Pulp and Nutrient	Make-up Water
Sludge	Anaerobic Sludge	401 g	15 g	1500 mL	1100 mL
Landfill	Landfill Contents	81.2 g	15 g	1500 mL	1450 mL
Rumen	Rumen Contents	116 g	15 g	1500 mL	1390 mL

The pH of the contents in the reactors was adjusted to about 7.0 - 7.1 at the starting point with 1 M NaOH or 1:1 HCl. The reactors were purged with nitrogen gas through side inlets during inoculation, and fed with paper pulp and nutrient solution. The purging process was continued until the reactors were sealed, when the contents and head space of the reactors were essentially air free. The reactors were incubated in the water

bath, maintained at 35 ± 1 °C by circulating 60°C water. The loading rate for the reactors was 500 mg volatile solids/L-d of unprinted newsprint pulp (about 650 mg COD/L-d). Both solid retention time (SRT) and hydraulic retention time (HRT) were 20 days.

Feeding and Maintenance Feeding and effluent wasting for a semicontinuous reactor were accomplished by manually removing one half volume of the reactor's contents and adding the same volume of fresh feeding materials every 1/2 HRT (10 days). This is referred to here as one round. Before effluent was wasted, the contents of the reactor were thoroughly mixed. As soon as the reactor was uncovered, one half of the uniformly mixed contents were removed and replaced with raw substrate (1 L in volume) along with nutrient solution (0.5 L in volume) while the reactor was continuously purged with nitrogen gas. By this procedure, less than 1.5% of the original inoculating material remained in the reactor after 3 retention periods, at which point bioconversion could be considered reaching a steady-state condition. At this time, analysis results from the starting and ending points of one experimental round should reflect only biological activity resulting from utilization of the unprinted newsprint paper (including yeast extract), fed to the seed reactor.

The 50% volume of the seed reactor contents remaining in the reactor served as inoculum for the next round of the experiment. Freshly wasted effluents were available as inoculating materials for other experiments.

Effects of Inoculating Materials The effects on bioconversion by different inoculating materials were investigated by monitoring the methane yields of the three seed reactors during semicontinuous reaction mode. Methane analysis with GC and

chemical analysis of the media were conducted to identify the effects of the three different microbial consortia on newsprint paper bioconversion.

Alkalinity Requirement It was found that the alkalinity consumption during a 10 day period of semicontinuous operation was relatively small. In order to find the minimum or optimum alkalinity requirement for the reactor system, the addition of $NaHCO_3$ was decreased gradually to quantify the relation between alkalinity supplement and methane production of the system.

Long-term Conversion Test When the semicontinuous reaction reached steady state conversion, it was found that only a small fraction of the cellulose in the newsprint was converted to biogas (results presented in Chapter VI). Since most plastic materials are somewhat air-permeable, the plastic reactors employed in this study might be subject to effects of permeating air and/or losing product methane and carbon dioxide. Therefore, the ultimate conversion rate of these seed reactors might depend on the reactors' air permeability. To investigate the ultimate conversion extent of the seed reactors, they were turned to long-term batch operation at the beginning of the Rounds 15 and 30. A set of parallel serum bottle test reactors which simulated conditions of the seed reactors at Round 15 were also initiated concurrently. Serum bottles were loaded with the same substrate, nutrient solution, inocula and substrate/nutrient/inocula ratio as in the seed reactors, as well as the same effective volume ratio, i.e. 3/4 total volume of media and 1/4 total volume as head space. Methane was analyzed every day for the first 10 days. As biogas production gradually slowed, the sampling duration was prolonged accordingly.

The long term tests were run until most reactors were no longer producing any biogas. Then the dissolved solids, dissolved VS, COD, pH and alkalinity of the media were analyzed to find the limiting factor(s) of the bioconversion in seed reactor conditions. The results are presented in Chapter IV.

Effects of Reactor Air Permeability Since bioconversion in the seed reactors stopped far earlier and produced much less methane than in the parallel serum bottles, the effect of the reactors' gas permeability was significant. To further differentiate the effects of permeating air versus losing precursor CO_2 to find which one was the predominant limiting factor, another set of serum bottle tests was initiated. The residue that was no longer producing biogas was distributed into serum bottles anaerobically. 50% of serum bottles were charged with pure nitrogen head space, while others were filled with 1/3 CO_2 and 2/3 nitrogen as head space. If the limiting factor was permeating air, the media should start producing methane after strict anaerobic conditions were reestablished, while if the limiting factor was losing precursor CO_2, adding CO_2 to the system should benefit methane production. By comparing the methane production of these bottles, the real limiting factor of the seed reactors with respect to gas permeability might be better defined. The experimental results are presented in Chapter IV.

Serum Bottle Experiment

Inocula Most often, the wasted effluent of the seed reactors were used as inocula for serum bottle experiments. These inocula were very active and were still producing a significant amount of biogas daily. At other times, wasted effluents of the long term

experiments and the contents of serum bottles were also used as inocula. Since these media underwent much longer periods without feeding than those of semicontinuous reactions, sometimes the microorganisms had entered stationary phase, and these inocula were generally less active. A longer lag period would be expected when these inocula were used. The volume of inocula used in serum bottle tests, 20% (v/v), followed the recommendation of Owens and Chynoweth (1993).

Paper Size Pretreatment of the paper samples by shredding or milling was investigated to quantify the effects of size reduction on paper wastes prior to anaerobic digestion. Three different physical sizes of paper described in Table 3.5 (ground, shredded, and unshredded) were used in the basic serum bottle experiments.

Table 3.5 Description of Paper Sizes

Sample	Description
Ground Paper	Ground with a coffee mill to powdery debris.
Shredded Paper	First cut paper in 2 inch strips, then cut the strips perpendicularly to 2 inch long and 1/4 inch wide strips.
Unshredded Paper	Whole piece of paper cut to the size of required weight.

Sample Size Owens and Chynoweth (1993) used 2 g VS/L in their modified BMP analyses for MSW, including waste paper. This sample size was employed for office paper digestion in this study. Previous studies (Owens and Chynoweth, 1993; Tong et al., 1990) found that newsprint produced much less methane than office paper. Very low methane yields of newsprint were also found in the preliminary serum bottle

test of this study. In order to compromise this low conversion extent, the sample size for the newsprints was increased to 5 g VS/L. Because of the extremely high COD value of the printing ink (see Chapter IV), sample size of 1 - 1.5 g VS/L of ink was used in this experiment.

Nutrients The same nutrient formula listed in Table 3.3 was employed for the serum bottle test. However, the concentration of the solution was adjusted according to substrate/nutrient/inoculum ratio of the serum bottle test.

Experimental Design Efficient experimental design will enable us to obtain unbiased estimates of treatment means, differences and of experimental error. The major objectives of this basic serum bottle test were to estimate the relative capacities of the three different inocula on waste paper, the bioconvertibilities of the three types of paper and the effects of the paper size on the anaerobic digestion. The basic serum bottle test was designed in such a way that it could be easily analyzed with the analysis of variance (AOV) procedure with subsamples (equal subsample numbers). The experimental matrix is outlined in Figure 3.2. Three different inocula, the types of paper and various paper sizes could be used as treatments, experimental units or sample units alternatively. All the samples were triplicated. Triplicates of controls (with inocula and nutrient solution only) were included to quantify the bioconversion due to the remaining cellulosic materials and other organic components in the inoculating materials. Triplicates of seed controls (with inocula but no nutrient solution) were also included to observe the effects on bioconversion due to the inocula only. Pure ink samples were included to investigate the anaerobic digestibility of ink itself under the same experimental conditions.

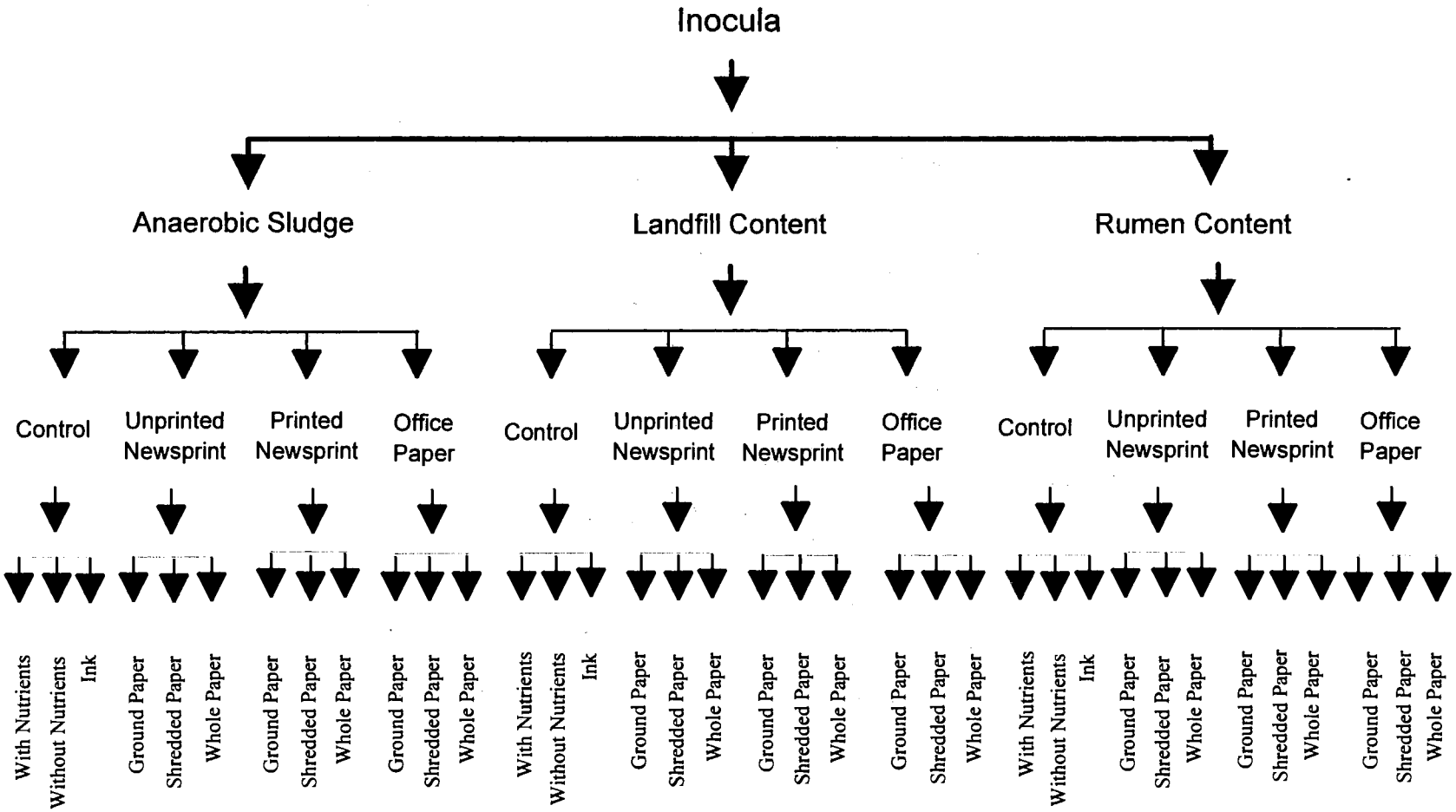

Figure 3.2 Basic serum bottle test profile.

The methane data obtained from these experiments can be analyzed with the statistical method AOV procedure to find if any significant differences exist among different treatments and among different experiments. A 95% confidence interval will be used in all the statistical analyses. If there exist significant differences, further analyses, such as Fisher's least significant difference procedure (LSD) and Tukey's procedure will be performed to identify sources of the differences. The reasons to use these two procedures are because they are extensively accepted procedures to compare population means, and are quick and easy to use. In LSD procedure, the α ($\alpha = 0.05$, i.e. at 95% confidence interval) is comparisonwise error rate, which means of all comparisons one makes, 5% will be incorrect on average if all treatments are the same, while in Tukey procedure, the α is experimentwise error rate, which means this procedure controls experimentwise error rate, 5% of all experiments on average will contain at least one bad comparison. So the Tukey's procedure is more conservative than the LSD procedure, which means it is more reluctant to declare a difference. All the procedures will be conducted with the statistical software SAS.

The above procedures can be used at different levels. For example, since the unprinted and printed newsprint were expected to give close methane yields, AOV procedure was used to analyze the methane data of these samples. The paper types were the treatments, the inocula were the experimental units and the paper sizes were sample units (Figure 3.3). The effect of the paper size on bioconversion of individual paper types was analyzed in the same way, the profile of analysis is shown in Figure 3.4.

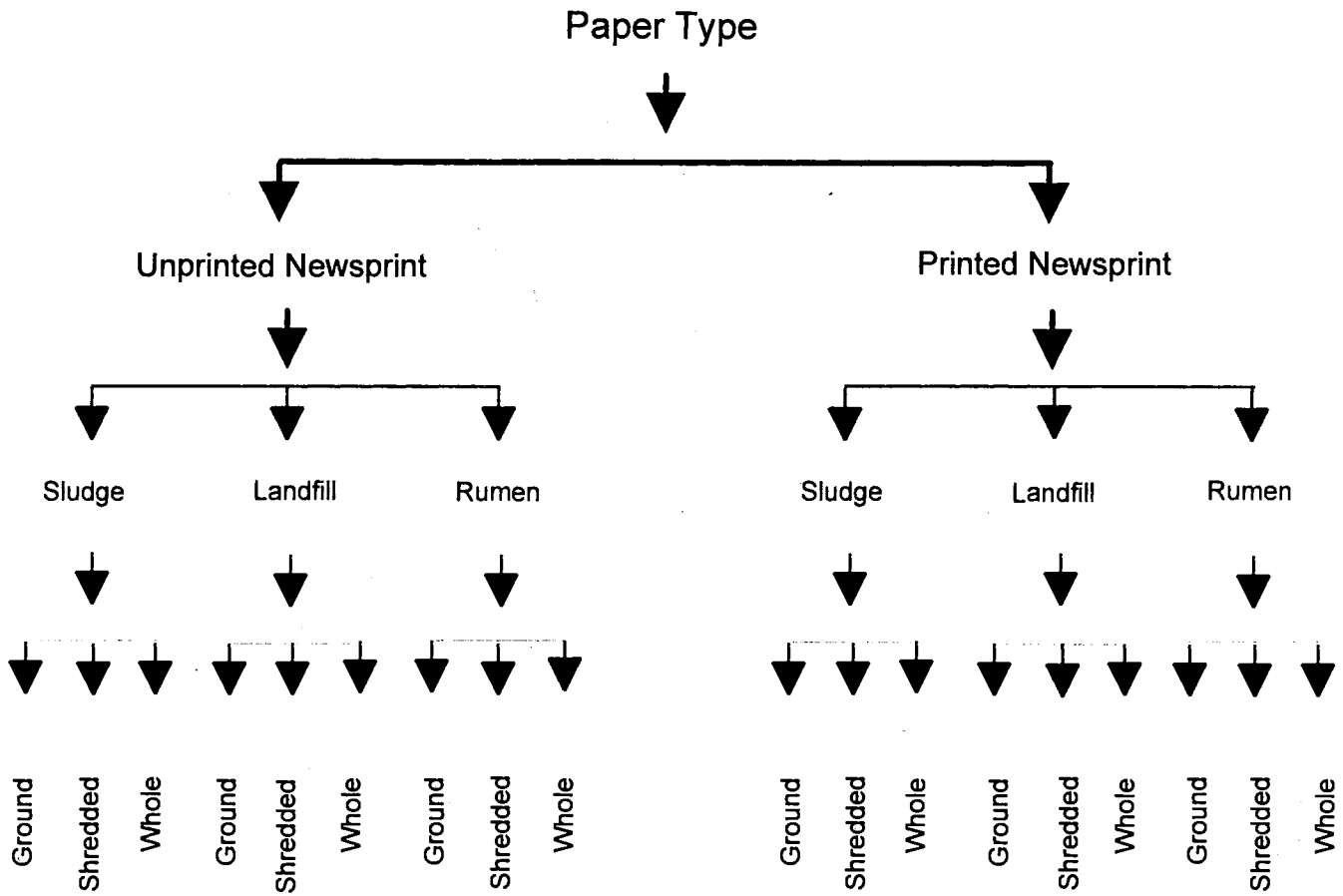

Figure 3.3 Analysis on effects of paper type and inocula type between unprinted and printed newsprint.

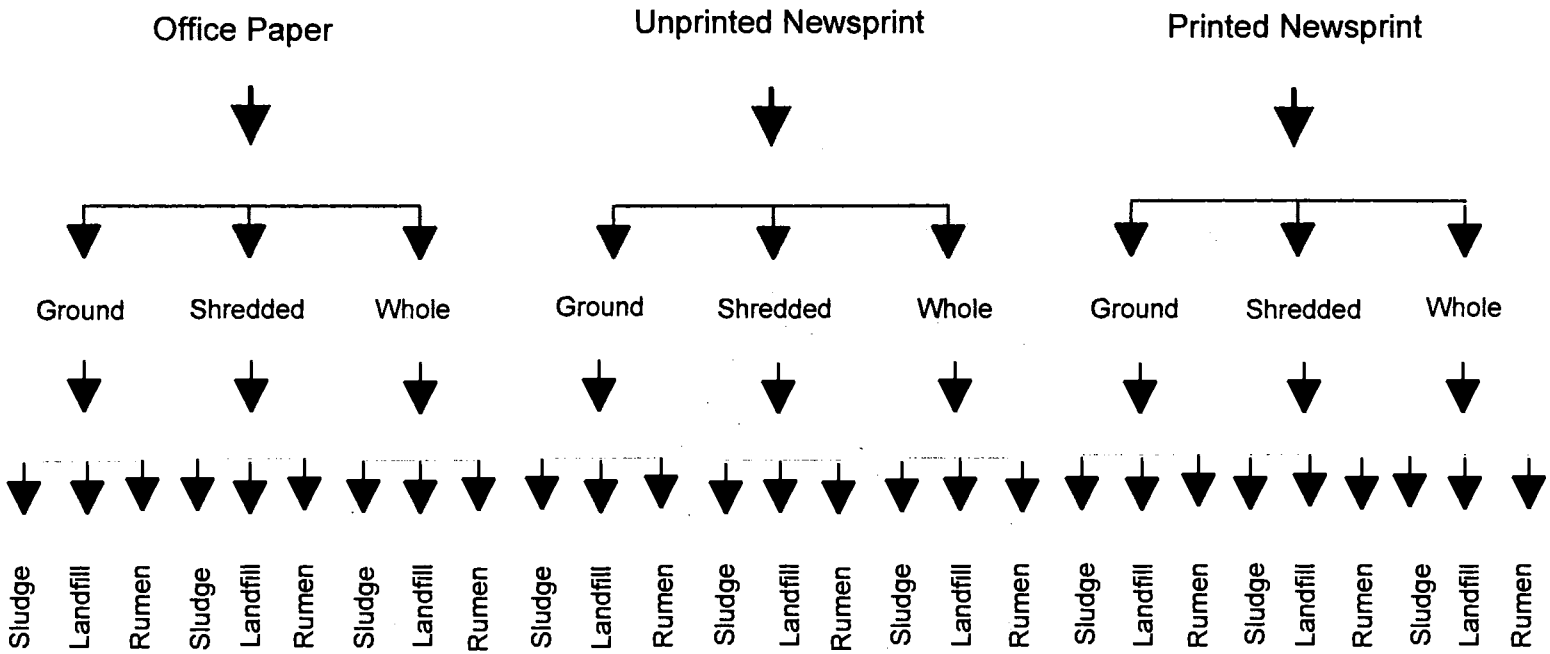

Figure 3.4 Analysis on effects of paper size and inocula type for each type of paper.

Start-up Before being loaded with substrate, the empty serum bottles were purged with pure nitrogen gas and capped with butyl rubber stoppers. The bottles were tared with an analytical balance, and the paper samples were added with care to avoid introducing too much air into the bottles. After weighing again after filling, serum bottles were placed into a nitrogen-gas-filled, ethylene oxide-treated 280-L two-hand glove bag "Atmosbag" (Aldrich). Then air-free nutrient solution and inoculating material were filled into the serum bottles with graduated cylinders inside the glove bag. Two drops of 0.1% resazurin were added to each bottle as a redox indicator. Before sealing the bottles, the contents of the serum bottles were purged with pure nitrogen gas by extending purging tips into the bottom of the bottles until the resazurin indicator turned from purple to light pink. The remaining trace amount of oxygen was consumed quickly after a short period of incubation. Since substrate concentrations adopted in this study were higher than those regularly used in the BMP test, the error caused by a small amount of air remaining inside the serum bottles should be negligible. After being sealed with air-impermeable butyl rubber stoppers held in place with Wheaton aluminum crimp seals, the serum bottles were incubated in the 35°C incubator.

The ink samples were treated basically in the same way, except for the sample filling process. Because ink is in a highly viscous form, it is difficult to weigh a fixed amount of it into a serum bottle directly. This difficulty was solved by weighing the ink onto a small piece of tared glass, which was then placed into a serum bottle.

To account for the possible uneven temperature distribution inside the incubator, triplicates were placed separately on three different levels. When the temperature of the

serum bottles reached 35°C in a couple of hours, the pressure built inside the bottles due to the temperature increase was released with a needle.

Yeast Extract Methane Production

Yeast extract can be biodegradable and produce a significant amount of methane under anaerobic conditions. To account for yeast extract's methane production in the paper bioconversion experiments, the amount of methane produced by yeast extract itself was determined and deducted from the total accumulated methane production.

This experiment employed serum bottles inoculated with supernatant from each of the three seed reactors. This test started with yeast extract as sole substrate and with the same nutrient solution as that of other serum bottle tests. In order to enhance the analytical accuracy and reduce the relative experimental error, the concentration of yeast extract was doubled in comparison with other serum bottle tests. To avoid introducing any other secondary substrate into this test, only a small amount (1 mL) of supernatant from each of the seed reactors was introduced into each bottle. In this way, the methane produced during the bioconversion process should reflect the methane potential only for the yeast extract used. This information is critical for the adjustment of the accumulated methane production of the semicontinuous reactors.

Alkali Pretreatment

Scope of Alkali Pretreatment To date, no economically sound physical, chemical or biological pretreatment methods are available to enhance bioconversion of lignocellulosic materials. Due to the high costs of processes like subdivision into micron-size particles, irradiation with high-energy electrons, and saccharification under high temperature and pressure conditions, these techniques are not promising for waste treatment in the near future. In recent years, considerable research effort has been expended on cellulases, hemicellulases, ligninases and on selective removal of lignin from lignocellulose. However, no industrial processes yet exist involving biological delignification (Kirk and Chang, 1990).

Alkali treatment is a proven technology which is used extensively for the removal of lignin and disruption of cellulose crystalline structure. Alkali swells cellulose and leads to an increase in cellulose internal surface area and decreases in degree of polymerization and crystallinity. However, most investigations of this treatment involve washing off the soaking reagents, losing digestible hemicelluloses, and creating strong caustic waste stream disposal problems (Millet et al., 1976; Tsao, 1984). It would be advantageous if the alkali dosage could be minimized during the soaking process and neutralized, instead of being washed off, after the process without sacrificing efficiency. Ideally, the whole treated media could be fed to an anaerobic digester directly, therefore avoiding losing digestible hemicelluloses and eliminating the need for disposal of reagents and wash waters. Lignin has the ability to absorb base, which means that less than equivalent amounts of acid will be needed to neutralize the base added. This makes

utilizing a weak acid like CO_2 to neutralize the soaking NaOH possible. Moreover, the whole process, from paper to methane, is an alkalinity-consuming process. We have to add alkalinity one way or another, and the Na_2CO_3 formed during neutralization can supply alkalinity for the digestion process. Even if hemicellulose and lignin associated with cellulose are only partially solubilized, cellulose should be made more accessible to microorganisms or enzymes released by the organisms.

Other potential advantages of this process are that in a continuous reaction, the extra NaOH added during the pretreatment can be neutralized by the CO_2 produced in the digestion process. The quality of biogas would be upgraded at the same time as CO_2 is scrubbed out due to the very low solubility of methane in water. The pretreatment will cost only NaOH as soaking reagent. After the selective removal of cellulose by the process, the high lignin content residue may be explored for other industrial usages.

The potential problems of feeding neutralized media to anaerobic digesters directly include the possible inhibition or adsorption of cellulases by solubilized lignin fractions (Converse, 1993). In a recent bioconversion study of newspaper and filter paper substrates, Stinson and Ham (1995) added milled wood lignin (MWL) to their BMP test to investigate whether that lignin would chemically inhibit cellulose decomposition. They found that differences between the amounts of methane generated by the samples with or without MWL addition were statistically insignificant. In fact, so long as the inhibition is insignificant, the adsorption problem may be overcome by adding a large quantity of inoculum.

Neutralizing of the soaking reagent, NaOH, will leave a higher concentration of Na^+ in the media. According to Kugelman and McCarty (1965), Na^+ concentration up to 0.20 M has little effect on acetate-utilizing methane bacteria. When soaking with 5 - 20% NaOH/paper (w/w), the Na^+ increment is between 0.05 - 0.20 M, so cation toxicity effects by Na^+ should be negligible for the methanogens of the microbial consortia.

Alkali treatment was employed as pretreatment in this study, followed with serum bottle tests of treated samples to determine the effectiveness of the treatment. Experimental approaches identical to those of the basic serum bottle tests were employed. The alkali pretreatment parameters investigated are listed in Table 3.6. Various combinations were tested, as shown in the Alkali Pretreatment and Bioconversion Test section of Chapter IV.

Besides methane and CO_2 analyses with GC, chemical analyses were performed to determine TS, VS, cellulose, AIL, COD and alkalinity of the residues of selected samples. Statistical analyses were performed to evaluate the effectiveness and the significance of different treatments.

Table 3.6 Parameters Tested in Alkali Pretreatment Experiment

Parameter	Range Investigated
Concentration, %	5, 10, 15 and 20
Durations, days	1, 6 and 12
Temperature, °C	25, 75 and 105
Neutralizing Acids	CO_2, H_2SO_4 and HCl

Start-up Unprinted newsprint was pretreated by sodium hydroxide over the range of experimental conditions outlined above (Table 3.6). To accomplish higher concentrations with certain amounts of sodium hydroxide, the water volume used to dissolve the sodium hydroxide must be minimized. It was found that one gram of torn newsprint could be barely wetted by 3 mL of water, so this water/paper ratio was used for the soaking process and a fixed amount of water (15 mL) was used to dissolve the different amounts of NaOH to make a series NaOH solutions with different concentrations.

For each treatment condition, 5 grams of paper were torn manually to about 1 × 1 cm pieces and placed into a 250 mL Erlenmeyer flask. After adding 15 mL NaOH solution, the paper was stirred with a glass rod to soak all the pieces. Then the flask was capped with a No. 6 black rubber stopper. For the samples treated at higher temperatures, 100 mL Corning Pyrex brand laboratory bottles (Baxter) were used to soak the samples. These bottles with caps can stand temperature as high as 140°C. For the samples treated with different NaOH concentrations, different soaking durations and different

neutralizing acids, a 25°C incubator was employed. For the samples treated at higher temperatures, a 35°C incubator, a 70°C water bath and a 105°C oven were used.

After incubation, soaked samples were quantitatively transferred into a Waring blender with 485 mL water. The sample was homogenized at 20,000 rpm for 2 minutes, then the pulp was transferred into a 500 mL beaker.

Pure CO_2 (Sooner Airgas, Inc.) was used to neutralize the homogenized sample. The CO_2 gas was charged into the pulp through an air distributor, which was extended to the bottom of the beaker. Initially, all the CO_2 charged into the pulp was absorbed. Later, foam was formed when CO_2 was close to saturation. The pH of the media dropped from around 11.0 to around 7.0 depending on the flow rate of the CO_2 stream and duration of the CO_2 charging process. Other samples were neutralized with strong acids, 6N H_2SO_4 or 1:1 HCl to pH 7.0.

After neutralization, the pulp was ready for anaerobic digestion. Since the CO_2 concentration in the treated pulp was expected to be much higher than that of other air components after CO_2 treatment, no air removing procedure was employed to this medium at this stage.

The control sample was soaked with water and homogenized in the same way as alkali treated samples. The same amount of $NaHCO_3$ as used in basic serum bottle tests was added to supply alkalinity for the bioconversion. Alkalinity was also supplied for the strong acid-neutralized samples.

Bioconversion for Alkali Treated Samples The same nutrient solution used in the basic serum bottle test was employed for bioconversion tests of the alkali-treated

samples, except that $NaHCO_3$ addition was omitted because a large amount of alkalinity, which was the product of reaction between NaOH with CO_2, remained in the media. Since this experiment would not be used to compare results from different groups of microorganism populations, mixed inoculating material (mixture of equal volumes of the three inocula) was used as inoculum for all samples. The sample incubation, sampling and analyses were the same as for the basic serum bottle test described before.

Different Neutralization Extent of Alkali Treated Samples A short term test was also performed to find the effect of the neutralization extent on the early stage of alkali treated sample bioconversion. The neutralization procedure of the alkali treated samples can be stopped over a pH range of 11.0 - 5.9. Since the well-known recommended pH range for anaerobic digestion is from 6.8-7.8, one sample was neutralized to this range, while another was neutralized until totally saturated with CO_2 (pH 5.9). The experimental protocol was the same as above, and run for 30 days.

Acid Pretreatment

Scope of Acid Pretreatment Acids have been reported to be used to pretreat lignocellulosic materials to hydrolyze cellulose and hemicellulose. The sugars resulting from treatment are then subject to enzymatic or microbial conversion. The most extensively used acids are H_2SO_4 and HCl (Grethlein, 1984; Han and Callihan, 1974; Millett, et al, 1976) of various concentrations. No reports were found using acetic acid as a reagent to pretreat the lignocellulosic materials except for analytical procedures.

In his semimicro-cellulose analysis method, Updegraff (1969) used a high concentration acetic-nitric acid reagent (80% acetic acid and 7.5% of nitric acid) to dissolve lignin from lignocellulosic materials. The remaining cellulose was then dissolved by 67% sulfuric acid for analysis. With this method, the removal of lignin is very effective and complete. The possibility of using this treatment to remove lignin before cellulose digestion will depend on the following factors: if the concentration of the reagent can be lowered enough to be accepted economically, if there are effective ways to separate the soaking solution with the cellulose completely, and if the dissolved lignin components can be separated from the soaking solution effectively (though not necessarily completely) without losing acetic acid after the treatment.

A group of experiments was initiated to find what would be the lowest acetic acid concentration that could dissolve lignin to a significant extent. Different acetic acid concentrations were used to soak the newsprint, then the weight loss (an indication of lignin removal) of paper due to the soaking and separation was determined. Nitric acid is part of the reagent used to solubilize lignin in the cellulose analysis method (Updegraff, 1969). In order to verify the function of the nitric acid, (as a catalyst, or just an acidifier), the efficiencies of lignin removal by different nitric acid concentrations in the soaking solution were also investigated. If nitric acid were used as acidifier only, it should be replaceable by other strong acids like HCl or H_2SO_4. Since nitric acid is more oxidative and expensive than other strong acids, its replacement with other strong acids would be preferred.

Possible methods for separating cellulose from the soaking solution include washing and centrifuging. To prepare samples for the bioconversion test, washing was used to remove the soaking solution from the residues. Efforts to find operations that can effectively separate solubilized lignin components from the soaking solution is obviously beyond the scope of this study but may deserve further investigation.

Weight Loss of the Acid Pretreatment Ground newsprint (0.15 - 0.2 g) was weighed into 16 × 125 mm Kimble Kimax tubes. Soaking solution (10 mL) was added into each tube. The tubes were heated in a boiling water bath for 30 minutes. After being heated, the tubes were centrifuged at 2500 rpm for 5 minutes, then the supernatants were discarded. Water was added into the tubes and mixed with a Vortex Genie mixer and centrifiged again. This procedure was repeated three times. The tubes were then put into a 105 C oven until dry. The moisture content of the newsprint was determined at the same time. The weight loss of the acid treatment was calculated according to the weight loss in the tubes.

