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ABSTRACT

ANAEROBIC DIGESTION AND ACID HYDROLYSIS OF NITROCELLULOSE

by
Fong-Jung Tai

In this investigation, studies were conducted to evaluate the biodegradation of nitrocellulose in anaerobic batch reactors with and without the supplemental carbon inducers, such as cellulose, cellobiose, and lactose. Results from the anaerobic study show that degradation of nitrocellulose alone is difficult and that nitrocellulose degradative enzymes could be induced by the three inducers tested. As high as 48.91% conversion could be obtained at Cellulose/Nitrocellulose ratio of 1 to 1. Studies also indicated that type 20 and 50 celluloses would be more effective and optimum pH was about 6.4 in biodegradation of nitrocellulose. Three testing systems, namely, single-stage, two-stage, and staged-feed anaerobic treatment were utilized in the biodegradation study. Results from this study showed that a two-stage anaerobic treatment did not clearly enhance biodegradation. Stage-feed system had a higher rate of gas production; unfortunately, the system sometimes was not stable. Experiments indicated that nitrocellulose affected the biodegradation of cellulose and decreased gas production at cellulose/nitrocellulose ratios lower than 1/1. Analysis of the data shows that the inhibitory effect of nitrocellulose on cellulose degradation behaved like competitive inhibition. This inhibitory effect can be overcome at higher cellulose concentrations.

In the second part of this study acid hydrolysis of nitrocellulose was conducted by using concentrated hydrochloric acid at intermediate temperatures. Results showed that

the end products from acid hydrolysis were mainly glucose and small molecular weight organic acids. Glucose yields ranged from 45 to 85 percent depending on acid concentration, acid/solid ratio, reaction time, and heating temperature. It was found that the higher the acid concentration and temperature, the faster the hydrolysis reaction. Nitrogen dioxide gas was the dominant species of nitrogen formed during the hydrolysis reaction. From a kinetics study of nitrocellulose hydrolysis and glucose degradation, it was found that the rate of the reaction is related to acid concentration, acid/solid ratio, and temperature.

A complete treatment system, including acid hydrolysis process to decompose nitrocellulose, electrodialysis system to recover the hydrochloric acid used in the acid hydrolysis process, and fermentation to finally convert glucose into ethanol, proved to be a technically feasible alternative to convert waste nitrocellulose into useful products.

ANAEROBIC DIGESTION AND ACID HYDROLYSIS OF NITROCELLULOSE

by
Fong-Jung Tai

A Dissertation
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APPROVAL PAGE

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This dissertation is dedicated to
my beloved family

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CHAPTER 1

INTRODUCTION

Of the various inorganic esters of cellulose which can be made, the only one that has achieved large commercial production is nitrocellulose. In addition to its wide use in industry, this inorganic ester is a versatile material for studying the chemistry of cellulose. Many advances in understanding the structure and properties of cellulose have been derived from studies of nitrocellulose.

Nitrocellulose, more correctly called cellulose nitrate since it is an inorganic ester, is the oldest cellulose derivative. The use of nitrocellulose as a propellant was the first major break from the use of black powder, which was used without change for centuries. At present nitrocellulose based powders are extensively used for the propulsion of bullets, shells, and various missiles for tube munitions. The next major step in the history of nitrocellulose was the development of celluloid, a thin and transparent material for film industry. Nitrocellulose is soluble in a wide variety of organic solvents, such as tetrahydrofuran, ether/alcohol mixtures, ethyl acetate, and acetone, and yields a clear and tough films which are compatible with many plasticizers and resins. Nitrocellulose has also been largely used in chemical industries, such as varnishes, films, adhesives, artificial leather, printing materials, and pharmaceuticals.

Nitrocellulose with high nitrogen content is a principal ingredient in propellants, smokeless powder, and some explosives. Nitrocellulose is currently manufactured by either a batch nitration process or a continuous nitration system. The Radford Army Ammunition Plant (RAAP) generates about 0.2 to 0.9 metric tons (500 to 2,000 lb.) per

day of waste nitrocellulose fines in process wastewater (Kim and Park, 1992). Because of the insolubility of nitrocellulose in water, the suspended solids from this process wastewater are primarily nitrocellulose fines, 50 percent of which are smaller than two micron, μ (Patterson, 1976). Two basic pollutants resulting from the nitrocellulose manufacturing process are nitrating acid rinses and nitrocellulose fines. Acidic rinse waters are discharged to the acid recovery plant for recovery of nitric and sulfuric acids. The recovery water is recycled and reused for the nitration and purification processes. The suspended solids from the manufacturing process are removed from the wastewater using a series of settling pits, lagoons, and a centrifugation system. Sometimes, nitrocellulose fines overflow the treatment reactors and reach the New River.

Nitrocellulose fines have created a water pollution problem for the manufacturing wastewater treatment process in RAAP. Based on the study conducted by Arthur D. Little, Inc., (1987) the US Army Toxic and Hazardous Materials Agency (USATHAMA) recommended removal of nitrocellulose by cross-flow microfiltration followed by alkaline digestion pretreatment, then biological treatment. Other potential alternatives, such as UV/Ozone oxidation, biological treatment by anaerobes, fungus, and composting, and other physicochemical treatments, are also attractive methods for nitrocellulose fines removal, treatment, and disposal.

CHAPTER 2

BACKGROUND

2.1 Characteristics of Nitrocellulose

2.1.1 Manufacturing Process of Nitrocellulose

Nitrocellulose is made by the mixed-acid (including nitric acid, sulfuric acid, and water) nitration of cellulose, a natural high polymer obtained from cotton linters or wood pulp. Figure 1 shows the nitrocellulose batch manufacturing process used in the American Military Munitions Industry (Patterson, 1976).

Pre-purified cotton linters or wood pulp are shredded and dried to remove excess moisture and then treated with mixed nitric and sulfuric acids in "dipping pots" fitted with agitators to esterify most of the hydroxyl groups. Since cellulose nitration is an equilibrium reaction, the extent of nitration at equilibrium is governed primarily by the composition of the mixed acid. Some researchers have found that the maximum nitrogen content that could be obtained was with sulfuric acid : nitric acid ratios between 0.25 : 1 and 3 : 1 (Ott et al., 1954). Enough water is required in this mixed acid solution. The extent of nitration is affected to a lesser degree by the ratio of mixed acid to cellulose (Lunge et al., 1901). The industrial mixture usually consists of 20-30% HNO_3, 55-65% H_2SO_4, and 8-20% water. The nitration temperature is between 10°C (dynamite type) and 36 °C (celluloid type). Even though the reaction is nearly completed after about five minutes, the mixture remains in the reactor for about 30 minutes. The temperature must remain constant (cooling), since hydrolytic degradation processes that lead to considerable losses in yield begin at a temperature as low as 40 °C. In theory, it should be possible to