An optimized concentration of the soaking solution, which would be able to remove significant amounts of lignin from newsprint with a relatively low acid concentration was determined. This solution was used to treat newsprint samples prior to the following bioconversion test.

Bioconversion for Acid Pretreated Sample Samples (10 grams) of newsprint were torn into 1 × 1 cm pieces and put into a 70 mL beaker with 300 mL optimized soaking solution. The beaker was heated on an electric heating plate to boil for 30 minutes. The residue of the treatment was transferred into a No. 40 (0.425 mm) sieve and rinsed with

tap water until pH neutral, then transferred into a Waring blender and homogenized for 2 minutes. The sample was allowed to stand overnight to eliminate entrapped air bubbles. This pulp was used as substrate for anaerobic digestion. The TS and VS of the pulp were determined to quantify the substrate concentration. The cellulose and AIL contents of the treated sample were also analyzed. The bioconversion of the treated samples was tested in the same way as that of alkali treated samples.

Volatile Acid Production and Consumption

For serum bottle tests, once the bottles are sealed, control over environmental conditions inside the bottles is totally lost, except for incubation temperature. All conclusions must be deduced from periodic analysis of methane and carbon dioxide productions and the final pH, alkalinity and COD changes in general. These deductions may not reflect microorganism activities and reactions that occur at earlier stages of the bioconversion.

The limiting steps of the anaerobic conversion of the waste paper were defined from the seed reactor and the basic serum bottle tests. However, the evidence from those experiments was obtained indirectly. Direct monitoring of the early stage reactions can be achieved by measuring the changes in acetic, propionic and butyric acids (specific volatile fatty acids, VFAs), pH, alkalinity, and TS and VS along with methane and CO_2 determinations in additional serum bottles which can be sacrificed regularly to perform those measurements. The following experiments were designed in this way to uncover some insights on the bioconversion process.

Alkali Treated and Untreated Sample Bioconversion It is well known that the cellulose, yeast extract and at least part of hemicelluloses in the media are biodegradable in anaerobic conditions. Previous studies had identified that the final limiting factor for lignocellulose bioconversion was caused by the lignin-cellulose association. However, this limiting factor might not be significant at the earlier stages of the bioconversion when there are plenty of easily convertible substrates like cellulose, yeast extract and some hemicelluloses available. In sealed serum bottles, it was not known whether the reactions, i.e., substrate hydrolysis, acid production or methane formation, had been well balanced, and if not, which one had predominated during earlier stages of the bioconversion.

To solve these problems, besides monitoring the methane and CO_2 production of the bioconversion, VFA, TS, VS, pH and alkalinity were also monitored to investigate the differences in reaction patterns of the alkali treated and untreated sample bioconversion. At each sampling period, duplicate bottles of both alkali treated and untreated samples were sacrificed for the chemical analyses, over an experimental duration of 60 days.

Sampling and Testing Procedures

Reactor Sampling

Both starting and final pH of the seed reactors on every round of feeding were measured. Starting from Round 5, gas accumulated in gas collectors was sampled every day for methane analysis. For Rounds 7 and 15 (long-term), TS and VS, cellulose, AIL,

and COD of the starting and final samples were analyzed. Alkalinity of the wasted effluent was analyzed since the 6th Round.

The biogas produced by the reactors was sampled from the gas collectors. First, the glass elbow was removed from a gas collector, then a sleeve rubber stopper was used to stop the mouth of the gas collector when it was still immersed. After recording the biogas volume, the bottle was turned up, and the sample was taken through the stopper with gas-tight GC syringes (20 mL for FID, and 160 mL for TCD). When taking samples, the syringe first was flushed three times with greater than the intended sample volume. Then 120% of the sample volume of gas was withdrawn into the syringe and the plunger was set back to the 100% mark. The volume was allowed to equilibrate for at least 30 seconds, then the needle was removed from the stopper and the sample injected into the GC immediately. The sample was taken at room temperature. A schematic diagram of the sampling process is shown in Figure 3.5.

After injection, the gas collector was uncovered and refilled with water. Then it was capped with the stopper again, turned upside down and put back into the water basin. Then the stopper was removed, and the glass elbow was inserted back into the collector.

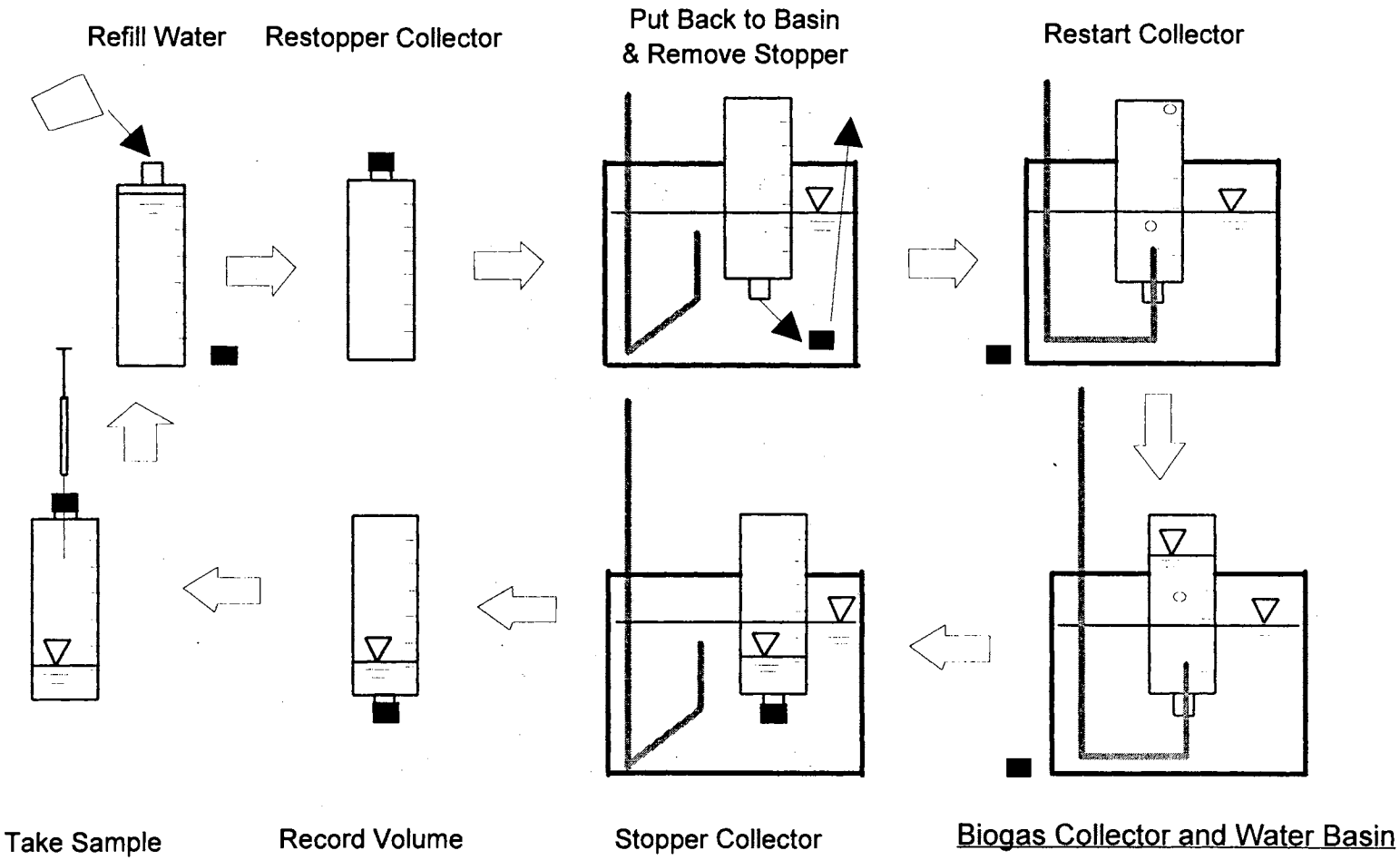

Figure 3.5 Gas collector sampling procedure (starting from lower right).

Serum Bottle Sampling

The sampling duration of the serum bottles was determined by the biogas production rate. Since the 30 mL glass syringe was used to collect the biogas produced and to release the pressure built inside the serum bottles, the biogas accumulated inside the bottles during a sampling period should not surpass this volume. The volume of biogas produced during the sampling period was directly read from the scale of the glass syringe. The sample was taken at 35°C. The temperature effect was corrected to the standard condition according to Tong et al. (1990) (0.395 liter methane at 35 °C and 1 atm can be obtained from 1 gram of COD).

After the biogas volume was noted, a sleeve stopper was used to cap the front end of the syringe as soon as the needle was removed. The needle was then removed from the serum bottle stopper immediately. Samples were taken from the glass syringe with a gas-tight GC syringe in a similar way as described before, the only difference being that the 30 mL glass syringe was always held vertically with its mouth downward. Under the weight of the plunger, there was a positive pressure inside the syringe and less equilibrium time for the injection syringe was needed. The gas samples were analyzed with a GC equipped with TCD for methane and carbon dioxide simultaneously.

Analytical Techniques

Biogas Composition

Routine monitoring of biogas composition was performed as a check on reactor performance and, in combination with gas volume measurements, to quantify methane production. To determine gas composition, samples were taken from the gas collectors or the glass syringe with a 250 mL Hamilton 1725RNW gas-tight syringe and injected directly into a Gow Mac model 350 thermal conductivity detector (TCD) gas chromatograph (GC). The chromatograph was fitted with a 6 foot stainless steel column (I.D. = 1/4 in.) packed with Porapak Q, 60/80 mesh. Column temperature was maintained at 55°C, the detector temperature was 170°C and that of the injection port was 105°C. The bridge current of the TCD was maintained at 70 mA and the attenuation was adjusted to full scale. Helium was used as carrier gas at a flow rate of 60 mL/min. The instrument was calibrated at each use with pure CH_4, CO_2 and N_2 gases as external standards.

The integrator used was a Hewlett Packard (HP) model 3380A. The attenuation of the integrator was also adjusted at full scale. The sample size and all settings on the GC and integrator described above were based on the best output possible with this combination of analytical instruments.

An HP GC Model 5890II fitted with Carbopack C, 60/80 mesh, 30% Carbowax column and FID was also used to determine methane in the early stages of the research. The length of the column was 4 feet with an I.D. of 1/4 inch. The integrator used was an

HP 3396II. The injection was made with a 25 mL Hamilton 1702RNW gas-tight syringe. Column temperature was maintained at 50°C. The temperature of the detector was 250°C while that of injector was 200°C. Helium was used as carrier gas at a flow rate of 20 mL/min. The instrument was calibrated with each use with pure CH_4 as external standard.

Total Solids

Total solids and total volatile solids analyses were conducted according to the methods described in Standard Methods (APHA et al., 1985), Section 209 A and 209 D. Porcelain drying dishes were exposed to 550°C before initial weighing. Samples were dried at 103-105°C for two hours and ashed at 550°C for 30 minutes. The drying oven used was a Thelco model 17, and the ashing oven used was a Lindberg Type 51894. Sample size for total solids was generally between 1.0000 - 2.0000 gram.

Cellulose

Cellulose was measured with the method developed by Updegraff (1969). Paper samples were homogenized with a Waring blender, and acetic/nitric acid reagent was used to solubilize lignin and hemicelluloses. After centrifugation and washing to remove solubilized lignin and hemicellulose, 67% sulfuric acid was used to dissolve cellulose. The anthrone method was then used to quantify the cellulose content. Pure cellulose (Sigma Chemical Co., Sigmacell type 50) was used to make standard curves and as an internal standard.

Acid-Insoluble Lignin

The method described in Analysis of Paper (Browning, 1977) Chapter 7, Part II, A, was adapted. In this procedure, dry or pre-dried sample was first ground by a Braun KSM 2 coffee mill, then dried in a 100°C oven. One gram oven-dried sample powder was placed in a 200 mL wide-neck, glass-stoppered bottle and reacted with 50 mL 38% HCl and 5 mL concentrated H_2SO_4 overnight to remove cellulose and hemicellulose in the sample. The mixture then was diluted with water and transferred to a 750 mL beaker with about 450 mL water. The solution was boiled for a few minutes and allowed to stand until the lignin settled out. The supernatant liquid was decanted into a tared Coors Gooch porcelain filtering crucible with a Whatman 2.4 cm glass microfibre filter, and then the lignin was transferred to the crucible and washed with hot water. The crucible was first dried at 100°C to a constant weight, then the residue was ashed at 550°C. The weight loss between drying and ashing reflects the AIL content.

Chemical Oxygen Demand

COD analysis was performed according to the Reactor Digestion Method described in Hach Water Analysis Handbook (Hach Company, 1992), but the volumes of the reagents were increased according to the method described by Clarkson (1986). In this procedure, 10 mL samples were mixed with 6 mL dichromate digestion reagent and 14 mL H_2SO_4-Ag_2SO_4 catalyst solution in sealed culture tubes (Kimax 25 × 100 mm, Kimble 45066-A, Fisher Scientific) with Teflon-lined screw caps (Kimble 45066-C). Reagents were dispensed from repeatable pipette containers. The tubes were incubated at

150°C for two hours, cooled and analyzed colorimetrically at 600 nm in a Milton Roy Spectronic 20 spectrophotometer. In this study, the oven for color development was a Lindberg Type 51894, which was set at 150°C for the COD analysis.

Calibration standards were prepared with standard potassium acid phthalate solution. The linear range of this method was found to be between 0 - 1,500 mg/L. When this method was used for analysis of solid samples, small amounts of samples were carefully measured and the digestion time had to be increased to at least 4 hours. The Open Reflux Method described in Standard Methods (APHA et al., 1985), Section 508A was also performed according to the needs of sample types and sizes (for example, ink COD analysis).

pH

Calibrated pH electrodes were placed directly into the reactors for most pH readings. Glass combination electrodes were used in conjunction with a Fisher Accumet model 900 pH meter (Fisher Scientific Co.). Buffer solutions (pH 7.0, 4.0 and 10.0, Fisher Scientific) were used to calibrate the meter before sample determination.

Alkalinity

Alkalinity was measured according to the procedures described in Standard Methods (APHA et al., 1985), Section 403. The procedure of potentiometric titration to preselected pH was employed. Standard 0.02 N sulfuric acid was used for titration. The end point of pH 4.5 was determined with a model 900 Accumet pH meter.

Volatile Fatty Acids

The volatile fatty acids were measured on a HP GC Model 5890II fitted with Carbopack C, 60/80 mesh, 30% Carbowax column and FID. The length of the column was 4 feet with an I.D. of 1/4 inch. The integrator used was an HP 3396II. Following 16 hours conditioning at 175°C, 1.5% formic acid was injected into the column repeatedly in 10 μL each time for at least 20 times. This formic acid injection was performed every time before the sample analysis. Samples were filtered with 0.45μm nylon syringe filter and acidified with 1.5% formic acid before injection of 1 μL liquid samples with a 10 μL Hamilton glass syringe. Column temperature was maintained at 145°C. The temperature of the detector was 250°C while that of injector was 200°C. Helium was used as carrier gas at a flow rate of 20 mL/min. The instrument was calibrated with each use with standard solutions containing known concentrations of acetic, propionic, and butyric acids.

Experiment Implementation Timeline

Planning, development of the experimental apparatus, collection of experimental materials, establishment of the reactors, conduct of the experiments, and experimental data analysis occupied approximately twenty-eight months. The sequence of activities is shown in the implementation timeline (Figure 3.6).

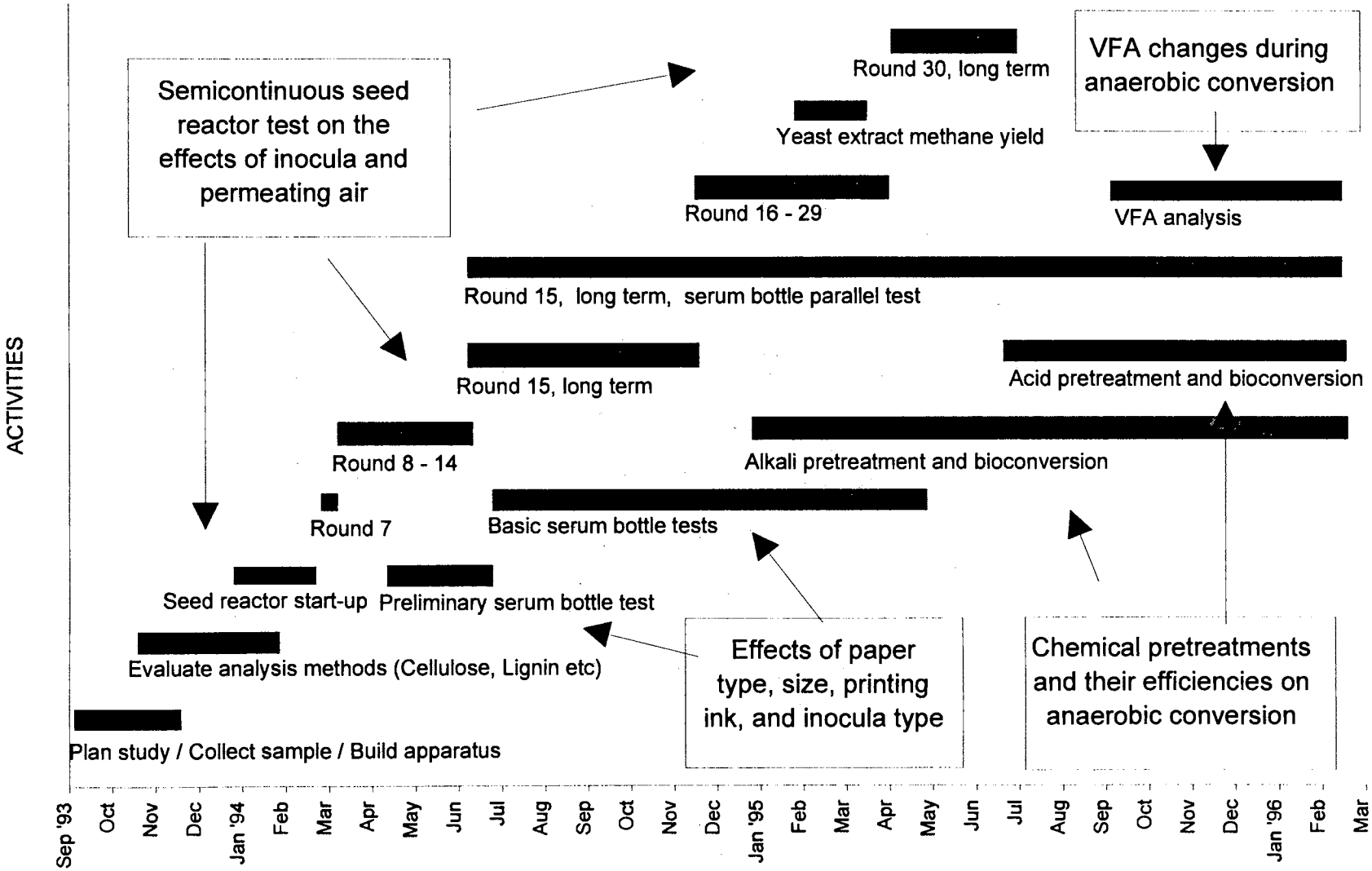

Figure 3.6 Chronological sequence of activities in the study.

CHAPTER IV

RESULTS AND DISCUSSION

Semicontinuous Seed Reactors

Start-up Phase

The establishment of semicontinuous seed reactors provided important qualitative and quantitative observations on the effects of different inoculating materials, different sources, physical sizes, previous usage of paper, and permeating air on the newsprint paper digestion. During the start-up phase, all three different inocula were acclimated to utilize newsprint paper as the substrate. pH changes during this phase were monitored. Biogas production and methane yield in the late period of this phase were also monitored.

Observation of these seed reactors at this start-up phase demonstrated that the abilities of the three inocula to adjust to new carbon and energy sources (especially for anaerobic sludge and rumen contents) and to a new mesophilic environment (in the case of anaerobic sludge and landfill contents) were quite different. The microorganisms from landfill contents seemed most adaptable to the new environment because they showed the least pH fluctuation and produced the most biogas during the start-up phase. This is understandable for the reason that only landfill microorganisms were exposed to a high proportion of waste paper substrate in their original habitat.

Limited data were collected during this start-up phase. Figure 4.1 shows the pH changes of the three reactors during the entire start-up phase and the biogas production at the latter period of this phase. pH values shown on the graph are following 10-day feeding cycles, with a starting pH value for all reactors at 7.0 - 7.1. After the first 3 HRT, equivalent to 6 feeding cycles (referred to here as "rounds"), inoculating materials remaining in the reactors had been reduced to less than 1.5% of their original levels. From this point, the gas production in each round can be considered to have been produced solely from the substrate added in that round. Figure 4.1 shows that all the three reactors achieved a steady state condition after 3 HRTs.

Semicontinuous Seed Reactor Test

Ability of the Semicontinuous Seed Reactor to Convert the Newsprint At this stage (following Round 7) complete analysis of the methane production and the changes in TS, VS, cellulose, AIL, pH, alkalinity and COD during a 10-day feeding cycle was undertaken to assess the performance of the semicontinuous reactors. The results are listed in Table 4.1. All the methane data reported here and subsequently (except where specified) were converted to milliliters of methane per gram of available paper COD (mL/g COD) under standard conditions (i.e., dry gas at 0°C, 1 atm) for the convenience of comparing with the theoretical COD methane potential.

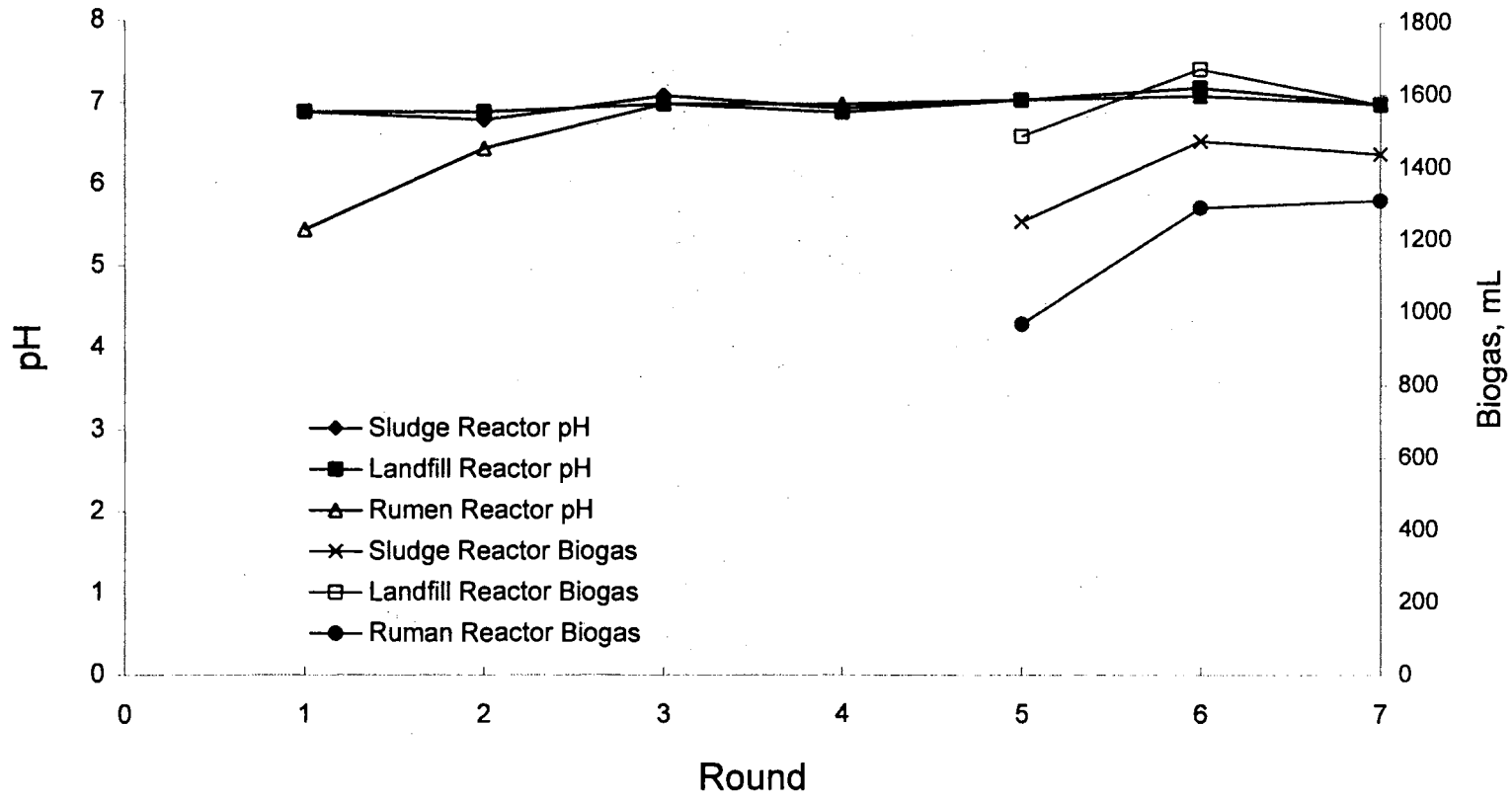

Figure 4.1 pH change and biogas production during the start-up phase.

Table 4.1 Analysis Results of Semicontinuous Reactor Test

Items			Seed Reactor		
			Sludge	Landfill	Rumen
Methane, mL/g COD			32.10	41.76	35.12
Total Solids, %	In		1.497(0.003)	1.471(0.032)	1.503(0.019)
	Out		1.377(0.008)	1.405(0.028)	1.382(0.007)
Volatile Solids, %	In		0.973(0.008)	0.956(0.032)	0.994(0.019)
	Out		0.873(0.011)	0.868(0.021)	0.876(0.002)
Cellulose, % of VS	In		41.05(0.36)	42.68(0.65)	41.95(0.12)
	Out		39.70(0.62)	38.31(0.17)	40.93(0.78)
AIL, % of VS	In		29.07(1.01)	28.71(0.19)	28.18(0.90)
	Out		32.04(0.85)	32.59(1.11)	32.37(0.41)
COD, mg/g VS	In		1387(60)	1319(16)	1266(88)
	Out		1307(18)	1267(1)	1230(18)
Cellulose/Lignin Ratio,		In	1.41	1.49	1.49
		Out	1.24	1.18	1.26
pH	In		7.05	7.05	7.05
	Out		7.0	7.0	7.0
Alkalinity, mg $CaCO_3$/L		In	3039	3046	2943
		Out	2719	2755	2765

Note: Test results of a 10-day steady state cycle of semicontinuous seed reactors. Values in parentheses are standard deviations of the parameter estimates.

Table 4.1 shows that only a small fraction of paper cellulose was converted in a 10-day cycle against the theoretical potential of 350 mL per gram of COD. These methane yields are roughly half of the yield obtained by Owens and Chynoweth (1993) via a 60 day biochemical methane potential (BMP) assay, and 40% of the yield obtained by Vermeulen *et al.* (1992) via a 42 day, 55°C anaerobic digestion. The total and volatile

solids, cellulose and COD concentrations of the seed reactors decreased along with the bioconversion, while the AIL concentration and its ratio to cellulose increased. Mass balance of the reaction revealed that the lignin component was kept untouched and likewise for most of the cellulose. The possible reasons for the relatively low conversion rate and extent may include the short HRT, gas permeability of the reactor wall and the high percentage of associating lignin, which physically hinders the cellulose bioconversion.

Alkalinity Consumption In addition to providing information on newsprint conversion rate and extent, as well as the effect of different inocula on newsprint digestion, an important function of these reactors was to supply inocula for succeeding experiments. Therefore, these semicontinuous reactors were maintained in steady state from Round 7 to 14. At steady state, it was found that these semicontinuous reactors consumed about 260 mg/L alkalinity in each 10-day feeding cycle. Since this alkalinity consumption was relatively low compared with the total available alkalinity, in the following feeding cycles the concentration of alkalinity was reduced gradually to find the minimum or optimum alkalinity requirements for these semicontinuous reactors. Figure 4.2 shows the alkalinity, pH and methane production relations during the following seven 10-day feeding cycles.

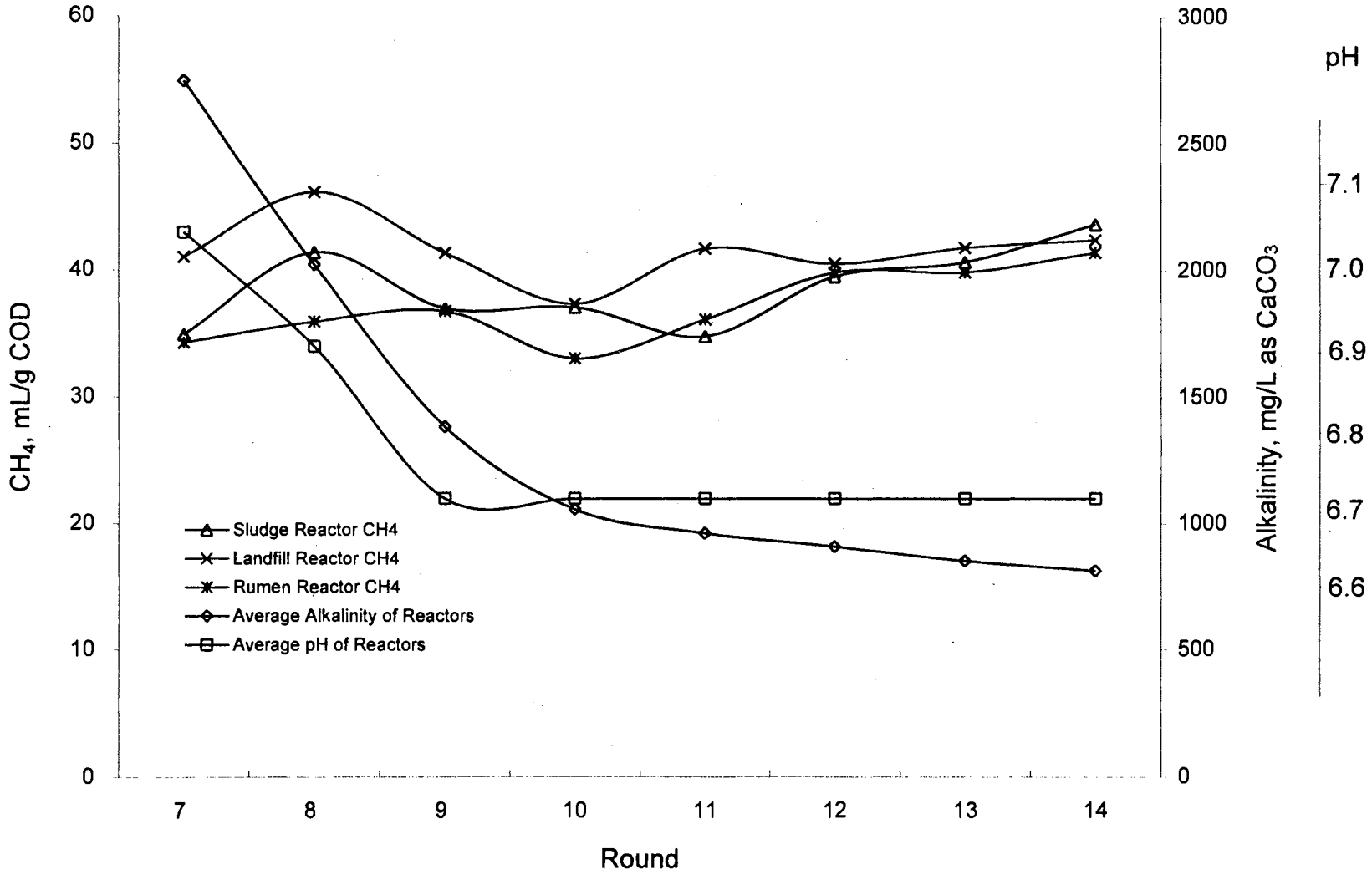

Figure 4.2 Methane production and pH change of seed reactors with various alkalinity concentrations.