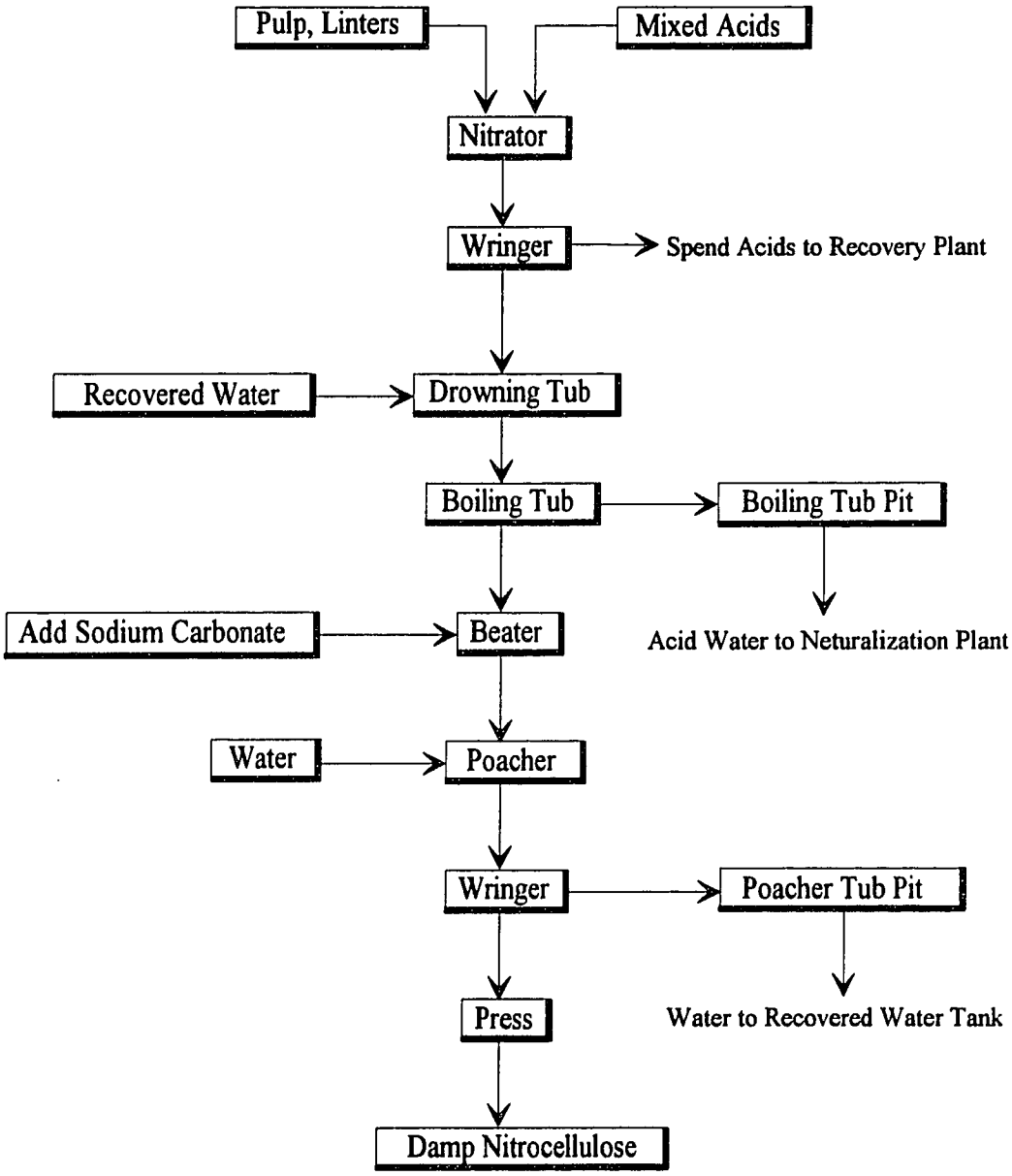


Figure 1 Batch Manufacturing Process of Nitrocellulose (Patterson, 1976)

nitrate all of the hydroxyl groups in cellulose for a nitrogen content of 14.14 percent; but in practice, the most desirable compositions fall between 10.5 to 13.8 percent nitrogen, representing a hydroxyl degree of substitution (D.S.) within 1.8 to 2.9 per glucose anhydride unit rather than the theoretical value 3.0.

After the nitration process the nitrocellulose/acid slurry is passed through a centrifugal wringer which removes the bulk of the spent acids for recovery. The crude nitrocellulose then is pumped as a water slurry to the purification area. The purification processes includes an elaborate series of water washes, boiling treatments (to destroy unstable sulfate esters and nitrates of partially oxidized cellulose by acid hydrolysis), neutralization (with dilute sodium carbonate solution), and heating steps to stabilize the nitrocellulose. After purification, the nitrocellulose is centrifuged to have approximately a 30 percent moisture. It is then processed in accordance with the specific end-use requirements of the batch.

2.1.2 Properties of Nitrocellulose

Nitrocellulose is yellowish-white, odorless, matted mass of filaments, with a specific gravity in the range of 1.58 to 1.65 for commercial usage and it has the appearance of raw cotton. The dry density of commercially available nitrocellulose is between 0.15 to 0.40 Kilogram/Liter and the specific surface of nitrocellulose is 1850-4700 cm^2/gram, depending on the fineness of the nitrocellulose. Its characteristics are dependent on the degree of substitution. Cellulose is a linear polymer composed of individual anhydroglucose units (also called glucopyranose units) linked at one and four position by

glucosidic bonds with beta configuration. The alcoholic hydroxyl groups of cellulose are polar and can be substituted by nucleophilic groups in strongly acid solution. The mechanism of esterification assumes the formation of a cellulose oxonium ion followed by the nucleophilic substitution of an acid residue and the splitting off of water. The esterification reaction from cellulose to nitrocellulose is shown in Figure 2.

The primary hydroxyl group on the C-6 atom reacts most readily, while the neighboring hydroxyl groups on the C-2 and C-3 atoms of the anhydorglucose react considerably slower due to steric hindrance. Basically, esterification is possible with all inorganic acids. Limiting factors are the type and the size of the acid residue as well as the varying degree of acid-catalyzed hydrolysis, which can lead to a complete cleavage of the cellulose molecule as the result of chain splitting. The three hydroxyl groups of cellulose can be completely or partially esterified by nitrating acid. The degree of nitration can be related to the following theoretical nitrogen contents:

Cellulose Mononitrate, $C_6H_7O_2(OH)_2(ONO_2)$	6.75% N
Cellulose Dinitrate, $C_6H_7O_2(OH)(ONO_2)_2$	11.11% N
Cellulose Trinitrate, $C_6H_7O_2(ONO_2)_3$	14.14% N

The degree of nitration is most commonly designated by the nitrogen content expressed as percent nitrogen or, less frequently, as the number of cubic centimeters of NO (at 0 °C and 760 mm pressure) evolved from one gram of nitrocellulose. It is often convenient to designate the degree of nitration by the "Degree of Substitution" (D.S.) which is the average number of hydroxyl groups nitrated per anhydroglucose unit. Nitrocellulose with a nitrogen content between 11.2 and 12.2% is a suitable raw material