Figure 4.2 shows that when media alkalinity was reduced from 2800 mg/L to around 800 mg/L, at which point $NaHCO_3$ addition was reduced to zero, the methane production of the semicontinuous reactors was not noticeably affected. During the same period, the pH in the reactors dropped significantly when the alkalinity was reduced from 2800 mg/L to 1400 mg/L. However, when the alkalinity was dropped further, the pH and methane production remained relatively steady during the entire tested alkalinity range. This means that carbonate or bicarbonate addition was not required to supply alkalinity for the system, with sufficient alkalinity being supplied by the nutrient solution prepared with tap water.

Long-term Batch Reaction in the Semicontinuous Seed Reactors Based on the initial experiments, the short HRT might be one of the reasons for slow newsprint digestion in the reactors. In order to investigate the extent of bioconversion and ultimate methane potential during prolonged incubation, these reactors were turned into long-term batch operation after 7 HRT periods (14 feed cycles) at Round 15. At the same time, a parallel serum bottle test was initiated to investigate the possible effect of strictly anaerobic conditions on the newsprint bioconversion, since serum bottles stoppered with air-impermeable stoppers can be considered strictly anaerobic, while the plastic seed reactors were not. Methane production from the seed reactors and the parallel serum bottles in the long-term batch test is shown in Figure 4.3. Since this experiment was started from active semicontinuous culture materials, the starting conditions of this experiment could only be obtained through sample analysis and mass balance.

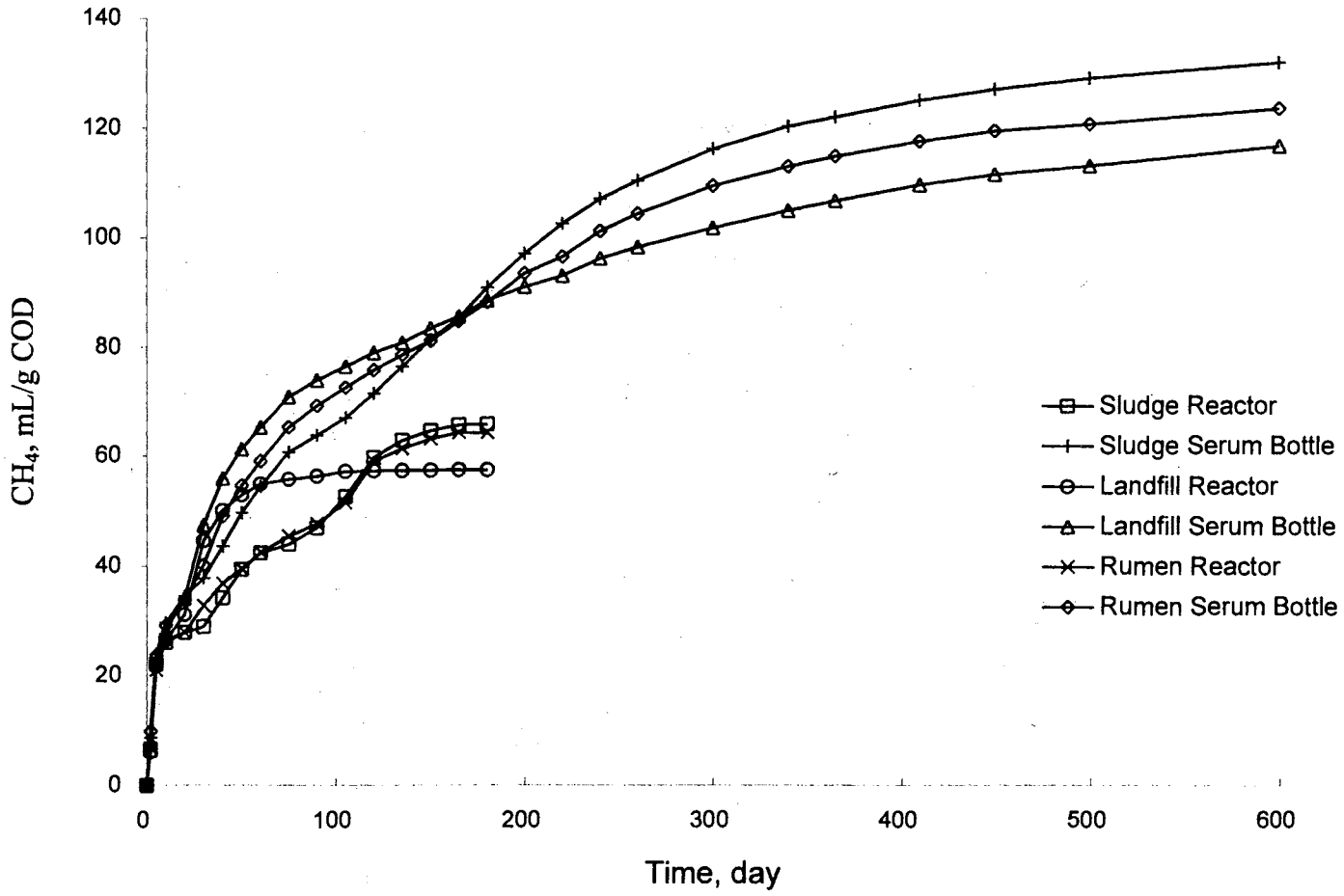

Figure 4.3 Newsprint conversion by seed reactors and parallel serum bottles.

For the seed reactors, the long-term biogas conversion virtually stopped after about 150 days, and monitoring of methane production was terminated on the 180th day. Residues from the three reactors after long term digestion were analyzed for TS, VS, cellulose, AIL, COD, pH and alkalinity changes during the whole experimental period; the results are listed in Table 4.2.

In contrast to the seed reactors, bioconversion in the parallel serum bottles was far from completion at that time, and monitoring continued with the aim of quantifying the ultimate methane potential. However, after 600 days incubation, methane production continued at slow but steady rates in each serum bottle reactor. Due to time constraints, the serum bottles were sacrificed on the 600th day for residue analysis. Since the differences in methane production among the three inocula were relatively small, and to obtain the necessary sample size for analyses, the residues of all the bottles were mixed. The analysis results are also listed in Table 4.2 to compare with those of the seed reactors.

Figure 4.3 shows that methane production for all the reactors and bottles was rapid and very close to each other for the first 7-8 days. After that, the conversion rates slowed down. The landfill reactor sustained a higher methane production rate than the other two reactors for a period of about 20 - 30 days. After that period, the conversion rate for the other two reactors began to increase gradually while the conversion rate for the landfill reactor began to decrease. At about the 110th day, cumulative methane yields for the other reactors surpassed that of the landfill reactor for the remainder of the test.

Table 4.2. Analysis Results and Mass Balance of the Long-term Test

Reactor	Total Solids, % In	Total Solids, % Out	Volatile Solids, % In	Volatile Solids, % Out	Cellulose, % VS In	Cellulose, % VS Out	AIL, %VS In	AIL, %VS Out	COD, mg/gVS In	COD, mg/gVS Out	Alkalinity,mg/L In	Alkalinity,mg/L Out	pH In	pH Out	Methane Extent mL/gCOD	Methane Extent mL/gVS
Sludge Seed	1.138	0.811	0.959	0.661	41.99	17.36	31.02	50.67	1374	1468	1143	653	7.10	6.50	68.7	94.3
Landfill Seed	1.134	0.872	0.956	0.716	40.47	29.58	32.88	42.54	1404	1392	1202	786	7.10	6.75	57.2	80.3
Rumen Seed	1.140	0.814	0.961	0.664	42.44	15.37	31.98	51.25	1380	1534	1173	689	7.10	6.65	64.9	89.6
Parallel Serum	1.137	0.730	0.959	0.561	41.63	12.11	31.96	66.90	1386	1628	1172	765	7.10	6.40	110.2	152.8

Mass Balance	Total Solids, g $W_{TS} = W_{media} \times TS\%$ In	Out	Removal	Volatile Solids, g $W_{VS} = W_{media} \times VS\%$ In	Out	Removal	Cellulose, g $W_{cellu} = W_{VS} \times Cellu\%$ In	Out	Removal	AIL, g $W_{AIL} = W_{VS} \times AIL\%$ In	Out	Removal	COD, g $W_{COD} = W_{VS} \times COD$ In	Out	Removal	Alkalinity Required,mg/L	Estimation, of Methane Loss, %	Methane Conversion mL/gCOD	mL/gVS
Sludge Seed	34.1	24.3	9.8	28.8	19.8	9.0	12.1	3.4	8.6	8.9	10.1	-12.5	39.5	29.1	10.4	490	25.7	260.0	303.3
Landfill Seed	34.0	26.2	7.8	28.7	21.5	7.2	11.6	6.4	5.3	9.4	9.1	3.1	40.3	29.9	10.4	416	36.6	221.9	320.1
Rumen Seed	34.2	24.4	9.8	28.8	19.9	8.9	12.2	3.1	9.2	9.2	10.2	-10.6	39.8	30.5	9.3	483	20.3	278.9	289.4
Parallel Serum	34.1	21.9	12.2	28.8	16.8	11.9	12.0	2.0	9.9	9.2	11.3	-22.5	39.9	27.4	11.8	407	-0.75	352.6	491.1

Note: (a) The serum bottle data were composite sample of all three inocula after 600 days incubation.
(b) Seed reactors were sampled separately after 180 days incubation.

Figure 4.3 also shows that for the first 15 days, methane conversion rates of the parallel serum bottles were similar to those of the seed reactors. However, after 15 days, the conversion rates of the bottles were higher than those of the reactors, and the methane yields were significantly higher. In contrast to the seed reactors, parallel serum bottles with all three inocula produced methane steadily with a nearly constant conversion rate from 60 to 300 days and from 300 to 600 days at a slower but still relatively constant rate. There was no significant rate reduction in biogas production even at the 600th day, as seen by observing the methane curves in Figure 4.3. Although the methane yields among the three groups of parallel serum bottles were significantly different during the period from day 30 to 130, in the long run the differences among methane production rates were relatively small for the three microbial inocula.

The methane extents listed in Table 4.2 were obtained by dividing the total COD or total VS (gram) added to individual reactors into the total methane (mL) generated from the corresponding reactor. The methane conversions were obtained by dividing the total COD or total VS removal of individual reactors from the total methane generated from the corresponding reactor. In the case of parallel serum bottles, assuming the mixture of all the serum bottles had the same volume of one reactor, then the average values of the parameters of the three reactors were used in calculating the equivalent methane production and mass removal of the serum bottles. The methane extent and conversion of the serum bottles thus can be calculated, and the conversion efficiency of the 600 days serum bottle experiment was calculated as only 31% of the theoretical value. The methane losses from each reactor and from serum bottles were calculated according

to the fractional shortage of the methane conversion (mL/g COD) compared with the theoretical value.

The negative lignin removals by sludge and rumen inocula shown in Table 4.2 were most probably caused by the experimental error in the AIL analysis. The AIL method used in this study was found to have good precision in AIL analysis. This can be seen from the relatively low standard deviations of the AIL results in paper (Table 4.9) or even in reactor media (Table 4.1) analysis. However, since the reactor media contain microorganisms and even some organic extractables, and the AIL method used in this study does not include an organic solvent extraction procedure, the accuracy of the method may be unsatisfactory. Comparing the relative lignin amounts obtained from a mass balance, the landfill reactor had the lowest lignin content at the end of the experiment, while it was the highest at the beginning. This might imply that landfill consortia have some ability to attack the lignocellulose structure under anaerobic, or at least under nonstrict anaerobic conditions.

Comparing the conversion of solid materials and methane production in the parallel serum bottle test with the seed reactor test, it is seen that strict anaerobic conditions were extremely important for the newsprint digestion. The methane conversion extent of the parallel bottles almost doubled that of seed reactors, while no potential methane loss could be documented. These losses were calculated to be as high as 20.3-36.6 percent for the seed reactors (Table 4.2). The mass balance revealed that there was still a significant amount of cellulose in the serum bottle residues even after 600 days incubation. This may suggest that the slow newsprint conversion rate was not

caused by the short HRT, nor by gas permeability of the seed reactors, but most probably by the physical barrier of the lignin cellulose association in the newsprint.

According to Table 4.2, the methane yield of the landfill reactor was significantly lower than that of the other two reactors during the long-term digestion, while in parallel serum bottles, the differences among the three groups of inocula were much smaller. Considering that the inoculating material contained as high as 50% reactor media, and since the reactors had produced different amounts of methane in the semicontinuous feeding rounds before the long-term experiment started, the contents of the 50% inocula are quite different. However, all contain a significant amount of cellulosic materials. Because the landfill reactor produced the largest amount of methane during the steady state condition, the cellulose content of its inoculating material was expected to be lower, while the lignin content was expected to be higher, than those of the other two reactors. This can be seen from Table 4.2. So it is most probably the lower cellulose/lignin ratio of the starting material that determined the lower final methane yield of the landfill reactor compared to the other two reactors in this experiment. Therefore, quantitative comparison of the effects by the different inocula should only be performed with continuous steady state or strictly batch conversion data, rather than from the results of this long-term experiment. Despite difficulties with a direct quantitative comparison, the qualitative discussion of the reaction patterns of the three reactors and comparison of the gas production from these reactors with parallel serum bottles are still valid.

Potential losses of methane from the reactors were found as high as 20.3 - 36.6%. In contrast, no methane potential loss was found in the serum bottle test. Due to its

relatively low solubility in water, the loss of methane through the water layer of the biogas collector should not be as great as that of carbon dioxide. The possible routes of losing methane were either through the plastic walls of the reactors as biogas exchanged with intruding air, or being converted to other final products, for example CO_2, because of the intruding air. It is impossible for this experiment to define how much methane potential loss was due to loss of methane and how much could have been converted to CO_2.

Restart Semicontinuous Loading after Long-term Operation After the long-term batch operation, the seed reactors were restarted with the semicontinuous loading. Since the substrate concentrations in the reactors were lowered, and the microorganisms were less active after the long-term test, when the seed reactors were turned back to the semicontinuous mode, the biogas productions were lower during the initial 10-day feeding cycles. After a 2-HRT recovery period when the steady state condition was resumed, it was interesting to note that the methane conversion rates for all three reactors were significantly higher than before the long-term batch experiment (Table 4.3). The methane yield increases for rumen and sludge reactors were as high as 61.0% and 50.3%, while the landfill reactor increased by 14.1%. These increases in methane conversion ability of the seed reactor microorganisms may have been caused by acclimation or mutation of the microbial consortia during long-term starvation conditions.

Table 4.3 Comparison of Semicontinuous Reactor Methane Yields (Before and After Long-term Batch Test)

Seed Reactor	(1) Methane Yield, mL/g COD Before	(2) Methane Yield, mL/g COD After	[(2)-(1)]÷(1) Increment %
Sludge	38.58 (2.46)	55.18 (2.09)	50.3
Landfill	41.65 (2.80)	45.25 (1.39)	14.1
Rumen	37.10 (2.50)	56.83 (1.91)	61.0

Note: The data presented are the average of at least five 10-day cycles before and after the long-term test. Values in parentheses are standard deviations of the parameter estimates.

Effect of Yeast Extract on Semicontinuous Reaction Yeast extract was added to supply trace minerals and organic growth factors to the waste paper bioconversion. However, its effect on the bioconversion was still unknown. To verify the effect of yeast extract on the semicontinuous reaction, its addition was totally stopped after Round 25. After another 2 HRT periods, the methane production during a 10-day cycle was measured and compared with comparable results with the yeast extract addition. The results are listed in Table 4.4.

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Seed Reactor	(1) Methane Yield, mL/g COD Before	(2) Methane Yield, mL/g COD After	[(2)-(1)]÷(1) Increment %
Sludge	38.58 (2.46)	55.18 (2.09)	50.3
Landfill	41.65 (2.80)	45.25 (1.39)	14.1
Rumen	37.10 (2.50)	56.83 (1.91)	61.0

Note: The data presented are the average of at least five 10-day cycles before and after the long-term test. Values in parentheses are standard deviations of the parameter estimates.

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Table 4.4 Comparison of Semicontinuous Reactor Methane Yields (With or Without Yeast Extract Addition)

Seed Reactor	(1) Methane Yield, mL/g COD With Addition	(2) Methane Yield, mL/g COD Without Addition	(1) - (2) Difference mL/g COD
Sludge	55.18 (2.09)	36.23	19.95
Landfill	45.25 (1.39)	26.24	19.01
Rumen	56.83 (1.91)	38.31	18.52

Note: The data of with yeast extract addition are the average of five 10-day cycles while that of without yeast extract addition is from Round 29 only. Values in parentheses are standard deviations of the parameter estimates.

Although the methane yields of the three reactor types were significantly different, the decreases in methane yield in a single reactor without yeast extract addition for all three reactors is very close, roughly 19 mL/g COD. It was not clear if this difference in methane production was partially due to the improvement by yeast extract addition on the methane conversion activity or solely due to use of yeast extract as an additional substrate. In order to verify the effect of yeast extract on the bioconversion, the methane yield of the yeast extract itself must be determined, and the following experiment was initiated for this purpose.

Methane Yield of Yeast Extract A set of serum bottle bioconversion tests was conducted with yeast extract as sole substrate (Chapter III, Yeast Extract Methane Production) to find the ultimate methane yield of yeast extract itself. The bioconversion test was run for 60 days, though biogas production was insignificant after about 35 days. The results of this test are shown in Figure 4.4 and Table 4.5.

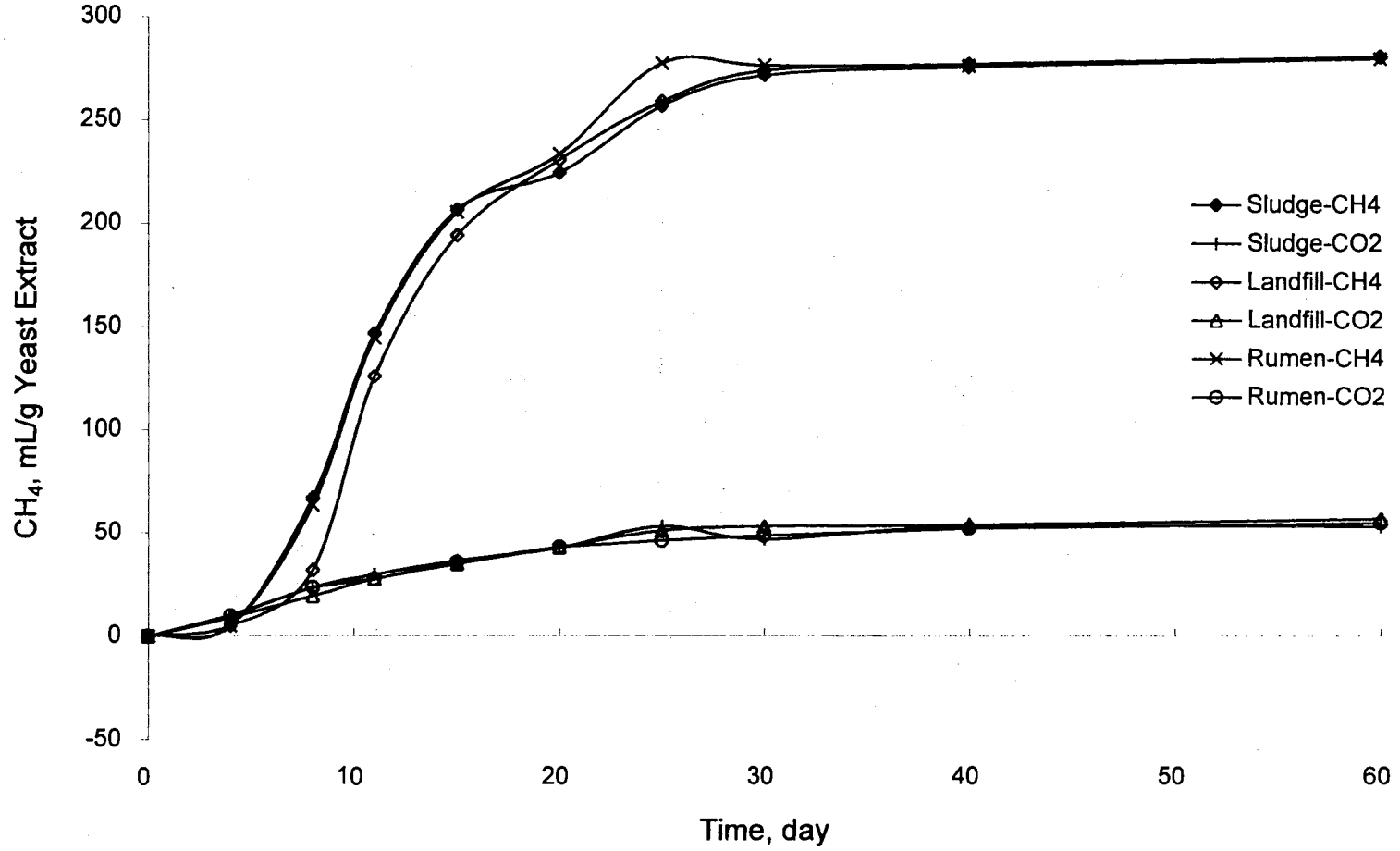

Figure 4.4 Yeast extract methane and carbon dioxide yields.

Table 4.5 Yeast Extract Methane and Carbon Dioxide Yields

Inocula	CH_4, mL/g	CO_2, mL/g
Sludge	281.7 (6.4)	54.79 (2.44)
Landfill	281.3 (7.0)	57.06 (1.64)
Rumen	280.6 (4.4)	54.95 (2.34)

Note: values in parentheses are standard deviations of the parameter estimates.

Figure 4.4 and Table 4.5 show clearly there are no differences in methane and carbon dioxide yields of yeast extract when inoculated with three different inocula, and the ultimate methane potential was about 281 mL/g yeast extract. Since the yeast extract addition to each reactor in a feeding cycle was known, and at steady-state the yeast extract added to each feeding cycle can be considered totally converted in that period, an equivalent methane production (in mL/g COD) of yeast extract could be obtained from a careful mass balance based on the ultimate yeast extract methane production. Such a mass balance indicated that the equivalent methane production of yeast extract was 16.2 mL methane per gram of available paper COD in steady state. Since the difference between periods with and without yeast extract addition was roughly 19 mL/g COD (Table 4.4), which is larger than the ultimate 16.2 mL/g COD methane yield of yeast extract, it seems that yeast extract addition did improve the methane conversion in the semicontinuous seed reactors, though the extent of acceleration was limited. However, considering there were some organics and trace minerals of yeast extract origin remaining

in the reactors even after the yeast extract addition was totally stopped, and the amounts of trace minerals and organic factors required are usually relatively small, the difference in methane production obtained in this experiment may be smaller than that if absolutely no yeast extract was added. Therefore, the improvement on methane production by yeast extract addition should not be overlooked.

Since yeast extract itself produces methane equivalent to 16.2 mL/g paper COD, this suggests that before the long-term experiment (in Round 7 - 14 and Table 4.1), the true methane yields of the semicontinuous seed reactors were very limited, only around 20 mL/g paper COD. However, after the long-term experiment, especially for the sludge and rumen reactors, the methane yields without yeast extract addition, as in the case of Round 29 (Table 4.4, third column), are comparable to those with yeast extract addition before the long-term experiment (Table 4.3, second column). This should not be interpreted that the yeast extract addition is not necessary but should be attributed to the conversion rate increases in the seed reactors due to the long-term experiment.

Define the Limiting Step of the Seed Reactors Comparing the results from long term seed reactor operation with the parallel serum bottle test, the permeability of the reactor wall was considered the major cause of the problem. However, there were still two major possible reasons of the slow conversion rate and the low ultimate methane potential of those seed reactors: permeating air inhibition on the bioconversion or loss of CO_2, which is a precursor of the final product, methane, through the reactors' plastic walls. Carbon dioxide may also have been lost through the gas collector water layer due to the high solubility of CO_2 in water.

In order to verify which of above hypotheses was legitimate, the seed reactors were turned to another long-term experiment at Round 30 until the methane production totally stopped. This experiment lasted 102 days and produced comparable methane with that of the first long-term experiment. Since analysis of the initial sample was not performed, no efforts would be made to compare its results with the first long-term test. After the seed reactors totally stopped producing biogas for at least six days, the residues were fed into serum bottles and sealed with butyl rubber stoppers. All the samples were triplicated as described in Chapter 3. In order to distinguish the effects caused by permeating air from the loss of precursor CO_2, half the bottles were filled with N_2 gas as head space atmosphere only, while the other half were filled with 2/3 N_2 and 1/3 CO_2. When incubated, almost all the bottles started to produce methane immediately; the methane and carbon dioxide yields of this test are shown in Table 4.6.

Statistical analysis with the general linear model (GLM) procedure failed to detect any significant difference in methane production between the two headspace groups from 3 to 38 days. The reason for using GLM procedure instead of AOV is because one bottle was lost during the incubation, which caused the imbalance of the sample number among different groups. In dealing with missing sample analysis, GLM is a more proper procedure than the AOV. These results clearly indicate that the differences in methane production between seed reactor and parallel serum bottles were mainly caused by permeating air inhibition, instead of losing precursor CO_2.

Table 4.6 Methane and Carbon Dioxide Production by Seed Reactor Residues

Reactor	Sludge		Landfill		Rumen	
Headspace	$N_2 + CO_2$	N_2	$N_2 + CO_2$	N_2	*$N_2 + CO_2$	N_2
3 Day, Methane,	0.39 (0.35)	0.22 (0.39)	0.79 (0.12)	0.43 (0.40)	0.28 (0.39)	0.00 (0.00)
Carbon Dioxide (mL/g COD)	7.49 (1.00)	1.99 (0.06)	8.44 (0.48)	2.39 (0.20)	7.73 (0.27)	1.84 (0.08)
10 Day, Methane	1.93 (0.02)	1.85 (0.03)	2.15 (0.02)	2.20 (0.02)	1.71 (0.03)	1.44 (0.09)
Carbon Dioxide (mL/g COD)	7.76 (0.81)	2.69 (0.11)	8.87 (0.04)	3.45 (0.04)	8.03 (0.39)	2.48 (0.12)
27 Day, Methane	4.94 (0.26)	4.64 (0.12)	5.48 (0.20)	5.69 (0.58)	3.75 (0.37)	3.99 (0.25)
Carbon Dioxide (mL/g COD)	9.25 (0.81)	4.14 (0.32)	10.30 (0.19)	5.05 (0.14)	8.28 (0.50)	3.47 (0.14)
38 Day, Methane	6.49 (0.12)	6.19 (0.16)	7.04 (0.35)	7.36 (0.17)	4.86 (0.51)	4.83 (0.41)
Carbon Dioxide (mL/g COD)	10.42 (0.80)	4.83 (0.33)	11.65 (0.29)	6.00 (0.05)	9.61 (0.57)	4.03 (0.16)

Note: Values in parentheses are standard deviations of the parameter estimates.
* Standard deviations were estimated from duplicate samples in this column.

Since the permeating air does affect the anaerobic biodigestion of all three inocula, this result can be in turn used to explain the differences between the first long-term experiment with its parallel serum bottle test. In average, the methane yields of the seed reactors were only 58% that of the serum bottles (the difference is as high as 73%). However, the extent of effects of permeating air to the various reactors is significantly different and the affecting patterns are complicated. According to Figure 4.3, during the first 7-8 days of the first long-term batch test, when plenty of easily digestible material was present and the biogas production was high, the effects of permeating air were negligible. However, when the biogas production slowed down, the effects of permeating air were manifest, further suppressing the microbial conversion rate. The microorganisms of landfill origin seemed most tolerant to the permeating air in the earlier stages. The air tolerance acclimation of the other two groups of microorganisms took a longer time to develop. Although the effects were complicated, these experiments show that beside the physical barrier caused by lignin cellulose association and the effect caused by low cellulose/lignin ratio, permeating air was another major limiting step for the seed reactors in this study.

Serum Bottle Test

Preliminary Serum Bottle Test

The purpose of these serum bottle tests was to evaluate the effects of different paper type, size, previous usage and different inocula on bioconversion. To make sure that the experimental design, procedures, sampling and analysis methods were valid, a

preliminary serum bottle test was conducted on one inoculum, from the sludge seed reactor, and on office paper and newsprint (both printed and unprinted) with 2 paper sizes (ground and shredded). Wheaton brand serum bottles (125 mL) were used as batch reactors. To each bottle, 2g/L volatile solids of paper was added with 80 mL nutrient solution (Table 3.3) and 20 mL inoculum. Controls included nutrient solution and inoculum only. All the samples were duplicated. This test was run for 60 days. The gas production pattern and the final results are shown in Figure 4.5 and Table 4.7.

Table 4.7 Methane Production of Preliminary Serum Bottle Test

Paper Type	Methane Production, mL/g COD	
	Ground Paper	Paper Strips
Unprinted Newsprint	27.99 (3.17)	27.18 (0.14)
Printed Newsprint	44.55 (1.08)	42.45 (2.81)
Waste Office Paper	297.0 (8.2)	292.4 (3.4)

Figure 4.5 shows a striking difference in methane production between office paper and newsprint. The bioconversion of office waste paper is completed within 40 days of incubation, while for newsprint paper, both printed and unprinted, the conversion extent was very limited, only about 27 - 45 mL/g COD, during the 60-day incubation.

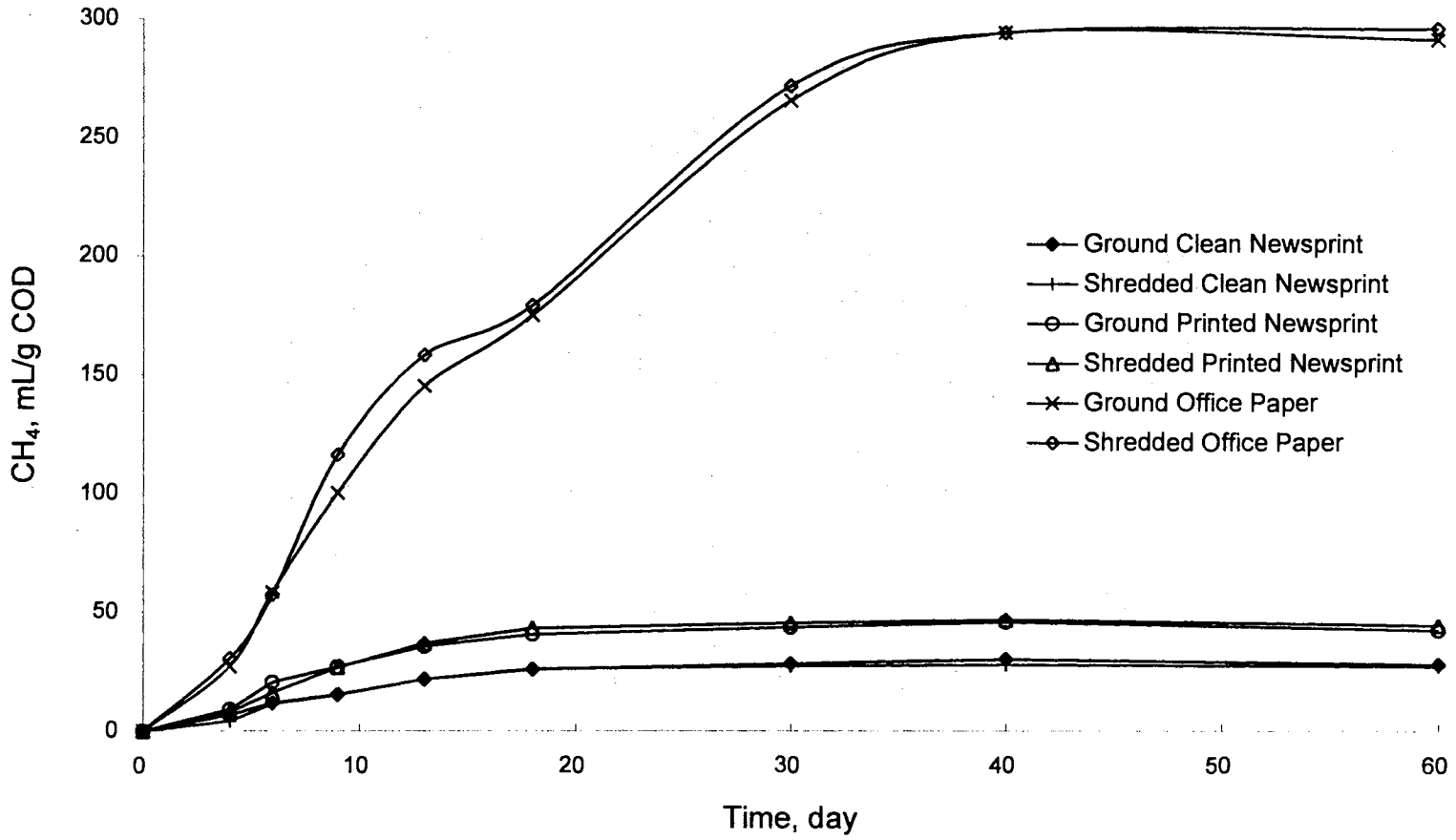

Figure 4.5 Methane production of preliminary serum bottle test.