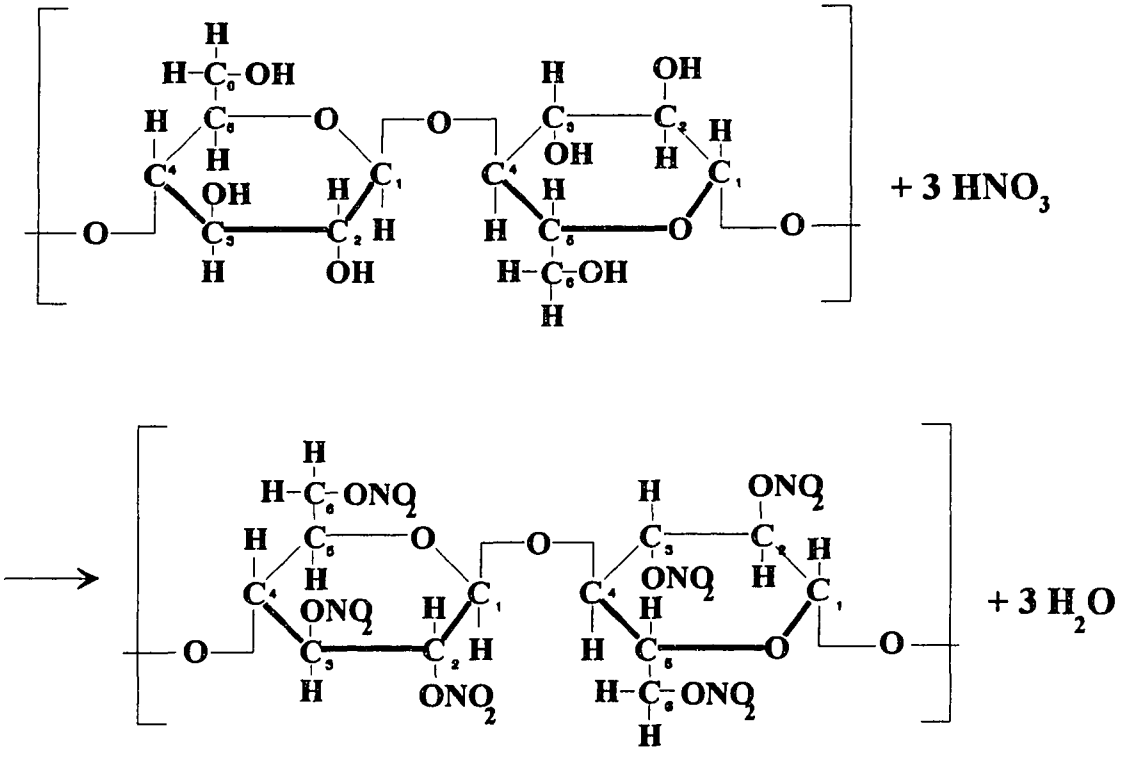


Figure 2 Esterification Reaction from Cellulose to Nitrocellulose

for lacquers, and nitrocellulose with nitrogen content 12.2% or higher is suitable for explosives exclusively (Conaway, 1938).

Dry nitrocellulose is a very powerful explosive and, very sensitive to shock and spark. Its explosive strength depends on the nitrogen content. The higher the nitrogen content the easier it is to explode. In addition, nitrocellulose in dry state is a rather poor conductor of electric static charge and can develop a strong charge which can cause a accidental ignition (Qunichon and Tranchant, 1989). Nitrocellulose mixes with at least 25 percent of water or alcohol are stabilized completely.

Nitrocellulose, like cellulose, is insoluble in water. This property easily allows its preparation, stabilization, and transportation by quenching with water. The solubility of nitrocellulose in organic solvents varies with its nitrogen content. Usually, increasing the solubility also increases the viscosity. Carbonyl compounds, like ketons - acetone, methyl ethyl keton, and cyclohexamone and esters - ethyl acetate, butly, and amyl acetate, are the good solvents for nitrocellulose in industrial use. All nitrate esters including nitrocellulose have poor resistance to acid, and are more stable in basic medium. Treating nitrocellulose with concentrated or slightly diluted acids or bases usually leads to denitration, even destruction (Quinchon and Tranchant, 1989).

2.1.3 Hazards of Nitrocellulose

Nitrocellulose is extremely flammable and, has a flash point of 12.8 °C (closed cup). The melting point and auto ignition range is from 160 to 170 °C (Kim and Park, 1992). Because of its low flash point and highly explosive potential, nitrocellulose is classified as

a highly flammable and explosive (or reactive) hazardous material. According to Resource Conservation and Recovery Act (RCRA), sludge from nitrocellulose manufacturing process wastewater treatment plant is also classified as hazardous waste by code K044 (from specific source). Data drawn from experiments feeding sheep nitrocellulose with regular food shows no negative effect based on blood analysis (Stoller, 1993). Results on the health risk study from contact with nitrocellulose has also shown negligible effect. In view of the nontoxic nature of nitrocellulose, turbidity and palatability have been used as the guidelines for drinking water standard. Nitrocellulose may blanket benthic habitats and limit available oxygen in receiving water producing significant abiotic environmental effects.

2.1.4 Waste from Nitrocellulose Manufacturing Process

Recent data indicated that Army Ammunition Plant generates about 0.2 to 0.9 metric tons (500 to 2,000 lb.) waste nitrocellulose fines from manufacturing process everyday. The volume of wastewater ranged from 16 to 100 gallons for every pound of nitrocellulose produced (Kim and Park, 1992). Since nitrocellulose is insoluble in water, the suspended solids are primarily fine nitrocellulose fibers, 50 percent of which are smaller than two micron (μ). Tables 1 and 2 show the detailed volume and characteristics of wastewater generated from the nitrocellulose manufacturing process (Patterson, 1976).

The two basic pollutants resulting from the nitrocellulose manufacturing process are nitrating acid rinses and nitrocellulose fines. Acidic rinse waters are discharged to the acid recovery plant for recovery of nitric and sulfuric acids. The recovered acidic wastes are recycled and reused for nitration. The suspended solids-laden wastewater from

Table 1 Volume of Wastewater Generated from NC Production (Patterson, 1976)

Source	Volume*, gpd	Percent Use
Nitration Cooling	1,000,000	29.1
Boiling Tub	998,000	29.0
Beaters	400,800	11.7
Poachers	343,000	10.0
Blender	423,000	12.1
Wringer	273,800	8.0
Total	3,438,600	100.0

* Flow per Manufactruing Line. NC capacity per line is 120,00-144,000 lb./day

Table 2 Characteristics of Wastewater Produced from NC Manufacturing Process (Patterson, 1976)

Source	pH	TSS, mg/l	COD, mg/l	NO_2+NO_3-N, mg/l
Boiling Tub	1.1-3.9	8.3-10.0	103.5-136.0	277.3-406.8
Beaters	7.2-9.1	140-580	31.0	0.6-4.0
Poachers	5.5-9.8	214-278	72-685	21.1-26.9
Blenders	6.0	463-495	-	30.0-34.0
Wringer	7.4-8.2	343-828	135	-

are recycled and reused for nitration. The suspended solids-laden wastewater from manufacturing processes is treated by a series of settling pits, lagoon, and centrifugation system. Currently, nitrocellulose fines in RAAP wastewater end up as sediments in settling pits or lagoons, some overflow into the New River. Waste nitrocellulose such as floor sweepings are collected and treated in a pit by alkaline digestion and given to hazardous waste disposal contractors for final disposal.

Since nitrocellulose is nontoxic, the measure of total suspended solids (TSS) is used as the water quality criteria. The general water quality criteria for TSS is that settleable and suspended solids should not reduce the depth of the compensation point for photosynthetic activity by more then 10% from the seasonally established average for aquatic life. The current TSS limitation set by the National Pollutant Discharge Elimination System (NPDES) is an average of 40 ppm for a 24-hour composite sample. Radford Army Ammunition Plant currently meets these permit requirements. However, the regulation may become more stringent in the future, and at that time additional removal and treatment technologies of nitrocellulose will be critically needed. Furthermore, Radford Army Ammunition Plant does not have the capability presently to further remove and treat nitrocellulose during mobilization (Kim and Park, 1992).

2.2 Decomposition of Nitrocellulose

The susceptibility of nitrocellulose to degradative processes is a reflection of both of the chemical nature of the cellulose chain molecule and of the substituents along the chain. The extent to which each of these factors contributes to the total effect is dependent upon the type and degree of substitution of the nitrocellulose. Since the material is composed of

macro-molecules, decomposition is caused by changes in physical properties due to the physical, chemical, or biological reactions.

2.2.1 Mechanical Decomposition

Grinding, milling, and cutting are common unit processes in the chemical industry and are employed in order to achieve both size reduction and an increase surface area of the treated substance. These mechanical processes are applied to high polymers such as cellulose and nitrocellulose. In this process the crystal lattice of cellulosic structure is deformed and the degree of polymerization (D.P.) is also reduced (Ott et al., 1954). The mechanism by which the mechanical decomposition occurs is not completely delineated, but it has been attributed to oxidation, hydrolysis, and mechanical rupture. Under the conditions of ball milling, cellulose and nitrocellulose undergoes a lattice structure deformation, chain scission, increase in solubility, and increase in moisture absorbability.

2.2.2 Thermal Decomposition

Nitrocellulose is relatively stable at moderate temperatures in high purity form. Thermal decomposition only becomes detectable at temperatures above 100 °C. The initial step (rupture of the O-NO_2 bond) is followed a series of oxidation reactions. The reaction is catalyzed by the production NO_2 which is responsible for the self-ignition phenomenon in nitrocellulose (Kennedy et al., 1970).

Fowler et al. (1954) tested nitrocellulose (D.S. = 2.2) in an oven with air at 130 °C for various periods of heating time. Results showed that after 17 hours very little change

occurred in the surface chemistry of nitrocellulose. Vandoni et al. (1954) measured thermal decomposition of nitrocellulose at 108 °C. Carbon monoxide and dioxide, nitric and nitrous oxides, methane and nitrogen were found as products of thermal decomposition. Hydrogen cyanide was found by Muraour et al. (1954) when nitrocellulose was ignited in a confined space. The propellant type of nitrocellulose (>12.6% nitrogen) was studied by Wolfrom et al. (1955). A solid residue was formed as a result of thermal decomposition which was characterized analytically. By analyzing homolytic bond scission, the residues were shown to be the fragmented type of oxycellulose nitrate in an extremely low degree of polymerization.

2.2.3 Photochemical Decomposition

Photochemical radiation is capable of cleaving C-C bonds. During photo-decomposition, chain scission, crosslinking, and monomer production, including other small molecular weight fractions, could occur. Random chain scission caused by photodecomposition in ethyl acetate and methanol solution has been found for nitrocellulose at high and low degrees of nitration. The quantum yields for chain scission were about 0.01-0.02 (Kennedy et al., 1970). Nitrocellulose, in film form breaks down under ultraviolet irradiation. The denitration reaction produces NO_2 and HNO_3 as well as organic reducing materials. The latter compound will cause further degradation of nitrocellulose and liberation of nitrogen oxides and instigate the autocatalytic process. Researchers reported that after UV irradiation for a period of time decomposition and certain degree of denitration took place in nitrocellulose. The decomposition products included carbon

monoxide, carbon dioxide, nitrogen gas, and Oxides of nitrogen (Berthelot and Gaudechon, 1965; Kraus, 1965; Oguri et al., 1965).

Some surface degradation of nitrocellulose caused by X-rays has been studied. The degradation process in the surface regions during X-ray exposure involved a decrease in the nitrate concentration and results in the concomitant evolution NO_x. On extended exposure a further nitrogen functionality became evident by the appearance of a peak centered at ~ 400 eV binding energy. After X-ray degradation, a sample showed slight yellowing and conversion from a fibrous character to a powdery form (Kennedy et al., 1970).

A study of destruction nitrocellulose by irradiation of pulsed lasers was conducted by Yang and Ramsey (1993). The laser induced denitration of nitrocellulose was investigated using an ion trap mass spectrometer for gaseous products. Results showed that shorter laser wavelengths seemed to be better for denitration of nitrocellulose. Results also indicated that laser detonation was undesirable for treating nitrocellulose because of a large number of by-products. Pulsed UV laser induced denitration with an appropriate laser intensity appeared to be a technical feasible alternative for nitrocellulose destruction.

2.2.4 Alkaline Decomposition

Previous workers have shown that the action of alkalies, especially potassium or sodium hydroxides, on aliphatic nitrates is not a simple saponification regenerating the alcohol and forming sodium nitrate, but is a profound decomposition yielding also sodium nitrite and

oxidation products of the aliphatic group. The products reported by various investigators on the action of alkalies on nitrocellulose included inorganic nitrate and nitrite, ammonia, oxides of nitrogen, cyanide, carbon dioxide and monoxide, organic acids (oxalic, malic, glycolic trihydroxyglutaric, dehydroxybutyric, malonic, and tartronic acids), sugars, modified celluloses and their nitrates and partially denitrated nitrocellulose (Kenyon and Gray, 1936).

The decomposition of nitrocellulose in aqueous sodium hydroxide was studied in a quantitative manner by Kenyon and Gray (1936). A relatively small amount of carbon dioxide was produced and a relatively large percentage of the nitrate groups was reduced to nitrite. The decomposition of nitrocellulose appeared to be related to time, concentration of alkali, ratio of alkali to nitrocellulose, and the temperature. The oxidative decomposition of the cellulose molecule was accompanied by reduction of the nitrate groups to nitrite groups. The time required to decompose a given weight of nitrocellulose decreased with increasing temperature and alkali concentration but appeared independent to the alkali-nitrocellulose ratio at constant alkali concentration.

Lure et al. (1991) conducted a study for heterophase alkaline hydrolysis of cellulose nitrate in aqueous sodium hydroxide by UV spectroscopy. He concluded that degradation of cellulose nitrate was significantly slower than denitration. And dissolution of the degradation products in the alkaline solution proceeded with higher rate than in neutral or acid solution. The main denitration step involved elimination of HNO_2.

Alleman et al. (1993) conducted an investigation on alkaline hydrolysis by using three types of alkalis at a variety of dosage levels and reaction temperatures. Results

showed that 4 % sodium hydroxide could digest most of the original carbon into a soluble form at 25 °C. The cyanide released was likely to be in the low ppm range. But some uncertainties still remained in this study. Total nitrgoens released from hydrolysis process were only up to 40%. Apparently some of nitrogen was still bounded with organic carbon residuals. The end products from the reaction were also unidentified at that time. The residual solids and liquor from alkaline hydrolysis were tested by a series of respirometric studies for biodegradability. Results show that the residual solids were still relatively recalcitrant to biodegradation. Although the BOD from residual liquor test was much higher than the solids' BOD, by comparing the total soluble organic carbon this BOD value was still too low. A similar study conducted by Wendt and Kaplan (1976) used a modified activated sludge process to treat NaOH-digested nitrocellulose solution. Results indicated a relatively good removal of BOD (88.6 %) but less satisfactory removal of TOC and COD (54.5 % and 55.2 %, respectively). From these two studies, obviously, the soluble form of the organics still exhibit resistance to biodegradation.