Table 4.7 shows that the methane yields of printed and unprinted paper are significantly different. It was found that although both paper samples were collected from the same printing plant, the printed and unprinted newsprint used in this experiment may have come from different suppliers and would have different compositions, especially the cellulose/lignin ratio. Analysis on AIL found that the unprinted newsprint used for this experiment and for the seed reactors contained 44.2 %VS AIL, while the printed newsprint paper contained only 30.3%VS AIL. So the difference in methane production of this preliminary serum bottle test was most probably caused by the different cellulose/lignin ratios of the unprinted and printed newsprint.

Only limited conversion of newsprint paper occurred during 60 days, which suggested that much longer experimental duration or some pretreatment must be applied to improve the bioconversion rate and/or ultimate extent. At times during regular sampling periods the biogas amount was too low to be accurately measured and determined, which might have caused larger experimental errors for newsprint results. So, to be successful in the succeeding experiments, 1) the experiment should be maintained for much longer duration than those previously expected, or some pretreatment should be tested, 2) the printed and unprinted newsprint must be from the same source so that the only difference between them is the printing process itself, 3) the newsprint paper loading to each serum bottle should be increased accordingly to increase the gas production and reduce the experimental error, and finally, 4) the TS, VS, cellulose, AIL and COD of all materials to be used in the following experiment, including the yeast extract and printing ink, must be determined.

The newsprint used in the following serum bottle test was collected from the Stillwater NewsPress Printing Plant. Both unprinted and printed paper were confirmed to be from a single paper web. The sample size for newsprint was increased from 2g/L to 5g/L. The analysis results of the different paper samples, printing ink and yeast extract are listed in Tables 4.8 and 4.9. Table 4.8 shows that the cellulose content of office paper is much higher than that of newsprint, while its AIL content is much lower. The most obvious difference between the office paper and the newsprint is that the cellulose to lignin ratio for office paper was found to be 23.4 while that of newsprint was only about 1.5. The COD/VS ratios were found to be about 1.45 for newsprint and 1.26 for office paper, while that of ink was as high as 2.72, which suggests ink itself is in a highly reduced chemical form.

Table 4.8 Characteristics of Paper Samples

Parameter	Paper Sample		
	Unprinted Newsprint	Printed Newsprint	Office Paper
Total Solids, %	93.79 (0.13)	93.87 (0.11)	95.68 (0.12)
Volatile Solids, %	90.73 (0.14)	90.80 (0.12)	87.95 (0.10)
COD/VS Ratio	1.44 (0.03)	1.46 (0.03)	1.26 (0.02)
Cellulose, % VS	49.8 (1.7)	46.2 (3.1)	82.4 (1.4)
AIL, % VS	30.3 (0.52)	31.3 (0.56)	3.6 (0.47)
Cellulose/AIL Ratio	1.64	1.47	23.4

Note: Values in parentheses are standard deviations of the parameter estimates.

Table 4.9. Characteristics of Other Media

Item	Other Media Sample	
	Ink	Yeast Extract
Total Solids(non-moisture material), %	99.19 (0.18)	90.68 (0.07)
Volatile Solids, %	98.19 (0.17)	76.15 (0.08)
COD/VS Ratio	2.72 (0.01)	1.26 (0.01)

Note: Values in parentheses are standard deviations of the parameter estimates.

Basic Serum Bottle Test

This experiment was designed to estimate the effects of inocula, paper type, paper size and printing ink (or the printing process) on waste paper digestion. The experimental profile is outlined in Figure 3.2. After the experiment started, the biogas volume and components, CH_4, CO_2 and N_2, were sampled and analyzed regularly. Statistical analysis was also performed regularly to find the significance of the effects of different paper types, paper sizes and inocula, and the printing ink or print process.

The basic serum bottle experiment was run for 220 days for office paper and 300 days for newsprint. Since the substrate control samples still contained a significant biodegradable materials and also were fed the same volume of nutrient solution as the paper samples, they also produced significant amounts of biogas. The averages of methane and carbon dioxide produced by each group of controls were subtracted from those of corresponding paper samples. The residues of newsprint and control samples

after 300 days incubation were analyzed for TS, VS, cellulose, AIL (AIL of control samples was not determined because the sample size could not satisfy the analysis requirement), COD, pH and alkalinity. The results are listed in Table 4.10.

One observation of the serum bottle test was that for the printed newsprint bioconversion, several bottles with either sludge or rumen inocula gave significantly lower biogas conversion. Their methane and carbon dioxide yields were only about half that of other bottles. This phenomenon only happened in the printed newsprint and with only two of the three inocula involved. Thus statistical analysis results (presented in Table 4.14) show that the standard deviations of the methane data for sludge and rumen inoculated samples are much higher than for landfill inoculated samples of printed newsprint. The behavior of the landfill inoculum in this study was coincident with the landfill cellulolytic bacterium studied by Cummings and Stewart (1995) that did not experience reduced cellulolysis of one-side-ink-coated filter paper. In order to verify that the low conversion in these sludge and rumen samples was caused by inhibition or physical blockage instead of biogas leakage, the residue of those samples was analyzed separately. The results of the low conversion sample analyses are also listed in Table 4.10. Since the TS, VS and cellulose of the low conversion residues were much higher than those of average samples, it clearly indicates the low conversion was caused by some kind of inhibition instead of biogas leakage.

Table 4.10 Serum Bottle Test Residue Analysis

Sample	Total solids %	Volatile Solids %	Cellulose % VS	AIL % VS	Cellulose Lignin Ratio	COD mg/g VS	pH	Alkalinity mg/L as $CaCO_3$
Sludge	0.580 (0.003)	0.388 (0.003)	15.65 (0.42))	49.24 (0.33)	0.32	1478	6.4	884
Landfill	0.616 (0.016)	0.425 (0.009)	18.10 (0.78)	47.49 (0.84)	0.38	1441	6.4	1002
Rumen	0.589 (0.003)	0.394 (0.000)	12.86 (0.69)	48.02 (0.09)	0.27	1459	6.4	983
Sludge Control	0.367 (0.004)	0.197 (0.005)	19.72 (0.81)	-	-	1385	6.8	1096
Landfill Control	0.344 (0.012)	0.176 (0.004)	14.04 (0.45)	-	-	1407	6.7	1079
Rumen Control	0.348 (0.010)	0.172 (0.007)	16.91 (0.44)	-	-	1391	6.8	968
Low Conversion.	0.703 (0.007)	0.512 (0.008)	31.40 (0.00)	-	-	1296	6.55	955

Note: Values in parentheses are standard deviations of the parameter estimates.

In their BMP test, Owens and Chynoweth (1993) found ink of carbon black in petroleum oils did not significantly affect the methane yields in newsprint digestion. However, that ink could act as a physical barrier to prevent bacterial adhesion and subsequent degradation was reported by Cummings and Stewart (1994). Since the low conversion bottles in this study were found only in the printed newsprint and with two of the three inocula, this abnormal phenomenon cannot be simply explained as being due to physical barrier or biochemical inhibition; it must be somehow related with the physical or chemical properties of printing ink and the characteristics of the microbial consortia involved in the digestion.

Since the low conversion samples were separated from the rest of the samples, the average TS, VS, cellulose data of sludge and rumen inoculated samples listed in Table 4.10 are slightly lower than if the low conversion samples were mixed with the rest and analyzed together.

Table 4.10 shows that the landfill group retained the highest cellulose content and the lowest AIL content in its residue. The lignin content in landfill controls was much lower than those of the other two groups. These results are consistent with the results obtained from the long-term seed reactor experiment. Statistical analysis proved the differences in remaining cellulose and AIL are significant among the three differently inoculated groups. It was known from the semicontinuous seed reactor experiment that landfill seed reactor gave the highest biogas and methane production (Table 4.1) during semicontinuous conversion. When used as inocula, it should contain the lowest cellulose content and highest lignin content (Table 4.2), and should not produce more methane than

the other two control groups. Considering the different behavior of the landfill seed reactor during the semicontinuous and long-term test, and that only landfill-inoculated printed newsprint digestion did not have low conversion samples in serum bottle tests and in substrate controls, it may suggest that landfill microbial consortia do have some different characteristics from the other two consortia tested with regard to high lignocellulosic material bioconversion. Since the serum bottle tests can be considered to contain strict anaerobic conditions, the low final AIL content of the landfill-inoculated group suggests the landfill microbial consortia have some special ability toward lignin structure even under strict anaerobic condition while leaving a higher cellulose content unutilized.

Since the gas productions by office paper and newsprint are so different, as can be observed directly from Figure 4.6, it is obvious that no statistical analysis is necessary to prove the significant difference between them. Therefore, the statistical analysis procedure of analysis of variance (AOV) was applied to the methane yields of newsprint (both unprinted and printed, as outlined in Figure 3.3) with paper type as treatments and inocula as experimental elements to find if there were any significant differences between the two kinds of paper and the effects of three different inocula on paper bioconversion. The results of this statistical analysis are shown in Table 4.11. Then, the AOV procedure was also applied to methane data of each type of paper individually, with paper size as treatment elements and inocula as experimental elements, as outlined in Figure 3.4, to find the effects of paper size and inocula on each type of paper. The analysis results are listed in Tables 4.12, 4.13 and 4.14.

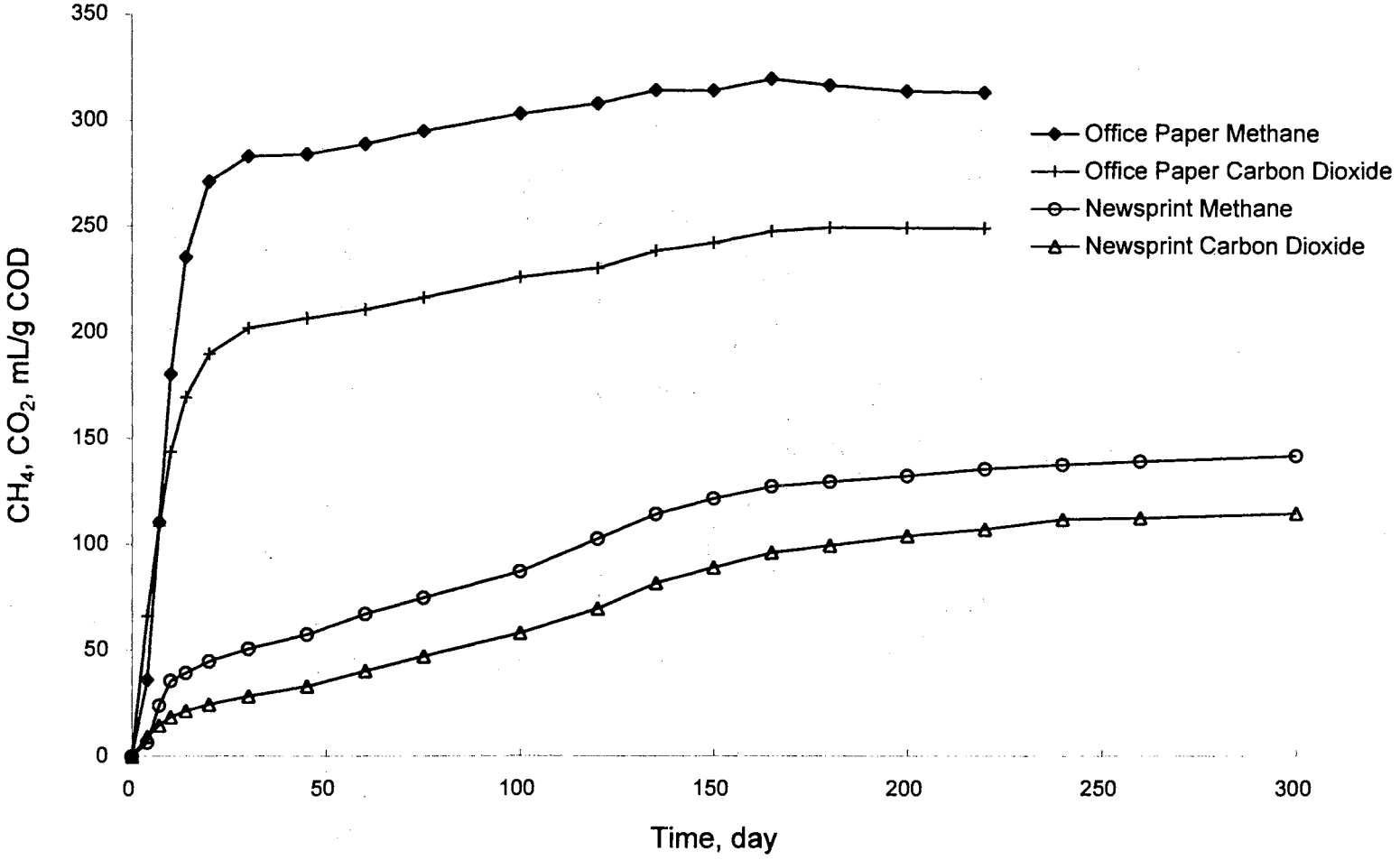

Figure 4.6 Methane and carbon dioxide productions by office paper and newsprint.

Table 4.11 AOV Analysis on Methane Yields of Newsprint

Day	Significance of Paper Type			Significance of Inocula		
(means)	Unprinted Newsprint (mL/g COD)	Printed Newsprint (mL/g COD)		Sludge (mL/g COD)	Landfill (mL/g COD)	Rumen (mL/g COD)
45	56.87 (3.52)	57.33 (3.93)	-	55.01 (2.85)	56.92 (4.12)	59.38 (2.73)
						+
60	67.38 (8.36)	66.25 (7.13)	-	60.87 (4.82)	72.75 (8.26)	66.81 (4.40)
						+
75	76.11 (11.75)	73.26 (10.98)	-	65.60 (6.47)	85.15 (10.88)	73.31 (6.07)
						+
100	90.88 (15.38)	83.38 (16.29)	-	73.09 (8.83)	99.55 (13.24)	88.75 (13.57)
						+
120	108.40 (15.97)	96.62 (22.13)	-	89.85 (19.35)	107.45 (9.28)	110.23 (18.57)
						+
150	129.47 (14.67)	113.80 (24.20)	-	117.71 (28.23)	118.01 (6.31)	129.18 (22.18)
						-
180	137.35 (12.58)	121.99 (27.42)	-	133.91 (30.90)	125.19 (6.15)	129.92 (23.37)
						-
220	144.47 (11.19)	126.76 (31.41)	-	141.37 (35.28)	132.65 (5.19)	132.83 (25.56)
						-
260	148.32 (13.34)	132.17 (33.95)	-	148.07 (37.65)	135.77 (4.55)	137.06 (41.20)
						-
300	151.77 (11.43)	132.73 (34.78)	+	147.32 (38.02)	139.96 (4.59)	138.88 (28.55)
						-

Note: + means there is significant difference, - means there is no significant difference.
Values in parentheses are standard deviations of the parameters estimates.

Table 4.12 AOV Analysis on Methane Yields of Office Paper

Day	Significance of Paper Size			Significance of Inocula		
(means)	Ground (mL/g COD)	Shredded (mL/g COD)	Whole (mL/g COD)	Sludge (mL/g COD)	Landfill (mL/g COD)	Rumen (mL/g COD)
10	163.37 (15.13)	188.54 (13.03)	185.16 (16.08)	173.55 (18.93)	181.22 (17.25)	186.31 (14.21)
		-			-	
20	263.68 (13.28)	273.17 (9.37)	276.88 (17.62)	260.04 (9.22)	271.01 (10.10)	282.69 (14.25)
		-			+	
30	279.75 (13.33)	281.82 (6.56)	288.23 (15.64)	276.83 (8.56)	279.31 (9.86)	293.65 (12.38)
		-			+	
45	283.57 (18.28)	283.55 (10.50)	285.40 (20.03)	281.63 (8.76)	269.28 (8.43)	301.60 (20.03)
		-			+	
60	287.83 (21.24)	288.50 (19.38)	291.33 (24.61)	290.06 (9.85)	265.98 (7.51)	311.63 (10.68)
		-			+	
75	292.90 (24.88)	295.49 (24.31)	297.43 (27.26)	297.16 (10.32)	266.95 (8.81)	321.71 (9.38)
		-			+	
100	298.95 (25.72)	305.47 (28.20)	307.11 (29.23)	306.56 13.21)	275.57 (18.49)	329.40 (13.94)
		-			+	
120	300.38 (26.72)	310.32 (31.09)	315.10 (38.87)	312.98 (12.29)	277.45 (23.37)	335.27 (25.11)
		-			+	
150	298.52 (27.11)	323.47 (48.45)	322.45 (43.68)	320.35 (29.77)	277.04 (30.39)	347.05 (28.65)
		-			+	
220	286.16 (22.89)	330.27 (49.09)	326.92 (45.22)	327.67 (39.92)	278.59 (35.30)	335.48 (44.74)
		-			+	

Note: same as Table 4.11.

Table 4.13 AOV Analysis on Methane Yields of Unprinted Newsprint

Day	Significance of Paper Size			Significance of Inocula		
(means)	Ground (mL/g COD)	Shredded (mL/g COD)	Whole (mL/g COD)	Sludge (mL/g COD)	Landfill (mL/g COD)	Rumen (mL/g OD)
45	55.59 (2.57)	56.69 (3.72) -	58.34 (3.95)	54.49 (2.99)	57.75 (3.87) +	58.39 (2.54)
60	59.18 (4.71)	64.95 (10.16) -	66.55 (8.03)	61.42 (6.30)	74.57 (8.29) +	66.14 (4.35)
75	70.85 (6.23)	78.22 (14.63) -	79.27 (12.19)	67.29 (8.01)	87.28 (10.80) +	73077 (5.85)
100	82.42 (8.4)	95.00 (15.78) -	95.22 (18.13)	77.59 (9.64)	101.06 (13.75) +	93.98 (12.67)
120	103.25 (15.36)	113.44 (11.79) -	108.52 (19.89)	97.60 (17.81)	108.36 (10.12) -	119.24 (12.08)
150	133.01 (17.62)	128.84.(12.75) -	126.56 (14.22)	129.72 (15.07)	118.12 (7.99) +	140.57 (11.17)
180	142.04 (18.40)	135.18 (9.35) -	134.84 (7.12)	145.90 (12.06)	125.86 (7.32) +	140.30 (8.62)
220	149.70 (17.98)	141.27 (8.02) -	142.46 (5.74)	154.99 (12.31)	134.32 (5.74) +	144.11 (6.54)
260	154.42 (19.06)	145.53 (9.29) -	145.34 (8.26)	160.82 (13.62)	136.51 (4.59) +	147.96 (6.26)
300	157.34 (16.11)	148.26 (7.06) -	148.55 (7.44)	161.41 (12.68)	141.97 (4.09) +	150.77 (5.84)

Note: same as Table 4.11.

Table 4.14 AOV Analysis on Methane Yields of Printed Newsprint

Day	Significance of Paper Size			Significance of Inocula		
(means)	Ground (mL/g COD)	Shredded (mL/g COD)	Whole (mL/g COD)	Sludge (mL/g COD)	Landfill (mL/g COD)	Rumen (mL/g COD)
45	55.88 (4.63)	56.68 (3.13) -	59.43 (3.35)	55.53 (2.78)	56.10 (4.43) +	60.36 (2.70)
60	63.93 (6.14)	66.55 (8.18) -	68.26 (7.05)	60.31 (2.99)	70.94 (8.29) +	67.49 (4.61)
75	69.47 (7.44)	74.04 (13.28) -	76.27 (11.55)	63.91 (4.26)	83.02 (11.16) +	72.85 (6.61)
100	76.82 (9.48)	83.22 (19.21) -	90.10 (17.43)	68.59 (5.24)	98.03 (13.35) +	83.52 (12.99)
120	91.31 (15.48)	95.39 (19.67) -	103.17 (22.13)	83.35 (18.52)	106.55 (8.88) +	101.22 (20.11)
150	106.27 (26.82)	113.74 (24.07) -	121.40 (21.81)	105.70 (33.79)	117.90 (4.56) -	117.80 (25.08)
180	109.09 (32.59)	126.64 (23.47) -	130.24 (83.37)	121.92 (39.50)	124.51 (5.08) -	119.54 (29.05)
220	111.26 (37.44)	133.52 (26.68) -	135.57 (27.75)	127.75 (45.57)	130.98 (4.24) -	121.56 (32.55)
260	115.40 (39.98)	140.13 (29.62) -	140.97 (30.17)	135.31 (49.74)	135.02 (4.06) -	126.17 (36.18)
300	114.77 (40.13)	141.99 (29.30) -	141.42 (30.29)	133.23 (49.64)	137.95 (4.36) -	127.00 (37.14)

Note: same as Table 4.11

The statistical analysis results listed in Table 4.11 indicate that no significant difference in methane yields between unprinted and printed newsprint could be detected, while the differences in methane yields caused by the three different inocula were significant before day 120. After day 150, the effects of inocula were reduced to insignificance. Finally, at 300 days, the effect of paper type caused a slight difference in methane yields between unprinted and printed newsprint. In fact, this difference could be attributed mainly to inclusion of the low conversion bottles in this analysis.

For office paper, Table 4.12 clearly indicates there were no significant differences caused by the paper size. However, the inocula seemed to have significant effects on the methane yields in most experimental periods analyzed. It was found that the biogas productions, before subtracting those produced by the controls for all three groups, were very similar. The differences in methane production among the three groups shown in Table 4.12 were mainly due to the three control groups' production of different volumes of methane. The landfill controls produced the highest volume of methane, while the sludge controls produced the lowest (Figure 4.7 and Tables 4.15a and 4.15b). The equivalent methane data listed in Table 4.15 are based on the assumption that the average amounts of paper added to the sample bottles were added to every control bottle with the equivalent amount of methane produced from the controls on a milliliter per gram of paper COD basis. Table 4.15a shows equivalent methane yields of controls for office paper, while Table 4.15b is for newsprint. The reason to do this is for easy comparison of the methane produced by controls with other methane data on the same basis.

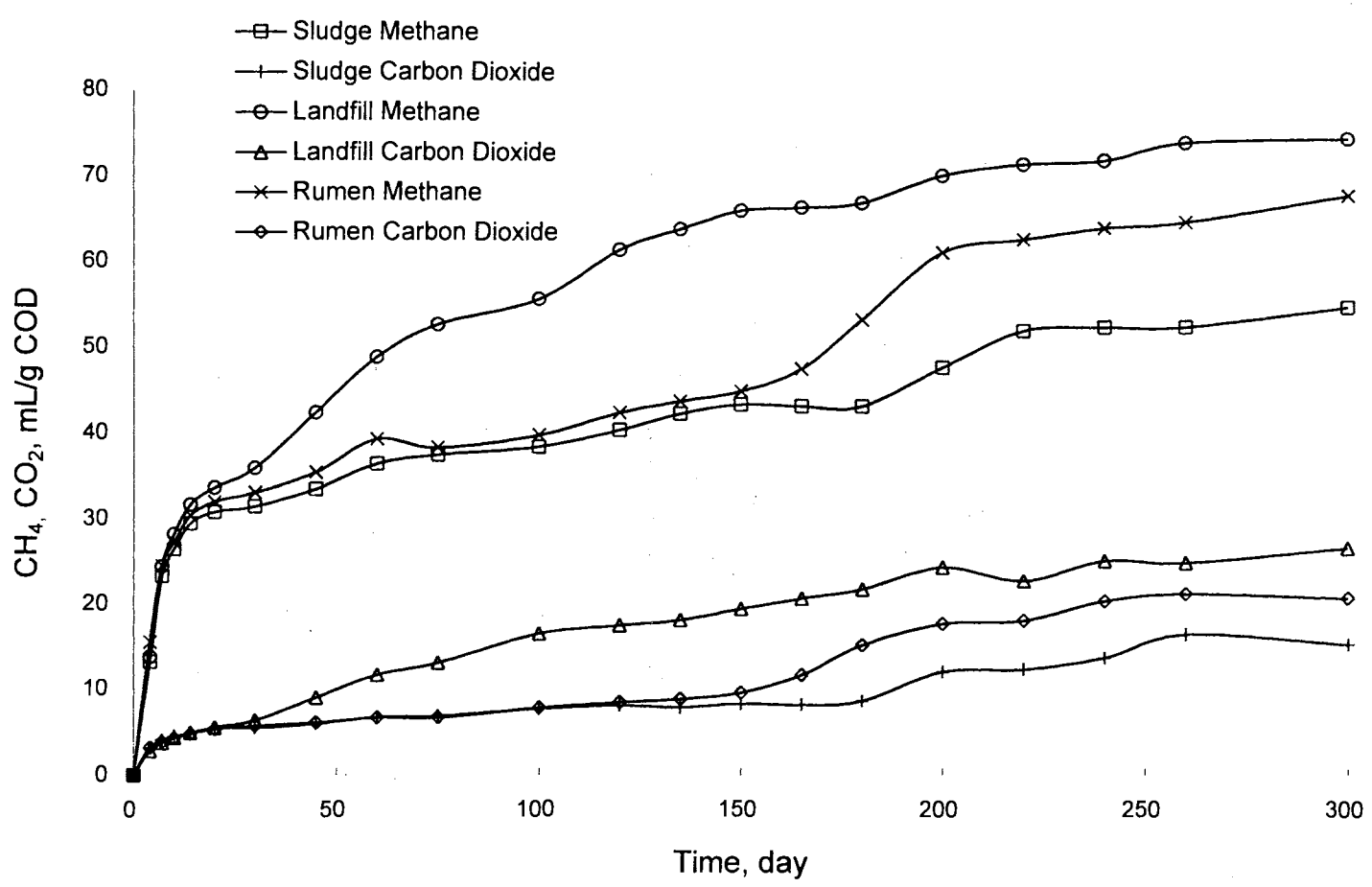

Figure 4.7 Methane and carbon dioxide productions by different controls.

Table 4.15a The Equivalent Methane Yields of Different Controls for Office Paper

Day	Equivalent Methane, mL/g Office Paper								
	Sludge Controls			Landfill Controls			Rumen Controls		
	Substrate	Nutrient	Difference	Substrate	Nutrient	Difference	Substrate	Nutrient	Difference
	111.4	33.83		145.7	46.86		111.1	31.85	
60	104.6	21.76		139.1	58.61		115.7	34.98	
	105.4	20.76		143.6	49.46		116.3	40.69	
Average	107.1	25.43	81.7	142.8	51.64	91.2	115.0	35.83	78.54
	132.1	66.26		212.1	88.26		163.1	90.32	
180	126.5	96.80		193.6	92.57		166.7	114.1	
	121.9	81.53		183.1	80.71		135.0	75.14	
Average	126.8	81.53	45.3	196.3	87.17	109.1	154.9	93.19	61.75
	179.2			230.8			201.9		
220	148.6			204.7			213.0		
	130.8			193.9			132.5		
Average	152.9			209.8			182.5		

Note: Nutrient controls were not determined at day 220.

Table 4.15b The Equivalent Methane Yields of Different Controls for Newsprint

Day	Equivalent Methane, mL/g Newsprint								
	Sludge Controls			Landfill Controls			Rumen Controls		
	Substrate	Nutrient	Difference	Substrate	Nutrient	Difference	Substrate	Nutrient	Difference
	37.92	11.52		49.37	15.88		37.99	10.89	
60	35.62	7.41		47.14	19.86		39.55	11.96	
	35.87	7.07		48.66	16.76		39.75	13.91	
Average	36.47	8.66	27.80	48.39	17.50	30.89	39.33	12.25	26.85
	44.99	22.56		71.87	29.91		55.76	30.88	
180	43.07	32.96		65.59	31.37		56.99	39.02	
	41.51	27.76		62.06	27.35		46.16	25.69	
Average	43.19	27.76	15.43	66.51	29.54	36.97	52.97	31.86	21.11
	71.74	25.02		80.32	40.64		74.27	33.05	
300	46.96	42.54		74.04	38.22		81.16	38.84	
	45.75	37.82		68.01	32.48		47.24	30.43	
Average	54.82	35.13	19.69	74.12	37.11	37.01	67.55	34.11	33.45

According to the data in Tables 4.15a and 4.15b, the methane yields were relatively close among the landfill control triplicates, while for the other two groups of controls, there were again low conversion bottles. By observing the methane data of nutrient control (control without adding nutrient solution), landfill controls still produced the highest amount of methane among the three groups. Mass balance revealed that the yeast extract added to the bottles should produce methane equivalent to 101.6 mL/g office paper COD and 34.6 mL/g newsprint paper COD. The landfill substrate controls produced somewhat more, while the sludge controls produced much less due to two of three of the controls experiencing unusually low conversion. Since the serum bottle test methane data presented were adjusted by the methane produced by the controls, it seems either the low methane yield of the sludge control caused a higher apparent methane yield of the sludge group samples or a high methane yield of the landfill controls caused a lower methane yield of the landfill group samples. A possible explanation of this abnormal behavior of the controls is that they received the same amount of nutrient solution as that of sample bottles so that the relative concentration of nutrients for the cellulosic materials in the controls was much higher than that for paper samples. In other words, the activities of the microorganisms at different substrate and nutrient ratios were inconsistent.

The statistical analysis on methane data of the unprinted newsprint (Table 4.13) indicates there are no significant differences in methane yields caused by the paper size but there are significant differences caused by the inocula. For the same reason as discussed with office paper, these differences were mainly caused by the differences in

methane production of the controls. The statistical analysis results on printed newsprint methane data failed to detect any significant difference in methane production between different paper sizes and among three inocula groups after 150 days incubation. (Table 4.14), although the mean values of methane production by ground paper were much lower than those of the other two paper sizes. Since there were low conversion samples in every different paper size of this group, high standard deviation and variance of the methane yields resulted from this trial.

From the statistical analysis results, there is no sufficient evidence to claim that there are significant differences in methane yields caused by the three different inocula, between unprinted and printed newsprint, nor for the three paper sizes for each type of paper investigated in this experiment in general. However, the consortia of landfill origin do demonstrate some different characteristics in high lignocellulosic materials bioconversion compared to the other two groups.

Figure 4.8 shows the methane and carbon dioxide productions by the three different sizes of office paper. General methane and carbon dioxide productions by unprinted and printed newsprint are shown in Figure 4.9. Methane and carbon dioxide productions of the three different inocula and three different paper sizes of newsprint are shown in Figures 4.10 and 4.11.

The experimental methane and carbon dioxide yields (220 days for office paper and 300 days for newsprint) of the basic serum bottle experiment are listed in Tables 4.16 and 4.17, and the methane conversion efficiencies against the theoretical methane yield per gram of COD under standard conditions are listed in Table 4.18.