2.2.5 Acid Denitration and Hydrolysis

Denitration of nitrocellulose also takes place with treatment by acids, but the reaction is much slower than that with alkalies. Acid denitration of nitrocellulose has been demonstrated by the treatment with mixed acid containing more water than the acid used to produce the nitrocellulose. In this case, the esterification equilibrium shifts in the direction of lower nitrogen content. One practical aspect of this behavior is observed in

the denitration of nitrocellulose which occurs while wringing out the spent acid. This denitration is caused by dilution of the spent acid with moisture from humid air.

Since the acid residue in the esterification process can cause a varying degree of acid-catalyzed hydrolysis, which can lead to decomposition or even a complete cleavage of cellulosic molecules as the result of statistical chain splitting, denitration and cleavage of the cellulosic structure caused by acids and acid hydrolysis shows attractive potential to treat the waste nitrocellulose fines. Little information is available for a detail study of acid hydrolysis to treat nitrocellulose fines, but acid hydrolysis has been used to convert cellulose, which has a formula structure similar to nitrocellulose, to useful products for a very long time.

Lure et al. (1991) conducted a study on chemical transformation of cellulose nitrate with aqueous sulfuric acid by UV spectroscopy. This study concluded that denitration occurred basically within the fibers and the rate of denitration was faster than rate of degradation. Denitration was accompanied by a series of oxidation-reduction reactions, the form of HNO_3 reduction products (NO, N_2O, N_2) and the oxidation of organic compounds (CO and CO_2).

In theory, any mineral acid is effective, but sulfuric and hydrochloric acids are widely used in the acid hydrolysis of cellulosic material because of their lower costs. Between sulfuric and hydrochloric acids, hydrochloric is used by most industries because it is easier to recover from the process. Glucose is the major product of acid hydrolysis of cellulosic materials. Glucose yields range from 40 % to almost 100 % depending on acid concentration, heating temperature, and reaction time (Goldstein et. al., 1985 and 1992).

Because it has the same crystal cellulosic structure as cellulose, nitrocellulose can be treated by the acid hydrolysis process to produce large amount glucose.

2.2.6 Biological Degradation

Biological degradation is chemical change in nature. However, it is not considered chemical degradation since the source of the attacking chemicals are microorganisms, such as fungi and bacteria. These chemicals are of a catalytic nature, e.g., enzymes. The susceptibility of a polymer to microbial attack generally depends on the enzyme availability for the polymer for, enzyme specificity of the polymer, and presence of a coenzyme, if required.

Little work has been done for biological treatment of nitrocellulose, because nitrocellulose was reported to be extremely bioresistant. Bokomy (1965) found that mold, e.g., aspergillus, grew on nitrocellulose in a medium comprising an aqueous solution of mineral salts. He suggested that nitrocellulose provides the mold with essential carbon, and perhaps nitrogen. Malenkovic (1965) and Jacque (1965) concluded that only the mineral salts dissolved in water and various organic substances, such as incompletely nitration cellulose were used by the mold. However, Hubregste (1978) conducted a feasibility study on treatment of nitrocellulose lime sludge and oxidation of nitroglycerin from wastewater stream. He found that nitrocellulose was only slowly degraded in landfills. Lacey (1980) reported fungal growth on gunpowder which caused deterioration.

Brodman et al. (1981) conducted a study using microorganisms for partial denitration of nitrocellulose-based small arms propellants, in order to gain burning rate

control. He reported that *Aspergillus fumigatus* was found to grow on gunpowder suspended in a nitrogen deficient, carbon supplemented medium, but no growth was observed under the same conditions when carbon source was absent. He concluded that nitrate was released from nitrocellulose by hydrolysis of the nitrocellulose nitrate ester group which was enhanced by the microorganisms. But *Aspergillus fumigatus* did not directly attack the nitrocellulose. Gallo et al. (1993) conducted an investigation using three different fungus, *Phanerochaete chrysosporium*, *Aspergillus fumagatus*, and *Actinomycetes*, to evaluate the potential degradative capability of fungus. Results showed that none of the tested organisms utilized nitrocellulose as a carbon source under the surveyed conditions. However, some nitrocellulose hydrolysis did occur when it was cultured with *Aspergillus fumagatus* and *Actinomycetes*.

Williams et al. (1989) also reported significant removal efficiency of nitrocellulose in soil by composting. A field demonstration of using static pile composting technique for nitrocellulose-contaminated soils was conducted by Roy F. Weston, Inc. (1993) at the Badger Army Ammunition Plant (BAAP) in Baraboo, Wisconsin. In this study, the contaminated soil and sediment is mixed with organic amendments (bulking agents/carbon sources) to enhance microbial metabolism and contaminants destruction. Results showed that the removal efficiency ranged from 26 % to almost 100 % for extractable nitrocellulose.

CHAPTER 3

OBJECTIVES

Although nitrocellulose production technology research and development has a long history, there has only been limited research on nitrocellulose waste treatment and disposal. Separation techniques employed in removing nitrocellulose fines from manufacturing wastewater include centrifugation, microfiltration, coagulation/ flocculation and sedimentation, and air flotation. However, the removal efficiency is low with these physical separation techniques because nitrocellulose fines are small and their sizes are distributed over a wide range. Another problem with the separation of nitrocellulose fines is that sludge produced from manufacturing process is listed as a hazardous waste because of its reactivity, which makes its disposal expensive.

Some early researches showed that even a very small degree of substitution in molecular structure of cellulose can render it resistant to microbial breakdown (Siu et al, 1949; Siu, 1951). Since nitrocellulose used by military has very high degree of substitution (about 2.3 to 2.9), it is believed the nitrocellulose is quite resistant to microbial attack. But, the biological studies conducted by Roy F. Weston, Inc., using static pile composting for treating nitrocellulose contaminated soils, showed that 26 % to 100 % nitrocellulose reduction was possible in more than one hundred days. The amendment mixtures used in this composting study included alfalfa leafs (chopped and whole), woodchips, and manure. Most of these amendments contained cellulose. Since cellulose has been shown to be more readily biodegradable in anaerobic conditions, it is possible that anaerobic microorganisms can be used to degrade nitrocellulose as well.

The residual acids remaining in nitrocellulose from the manufacturing process can cause nitrocellulose to be unstable and accelerate the rate of decomposition (Ott et al., 1954). This decomposition results from the cleavage of the cellulosic molecule and chain splitting. Therefore, acid is a possible alternative way to treat nitrocellulose due to its ability to accelerate the hydrolysis process. Acid catalyzed hydrolysis has been used for a long time in wood industry and agricultural waste treatment to convert cellulose to useful products (Goldstein et al., 1985 and 1992). Additionally, nitrocellulose is a cellulose derivative. They have similar chemical and physical crystal structure. Acid hydrolysis should be as effective treating nitrocellulose as it is for cellulose.

In order to study these two potential alternatives mentioned above, this investigation was divided into three major phases and the objectives of study are as follows:

3.1 Anaerobic Treatment Process

- To study the effects of substrate and inducers concentration on anaerobic biodegradation of nitrocellulose. This treatability study used both serum bottle and biochemical methane potential technique by controlling the concentrations of nitrocellulose and inducers/ nitrocellulose ratios at neutral pH condition. Lactose, cellobiose, and cellulose were selected as enzymatic inducers in this study. The biogas, volatile acids, nitrite, nitrate, and ammonia produced were used as the parameters to evaluate the system performance.
- To investigate the nitrocellulose biodegradation with co-substrates by using two batch type of single-stage anaerobic reactors.