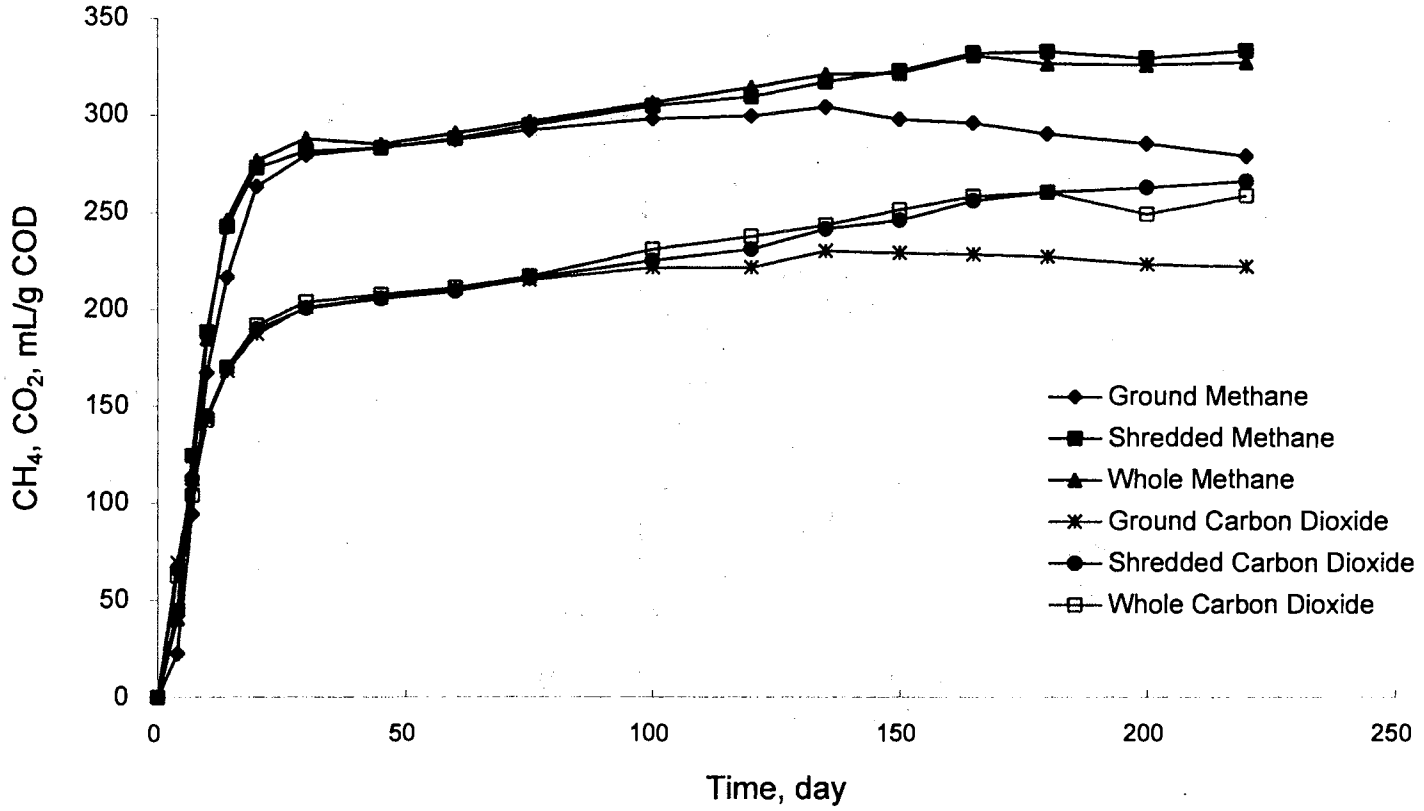

Figure 4.8 Methane and carbon dioxide productions by different size office paper (ground, shredded into strips, and whole pieces).

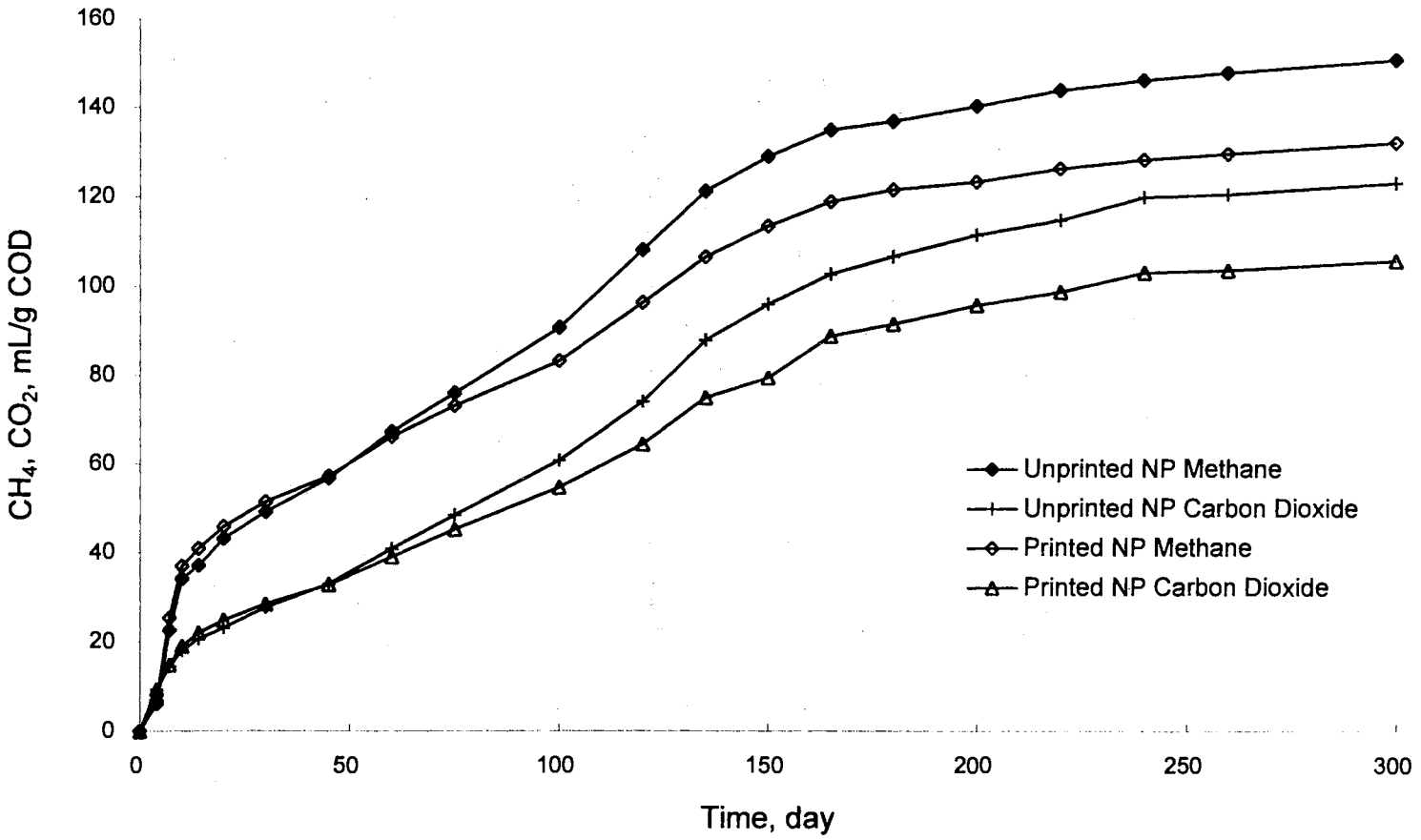

Figure 4.9 Methane and carbon dioxide productions by printed and unprinted newsprint.

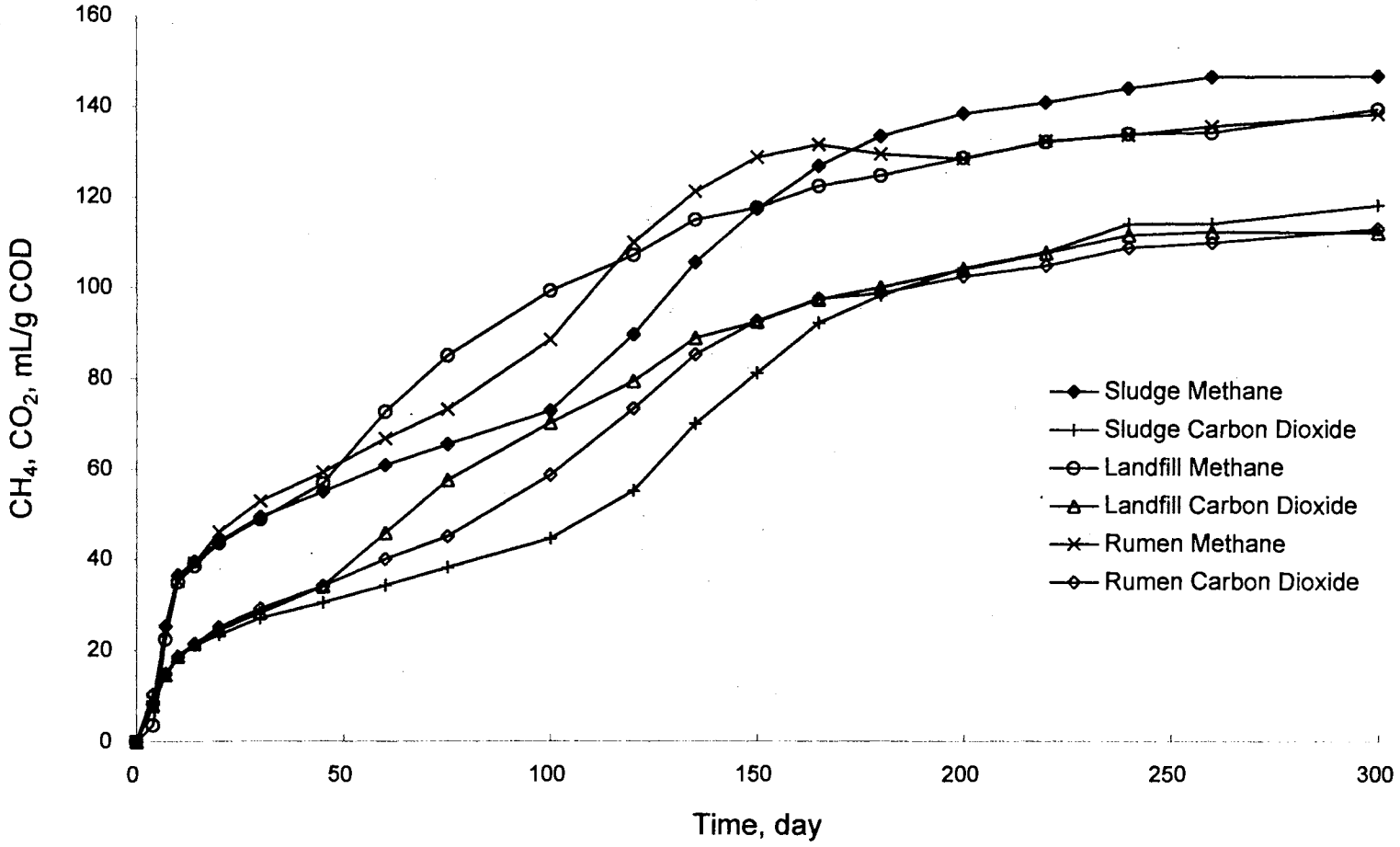

Figure 4.10 Methane and carbon dioxide productions by newsprint with different inocula.

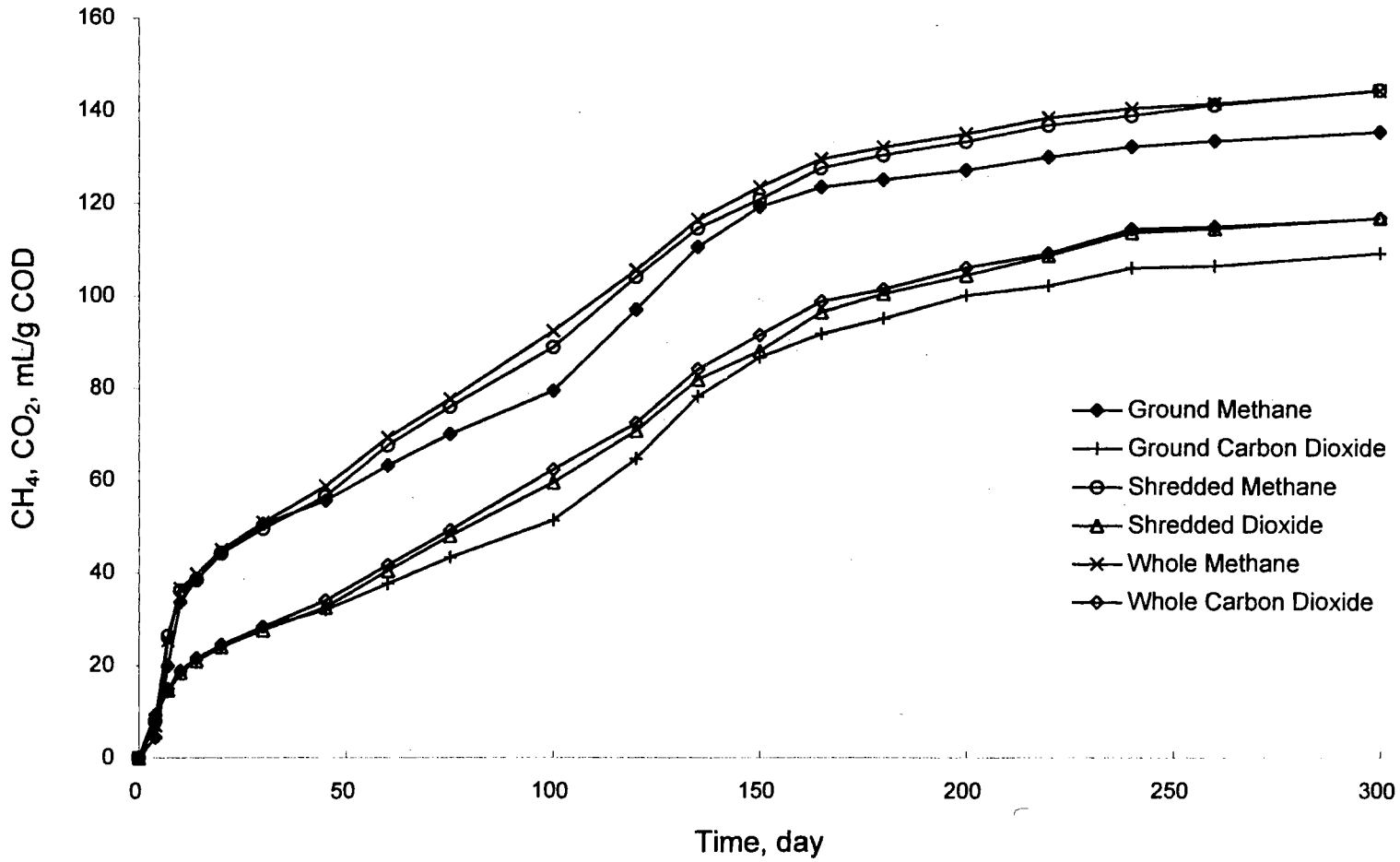

Figure 4.11 Methane and carbon dioxide productions by different size newsprint.

Table 4.16 Estimation of Methane Yields for Different Paper Type, Size, and Previous Use with Three Different Inocula (220 Days for Office Paper, 300 Days for Newsprint)

Sample	Methane Yield, mL/g COD		
	Sludge	Landfill	Rumen
U.G.N.	176.18 (4.70)	139.47 (1.15)	156.36 (1.46)
U.S.N.	154.43 (5.09)	141.11 (5.26)	149.25 (3.28)
U.W.N.	153.62 (10.20)	145.34 (3.20)	146.68 (6.94)
P.G.N.	104.20 (61.34)	134.42 (6.55)	105.67 (42.01)
P.S.N.	162.78 (12.81)	139.50 (2.14)	123.69 (45.89)
P.W.N.	132.71 (57.94)	139.92 (0.52)	151.63 (6.26)
G.O.P.	296.80 (12.64)	257.40 (14.28)	284.70 (22.60)
S.O.P.	348.31 (41.64)	289.45 (53.22)	364.48 (19.84)
W.O.P.	337.88 (47.51)	288.91 (30.64)	357.25 (35.53)

Note: N.: newsprint O.P.: office paper
U.: unprinted P.: printed
G.: ground S.: shredded W.: whole piece
Values in parentheses are standard deviations of the parameter estimates.

Table 4.17 Estimation of Carbon Dioxide Yields for Different Paper Type, Size, and Previous Use with Three Different Inocula (220 Days for Office Paper, 300 Days for Newsprint)

Sample	CO_2 Yield, mL/g COD		
	Sludge	Landfill	Rumen
U.G.N.	145.35 (3.84)	112.41 (3.42)	126.34 (4.56)
U.S.N.	126.11 (6.31)	114.77 (4.03)	123.92 (3.13)
U.W.N.	125.28 (12.87)	117.02 (2.84)	121.28 (7.60)
P.G.N.	78.74 (55.19)	109.38 (8.87)	86.58 (33.51)
P.S.N.	133.12 (6.65)	110.40 (0.09)	96.34 (33.17)
P.W.N.	103.38 (51.67)	111.38 (1.99)	125.69 (3.04)
G.O.P.	236.66 (15.21)	212.75 (4.53)	218.70 (17.23)
S.O.P.	271.11 (34.46)	237.05 (43.91)	292.14 (3.07)
W.O.P.	263.46 (38.78)	231.37 (27.30)	283.05 (34.58)

Note: same as Table 4.16.

Table 4. 18. Methane Conversion Efficiency
(220 Days for Office Paper, 300 Days for Newsprint)

Sample	Percent Conversion to Methane, %			Average
	Sludge	Landfill	Rumen	
U.G.N.	44.60% (1.19%)	35.31% (0.29%)	39.59% (0.37%)	39.83% (4.08%)
U.S.N.	39.10% (1.29%)	35.72% (1.33%)	37.79% (0.83%)	37.54% (1.79%)
U.W.N.	38.89% (2.58%)	36.79% (0.81%)	37.14% (1.76%)	37.61% (1.88%)
Average	40.86% (3.21%)	35.94% (1.03%)	38.17% (1.48%)	38.32% (2.89%)
P.G.N.	26.38% (15.53%)	34.03% (1.66%)	26.75% (10.63%)	29.05% (10.16%)
P.S.N.	41.21% (3.24%)	35.32% (0.54%)	31.31% (11.62%)	35.95% (7.42%)
P.W.N.	33.60% (14.67%)	35.42% (0.13%)	38.39% (1.59%)	35.80% (7.67%)
Average	33.73% (12.57%)	34.92% (1.10%)	32.15% (9.40%)	33.60% (8.80%)
G.O.P.	75.14% (3.20%)	65.16% (3.62%)	72.07% (5.72%)	70.79% (5.80%)
S.O.P.	88.18% (10.5%)	73.28% (13.5%)	92.27% (5.02%)	84.58% (12.43%)
W.O.P.	85.54% (12.0%)	73.14% (7.76%)	90.44% (9.00%)	83.04% (11.45%)
Average	82.95% (10.1%)	70.53% (8.94%)	84.93% (11.3%)	79.47% (11.73%)

Note: same as Table 4.16.

Table 4.18 shows that the conversion efficiencies of office paper are as high as 80% of available COD, and the reaction was virtually finished during the first 20 days, although there was some methane production through 165 days.

The 300 days duration for newsprint digestion is much longer than that tested in all the other available studies. Even so, the ultimate methane potential had not been reached, as indicated by continuing slow biogas production. The 133 days conversion efficiency of newspaper obtained by Tong et al. (1990) was 21% (2%) when anaerobic sludge was used as inoculum. Table 4.18 shows that the conversion efficiencies of newsprint with all three inocula at the 300th day in this experiment (32.15 - 40.86%) are much higher than that of Tong et al. (1990) for 133 days. The biochemical methane potentials (BMPs) of newsprint obtained by Owens and Chynoweth (1993) are also around 20%, and the ultimate methane yields, $Y_{\mu}s$, were reported as 0.084 and 0.100 mL/gVS, about 21% and 22.8% against the maximum methane potential of 438 mL/gVS (Tong et al., 1990). Since the methane potentials predicted by other researchers are much lower than that obtained in this study, it is obvious that the results obtained from those BMP tests on newsprint were far from complete or ultimate. Beyond our 300th day's incubation, the conversion efficiency for unprinted newsprint is between 35 - 41%, which approaches complete cellulose conversion, if methane was produced only from conversion of cellulose. This suggests that as long as the other conditions are satisfied, complete cellulose conversion may be possible by properly acclimated anaerobic consortia, although the process is a rather slow one. Application of first order models to these results is discussed in Appendix C.

Printing Ink Biogas Conversion

The ink made with soy bean oil is readily bioconvertable, and no toxic effect on the microbial bioconversion was observed. This agrees with the results showing no significant difference in terms of methane yields between printed and unprinted newsprint paper. The methane and carbon dioxide yields of ink itself are shown in Figure 4.12 and Table 4.19.

Table 4.19 Methane Yields and Conversion Efficiencies of Ink

Parameter	Inocula		
	Sludge	Landfill	Rumen
Methane Yields, mL/g Ink COD	209.65 (28.91)	200.61 (15.14)	199.76 (42.48)
Conversion Efficiencies, %	53.08 (7.32)	50.79 (3.83)	50.57 (10.75)

Figure 4.12 shows that ink bioconversion displayed a short lag period, then proceeded in much the same way as the cellulosic materials. Table 4.19 shows that the conversion efficiencies for available COD of ink were only around 50%, which suggests that about 50% of oily material could not be accessed by the microorganisms. Also, the ink is not easily dispersed evenly in the water phase, adhering to glass and forming a layer on surfaces, which would reduce bioavailability. No hints of toxic effects on the microorganisms were found, and the low conversion efficiencies were most probably caused by physical blockage of the filling materials of the ink. This is consistent with the phenomenon observed from printed newspaper bioconversion.

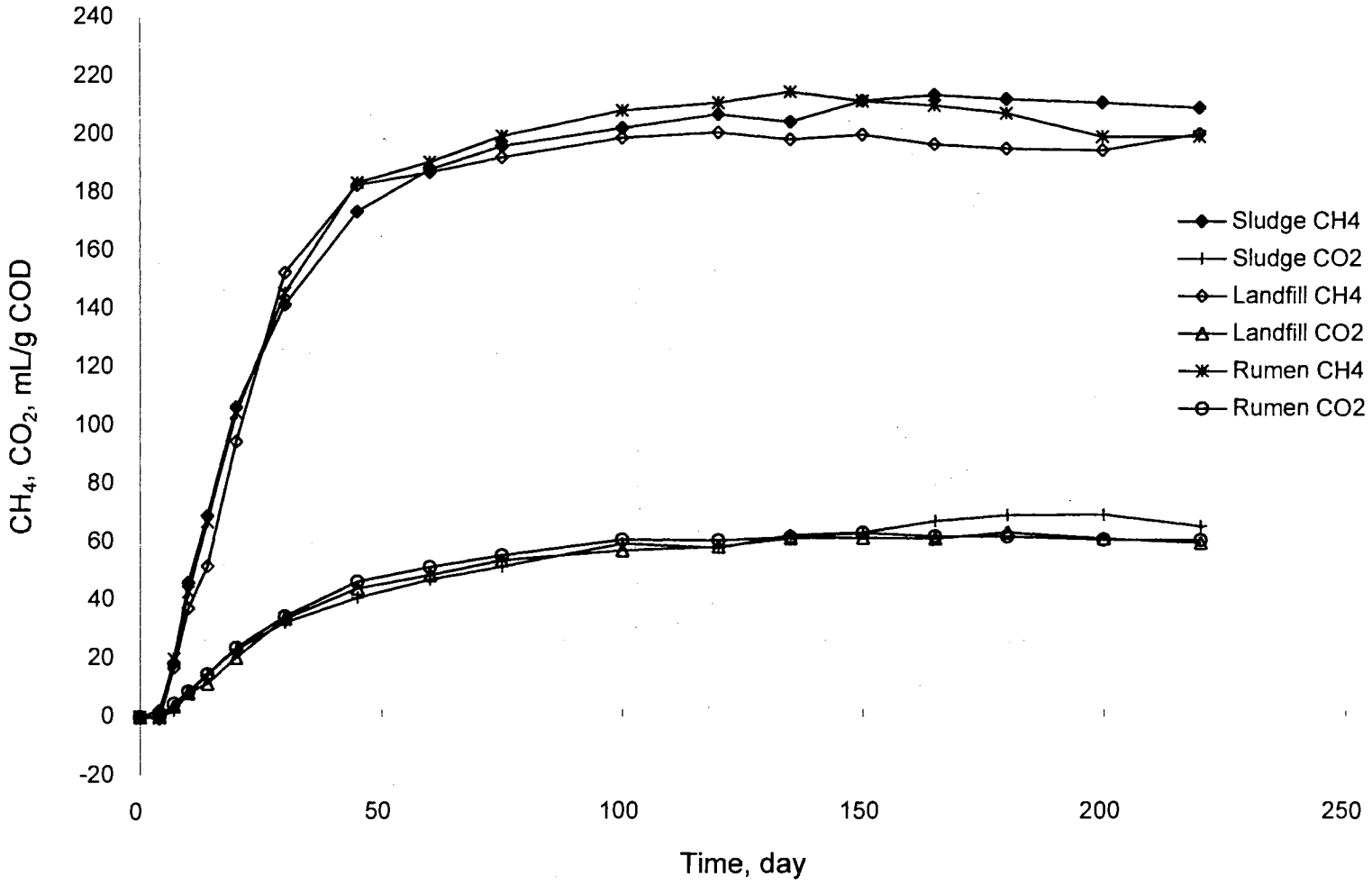

Figure 4.12 Methane and carbon dioxide productions from printing ink.

Chemical Pretreatment and Bioconversion

Alkali Pretreatment and Bioconversion Test

Alkali Pretreatment Four different concentrations of NaOH solution were used to soak the unprinted newsprint. Three different temperatures and three different soaking durations were also performed at NaOH concentrations of 5% and 10%. For comparison, baseline conditions for the soaking were set at room temperature and 1 day duration. To quantify the amount of solids solubilized by the soaking process, samples were homogenized, and filtered after alkali treatments. The TS and VS of the filtrates were determined. The results are listed in Table 4.20. Since the remaining NaOH would also be detected as TS, its amount in the filtrates was quantified by titrating with standardized H_2SO_4, and then subtracted from the TS.

Table 4.20 shows that the TS, VS and VS/TS ratio of the filtrates increased for all different treatments. The TS and VS of the filtrates generally increase with the NaOH concentrations, the soaking temperatures, and the soaking durations. The higher the NaOH concentration and the higher the temperature, the higher the solubilization. However, the dissolved solids of 6 day and 12 day soaking times were almost the same, which indicates that soaking for longer than 6 days would not improve solubilization of the lignin from newsprint. Although the dissolved solids were increased with the NaOH concentrations, it is interesting to note that the volatile to total solids ratios of the filtrates for different concentrations are in the same range, and likewise for two different elevated temperatures, and for two different soaking durations. Both soaking at elevated temperatures and for longer durations significantly increased filtrate VS/TS ratios.

Table 4.20 Dissolved Solids in Alkali-Treated Sample Filtrates

Sample	(1) Apparent TS, %	(2) VS, %	(3) NaOH, %	(4) = (1) - (3) Real TS, %	(5) = (2) ÷(4) VS/Real TS, %
Untreated	0.024	0.005	0.000	0.024	21.57
5% NaOH	0.112	0.033	0.009	0.103	32.37
10% NaOH	0.177	0.042	0.064	0.113	36.87
15% NaOH	0.246	0.042	0.112	0.134	31.62
20% NaOH	0.319	0.053	0.161	0.158	33.40
75°C, 10% NaOH	0.249	0.091	0.037	0.212	43.00
105°C, 10% NaOH	0.285	0.119	0.023	0.262	45.38
6 Day, 10% NaOH	0.219	0.085	0.061	0.158	53.53
12 Day, 10% NaOH	0.217	0.082	0.063	0.154	53.00

Another interesting observation from this experiment is that when the newsprint was soaked with 5% NaOH, almost all the NaOH added was neutralized by the newsprint or its solubilized components, which made the NaOH concentration in the sample filtrate as low as 0.009%. The remaining NaOH concentrations of the filtrates, at elevated temperature, were much lower than those at room temperature, while in prolonged soaking, the NaOH concentrations were the same as those of one day soaking time. The solubilization process significantly absorbed alkali from solution, and the higher the temperature, the greater the absorption. This alkali absorption may also explain why the newsprint bioconversion usually ended with relatively low pH (Table 4.2 and most of the serum bottle tests) although the alkalinity was still high.

Before the bioconversion test, the soaked samples were homogenized with a Waring blender. Then they were neutralized with acids. Carbon dioxide was used to neutralize a majority of the samples, while H_2SO_4 and HCl were used for comparing the effects of neutralizing acids. When carbon dioxide was used as neutralizing acid, the final pH of the media could be controlled at different stages. If the neutralizing process is carried out for a sufficient length of time, the pH of the pulp can be lowered to below 6.0.

Treated Sample Bioconversion The treated sample was then used as substrate for bioconversion without going through any waste removal steps. The same nutrient solution that was used in the previous serum bottle test was used in all the alkali treated samples, except that no Na_2S was added as reducing agent in Test 1. Since generally there were no significant differences among the three inocula in production of methane, mixed inocula (1/3 by volume of each inoculum) were used as seed to avoid the

difference caused by the inocula control. In Test 1, active inocula were used while in Test 2, somewhat aged inocula were used because the seed reactors had already been stopped. Unprinted newsprint soaked with water for one day was used as substrate control, $NaHCO_3$ was added to supply alkalinity for the substrate control, and for those samples neutralized by H_2SO_4 and HCl. Both Test 1 and Test 2 on the alkali treated samples was monitored for 200 days. The results are shown in Figures 4.13 through 4.16 and Table 4. 21.

In Test 1, all the different conditions were tested, however, since it was uncertain what would happen to the bioconversion of the treated samples when they were treated at the high temperatures, soaking at 70°C was carried out for 4 hours while 105°C samples were only soaked for half an hour. Test 2 increased soaking time at elevated temperatures to 1 day. At the same time, treatments of different concentrations and durations were repeated to check the reproducibility between two tests. The nutrients added in Test 2 included the Na_2S reductant addition.

The statistical analysis procedure AOV was used again, and strong evidence supports that there are significant differences in methane yield for most treated samples compared with untreated samples. To further distinguish the differences in methane yields caused by the different treatments, the Fisher's least significant difference (LSD) and Tukey procedures were also performed (Appendix A). The results are listed in Table 4.22.