- To study the effects of pH values in enzymatic inducers. A treatability study using anaerobic biodegradation on nitrocellulose was performed by controlling the concentrations of nitrocellulose and inducers at different pH values. The biogas, soluble chemical oxygen demand, volatile acids, nitrite, nitrate, and ammonia produced were used as the parameters to evaluate the system performance.
- To evaluate a batch two-stage anaerobic system to enhance the biodegradation of nitrocellulose by separation of acidogenesis and methanogenesis phases.
- To evaluate a batch staged-feed anaerobic system to study the enhancement of the biodegradation of nitrocellulose.
- To investigate the inhibition caused by adding nitrocellulose into an anaerobic biological system.

3.2 Hydrochloric Acid Hydrolysis of Nitrocellulose

- To study the feasibility of acid hydrolysis of nitrocellulose, a series of tests were conducted by controlling the concentration of acid, reaction time and heating temperature, within a moderate temperature range at ambient pressure.
- To predict the hydrolysis reaction of nitrocellulose with hydrochloric acid using the kinetic model modified from Saeman's work (1945).
- To identify the degradative intermediate and end products in acid hydrolysis of nitrocellulose, and to analyze the material balances for both carbon and nitrogen contents.

- To study the mechanism of hydrolysis reaction and, to evaluate the optimal operational condition. Glucose yield, other small molecular weight organic acids, nitrite, nitrate, and ammonia were measured.

3.3 Proposed Nitrocellulose Treatment Method

- To recommend a complete treatment process that fully convert waste nitrocellulose into useful products, by combining the results of this study with the currently available technologies.

CHAPTER 4

MATERIALS AND METHODS

Pure nitrocellulose was obtained from Radford Army Ammunition Plant (RAAP) in Virginia. The nitrogen content was about 13.5 %. Nitrocellulose received from RAAP was mixed with a large amount of water. Deionized water was added to nitrocellulose mix and allowed to sit to expel alcohol overnight. Then it was dried at room temperature for 12 to 16 hours. The air dried nitrocellulose was then put into a vacuum oven (NAPCO vacuum oven model 5831, Fisher Scientific Inc.) at a pressure of two to five cm of mercury at 65 °C for four hours to evaporate all water then placed in a desiccator (White, 1962). After the drying process, the nitrocellulose was ready to be used for all tests in this investigation. Cellulose (type 20, 20 μ average particle size), crystalline D-(+)-cellobiose, lactose (sugar milk), and other chemicals used in this study were obtained form Sigma Chemical Company, St. Louis, MO.

4.1 Anaerobic Treatment Process

Two types of enzyme systems are usually utilized by bacteria for their cellular activities and energy conversion. Biosynthetic enzyme systems provide essential amino acids and other intermediates which are essential for growth and other cellular activities. Organic compounds are converted by the second enzyme system, the catabolic enzyme system, into simpler growth substrates and energy. Biosynthetic enzymes are often produced continuously, while on the other hand catabolic enzymes usually require induction by the degradation products of interested compounds. These kinds of enzyme systems can be substrate specific but some are not. Sometimes compounds of similar structure,

degradation products, or earlier precursors may induce these enzymes (Babcock and Stenstrom, 1993).

Because its chemical structure is similar to that of nitrocellulose, cellulose was utilized as the inducer in this study. Both cellulose and nitrocellulose are linear polymers composed of individual anhydroglucose units linked at 1 and 4 positions through glucosidic bonds with beta configuration. The only difference is that the hydroxyl groups of cellulose were esterfied by nitro- groups in nitrocellulose. As it was mentioned earlier, it is possible to treat nitrocellulose contaminated soil with a composting technique. In this application, contaminated soil was mixed with bulking agent and amendment materials which contained large amount of cellulose. Therefore, cellulose would be a good candidate for a co-substrate to enhance biodegradability in nitrocellulose treatment. Cellobiose consists of two anhydroglucose units and is the degradation product of cellulose. To provide the degradation products for biological hydrolysis, cellobiose was chosen as another enzymatic inducer in this investigation. Upon hydrolysis, the molecule of lactose, or milk sugar, is split to yield a molecule of glucose and a molecule of galactose. Glucose is the major degradation product from the hydrolysis of cellulose. Therefore, lactose was considered as the third inducer in this study.

For many enzymes, the rate of catalysis, V, varies with the substrate concentration, [S]. V is defined as the number of moles of product formed per unit time. At fixed concentration of enzyme, V is almost linearly proportional to [S] when [S] is small. At high [S], V is nearly independent of [S]. In 1913, Leonor Michaelis and Maud Menten proposed a simple model to account for these kinetic characteristics. The critical feature

in their system is that a specific ES complex is a necessary intermediate in catalysis. The model proposed, which is the simplest one that accounts for the kinetic properties of many enzymes, is

$$E + S \underset{k_2}{\overset{k_1}{\Leftrightarrow}} ES \xrightarrow{k_3} E + P$$

An enzyme, E, combines with S to form an ES complex, with a rate constant k_1. The ES complex has two possible fates. It can dissociate to E and S, with a rate constant k_2 , or it can proceed to form product P, with a rate constant k_3. After rearrangement and substitution, the Michaelis-Menten equation results :

$$V = V_{max} \frac{[S]}{[S] + K_M} \qquad \text{Eq.(1)}$$

where K_M is Michaelis constant and V_{max} is the maximal rate. K_M is equal to the substrate concentration at which the reaction rate is half of its maximal value. The Michaelis constant and the maximal rate can be readily derived from rates of catalysis measured at different substrate concentrations. A plot of 1/V versus 1/[S], called a Lineweaver-Burk plot, yields a straight line with an intercept of $1/V_{max}$ and a slope of K_M/V_{max}.

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} x \frac{1}{[S]} \qquad \text{Eq.(2)}$$

In enzyme catalysis, some specific molecules and ions can inhibit the enzymatic activity. In the presence of competitive inhibitor, the Michaelis-Menten equation is replaced by

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}(1 + \frac{[I]}{K_i})(\frac{1}{[S]}) \qquad \text{Eq.(3)}$$

in which [I] is the concentration of inhibitor and Ki is the dissociation constant of the enzyme-inhibitor complex.

The master culture of mixed anaerobes was taken from an anaerobic digester at Bergen County Wastewater Treatment Plant, in Little Ferry New Jersey and acclimated to a defined synthetic wastewater, as shown in Table 3. This defined media provided sufficient amounts of nitrogen and phosphate for organisms metabolism. The necessary mineral materials were also provided to insure anaerobes' growth. The acclimation system consisted of a four-liter flask reactor and gas collection devices as depicted in Figure 3. Gas produced was measured using a wet tip gas meter. The reactor was maintained at 35 °C by a constant-temperature waterbath or a temperature-controlled chamber. The pH was controlled in neutral condition by the addition of sodium bicarbonate as a buffer. Hydraulic Residence Time and Sludge Retention Time of anaerobic system were sustained at 20 days in this study.

After a period of microbial acclimation to cellulose as the sole carbon source, Biochemical Methane Potential (BMP) and Serum-Bottle Technique were used to test the anaerobes' activeness and substrate toxicity for all experiments (Owen et al. 1979). The BMP assay was conducted by introducing 80 ml of deoxygenated defined media, as shown in Table 3, into a 125 ml serum bottle and the bottle was sealed with butyl rubber septum stopper and aluminum seal to prevent further oxygen contamination. Then 20 ml of anaerobic sludge from the master culture was injected into the deoxygenated and negative pressure serum bottle with air-tight syringe. After 1 hour of equilibration in a 35 °C

Table 3 Composition of Defined Media

Ingredient	**Conc, mg/l**	**Ingredient**	**Conc, mg/l**
Nutrients		**Minerals**	
KH_2PO_4	500	$CaCl_2$	150
Na_2SO_4	150	$MgCl_2.6H_2O$	200
NH_4Cl	530	$FeCl_2.4H_2O$	20
		$MnCl_2.4H_2O$	0.50
Buffer		H_3BO_3	0.25
$NaHCO_3$	3000	$ZnCl_2$	0.25
		$CuCl_2$	0.15
		$Na_2MoO_4.2H_2O$	0.05
		$CoCl_2.6H_2O$	2.50
		$NiCl_2.6H_2O$	0.25

waterbath, gas volume was "zeroed" to ambient pressure by a pre-lubricated syringe and the bottle was ready for further testing.

Currently, there is no "standard" analytical method for the measurement of nitrocellulose in soil, compost, and sludge. An indirect method is used to extract nitrocellulose from soil, compost, and sludge. It hydrolyzes the nitro-groups in nitrocellulose, separates nitrate or nitrite, and measures the liberated nitrite colorimetrically. The disadvantage of this method is that the nitrogen content or degree of substitution (D.S.) of the nitrocellulose must be known. It converts the nitrite

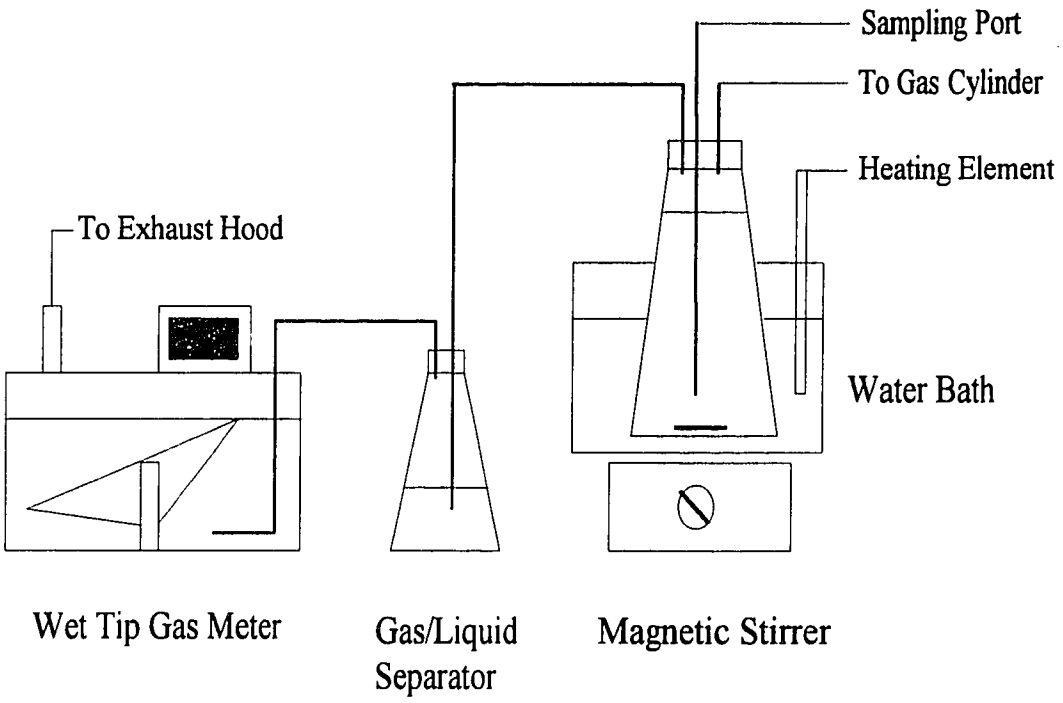


Figure 3 Schematic Diagram of Anaerobic Treatment System for Master Culture of Mixed Anaerobes

measurements to nitrocellulose concentrations. Incomplete separation of nitrate/nitrite ions coextracted from the residue leads to over-estimation of nitrocellulose, and incomplete extraction and/or hydrolysis of the nitrocellulose causes a low bias to the nitrocellulose estimations. Additionally, this method provides no information about the condition of nitrocellulose. Griest (1993) proposed a size exclusion chromatography (SEC)-base method to analyze nitrocellulose in soil, compost, or sludge. The method has the potential of providing both quantitative (e.g. concentration of nitrocellulose) and qualitative (e.g. molecular weight distribution, functional groups) information. This method is still under investigation and has some technical difficulties to overcome. Because of the reasons mentioned above, indirect parameters, such as biogas production and volatile organic acids contained in solution, were used to evaluate the biodegradability of nitrocellulose by anaerobic microorganisms in this study.

The concentration of volatile organic acids were measured by the distillation method in accordance with Standard Methods (Method 504 B). The sample was first filtered and 100 ml of filtrate was distilled with 5 ml of concentrated sulfuric acid and 100 ml of deionized water. Exact 150 ml of distillate was then titrated with 0.1 N standard sodium hydroxide solution. Volatile organic acids were expressed as mg volatile acids as acetic acid per liter. This technique can recover acids containing up to six carbon atoms.

The biogas produced in the anaerobic reactor was collected in a gas collection tube. The retaining solution contained saturated sodium chloride and five percent of sulfuric acid to prevent the biogas from dissolving in the solution. The volume of gas produced was measured as the volume of liquid displaced. The gas produced in the BMP

test was determined by the pressure change in the bottle with a 35 ml pre-lubricated syringe equipped with a 20-gauge needle (Owen et al. 1979). The compositions of biogas were analyzed by Gow-Mac Series 550P Gas Chromatograph equipped with Thermal Conductivity Detector, CTR1 Alltech Column, using helium as the carrier gas.

The amounts of nitrate and nitrite from biotransformation of nitrocellulose were measured by EPA method B-1011 (EPA Test Method 300.0) using single column Ion Chromatograph (Water Series 600E controller and pump, 715 WISP sample injector, and UV detector set at 214 μm wavelength). Sample was filtered through 0.22 μ filter disk paper, C18, and Hg-Ag pretreatment cartridges to remove organics and chloride ion. One hundred μL of pretreated sample was injected into IC for analysis. The concentration of ammonia was analyzed following Standard Methods-Nesslerization method (Method 417). An Orion 407A pH meter was used to measure the pH.

4.2 Acid Hydrolysis

4.2.1 Hydrochloric Acid Hydrolysis of Nitrocellulose

Acid hydrolysis of cellulosic materials has been studied for many years. The degradation of cellulosic materials to sugar seems, at first, to be a hydrolytic cleavage of the glucosidic bonds. However, cellulosic materials behave fundamentally different from other carbohydrates in hydrolysis. The glucosidic bonds are cleaved relatively easily, but the crystalline structure is far more resistant to heterogeneous hydrolysis by dilute acids than similar, but non-crystalline, carbohydrates. Over a hundred years ago, it was found that highly concentrated hydrochloric acid is a very effective hydrolytic agent. A considerable

amount of experimentation has been performed to study the kinetics of acid hydrolysis of pure cellulose substrates. In a cellulose study, researchers depicted the acid hydrolysis of cellulose as a pseudo-first-order sequential process (Saeman, 1945). The reactions can be described by the following equation.