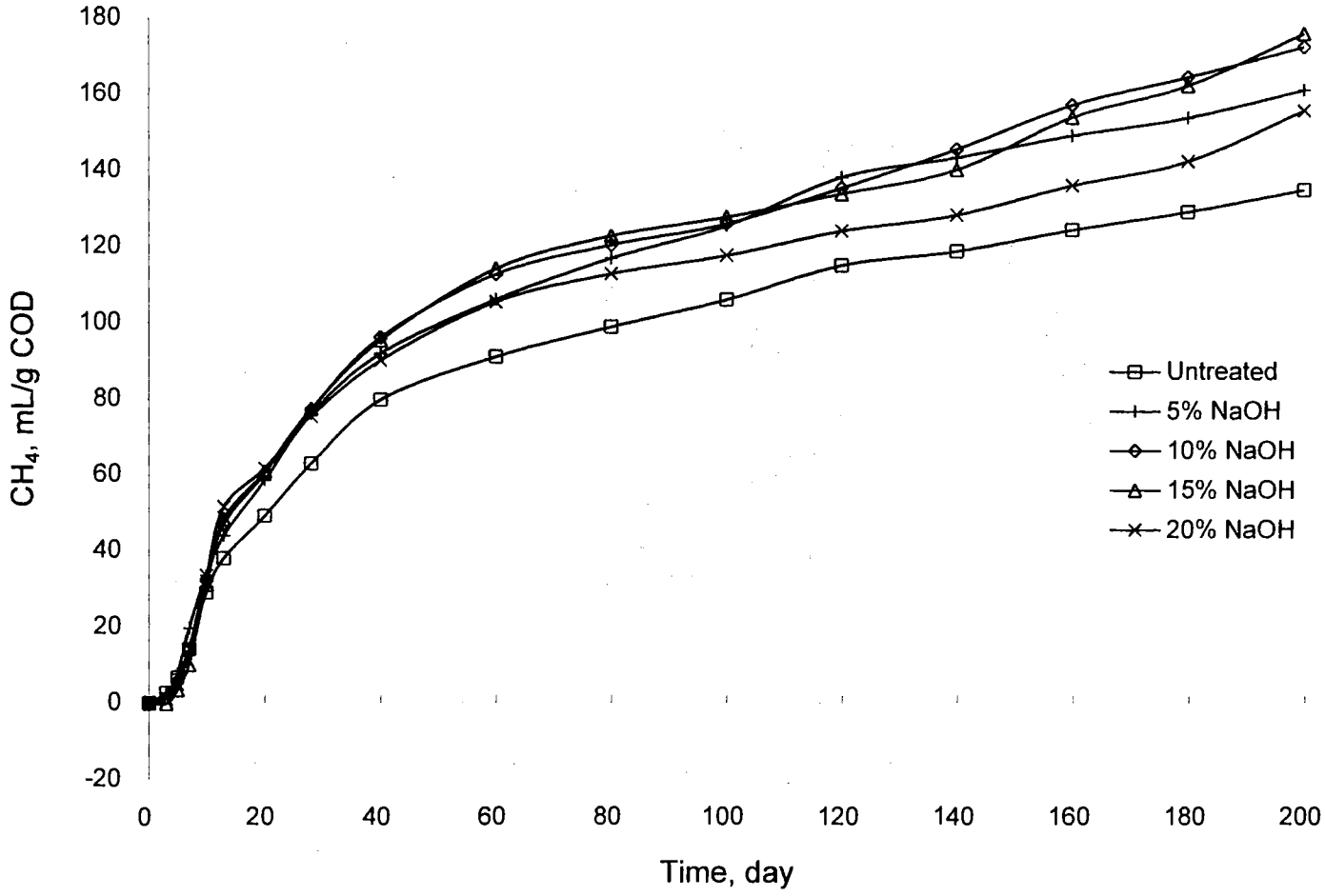

Figure 4.13 Methane production by alkali treated samples (with different NaOH concentrations).

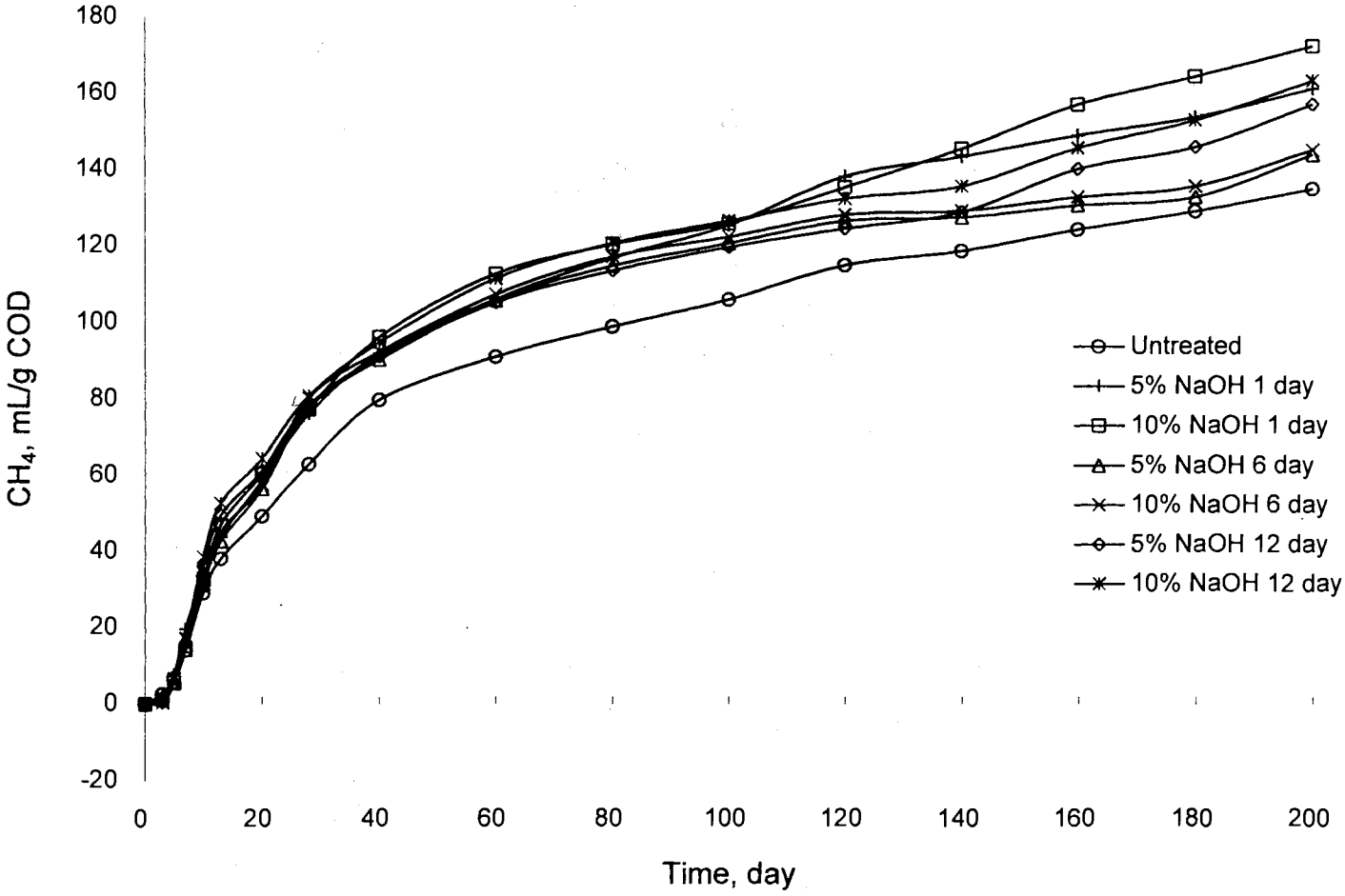

Figure 4.14 Methane production by alkali treated samples (with different soaking durations).

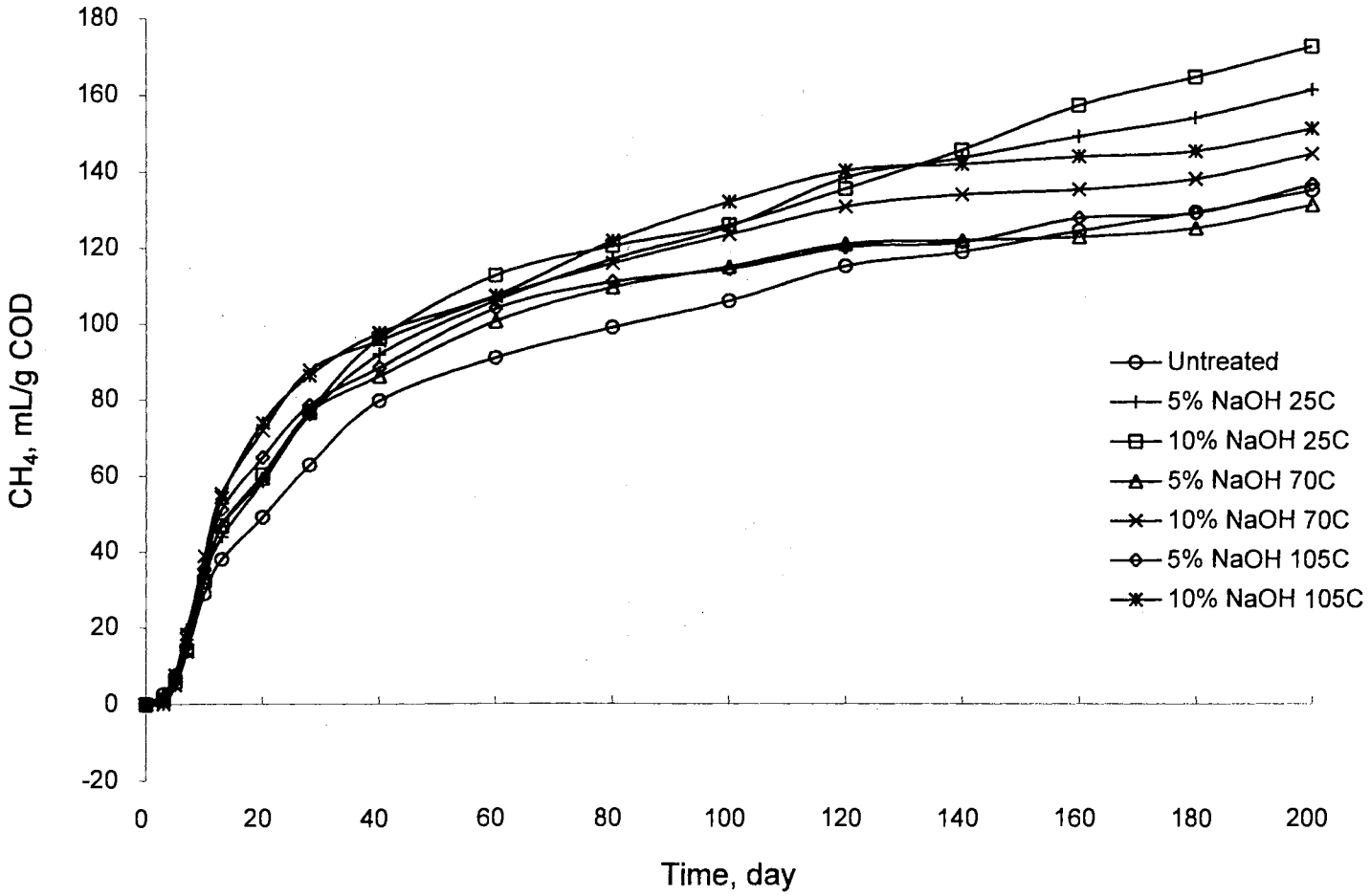

Figure 4.15 Methane production by alkali treated samples (with different soaking temperatures).

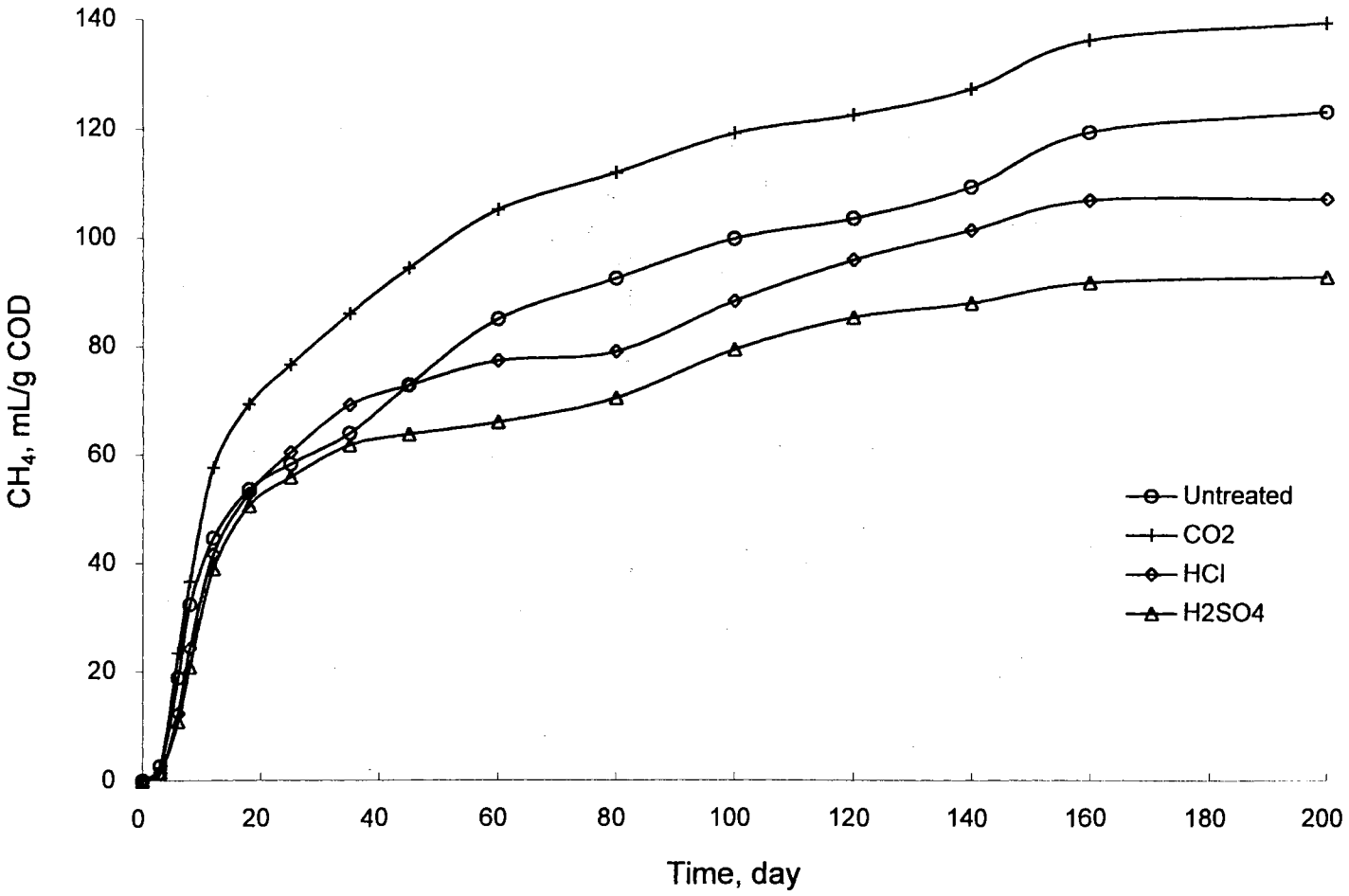

Figure 4.16 Methane production by alkali treated samples (with different neutralizing reagents).

Table 4.21 Estimation of Methane Yields for Alkali Pretreatment

Different Concentrations(mL/g COD)		Different Soaking Time (mL/g COD)			Different Soaking Temperature (mL/g COD)			Different Neutralizing Reagent (mL/g COD)	
Conc.		Time	5% NaOH	10% NaOH	Temp.	5% NaOH	10% NaOH	Acid	
TEST 1									
0% NaOH	121.9 (5.3)								
5% NaOH	140.6 (5.2)	1 Day	140.6 (5.2)		25 °C	140.6 (5.2)		CO_2	140.60 (5.19)
10% NaOH	150.0 (2.4)	6 Days	142.8 (2.2)		70 °C*	138.2 (3.9)		H_2SO_4	93.02 (27.57)
15% NaOH	144.7 (12.7)	12 Days	149.2 (1.9)		105 °C**	126.3 (12.8)		HCl	107.37 (20.43)
20% NaOH	154.7 (15.7)								
TEST 2									
0% NaOH	135.3 (7.2)								
5% NaOH	161.6 (6.5)	1 Day	161.6 (6.5)	172.9 (3.3)	25 °C	161.6 (6.5)	172.9 (3.3)		
10% NaOH	172.9 (3.3)	6 Days	144.3 (9.4)	145.5 (4.2)	70 °C	131.5 (4.9)	144.8 (6.6)		
15% NaOH	176.4 (12.7)	12 Days	157.5 (18.3)	163.7(19.9)	105 °C	136.8 (17.1)	151.4 (3.1)		
20% NaOH	156.1 (21.2)								

Note: values in parenthesis are standard deviations of the parameter estimates.

* 4 hour treatment; ** half an hour treatment.

Table 4.22 Statistical Analysis Results of Alkali Treated Sample Bioconversion

Different Concentrations (mL/g COD)			Different Soaking Time (mL/g COD)					Different Soaking Temperature (mL/g COD)					Different Neutralizing Reagent (mL/g COD)	
Conc			Time	5% NaOH		10% NaOH		Temp	5% NaOH		10% NaOH		Acid	
TEST 1	LSD	Tukey		LSD	Tukey				AOV					AOV
0% NaOH	B	B												
5% NaOH	A	B	1 Day	B	A			25 °C	N				CO_2	N
10% NaOH	A	A	6 Days	B A	A			70 °C*	N				H_2SO_4	N
15% NaOH	A	B A	12 Days	A	A			105 °C**	N				HCl	N
20% NaOH	A	A												
TEST 2	LSD	Tukey		LSD	Tukey	LSD	Tukey		LSD	Tukey	LSD	Tukey		
0% NaOH	B	B												
5% NaOH	A	B A	1 Day	B A	B A	A	A	25 °C	B A	B A	A	A		
10% NaOH	A	A	6 Days	B C	B A	B C	B A	70 °C	D	C	D C	B C		
15% NaOH	A	A	12 Days	B A	B A	B A	B A	105 °C	D	C	B C	ABC		
20% NaOH	B A	B A												

Note: In LSD and Tukey analyses, the means of treatments with same letter are not significantly different.
In AOV test, the means of all treatments with letter N are not significantly different.
* 4 hour treatment; ** half an hour treatment.

The LSD analysis results in Table 4.22 indicate that in 200 days incubation, all four concentrations of alkali treatment significantly improved methane production, except that no significant difference between the untreated samples and those treated with 20% NaOH was detected in Test 2. In both tests, no significant differences in methane production were found among the samples treated with four different NaOH concentrations. Therefore, a NaOH concentration higher than 10% is obviously unnecessary.

Soaking at prolonged durations caused slight differences in both tests. In Test 1, the samples soaked for 12 days produced more methane than those soaked for 1 days, however, in Test 2, the samples soaked for 6 days produced less methane than those soaked for 1 and 12 days in both 5% and 10% NaOH concentrations. There were no significant differences in methane production detected between the samples treated by 5% and 10% NaOH concentrations in prolonged soaking durations.

In Test 1, no differences in methane production were detected when the samples were treated at elevated temperatures for shorter durations; while in Test 2, significantly less methane production was caused by treating the samples at elevated temperatures in both 5% and 10% NaOH concentrations for 1 day. Notably the samples soaked with 5% NaOH produced significantly less methane than those treated with 10% NaOH and produced no more methane than the untreated samples.

The Tukey analysis gave slightly different results since it is generally reluctant to declare differences as discussed in Chapter 3. The basic conclusions drawn from both

analyses are the same. The slight difference between Test 1 and Test 2 may be attributed to the different ages of the inocula used and whether there was Na_2S reductant addition.

Although prolonged soaking durations solubilized more solid materials than the 1 day soaking, the improvement on 200 days methane conversion was very limited. And although the elevated temperature treatments improved the methane conversion in the early stages, it did not improve methane conversion in the long run. Neutralizing with strong acid after soaking with 5% NaOH did not show improvement on bioconversion in 200 days incubation, and the H_2SO_4 neutralized samples gave the lowest average methane conversion extent, even lower than the untreated samples. This result was expected because H_2SO_4 is an alternative electron acceptor to CO_2 under reduced anaerobic conditions and would be preferred by the sulfur-reducing organisms. Neutralizing with HCl also gave a low methane conversion extent; the reason is still unknown. Therefore, the most important factor in alkali treatment is the NaOH concentration. Soaking at prolonged durations or at elevated temperatures is unnecessary. And, carbon dioxide is the preferred neutralizing reagent. This is of great advantage because the CO_2 produced by the process itself could satisfy the requirement of neutralization, and the biogas could be purified at the same time.

The increases of the methane production by alkali treatment suggest that solubilizing part of the lignin and/or hemicellulose improves newsprint bioconversion because, when part of the lignin was solubilized, more cellulose would be exposed or accessible to the cellulose hydrolysis enzymes. However, higher solubilization did not guarantee higher conversion to methane. At NaOH concentration of 20% and at elevated

temperatures, the dissolved solids were higher than those at room temperature with a concentration of 10% NaOH; however the methane production in the former case was lower than that of the latter. It seems there is a threshold in the beneficial extent of lignin solubilization. When the solubilized lignin components were lower than the threshold, the methane conversion would be significantly increased without inhibition, while at higher solubilization than the threshold, inhibition became obvious. Some solubilization products of hemicellulose, or even of lignin, might be bioconvertable to methane, so at the earlier stage, methane conversion of the treated samples was still higher than that of untreated samples due to the higher accessible substrate concentrations. However, in the long run, inhibition would play a role. This result seems contrary to the result reported by Stinson and Ham (1995) that addition of lignin did not affect the rate of cellulose decomposition. However, in their experiments, physically milled wood lignin was added separately, whereas here the lignin components in this experiment were solubilized from complex material via alkali treatment at different concentrations and temperatures. Therefore, different conclusions were quite possible.

Comparing the methane yields of alkali pretreatment tests with the basic serum bottle test, the 200 day methane yields (Table 4.21) of untreated samples were a little lower than those of unprinted newsprint in basic serum bottles tested over 300 days (Table 4.13). Most alkali treated samples produced comparable methane to those basic serum bottle tests in 300 days, while those treated with 10% NaOH at room temperatures produced a little more. This indicates that the reaction rates of the alkali-pretreated samples were significantly increased.

Controlled Neutralization by Carbon Dioxide Since the alkali-treated sample can be neutralized to different extents, the effect of different neutralization extent on the earlier stage of bioconversion was studied by conducting a parallel serum bottle test for 30 days. The newsprint was treated with 5% NaOH at 105°C for 24 hours, and after homogenization, part of the sample was neutralized with CO_2 to pH 6.9. The rest was charged with CO_2 until fully saturated, at which point pH dropped to 5.9. The remainder of the experiment was performed in exactly the same way on the 2 samples, and the same as other serum bottle tests. The result is shown in Figure 4. 17. Statistical analysis found there was no significant difference in methane production between the two conditions. The only difference is that the CO_2-saturated treatment released more CO_2 than the CO_2 unsaturated treatment. This suggests that in neutralized alkali-treated samples, the extent of CO_2 charging is not critical for the bioconversion.

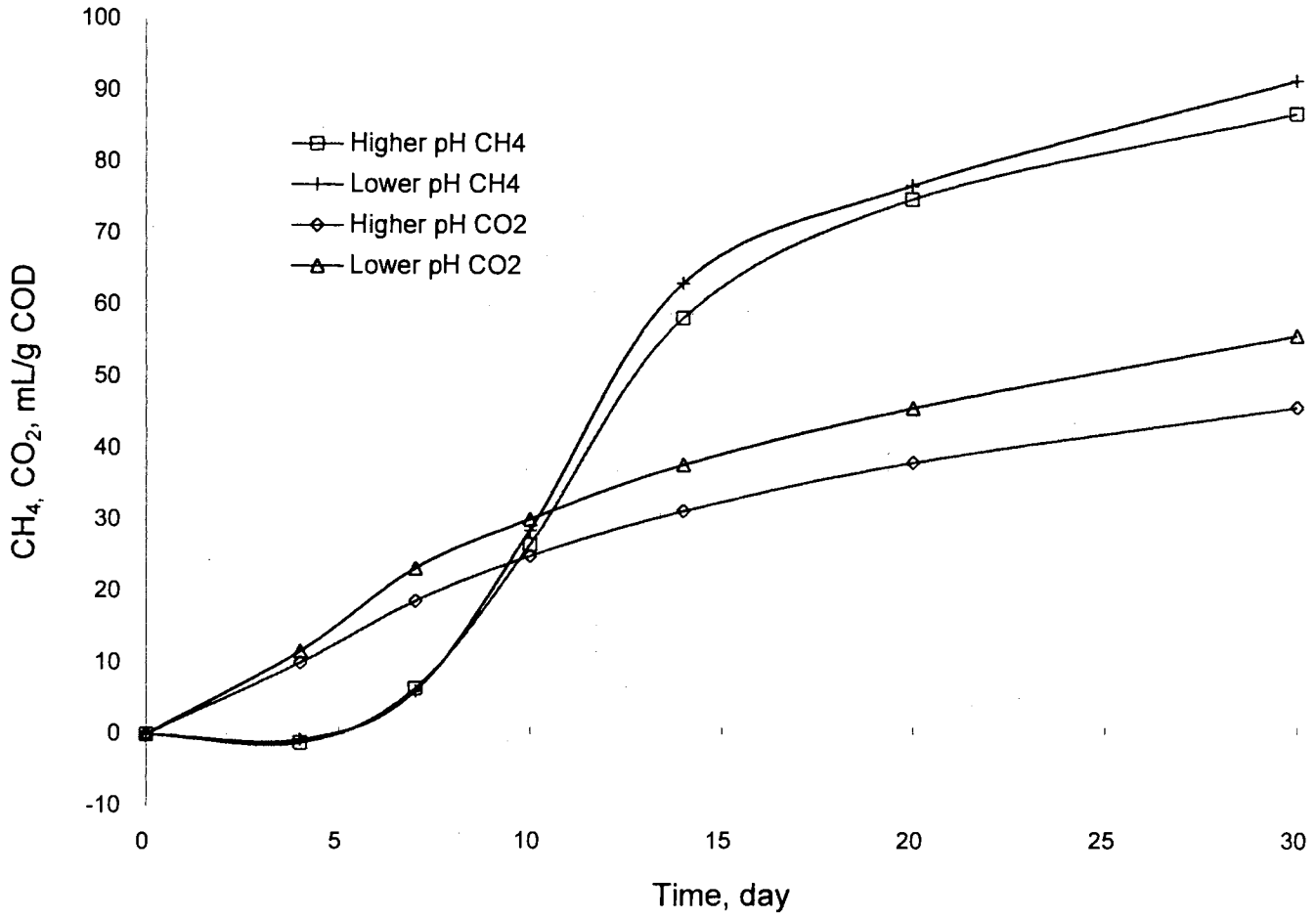

Figure 4.17 Bioconversion of contolled neutralization with carbon dioxide.

Acid Pretreatment and Bioconversion Test

Acid Pretreatment Acetic-nitric acid reagents are used to remove lignin from lignocellulosic material for the cellulose analysis (Updegraff, 1969). The concentration of acetic acid used is as high as 80%, while the concentration of nitric acid is 7.5%. Obviously such a high acid concentration is not economically practical for lignocellulosic material pretreatment on a large scale. In order to find if acetic acid alone can solubilize lignin and the relationship between acetic acid concentration and lignin removal, newsprint paper was treated with an acetic acid solution in a series of different concentrations. The weight loss of the newsprint was determined to reflect the lignin removal extent. The results are listed in Table 4.23.

Table 4.23 Weight Loss with Acetic Acid Pretreatment

Acetic Acid Concentration, (% v/v)	Weight Loss, (%)
0	6.04
20	6.16
40	7.16
60	7.15
80	7.19

The result in Table 4.23 clearly shows that acetic acid itself can not solubilize the lignin even at a concentration as high as 80%. So different concentrations of acetic-nitric

acid solutions were used to treat the newsprint as the next step. The results are listed in Table 4.24.

Table 4.24 Weight Loss with Acetic-Nitric Acid Pretreatment

Acids Concentration, (% v/v)		Weight Loss, (%)
Acetic	Nitric	
0	0	5.18
14.5	1.3	25.61
29	2.5	37.19
44	3.9	43.73
58	5.1	46.52

Table 4.24 shows when a low concentration of nitric acid was added, the weight loss of the newsprint was very significant even at relatively low acetic acid concentrations. According to Table 4.24, in order to achieve 40% weight loss (removing most of the lignin and some hemicellulose), the acetic acid concentration should be at about 35% in the presence of nitric acid. The nitric acid may function as a catalyst or just as an acidifier for the solubilization of the lignin, since nitric acid is a strong, oxidative acid, if it could be replaced by a non-oxidative, more economical acid like hydrochloric acid the practicality of acid pretreatment would be enhanced. To find the optimum nitric acid concentration and to test whether it can be replaced by hydrochloric acid, the

following test was conducted at a constant acetic acid concentration with nitric or hydrochloric acid at three different levels. The results are shown in Table 4.25.

Table 4.25. Weight Loss with Alternative Acids Pretreatment

Acids Concentration, (% v/v)			Weight Loss, (%)
Acetic	Nitric	Hydrochloric	
35	3	0	43.38
35	2	0	39.70
35	1	0	30.47
35	0	3	28.12
35	0	2	26.24
35	0	1	23.35

The results listed in Table 4.25 clearly indicate that nitric acid significantly increases lignin solubilization, compared with HCl. This suggests that the nitric acid most probably acts as a catalyst instead of an acidifier because at the same strong acid concentrations the weight loss of the samples is significantly different. At a 2% nitric acid level, the weight loss of sample was about 40%, roughly the percentage of the lignin component of the newsprint.

Although it is obvious that nitric acid cannot be totally replaced by the hydrochloric acid, the next experiment was conducted to verify whether partial

substitution of HCl would be feasible. The acetic acid concentration was still fixed at 35%, while the total concentration of the strong acids was 2% but the ratio of nitric to hydrochloric acid varied. The results are listed in Table 4.26.

Table 4.26 Weight Loss with Mixed Acids Pretreatment

Acids Concentration, volume (% v/v)			Weight Loss, (%)
Acetic	Nitric	Hydrochloric	
35	2	0	39.68
35	1	1	38.28
35	0.5	1.5	36.58
35	0.25	1.75	35.48
35	0.125	1.875	30.78
35	0	2	26.07

It is interesting to find that when the nitric concentration was as low as 0.125%, with a total strong acid concentration of 2%, it still caused a higher weight loss than using 3% hydrochloric acid only (Table 4.25). When 50% of nitric acid was replaced by hydrochloric acid, the weight loss was only 1.4% lower. Increasing the incubation time might produce a higher weight loss, based on previous experience with the elevated temperature trials.

Cellulose and AIL in the Treated Newsprint The cellulose and AIL components in the acid pretreated samples were determined according to the methods described in Chapter III. The sample to be analyzed and also to be used in the following bioconversion test was treated with 35% acetic acid with 2% nitric acid. The residue of the pretreatment was rinsed in a No. 40 sieve with tap water until neutral (the rinse water pH was 7.0), then homogenized with the Waring blender. The results of this determination are listed in Table 4.27. The TS and VS were also determined to quantify the cellulose and AIL components as % of VS.

Table 4.27 Cellulose and AIL in the Acid Pretreated Sample

Parameter	Result
Cellulose, %VS	83.41 (5.80)
AIL, %VS	9.97 (0.34)
Cellulose to AIL Ratio	8.37

Note: values in parenthesis are standard deviations of parameter estimates.

Comparing the cellulose and AIL in Table 4.27 with untreated samples in Table 4.8, more than 80% of the AIL was removed by the acid treatment while the cellulose percentage increased from 49.8 to 83.4 %VS. Comparing the cellulose and AIL contents with those of office paper in Table 4.8, we can find that both cellulose and AIL concentrations in the treated newsprint sample are higher than those of office paper. This suggests that under the conditions adopted for the above treatment, lignin removal is not

complete and a certain part of the weight loss is due to components in the newsprint other than cellulose and AIL, such as hemicelluloses.

Treated Sample Bioconversion The pretreated newsprint sample was used as substrate for anaerobic conversion. The experimental setting was the same as the former serum bottle test, and the inocula used were the same as the alkali pretreated sample bioconversion Test 2. The bioconversion results and a comparison of the acid pretreated sample with untreated newsprint and office paper are shown in Table 4. 28 and Figure 4.18.

Table 4.28 Comparison of Acid Treated Newsprint Sample with Untreated Newsprint and Office Paper

Parameter		Untreated	Acid Pretreated	Office Paper
Cellulose, %VS		49.8 (1.7)	83.4 (5.8)	82.4 (1.4)
AIL, %VS		30.0 (0.5)	9.97 (0.34)	3.6 (0.5)
Cellulose/Lignin Ratio		1.64	8.37	22.9
Methane, mL/gVS	(60 day)	97.03 (12.04)	271.0 (10.4)	364.4 (26.6)
Carbon Dioxide, mL/gVS	(45 day)	47.49 (3.54)	209.3 (6.1)	260.4 (10.3)

Note: 60 day methane and 45 day carbon dioxide data (no CO_2 data after 45 days) are compared.

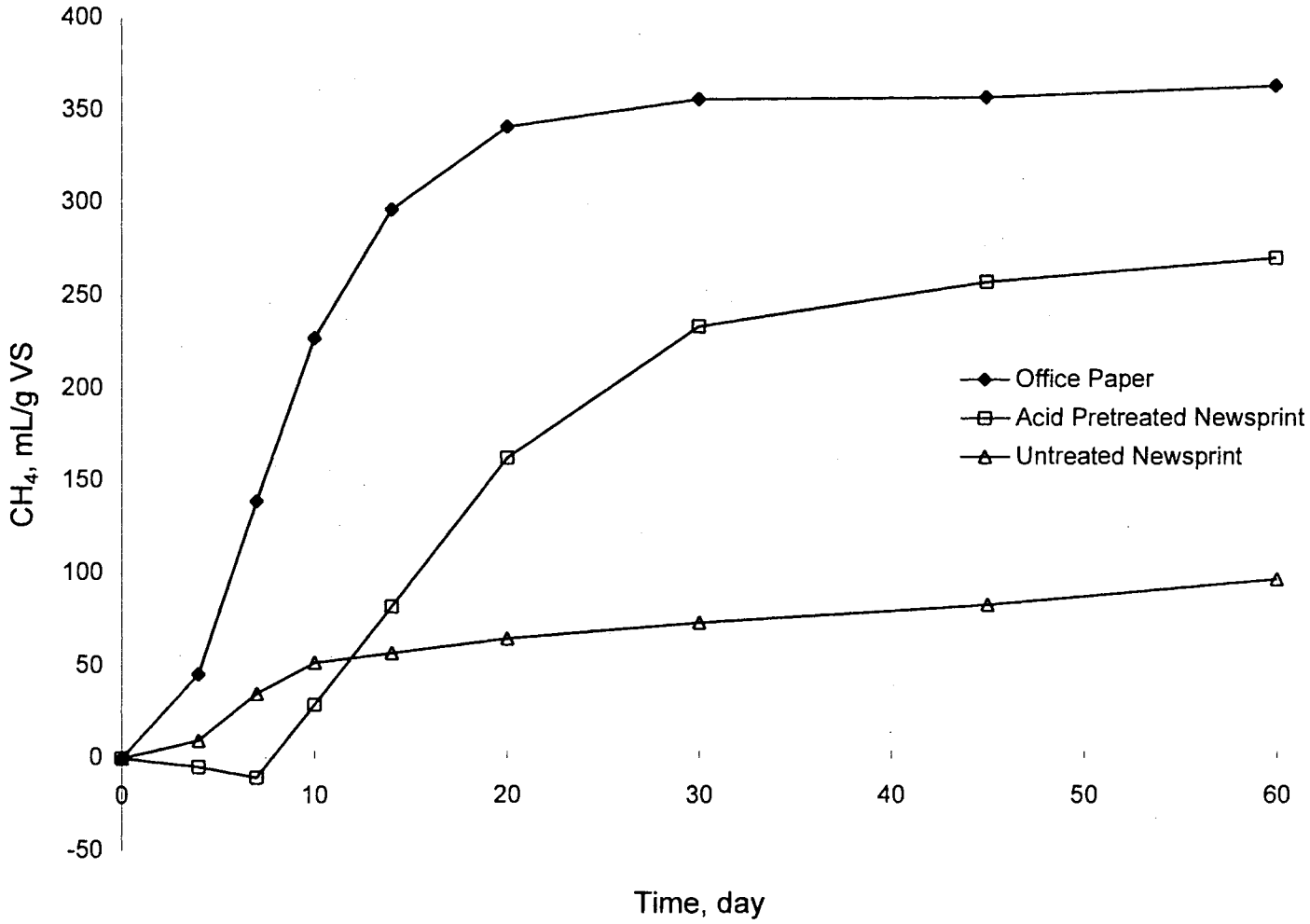

Figure 4.18 Comparison methane yield of acid pretreated newsprint with untreated newsprint and office paper.

Table 4.28 and Figure 4.18 clearly show acid pretreatment significantly improved the bioconversion of the newsprint. The bioconvertability of the treated sample was increased by close to 3 times on a volatile solids basis during the 60 days incubation. Table 4.28 also indicates the importance of the relative cellulose and lignin contents or cellulose to lignin ratio on the bioconversion. Even though the cellulose content of the acid pretreated sample is in the same range as office paper, because of its higher lignin content, the conversion rate and extent are much lower.

Possible Application of Acid Pretreatment Experimental results indicate that acetic-nitric acid can effectively remove significant amounts of lignin from newsprint with a much lower acid concentration than previously known. However, the acid concentration needed for achieving substantial lignin removal is still too high from a practical or economical point of view. It could not be put in practical application unless the acid can be utilized repeatedly and the solubilized lignin fraction could be separated effectively while acetic acid loss could be avoided during the separation.

When paper is treated, it absorbs a large volume of solution, so the amount of paper that can be treated with a certain volume of solution in each batch is limited. After the treatment results in partial solubilization, the volume of solids is greatly reduced. Thus the reuse of treatment liquid is quite possible after the remaining solids are removed. A test on whether the treatment liquid could be reused was conducted by using centrifugation to separate the treatment liquid from the solids and reusing it for the next treatment. It was found that the used liquid was as effective in lignin solubilization for up to (but not limited to) three uses. One of the remaining problems is how to separate the

lignin fraction from used treatment liquid efficiently without losing acetic acid. Possible methods include chromatographic separation by adsorption or size exclusion, flocculation or crystallization of particular components in the treatment liquid, and ultra-filtration or reverse osmosis separation. Of course any separation process must be performed in sealed utensils to avoid vaporization of the acetic acid.

Volatile Fatty Acid Production and Consumption

Most studies of bioconversion of cellulosic materials to date have used the biological methane potential (BMP) test developed by Owen et al. (1979). During the incubation period, only the gas phase was sampled and determined. In this experiment, since the incubation time for the bioconversion was maintained much longer than in other studies, unbalanced growth with abrupt changes in kinetics was frequently observed. To better understand the different steps of bioconversion, especially the difference between pretreated and untreated samples, an experiment was initiated using a group of serum bottles. The identical bottles were sacrificed periodically for sample analysis. When other analyses were performed on the sacrificed samples, the methane yields calculated were the averages of all the available bottles, including the sacrificed bottles. Volatile fatty acids (VFAs) were determined along with the methane and carbon dioxide, TS and VS, pH and alkalinity changes. Methane conversion with changes of VFAs and pH during the 60 day incubation are shown in Figure 4.19, and the remaining analysis results are listed in Table 4.29. Acetic acid usually predominated during the whole period, which is indicative of a balanced methane-producing process.

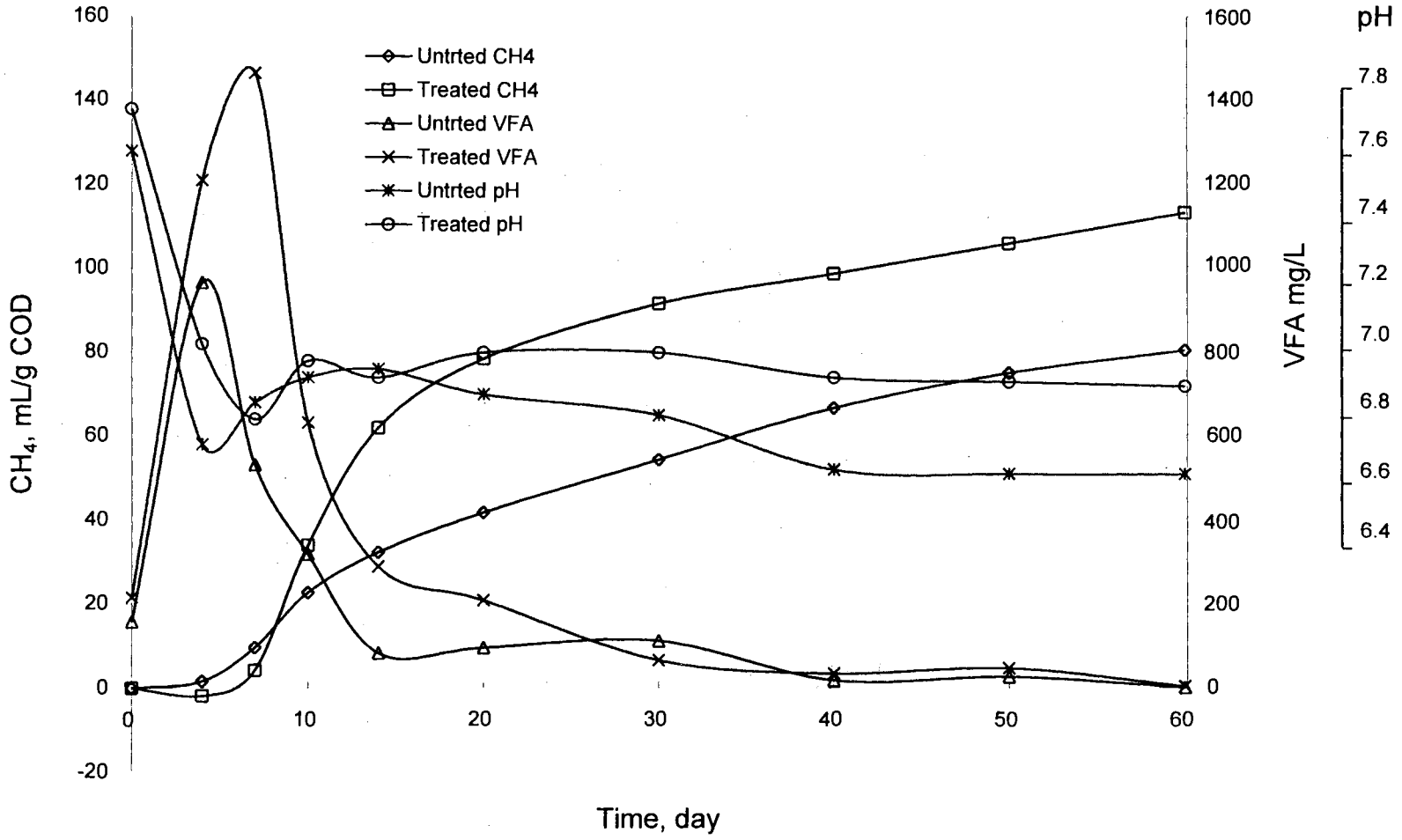

Figure 4.19 Methane production with VFA and pH changes.

Table 4.29 Analysis Results of VFA Production and Consumption Test

Parameter	Duration		
	10 day	30 day	60 day
Methane, mL/gCOD			
Untreated	22.72	54.46	80.59
Treated	33.99	91.73	113.51
Carbon Dioxide, mL/gCOD			
Untreated	17.48	35.38	(not tested)
Treated	33.47	52.62	(not tested)
Acetic Acid, mg/L			
Untreated	467	33.0	0.5
Treated	1174	91.9	4.0
Propionic Acid, mg/L			
Untreated	30.9	62.2	0.4
Treated	262	117	0
Butyric Acid, mg/L			
Untreated	12.2	0	0
Treated	34.6	0	0
Total Solids, %			
Untreated	0.83	0.79	0.76
Treated	0.95	0.91	0.89
Volatile Solids, %			
Untreated	0.60	0.57	0.54
Treated	0.58	0.53	0.52
pH			
Untreated	6.85	6.9	6.6
Treated	6.8	7.0	6.9
Alkalinity, mg/L as $CaCO_3$			
Untreated	1273	1332	1321
Treated	2028	2273	2366

Figure 4.19 shows that there is a period of acid formation associated with hydrolysis during the first 7 days when methanogens were subjected to a lag period. After 7 days, the acid concentration dropped abruptly, coincident with the highest methane formation rate. During the 60-day monitoring period, no further VFA accumulation was observed, which indicates that the methanogens remained active in a balanced reaction environment.

The alkali-treated sample had a higher initial VFA concentration, which suggests some of the solubilized material in the alkali pretreatment can be categorized as VFA. In the succeeding period, the alkali-treated sample produced significantly higher VFAs associated with methane production than the untreated samples. However, the leading position for the alkali-treated samples is relatively short. In both treated and untreated samples, the VFA concentrations were almost the same during the remaining period. This indicated that alkali treatment in the tested conditions only disrupted a small portion of the cellulose lignin association. This explains why the alkali treated samples showed a faster growth rate in the earlier stage, while in the long run, when the reactions were slowed by physical limitations, both treated and untreated samples kept synchronized reaction rates.

CHAPTER V

CONCLUSIONS

General Conclusions

1. This study is the first to focus on waste paper anaerobic biodegradation with three different inocula and three different paper sizes to find the ultimate methane potential in prolonged incubations.
2. Bioconversion of office paper was virtually completed during the first 20 days with conversion efficiency as high as 80%, while that of newsprint was not completed even after incubation for 300 to 600 days with conversion efficiencies ranging from 31 - 41%.
3. Although the conversion efficiencies of newsprint observed in these experiments were relatively low compared with that of office paper, they were significantly higher than the generally believed 20% level obtained in other shorter-term studies to date.
4. Even though conversion efficiencies were much higher than previously believed, the ultimate methane potential was still not reached. This suggests that with properly acclimated inocula, complete cellulose digestion under anaerobic conditions may be achieved, though the reaction is a very slow one.

5. Since the ultimate methane potential of newsprint was found to be much higher than previously believed, when the first order kinetic model was applied, the calculated newsprint initial conversion rates of this study were much lower than previously reported even though the apparent reaction rates were in the same range, as discussed in Appendix C.
6. Different sources of inocula (i.e. microbial consortia from anaerobic sludge, landfill and rumen contents) did not exhibit significantly different effects on either office paper or newsprint digestion in general.
7. Size reduction did not exert significant effects on bioconversion rate and extent, based on comparison of ground, shredded strips, and whole pieces of both office and newsprint paper. Therefore, extensive grinding or shredding may not be necessary to enhance bioconversion of waste paper to biogas, if such a process is to be established.
8. Soy-based ink does not exert any toxic effect on printed newsprint digestion; in fact, ink itself is readily bioconvertable. The low conversion associated with printed newsprint may be caused by a physical barrier of the ink layer or by the printing process. Deinking would most probably not be necessary for enhancement of bioconversion processes.
9. Chemical pretreatments have potential to increase initial bioconversion rates of newsprint. Both alkali and acid pretreatments have significant effects on newsprint bioconversion.

10. The major limiting factor on bioconversion of lignocellulosic material like newsprint is the physical barrier of the cellulose-lignin association.

Semicontinuous Seed Reactors

1. Steady state can be achieved in 3 HRTs for semicontinuous seed reactors inoculated with anaerobic sludge, landfill or rumen contents at a loading rate of 500 mg VS/L-d of newsprint as substrate with 10-day feeding cycles.
2. Under the above conditions, the methane yield of high lignin-content newsprint was limited to about 20 mL/g added paper COD when reaction vessels were not gas-tight, allowing some contact with air.
3. Also under the above conditions, the alkalinity requirement was about 260 mg/L as $CaCO_3$ in each feeding cycle. No additional carbonate or bicarbonate was required to supply alkalinity besides the nutrient solution made with tap water.
4. Strict anaerobic conditions are extremely important in waste paper biodigestion. The difference in methane yield between air-permeable plastic seed reactors and strictly anaerobic serum bottles with butyl rubber stoppers was found to be as high as 73%.

Serum Bottle Tests

1. Statistical analysis failed to detect significant differences in methane production caused by different inocula and different paper sizes.

2. Statistical analysis failed to detect significant differences in methane production between unprinted and printed newsprint.
3. Printing ink made from carbon black and soybean oil is readily bioconvertable. The apparent difference observed between the methane yields of printed and unprinted newsprint was probably caused by the coating effect of the printing ink which blocked microbial access.
4. It was found that the printed newsprint exhibited large variability in bioconversion when inoculated with either anaerobic sludge or rumen contents. This variability may be due partially to the ink coating of fibers during the printing process.
5. Methane production by microbial inocula controls in serum bottle studies gave inconsistent results, therefore care must be taken when using these controls to analyze results fro the serum bottle tests.

Chemical Pretreatment Tests

1. Alkali pretreatment improved newsprint bioconversion, apparently by partially solubilizing lignin and hemicellulose. NaOH (10% based on the paper weight) gave the highest methane production on treated sample bioconversion; higher concentrations were not necessary.
2. Prolonged soaking with NaOH improved solubilization of lignin and hemicellulose but did not significantly improve newsprint bioconversion.

3. Alkali treatment at elevated temperatures (70°C and 105°C, compared to 25°C room temperature) improved lignin and hemicellulose solubilization, but did not improve newsprint bioconversion statistically.
4. Based on the previous observations, a threshold of lignin solubilization for anaerobic digestion of alkali-pretreated newsprint might exist. If solubilization were lower than the threshold, bioconversion would not be inhibited, while if solubilization were higher than the threshold, the bioconversion reactions would be inhibited at some point.
5. Alkali-treated samples neutralized with CO_2 gave higher methane yields than those neutralized with other acids. Samples neutralized with strong acids like H_2SO_4 and HCl produced no more methane than the untreated samples.
6. Solubilized newsprint components or newsprint itself absorbed significant amounts of NaOH, which might account for the low pH of the bioconversion media, even though alkalinity was still high.
7. Statistical analysis proved that the extent of neutralization following alkali pretreatment did not have a significant effect on methane production during the first 30 days bioconversion, which suggests that the neutralization extent is not critical when CO_2 is utilized.
8. The ability of alkali pretreatment to disrupt the newsprint cellulose-lignin association is limited. By monitoring volatile fatty acids in the reaction media, it was found that the hydrolysis of newsprint components to acids was significantly improved by the alkali treatment at the early stage of

bioconversion. However, in the long run, the physical barrier caused by the cellulose lignin association would predominate.

9. Efforts to establish an acid pretreatment protocol based on analytical methods for lignin determination (80% acetic acid, with 7.5% nitric acid in a boiling water bath for 30 minutes) did not result in a practical pretreatment alternative.
10. Without adding nitric acid, 80% acetic acid by itself could not solubilize lignin, even under elevated temperature conditions. The nitric acid, which could not be replaced by another strong acid like hydrochloric acid, must also be added to effect lignin solubilization.
11. About 94% of lignin was removed from newsprint by treatment with a combination of 35% acetic and 2% nitric acids. The bioconversion of the treated sample volatile solids was increased by close to 3 times over untreated newsprint in 60 days incubation.
12. A portion of the nitric acid can be replaced by hydrochloric acid. A longer reaction time may be required for treatments with lower nitric acid concentrations.

Microbial Aspects

1. Statistical analysis demonstrated that after proper acclimation, the three inocula tested in this study performed equally well in methane production from waste paper under strict anaerobic reaction conditions.

2. In seed reactors where strict anaerobic conditions could not be maintained, microbial inoculum of landfill origin adapted to the system much earlier than the other two inocula (sewage sludge and rumen contents).
3. In either strict or nonstrict anaerobic conditions, the microbial consortium of landfill origin seemed to have greater potential to disrupt lignin structure, while leaving a higher cellulose concentration unutilized.
4. During long-term batch operation, the microorganisms might have gone through series of starvation and survival processes. When semicontinuous operation was restarted, all the microbial consortia showed higher conversion rates on newsprint digestion. However, the extent of rate increases was quite different. Sewage sludge and rumen populations demonstrated significantly higher newsprint conversion, while the microbial consortium of landfill origin was most stable following the long term batch operation.
5. During serum bottle tests, the three different microbial consortia performed equally well in office paper and unprinted newsprint digestion. However, the landfill consortium exhibited greatest stability in printed newsprint digestion, while those of other two origins appeared unstable in the same situation.
6. In summary, although the three inocula exhibited no significant differences in methane production potential from waste paper, the apparent stability and oxygen tolerance of the microbial consortium of landfill origin confer advantages for printed or mixed paper biodegradation, as well as during start-up of a new reactor.

CHAPTER VI

SIGNIFICANCE OF THE STUDY

Results of this investigation indicate that the anaerobic bioconversion of cellulosic materials exhibits significant potential for efficient conversion from waste to energy. Conversion efficiency of waste office paper is observed as high as 80%, which can be achieved within 20 days under normal mesophilic anaerobic digestion conditions with any of the three tested inocula. No physical size reduction nor deinking process is needed to enhance the bioconversion. This is considered a great advantage because for waste treatment, the simpler the process, the better its chance to succeed. At locations where the waste office paper supply exceeds demand for recycling by conventional means, such an anaerobic bioconversion facility could be considered. Waste office paper could also be fed with settled activated sludge in wastewater treatment plants into existing anaerobic digestors to increase their biogas production.

The newsprint takes more landfill space than any other paper category and is the most recalcitrant cellulosic material for bioconversion from waste to energy. Generally, a 20% conversion efficiency is believed to be the ultimate methane potential of the newsprint anaerobic bioconversion. However, this study achieved much higher methane conversion extents from newsprint (31 - 41%) than originally expected, over extended

reaction times. Continued low-level biogas production supports the observation that the true ultimate methane potential of newsprint can be even higher.

Due to the high proportion of lignin and its physical association with cellulose in the newsprint, the bioconversion rate is very low. This low conversion rate is the bottleneck on potential application of newsprint bioconversion. It is important to note that this low conversion rate was obtained under "ideal" laboratory conditions where active microorganisms are inoculated, anaerobic and steady mesophilic temperature conditions are well maintained, and nutrients, alkalinity, moisture and reducing power are adequately supplied. On the other hand, waste paper conventionally goes directly to landfills, where bioconversion is under unfavorable conditions such as inadequate moisture content and nutrient levels, and haphazard redox, temperature, and pH conditions. It should not be a surprise that intermittent reports of the extremely slow decomposition rates of landfill contents which are predominated by newsprint. Therefore, from a technical point of view, using landfilling as an alternative technology for lignocellulose bioconversion will be inefficient unless some special measures are taken to improve the landfill environmental conditions and enhance biogas production.

Effective and economical pretreatment processes have to be developed to disrupt the lignin-cellulose association before efficient bioconversion of newsprint can be achieved in anaerobic digesters. Chemical pretreatments like alkali and acid soaking can partially or completely dissolve lignin in newspaper and increase both reaction rate and extent of the newsprint bioconversion. To achieve significant increases in both bioconversion rate and extent, a treatment with 10% NaOH (alkali vs. paper, w/w) with

minimum water volume is recommended, based on these experiments. Lower concentrations of NaOH will limit the potential efficiency of this treatment, while higher concentrations may have negative effects on bioconversion of the treated samples. The alkali pretreatment can be conducted under ambient temperature and in a short duration (1 day). Extreme conditions like elevated temperature and prolonged soaking durations will not necessarily exert positive results. The alkali-pretreated samples are best neutralized with carbon dioxide rather than other strong acids. In a continuous reaction, the remaining alkali in the media can be neutralized by the carbon dioxide produced in the digestion process. The methane content of biogas would be upgraded at the same time as carbon dioxide is scrubbed out, due to the very low solubility of methane in water, resulting in higher quality biogas. The pretreatment will cost only the NaOH used as soaking reagent. The treated newsprint can be fed into the reactor directly without any washing or waste removal process, therefore no waste treatment or disposal is required for the pretreatment process. The high lignin residue may be explored for other industrial uses.

The limitation of the alkali pretreatment is that it only involves limited cellulose-lignin structure disruption. More severe soaking conditions like elevated temperature or prolonged duration can increase the solubilization but may also cause inhibition during the bioconversion. Therefore, some other treatment processes may be worth investigating for the possibility of coupling with simple alkali soaking to improve the disruption of the cellulose-lignin structure.

Pretreatment with acetic-nitric acid mixtures, investigated in this study, is much more effective than that with alkali alone. The lignin removal can be as high as 80% with a moderate acid concentration. However, the remaining solid materials must be effectively separated from the soaking solution for bioconversion, and the dissolved lignin components should also be effectively removed from the soaking solution for possible reuse. These separation processes will be both technical and economic challenges toward a possible solution of problems inherent with newsprint treatment. It is still too early to speculate on the engineering significance of this pretreatment before further research activities are carried out. Additional investigation on such separation processes is highly recommended.

CHAPTER VII

RESEARCH NEEDS

Interest in research on effective utilization of lignocellulose has existed for more than 100 years, although research on bioconversion of waste paper and newsprint to biogas is still relatively new. Earlier research activities in this area mainly focused on paper manufacturing and feedstuff industrial applications. The use of biotechnology to couple with traditional techniques is still at an early stage. The most difficult problems encountered in this area are cellulose-lignin separation and/or the recalcitrance of lignin to biodegradation, so lignin-cellulose separation and lignin biodegradation are naturally the research focuses in this area.

The much higher newsprint bioconversion extent obtained in this study suggests that newsprint anaerobic bioconversion is not limited to about 20% as generally accepted up to this time. A much higher bioconversion extent can be expected with longer incubation. Therefore, the bioconversion rate of lignocellulosic materials deserves more emphasis than the conversion extent, even though the two are closely interrelated.

Physical, chemical and biological pretreatments developed so far may improve both rate and extent of lignocellulosics bioconversion. However, they are either economically impractical or not powerful enough to effectively disrupt the lignin-

cellulose association. Thus, further research is warranted in the area of pretreatment methods.

The need for further research leading to practical application of the technology of waste paper and newsprint bioconversion comprises at least four areas of inquiry: economically sound pretreatments to improve the bioconversion rate and extent; selective removal of lignin from lignocellulose while leaving cellulose for bioconversion; microbial varieties and activities in lignocellulose bioconversion; and development of valid kinetic models to effectively describe lignocellulose bioconversion. Suggested specific research directions are as follows:

1. Investigate economically sound pretreatments or pretreatment combinations to effectively disrupt the lignin-cellulose association, and in turn to improve newsprint bioconversion rate and extent. Specifically, follow up on promising results of this study: alkali pretreatment with carbon dioxide neutralization, and the use of acetic acid in combination with other acids for lignin solubilization.
2. Define the digestibility of dissolved lignin components (mono- or oligo-aromatic compounds). This will aid in determining how much lignin can be converted to biogas after certain pretreatments, and whether the dissolved lignin components should be removed from treatment processes.
3. Define any inhibition effects on bioconversion caused by dissolved lignin components. This will determine what pretreatments can be applied to waste paper and newsprint, and whether methods for efficient and economical removal of dissolved lignin components should be investigated.

4. Optimize nutrients, organic factors and environmental conditions for bioconversion of waste paper and newsprint.
5. Identify and characterize the microbial consortia of different origins, especially that from landfill populations, and any possible anaerobic lignin biodegradation mechanism with selected species from landfill contents.
6. Expand research in describing the lignin-degrading enzyme system from white-rot fungi and other lignin degraders, as applied to bioconversion of waste paper and newsprint.
7. If feasible, scale up cultivation of white-rot fungi or other organisms capable of selectively removing lignin; and investigate bioconversion of the remains to biogas.
8. Develop more reliable analysis methods for determination of lignin in reaction media.
9. Develop effective methods for determination of microbial mass in lignocellulose bioconversion media.
10. Develop effective kinetic models to describe the behavior of the anaerobic bioconversion of lignocellulose with different cellulose-lignin ratios and with different pretreatment and bioconversion conditions to aid further research in improving bioconversion rate and extent.
11. Study the economic feasibility of waste paper and newsprint bioconversion to biogas and/or other fermentation products.

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APPENDIXES

Appendix A. Statistical Analysis Methods Used in This Study

The statistical analysis software SAS for PC DOS was used to perform all statistical analyses in this study. Statistical analysis was performed mainly for basic serum bottle tests and alkali pretreatment tests. Effects of paper type, size and inocula type on accumulated methane conversion data in basic serum bottle tests, and effects of NaOH concentrations, soaking durations, temperatures and neutralization with different acids on 200 days methane conversion extent in alkali pretreatment tests were analyzed. For all statistical analyses, the significance level used was 95% confidence interval (α = 0.05).

On most occasions, more than two populations of data and/or more than one level (in subsampling case) were analyzed. Under the assumptions that: 1) treatment and environmental effects are additive and 2) experimental errors are random, independently and normally distributed about zero mean and with a common variance, analysis of variance (AOV) procedure was used at first to determine whether means from two or more samples were drawn from populations with the same mean. When the AOV is performed for two or more groups (Appendix B.8), the basic steps are:

Suppose two hypotheses:

H_0: $\mu_1 = \mu_2 = ... = \mu_i = ... = \mu_n$ (I = 1, 2, ..., i, ..., n)

H_A: H_0 is not true.

where,

H_0 = null hypothesis (all population means are equal)
H_A = alternative hypothesis (at least one population mean is different)
μ_i = mean of ith population
n = the numbers of different populations sampled.

In subsampling cases (Appendix B.1-4), the test for the experimental unit means is basically the same as above (for example, in Appendix B.1, to test the effect of inocula on methane yield). However, there are two sources of variation which contribute to the variance applicable to comparisons among treatment means (to test the effect of paper type): 1) the variation among bottles treated alike, that is, among bottles within triplicates and 2) the variation among bottles in different triplicates treated alike, that is, variation among bottles within treatments. Mean squares for the two types of variation above are generally referred to as sampling error and experimental error, respectively. The experimental error usually is expected to be larger in random sampling since it contains an additional source of variation. So that in testing a hypothesis about population treatment means, the appropriate divisor for F (see Appendix B.1-4) is the experimental error mean square since it includes variation from all sources that contribute to the variability of treatment means except treatments.

If the observed significance level (OSL, or Pr>F in statistical analysis output) from analysis output is larger than α (= 0.05), then null hypothesis H_0 is accepted and it can be concluded that there is no significant difference among population means. Otherwise, if the OSL is less than α , then H_0 is rejected (or alternative hypothesis H_A is accepted) which indicates at least one population mean is significantly different.

AOV can only be used when above assumptions are true and to detect if there were significant differences among different experimental units or treatments, but can not be used to find the sources of the differences. If there is no detectable significant difference, the analysis is complete. Otherwise, further analyses have to be performed to identify the sources of the differences.

Once a significant difference was detected from AOV, to further identify which means are alike and which means are significantly different, Fisher's least significant difference procedure (LSD) and Tukey's procedure were performed in this study (Appendix B. 5-7, 9-12). The reasons to use these two procedures have been discussed in Chapter III. Each of these procedures requires calculating a critical value and applying it to differences between all pairs of means.

For LSD procedure, the critical value:

$$LSD = t_{\alpha/2}\sqrt{MSE(\frac{1}{r_i}+\frac{1}{r_j})}$$

where,

$t_{\alpha/2}$ = Tabulated value
MSE = Error mean square from AOV
r_i = Sample size for population i
r_j = Sample size for population j.

For Tukey's procedure, the critical value:

$$\varpi = q_\alpha(p,f)\sqrt{\frac{MSE}{2}(\frac{1}{r_i}+\frac{1}{r_j})}$$

where,

$q_\alpha(p,f)$ = Tabulated value
p = Number of treatments in experiment
f = Error degree of freedom
MSE = Error mean square from AOV
r_i = Sample size for population i
r_j = Sample size for population j.

A significant difference is concluded only if the difference between a pair of means is larger than the critical value. Following in Appendix B are the selected statistical analysis outputs and results.

Appendix B. Selected Statistical Analysis Outputs and Results

1. AOV on 300 day basic serum bottle tests - type of paper
   Treatments: effects of unprinted and printed newsprint;
   Reject, slightly different.
   Experimental units: effects of sludge, landfill and rumen inocula;
   Fail to reject, no significant difference.

```
TITLE 'AOV FOR 300-D BASIC SERUM BOTTLE TEST NEWSPRINT';
DATA METHANE;     INPUT TYPE$ INOCULA$ YIELD;
CARDS;
UNP SL 176.18     UNP SL 180.89     UNP SL 171.48
UNP SL 149.11     UNP SL 154.91     UNP SL 159.26
UNP SL 152.68     UNP SL 143.92     UNP SL 164.25
UNP LA 140.59     UNP LA 139.52     UNP LA 138.29
UNP LA 137.64     UNP LA 147.16     UNP LA 138.52
UNP LA 148.14     UNP LA 146.03     UNP LA 141.84
UNP RU 157.77     UNP RU 156.45     UNP RU 154.86
UNP RU 147.01     UNP RU 153.01     UNP RU 147.74
UNP RU 138.91     UNP RU 152.25     UNP RU 148.89
PNP SL 174.65     PNP SL 75.29      PNP SL 62.67
PNP SL 167.26     PNP SL 172.75     PNP SL 148.33
PNP SL 161.07     PNP SL 171.01     PNP SL 66.05
PNP LA 137.16     PNP LA 126.95     PNP LA 139.15
PNP LA 141.80     PNP LA 139.15     PNP LA 137.55
PNP LA 139.53     PNP LA 139.73     PNP LA 140.51
PNP RU 154.09     PNP RU 84.03      PNP RU 78.90
PNP RU 151.37     PNP RU 70.72      PNP RU 148.99
PNP RU 157.86     PNP RU 151.68     PNP RU 145.34;
PROC ANOVA;       CLASS INOCULA TYPE;
MODEL YIELD=TYPE INOCULA(TYPE);     TEST H=TYPE E=INOCULA(TYPE);
RUN;
```

AOV FOR 300-DAY BASIC SERUM BOTTLE TEST

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
INOCULA	3	LA RU SL
TYPE	2	PNP UNP

Number of observations in data set = 54

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	6947.138120	1389.427624	2.05	0.0889
Error	48	32596.784511	679.099677		
Corrected Total	53	39543.922631			

R-Square	C.V.	Root MSE	YIELD Mean
0.175682	18.34488	26.05954	142.053519

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TYPE	1	4698.588224	4698.588224	6.92	0.0114
INOCULA(TYPE)	4	2248.549896	562.137474	0.83	0.5141

Tests of Hypotheses using the Anova MS for INOCULA(TYPE) as an error term

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TYPE	1	4698.588224	4698.588224	8.36	0.0445

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

2. AOV on 220 day basic serum bottle tests - office paper
Treatments: effects of ground, shredded, whole piece of paper;
Fail to reject, no significant difference.
Experimental units: effects of sludge, landfill and rumen inocula;
Reject, significantly different.

```
TITLE 'AOV FOR 220-D BASIC SERUM BOTTLE OP';
DATA METHANE;
INPUT SIZE$ INOCULA$ YIELD;
CARDS;
GROUND SL 294.43        GROUND SL 285.51        GROUND SL 310.46
GROUND LA 264.79        GROUND LA 266.47        GROUND LA 240.94
GROUND RU 275.29        GROUND RU 268.32        GROUND RU 310.48
SHRED SL 396.21         SHRED SL 320.64         SHRED SL 328.10
SHRED LA 339.18         SHRED LA 233.33         SHRED LA 295.84
SHRED RU 359.53         SHRED RU 347.59         SHRED RU 386.34
WHOLE SL 363.69         WHOLE SL 366.89         WHOLE SL 283.06
WHOLE LA 324.14         WHOLE LA 274.17         WHOLE LA 268.44
WHOLE RU 381.12         WHOLE RU 374.22         WHOLE RU 316.41
;
PROC ANOVA;
CLASS INOCULA SIZE;
MODEL YIELD=SIZE INOCULA(SIZE);
TEST H=SIZE E=INOCULA(SIZE);
RUN;
```

AOV FOR 220-D BASIC SERUM BOTTLE OP

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
INOCULA	3	LA RU SL
SIZE	3	GROUND SHRED WHOLE

Number of observations in data set = 27

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	35271.73132	4408.96641	3.86	0.0083
Error	18	20584.49567	1143.58309		
Corrected Total	26	55856.22699			

R-Square	C.V.	Root MSE	YIELD Mean
0.631474	10.77278	33.81691	313.910741

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SIZE	2	16028.46859	8014.23429	7.01	0.0056
INOCULA(SIZE)	6	19243.26273	3207.21046	2.80	0.0417

Tests of Hypotheses using the Anova MS for INOCULA(SIZE) as an error term

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SIZE	2	16028.46859	8014.23429	2.50	0.1624

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

3. AOV on 300 basic serum bottle tests - unprinted newsprint
Treatments: effects of ground, shredded and whole piece paper;
Fail to reject, no significant difference.
Experimental units: effects of sludge, landfill and rumen inocula;
Reject, significantly different.

```
TITLE 'AOV FOR 300-D BASIC SERUM BOTTLE UNP';
DATA METHANE;
INPUT SIZE$ INOCULA$ YIELD;
CARDS;
GROUND SL 176.18        GROUND SL 180.89        GROUND SL 171.48
GROUND LA 140.59        GROUND LA 139.52        GROUND LA 138.29
GROUND RU 157.77        GROUND RU 156.45        GROUND RU 154.86
SHRED SL 149.11         SHRED SL 154.91         SHRED SL 159.26
SHRED LA 137.64         SHRED LA 147.16         SHRED LA 138.52
SHRED RU 147.01         SHRED RU 153.01         SHRED RU 147.74
WHOLE SL 152.68         WHOLE SL 143.92         WHOLE SL 164.25
WHOLE LA 148.14         WHOLE LA 146.03         WHOLE LA 141.84
WHOLE RU 138.91         WHOLE RU 152.25         WHOLE RU 148.89
;
PROC ANOVA;
CLASS INOCULA SIZE;
MODEL YIELD=SIZE INOCULA(SIZE);
TEST H=SIZE E=INOCULA(SIZE);
RUN;
```

AOV FOR 300-D BASIC SERUM BOTTLE UNP
Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
INOCULA	3	LA RU SL
SIZE	3	GROUND SHRED WHOLE

Number of observations in data set = 27

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2894.588207	361.823526	12.91	0.0001
Error	18	504.636333	28.035352		
Corrected Total	26	3399.224541			

R-Square	C.V.	Root MSE	YIELD Mean
0.851544	3.497681	5.294842	151.381481

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SIZE	2	479.128363	239.564181	8.55	0.0025
INOCULA(SIZE)	6	2415.459844	402.576641	14.36	0.0001

Tests of Hypotheses using the Anova MS for INOCULA(SIZE) as an error term

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SIZE	2	479.1283630	239.5641815	0.60	0.5811

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

4. AOV on basic serum bottle tests - printed newsprint
Treatments: effects of ground, shredded and whole piece paper;
Fail to reject, no significant difference.
Experimental units: effects of sluge, landfill and rumen inocula;
Fail to reject no significant difference.

```
TITLE 'AOV FOR 300-D BASIC SERUM BOTTLE PNP';
DATA METHANE;
INPUT SIZE$ INOCULA$ YIELD;
CARDS;
GROUND SL 174.65        GROUND SL 75.29         GROUND SL 62.67
GROUND LA 137.16        GROUND LA 126.95        GROUND LA 139.15
GROUND RU 154.09        GROUND RU 84.03         GROUND RU 78.90
SHRED SL 167.26         SHRED SL 172.75         SHRED SL 148.33
SHRED LA 141.80         SHRED LA 139.15         SHRED LA 137.55
SHRED RU 151.37         SHRED RU 70.72          SHRED RU 148.99
WHOLE SL 161.07         WHOLE SL 171.01         WHOLE SL 66.05
WHOLE LA 139.53         WHOLE LA 139.73         WHOLE LA 140.51
WHOLE RU 157.86         WHOLE RU 151.68         WHOLE RU 145.34
;
PROC ANOVA;
CLASS INOCULA SIZE;
MODEL YIELD=SIZE INOCULA(SIZE);
TEST H=SIZE E=INOCULA(SIZE);
RUN;
```

AOV FOR 300-D BASIC SERUM BOTTLE PNP

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
INOCULA	3	LA RU SL
SIZE	3	GROUND SHRED WHOLE

Number of observations in data set = 27

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	8964.045133	1120.505642	0.90	0.5390
Error	18	22482.064733	1249.003596		
Corrected Total	26	31446.109867			

R-Square	C.V.	Root MSE	YIELD Mean
0.285061	26.62731	35.34124	132.725556

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SIZE	2	4356.049356	2178.024678	1.74	0.2031
INOCULA(SIZE)	6	4607.995778	767.999296	0.61	0.7158

Tests of Hypotheses using the Anova MS for INOCULA(SIZE) as an error term

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SIZE	2	4356.049356	2178.024678	2.8	40.1358

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

5. LSD and Tukey on alkali pretreatment Test 2 - concentration
Treatments: effects of 0%, 5%, 10%, 15% and 20% NaOH concentrations.

```
TITLE 'LSD AND TUKEY FOR ALK TEST2 CONCENTRATION';
DATA METHANE;      INPUT CONC$ YIELD;  CARDS;
0% 128.07          0% 135.38           0% 142.46
5% 158.00          5% 157.78           5% 169.15
10% 175.90         10% 169.33          10% 173.48
15% 191.03         15% 170.13          15% 168.15
20% 132.05         20% 164.61          20% 171.71;
PROC ANOVA;        CLASS CONC;         MODEL YIELD=CONC;
MEANS CONC/LSD;    MEANS CONC/TUKEY;   RUN;
```

LSD AND TUKEY FOR ALK TEST2 CONCENTRATION

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
CONC	5	0% 5% 10% 15% 20%

Number of observations in data set = 15

Dpendent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3189.458507	797.364627	5.59	0.0125
Error	10	1426.076733	142.607673		
Corrected Total	14	4615.535240			

R-Square	C.V.	Root MSE	YIELD Mean
0.691027	7.441237	11.94185	160.482000

T tests (LSD) for variable: YIELD

NOTE: This test controls the type I comparisonwise error rate not Cthe experimentwise error rate.

Alpha= 0.05 df= 10 MSE= 142.6077
Critical Value of T= 2.23
Least Significant Difference= 21.725
Means with the same letter are not significantly different.

T Grouping	Mean	N	CONC
A	176.437	3	15%
A	172.903	3	10%
A	161.643	3	5%
B A	156.123	3	20%
B	135.303	3	0%

Tukey's Studentized Range (HSD) Test for variable: YIELD

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 10 MSE= 142.6077
Critical Value of Studentized Range= 4.654
Minimum Significant Difference= 32.091
Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	CONC
A	176.437	3	15%
A	172.903	3	10%
B A	161.643	3	5%
B A	156.123	3	20%
B	135.303	3	0%

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

6. LSD and Tukey on alkali pretreatment Test 2 - soaking duration
Treatments: effects of 1 day 0%, 1 day 5%, 1 day 10%, 6 day 5%, 6 day 10%, 12 day 5% and 12 day 10% NaOH.

```
TITLE 'LSD AND TUKEY FOR ALK TEST2 TIME';
DATA METHANE;     INPUT TIME$ YIELD;        CARDS;
1d0% 128.07       1d0% 135.38               1d0% 142.46
1d5% 158.00       1d5% 157.78               1d5% 169.15
1d10% 175.90      1d10% 169.33              1d10% 173.48
6d5% 133.70       6d5% 151.45               6d5% 147.64
6d10% 143.56      6d10% 142.59              6d10% 150.37
12d5% 136.39      12d5% 166.46              12d5% 169.55
12d10% 140.96     12d10% 172.67             12d10% 177.59;
PROC ANOVA;       CLASS TIME;               MODEL YIELD=TIME;
MEANS TIME/LSD;   MEANS TIME/TUKEY;         RUN;
```

LSD AND TUKEY FOR ALK TEST1 TIME
Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TIME	7	12d10% 12d5% 1d0% 1d10% 1d5% 6d10% 6d5%

Number of observations in data set = 21

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	3114.015695	519.002616	3.86	0.0175
Error	14	1882.366600	134.454757		
Corrected Total	20	4996.382295			

R-Square	C.V.	Root MSE	YIELD Mean
0.623254	7.509829	11.59546	154.403810

T tests (LSD) for variable: YIELD

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.
Alpha= 0.05 df= 14 MSE= 134.4548
Critical Value of T= 2.14
Least Significant Difference= 20.306
Means with the same letter are not significantly different.

T Grouping		Mean	N	TIME
	A	172.903	3	1d10%
B	A	163.740	3	12d10%
B	A	161.643	3	1d5%
B	A	157.467	3	12d5%
B	C	145.507	3	6d10%
B	C	144.263	3	6d5%
	C	135.303	3	1d0%

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

6. continuoued

Tukey's Studentized Range (HSD) Test for variable: YIELD

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 14 MSE= 134.4548
Critical Value of Studentized Range= 4.829
Minimum Significant Difference= 32.329

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	TIME
	A	172.903	3	1d10%
B	A	163.740	3	12d10%
B	A	161.643	3	1d5%
B	A	157.467	3	12d5%
B	A	145.507	3	6d10%
B	A	144.263	3	6d5%
B		135.303	3	1d0%

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

7. LSD and Tukey on alkali pretreatment Test 2 - temperature
Treatments: effects of 25°C 0%, 25°C 5%, 25°C 10%, 70°C 5%, 70°C 10%, 105°C 5% and 105°C 10% NaOH concentrations.

```
TITLE 'LSD AND TUKEY FOR ALK TEST2 TEMPERATURE';
DATA METHANE;      INPUT TEMP$ YIELD;        CARDS;
0%25C 128.07       0%25C 135.38              0%25C 142.46
5%25C 158.00       5%25C 157.78              5%25C 169.15
10%25C 175.90      10%25C 169.33             10%25C 173.48
5%70C 127.03       5%70C 130.72              5%70C 136.71
10%70C 139.74      10%70C 142.44             10%70C 152.29
5%105C 122.08      5%105C 132.90             5%105C 155.55
10%105C 148.35     10%105C 154.59            10%105C 151.34;
PROC ANOVA;        CLASS TEMP;               MODEL YIELD=TEMP;
MEANS TEMP/LSD;    MEANS TEMP/TUKEY;         RUN;
```

LSD AND TUKEY FOR ALK TEST1 TEMPERATURE
Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TEMP	7	0%25C 10%105C 10%25C 10%70C 5%105C 5%25C 5%70C

Number of observations in data set = 21

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	4158.493248	693.082208	10.23	0.0002
Error	14	948.105267	67.721805		
Corrected Total	20	5106.598514			

R-Square	C.V.	Root MSE	YIELD Mean
0.814337	5.568795	8.229326	147.775714

T tests (LSD) for variable: YIELD

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 14 MSE= 67.7218
Critical Value of T= 2.14
Least Significant Difference= 14.411

Means with the same letter are not significantly different.

T Grouping		Mean	N	TEMP
	A	172.903	3	10%25C
B	A	161.643	3	5%25C
B	C	151.427	3	10%105C
D	C	144.823	3	10%70C
D		136.843	3	5%105C
D		135.303	3	0%25C
D		131.487	3	5%70C

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

7. continuoued

Tukey's Studentized Range (HSD) Test for variable: YIELD

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 14 MSE= 67.7218
Critical Value of Studentized Range= 4.829
Minimum Significant Difference= 22.944

Means with the same letter are not significantly different.

Tukey Grouping			Mean	N	TEMP
	A		172.903	3	10%25C
B	A		161.643	3	5%25C
B	A	C	151.427	3	10%105C
B		C	144.823	3	10%70C
		C	136.843	3	5%105C
		C	135.303	3	0%25C
		C	131.487	3	5%70C

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

8. AOV on alkali pretreatment Test 1 - neutralizing acids
Treatments: effects of carbon dioxide, sulfuric acid and hydrochloric acid;
Fail to reject, no significant difference.

```
TITLE 'AOV FOR ALK TEST1 ACID';
DATA METHANE;
INPUT ACID$ YIELD;
CARDS;
CO2 119.02
CO2 127.97
CO2 118.56
H2SO4 73.52
H2SO4 112.51
HCl 121.82
HCl 92.92
;
PROC ANOVA;
        CLASS ACID;
        MODEL YIELD=ACID;
RUN;
```

AOV FOR ALK TEST1 ACID
Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
ACID	3	CO_2 H_2SO_4 HCl

Number of observations in data set = 7

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1010.147121	505.073561	1.64	0.3024
Error	4	1234.002450	308.500612		
Corrected Total	6	2244.149571			

R-Square	C.V.	Root MSE	YIELD Mean
0.450125	16.04412	17.56419	109.474286

Source	DF	Anova SS	Mean Square	F Value	Pr > F
ACID	2	1010.147121	505.073561	1.64	0.3024

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

9. AOV for 38 day seed reactor residue
Treatments: effects of nitrogen and nitrogen-carbon dioxide headspaces;
Fail to reject, no significant difference.
Experimental units: effects of sludge, landfill and rumen inocula;
Reject, significantly different.

```
TITLE 'AOV FOR 38-D SEED REACTOR RESIDUE';
DATA METHANE;
INPUT HEADSP$ INOCULA$ YIELD;
CARDS;
N2CO2 SL 6.36      N2CO2 SL 6.52      N2CO2 SL 6.59
N2CO2 LA 6.70      N2CO2 LA 7.01      N2CO2 LA 7.41
N2CO2 RU 4.49      N2CO2 RU 5.22      N2CO2 .
N2 SL 6.16         N2 SL 6.37         N2 SL 6.04
N2 LA 7.21         N2 LA 7.55         N2 LA 7.31
N2 RU 4.46         N2 RU 4.75         N2 RU 5.27;
PROC GLM;
CLASS HEADSP INOCULA;
MODEL YIELD=HEADSP INOCULA(HEADSP);
TEST H=HEADSP E=INOCULA(HEADSP);
RUN;
```

AOV FOR 38-D SEED REACTOR RESIDUE

General Linear Models Procedure

Class Level Information

Class	Levels	Values
HEADSP	2	N2 N2CO2
INOCULA	3	LA RU SL

Number of observations in data set = 17

NOTE: Due to missing values, only 16 obsservations can be used in thi analysis.

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	15.65874167	3.13174833	31.32	0.0001
Error	10	1.00003333	0.10000333		
Corrected Total	15	16.65877500			

R-Square	C.V.	Root MSE	YIELD Mean
0.939970	5.097450	0.316233	6.20375000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
HEADSP	1	0.11222500	0.11222500	1.12	0.3144
INOCULA(HEADSP)	4	15.54651667	3.88662917	38.86	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
HEADSP	1	0.00000476	0.00000476	0.00	0.9946
INOCULA(HEADSP)	4	15.54651667	3.88662917	38.86	0.0001

Tests of Hypotheses using the Type III MS for INOCULA(HEADSP) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
HEADSP	1	0.00000476	0.00000476	0.00	0.9992

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

10. LSD and Tukey for basic serum bottle tests residue cellulose
Treatments: effects of sludge, landfill and rumen inocula.

```
TITLE 'LSD AND TUKEY FOR BASIC SERUM BOTTLE RESIDUE CELLULOSE';
DATA CELLU;
INPUT INOCULA$ REMAIN;
CARDS;
SL 15.35          SL 15.95
LA 18.65          LA 17.54
RU 13.35          RU 12.37;
PROC ANOVA;
CLASS INOCULA;
MODEL REMAIN=INOCULA;
MEANS INOCULA/LSD;
MEANS INOCULA/TUKEY;
RUN;
```

AOV FOR BASIC SERUM BOTTLE RESIDUE CELLULOSE

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
INOCULA	3	LA RU SL

Number of observations in data set = 6

Dependent Variable: REMAIN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	27.44490000	13.72245000	32.26	0.0094
Error	3	1.27625000	0.42541667		
Corrected Total	5	28.72115000			

R-Square	C.V.	Root MSE	REMAIN Mean
0.955564	4.198518	0.652240	15.5350000

T tests (LSD) for variable: REMAIN

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 3 MSE= 0.425417

Critical Value of T= 3.18

Least Significant Difference= 2.0757

Means with the same letter are not significantly different.

T Grouping	Mean	N	INOCULA
A	18.095	2	LA
B	15.650	2	SL
C	12.860	2	RU

Tukey's Studentized Range (HSD) Test for variable: REMAIN

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 3 MSE= 0.425417

Critical Value of Studentized Range= 5.998

Minimum Significant Difference= 2.7665

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	INOCULA
A	18.095	2	LA
A	15.650	2	SL
B	12.860	2	RU

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

11. LSD and Tukey for basic serum bottle test control residue cellulose Treatments: effects of sludge, landfill and rumen inocula.

```
TITLE 'LSD AND TUKEY FOR BASIC SERUM BOTTLE CONTROL RESIDUE
CELLULOSE';
DATA CELLU;
INPUT INOCULA$ REMAIN;
CARDS;
SL 20.29          SL 19.15
LA 13.72          LA 14.36
RU 17.22          RU 16.60;
PROC ANOVA;       CLASS INOCULA;
MODEL REMAIN=INOCULA;
MEANS INOCULA/LSD;
MEANS INOCULA/TUKEY;     RUN;
```

LSD AND TUKEY FOR BASIC SERUM BOTTLE RESIDUE CELLULOSE

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
INOCULA	3	LA RU SL

Number of observations in data set = 6

Dependent Variable: REMAIN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	32.26360000	16.13180000	46.23	0.0056
Error	3	1.04680000	0.34893333		
Corrected Total	5	33.31040000			

R-Square	C.V.	Root MSE	REMAIN Mean
0.968574	3.497370	0.590706	16.8900000

T tests (LSD) for variable: REMAIN

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 3 MSE= 0.348933

Critical Value of T= 3.18

Least Significant Difference= 1.8799

Means with the same letter are not significantly different.

T Grouping	Mean	N	INOCULA
A	19.720	2	SL
B	16.910	2	RU
C	14.040	2	LA

Tukey's Studentized Range (HSD) Test for variable: REMAIN

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 3 MSE= 0.348933

Critical Value of Studentized Range= 5.998

Minimum Significant Difference= 2.5055

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	INOCULA
A	19.720	2	SL
B	16.910	2	RU
C	14.040	2	LA

Appendix B. Selected Statistical Analysis Outputs and Results (Cont'd)

12. LSD and Tukey for basic serum bottle test residue lignin
Treatments: effects of sludge, landfill and rumen inocula.

```
TITLE 'LSD AND TUKEY FOR BASIC SERUM BOTTLE RESIDUE LGNIN';
DATA LIGNIN;
INPUT INOCULA$ REMAIN;
CARDS;
SL 49.01          SL 49.47
LA 48.08          LA 46.89
RU 48.09          RU 47.95;
PROC ANOVA;
CLASS INOCULA;
MODEL REMAIN=INOCULA;
MEANS INOCULA/LSD;
MEANS INOCULA/TUKEY;
RUN;
```

LSD AND TUKEY FOR BASIC SERUM BOTTLE RESIDUE LIGNIN

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
INOCULA	3	LA RU SL

Number of observations in data set = 6

Dependent Variable: REMAIN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	3.23643333	1.61821667	5.89	0.0914
Error	3	0.82365000	0.27455000		
Corrected Total	5	4.06008333			

R-Square	C.V.	Root MSE	REMAIN Mean
0.797135	1.085996	0.523975	48.2483333

T tests (LSD) for variable: REMAIN

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 3 MSE= 0.27455
Critical Value of T= 3.18
Least Significant Difference= 1.6675
Means with the same letter are not significantly different.

T Grouping	Mean	N	INOCULA
A	49.240	2	SL
B A	48.020	2	RU
B	47.485	2	LA

Tukey's Studentized Range (HSD) Test for variable: REMAIN

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 3 MSE= 0.27455
Critical Value of Studentized Range= 5.998
Minimum Significant Difference= 2.2224
Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	INOCULA
A	49.240	2	SL
A	48.020	2	RU
A	47.485	2	LA

Appendix C. Analysis of Reaction Extent and Kinetics in This Study

Reaction rate and extent are two of the most important factors to be considered in lignocellulosic material bioconversion. The reaction rates can be qualitatively compared according to the methane production curves or the apparent initial conversion rates. However, to quantify the reaction rate constants requires use of some form of kinetic model. All the statistical analyses and quantitative comparisons among different tests in this thesis are based on the conversion extent only. The reason for this is that no available model was found to be able to objectively compare the initial conversion rates obtained from bioconversions of different durations or from differently pretreated samples, where the extent of bioconversion may be different.

The kinetic behavior of microbial conversion has been traditionally described with the Monod equation, one form of which is given as follows:

$$-r_s = \frac{K_1 C_m C_s}{K_2 + C_s}$$

where $-r_s$ = reaction rate;

C_m = microorganism concentration;

C_s = substrate concentration; and

K_1, K_2 are constants.

Many modifications of this equation have been suggested to accommodate complex reactions or substrates. However, in anaerobic digestion of solid materials like cellulose or lignocellulose, the measurement of the microbial biomass is not trivial. Therefore, many researchers (Stinson and Ham, 1995; Owens and Chynoweth, 1993; and Tong et al., 1990) have used first order kinetics to describe the initial reaction rate.

The expression of the first order kinetics is independent of microorganism concentration:

$$-\frac{dC}{dt} = KC$$

where C is the substrate concentration represented by methane potential remaining at time t, obtained from subtracting the methane yield at time t from the ultimate methane potential. Therefore, when this model is used, some value of ultimate methane potential must be specified. Also, the lag phase of microbial conversion has to be averted during the rate constant determination. In previous studies, the ultimate methane potential remaining was obtained from incubations of relatively limited duration, seldom greater than 60 days.

In calculating the reaction rate, it is critical what ultimate methane potential value is selected. When ultimate methane potentials are different for similar tests, comparison among calculated rate constants will not be valid. The ultimate methane potential used by Tong et al. (1990) was 92 mL/gVS, while Owens and Chynoweth (1993) estimated the ultimate methane potentials at 84 and 100 mL/gVS for unprinted and printed newsprint, respectively. A nonlinear regression model was used to fit their methane yield data. These estimated methane potentials are less than one-half of the actual methane yield obtained in this study. In the basic serum bottle test of this study, the methane yield for unprinted newsprint with sludge as inoculum at 300 days was 204 mL/gVS. If this methane yield were used as ultimate methane potential, then the initial first order rate constant would be 0.041 d^{-1}. However, if 100 mL/gVS (as Owens and Chynoweth estimated) were used as ultimate methane potential, that rate constant would be 0.100 d^{-1}. If the maximum methane potential of newsprint were 438 mL/gVS (as Tong et al.

reported), then the rate constant would be only 0.017 d^{-1}. Since lignin, which is basically refractory under anaerobic reaction conditions, is associated with cellulose in the newsprint, it is still unknown whether the maximum methane potential of all available volatile solids can be reached or not.

Theoretically, the ultimate methane potential should be at most the methane potential from all other volatile solids except lignin, if nutrients and substrates are fully balanced. However, due to the cellulose-lignin association, it is unknown how much cellulose would be available. Therefore, experimental determination of the true ultimate methane potential would be time consuming and uncertain. Although the basic serum bottle tests in this study were run for 300 days, and the long term test in serum bottles was run for 600 days, the ultimate methane potential of newsprint had not been reached, as indicated by continued biogas production at a low but steady rate.

Even if there is a definite ultimate methane potential, its determination may still rely on several factors like the composition of the newsprint, the inocula used, the bioconversion environment, and so on. For example, in the preliminary serum bottle test of this study, the compositions of printed and unprinted newsprint were significantly different even when the samples were collected from the same printing plant. Comparison of the reaction rates of those samples, even if the ultimate methane potential were set at the same level, would still be invalid. Likewise in the alkali-pretreated sample bioconversion tests, since the newsprint was treated under various conditions, the ultimate methane potential for differently treated samples may be quite different.

Taking alkali pretreated sample bioconversion as an example, Table C1 lists the apparent initial conversion rates, the ultimate methane conversion extents and the

calculated first-order initial conversion rates. In Test 1, when 200 days ultimate methane yields are relatively close for most samples, the apparent initial conversion rates agree with the calculated first-order initial conversion rates. Both apparent and calculated conversion rates of newsprint treated with different NaOH concentrations, under prolonged durations or elevated temperatures, were significantly higher than that of untreated samples. However, in the case of neutralization with sulfuric acid, although the apparent initial conversion rate of newsprint was significantly lower than most other treatments, its calculated first-order initial conversion rate was as high as or even higher than most of other treatments because its ultimate methane yield at 200 days was much lower.

In Test 2, differently pretreated samples still have significantly higher apparent initial conversion rates and ultimate methane yields than that of untreated sample at 200 days; however, their calculated first-order conversion rates were in the same range or even lower than that of the untreated samples. It is obvious that comparisons made according to these first-order conversion rates would be invalid.

All of the above observations make the use of reaction rates, as determined by traditional first-order rate constant analysis, to compare different reaction conditions or effects of treatment impractical. Therefore, besides determination of the methane yields, other analyses, like determinations of CO_2 yields, COD, TS, VS, cellulose and AIL, were also performed to obtain a more complete description of biological activity, and the statistical analyses were mainly performed on methane conversion extent instead of initial first-order conversion rates.

Table C1. Alkali Pretreatment Bioconversion Rates and Extents

Treatment	Test 1			Test 2		
	Apparent Initial Rate (mL CH4/g-D)	Ultimate CH_4 (200day) (mL/g COD)	Calculated 1st order Rate (d^{-1})	Apparent Initial Rate (mL CH4/g-D)	Ultimate CH_4 (200day) (mL/g COD)	Calculated 1st order Rate (d^{-1})
(Conc.)						
Untreated	4.71 (0.33)	121.8 (5.3)	0.049 (0.003)	4.49 (0.50)	135.3 (7.2)	0.041 (0.005)
5% NaOH	6.12 (0.22)	140.6 (5.2)	0.057 (0.002)	5.13 (0.24)	161.6 (6.5)	0.039 (0.002)
10% NaOH	6.90 (0.37)	150.0 (2.4)	0.062 (0.003)	5.36 (0.35)	172.9 (3.3)	0.037 (0.003)
15% NaOH	6.42 (0.26)	144.7 (12.7)	0.060 (0.002)	5.65 (0.53)	176.4 (12.7)	0.039 (0.004)
20% NaOH	5.97 (0.31)	154.7 (15.7)	0.049 (0.002)	5.78 (0.28)	156.1 (21.2)	0.047 (0.002)
(Time, Conc.)						
6 day, 5% NaOH	5.96 (0.25)	142.8 (2.2)	0.057 (0.001)	5.13 (0.27)	144.3 (9.4)	0.045 (0.002)
6 day, 10% NaOH				5.25 (0.21)	145.5 (4.2)	0.046 (0.002)
12 day, 5% NaOH	5.93 (0.24)	149.2 (1.9)	0.052 (0.002)	6.12 (0.27)	157.5 (18.3)	0.049 (0.003)
12 day, 10% NaOH				6.45 (0.25)	163.7 (19.9)	0.050 (0.002)
(Temp., Conc.)						
70°C, 5% NaOH	5.73 (0.45)*	138.2 (3.9)*	0.055 (0.003)*	5.22 (0.21)	131.5 (4.9)	0.050 (0.002)
70°C, 10% NaOH				6.31 (0.45)	144.8 (6.6)	0.056 (0.004)
105°C, 5% NaOH	5.59 (0.40)**	126.3 (12.8)**	0.061 (0.003)**	5.54 (0.18)	136.8 (17.1)	0.052 (0.002)
105°C, 10% NaOH				5.82 (0.37)	151.4 (3.1)	0.047 (0.003)
(Neutralizing Acid)						
CO2	6.12 (0.22)	140.6 (5.2)	0.057 (0.002)			
H2SO4	4.22 (0.19)	93.0 (27.6)	0.060 (0.004)			
HCl	4.56 (0.22)	107.4 (20.4)	0.054 (0.003)			

Note: * Treated for 4 hours
Treated for 0.5 hour
Rate data in parentheses are standard error of coefficients of the parameter estimates.

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