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \text{Decomposed Products}$$

Hydrolysis of cellulosic materials and its product, glucose, play a central role in the conversion of renewable resources to foods, fuel, and chemical feedstocks. Cheap glucose would not only find demand in the food sweetener market but could serve as a substrate for the production of fuel, alcohol, and protein. There are many organisms that can grow on glucose compared with other substrates. Also, with glucose substrates there should be less problem with undesirable or toxic residues.

Little experiment has been performed on acid hydrolysis of nitrocellulose. Also because of their similar chemical structure, acid hydrolysis would seem to be an attractive treatment process for nitrocellulose. However, there is a drawback in this process. Since large amount of concentrated hydrochloric acid is used in this process, economically, it is not practical. Therefore, recovery the acid and conversion of the glucose to useful final products will be the critical factors for implementing this process economically. Several technologies have been studied and developed to recover the concentrated acid from hydrolysis process. Some of them have been proved to be successful. The research conducted by Goldstein and Easter (1992) showed potentially large saving in recovery costs by electrodialysis. Since fermentation of glucose and acid recovery have been

proved to be feasible, the experiments along these lines were not conducted in this investigation.

Chemical reaction rates generally increase with increase temperature. In general, variations in reaction rate as a function of temperature can be represented by the Arrhenius equation,

$$k = A_f(e^{-Ea/RT}) \qquad \text{Eq. (4)}$$

or

$$\ln k = \ln A_f - Ea/RT \qquad \text{Eq. (5)}$$

where k = the rate constant; time^{-1}

A_f = Arrhenius frequency factor;

Ea = activation energy; Kcal/mole

R = universal gas constant; 1.987 g-cal / g-mole-°K, and

T = absolute temperature, °K

The energy of activation determines that fraction of the total number of molecules which can be sufficiently activated at a given temperature that will react; therefore the magnitude of activated energy is a direct determination of the rate of particular chemical reaction. The study of chemical hydrolysis involves the effect of retention time, reaction temperature, substrate/acid ratio and the concentration of hydrochloric acid.

In this experimental study, 0.4 gram nitrocellulose was placed in glass tubes with various amounts of concentrated hydrochloric acid (about 38%) with predetermined acid/solid ratios. These tubes were put into a water bath controlled at a designated temperature. Tubes were then removed from the water bath at various intervals, quenched in ice water, and analyzed for glucose content.

The concentrations of glucose were determined using a Sigma glucose diagnosis kit (enzymatic-colorimetric) with spectrophotometer at wavelength 425 μm. A standard glucose solution of 1,000 mg/l was also used as standard to calibrate the measurements. Small molecular weight organic acids were measured by a HPLC (Water 6000A solvent delivery system, Water 410 Differential Refractometer equipped with Refractive Index Detector and carbohydrate column and Varian 4270 Integrator). Organic acids were identified by comparing them with standard organic acids in terms of retention time for each peak. Nitrite, nitrate, and ammonia were determined essentially the same methods used in anaerobic treatment process.

4.2.2 Approach to Estimate the Kinetic Constants of Acid Hydrolysis

A considerable amount of study has been done on the kinetics of acid hydrolysis of pure cellulose substrates. In a cellulose study, the researchers depicted the acid hydrolysis process of cellulose as a pseudo-first-order sequential process (Saeman, 1945 and Fagan et al, 1971). These theories and models were adapted and compared in this study. Since nitrocellulose concentration is not easy to measure directly, the concentration of glucose was used to develop this kinetic model. the Method of Residuals were employed to estimate the reaction rate constant. The reactions and rate constants can be described by the following equations:

$$\text{Nitrocellulose } (C_x) \xrightarrow{k_1} \text{Glucose } (C_1) \xrightarrow{k_2} \text{Decomposed Products } (C_0)$$

$$dCx/dt = -k_1Cx \qquad \text{Eq. (6)}$$

$$dC_1/dt = +k_1C_x - k_2C_1 \qquad \text{Eq. (7)}$$

$$dC_0/dt = + k_2C_1 \qquad \text{Eq. (8)}$$

where k_1 = rate constant of nitrocellulose hydrolyzed to glucose

k_2 = rate constant of glucose degraded to decomposed products

In these equations, C_x = concentration of nitrocellulose (M), C_1 = concentration of glucose (M), and C_0 = concentration of decomposed glucose products (M); k_1 and k_2 are the rate constants for each individual reaction ($time^{-1}$).

The hydrolysis of nitrocellulose follows a first-order rate equation, hence,

$$C_x = C^0_x e^{-k_1 t}. \qquad \text{Eq. (9)}$$

To find the dependence of C_1 on time, Eq. (7) can be solved by using the integrating factor method. First write Eq. (7) as:

$$dC_1/dt + k_2C_1 = k_1C^0_x e^{-k_1 t}$$

and multiply both sides by $e^{k_2 t}$, the integrating factor, the following expression is obtained:

$$(dC_1/dt + k_2C_1)e^{k_2 t} = k_1C^0_x e^{-k_1 t}e^{k_2 t}. \qquad \text{Eq. (10)}$$

Next notice that

$$dC_1e^{k_2 t}/dt = (dC_1/dt + k_2C_1)e^{k_2 t}. \qquad \text{Eq. (11)}$$

Comparing Eqs. (10) and (11)

$$dC_1e^{k_2 t}/dt = k_1C^0_x e^{(k_2-k_1)t}$$

which can be integrated to yield

$$C_1e^{k_2 t} = k_1C^0_x e^{(k_2-k_1)t} / (k_2-k_1) + \text{Constant}$$

The constant can be determined by the boundary conditions. Set $C_1 = 0$ at $t = 0$; then the constant equals to $-k_1 C^0_x / (k_2-k_1)$, and the integrated equation becomes:

$$C_1 = k_1 C^0_x (e^{-k_1 t} - e^{-k_2 t}) / (k_2-k_1) \qquad \text{Eq. (12)}$$

For the initial conditions $C^0_1 = 0$ and $C^0_0 = 0$, the mass balance relationship is

$$C^0_x = C_x + C_1 + C_0. \qquad \text{Eq. (13)}$$

Substituting Eqs. (9) and (12) into Eq. (13) and rearranging this equation

$$C_0 = C^0_x + C^0_x (k_2 e^{-k_1 t} - k_1 e^{-k_2 t}) / (k_1-k_2). \qquad \text{Eq. (14)}$$

Clearly, Eqs. (12) and (14) are inapplicable in the special case $k_2 = k_1$. The concentration of glucose is a function of time and the smaller rate constant, k_2, can be estimated from a semi-logarithmic plot of C_1 at later times when C_x is negligible. This plot is extrapolated back to $t = 0$. This line is described by the equation [from Eq (12)],

$$\ln C_1^{ext} = \ln [k_1 C^0_x / (k_1-k_2)] - k_2 t. \qquad \text{Eq. (15)}$$

Combining Eqs. (15) and (12),

$$\ln (C_1^{ext} - C_1) = \ln [k_1 C^0_x / (k_1-k_2)] - k_1 t \qquad \text{Eq. (16)}$$

Graphically, Eq. (16) represents the logarithm of the differences between the experimental C_1 values at early times and values extrapolated from late times (C_1^{ext}). The plots of Eqs. (15) and (16) should have the same intercepts and their slopes yield estimates of the rate constants. Figure 4 shows this technique.

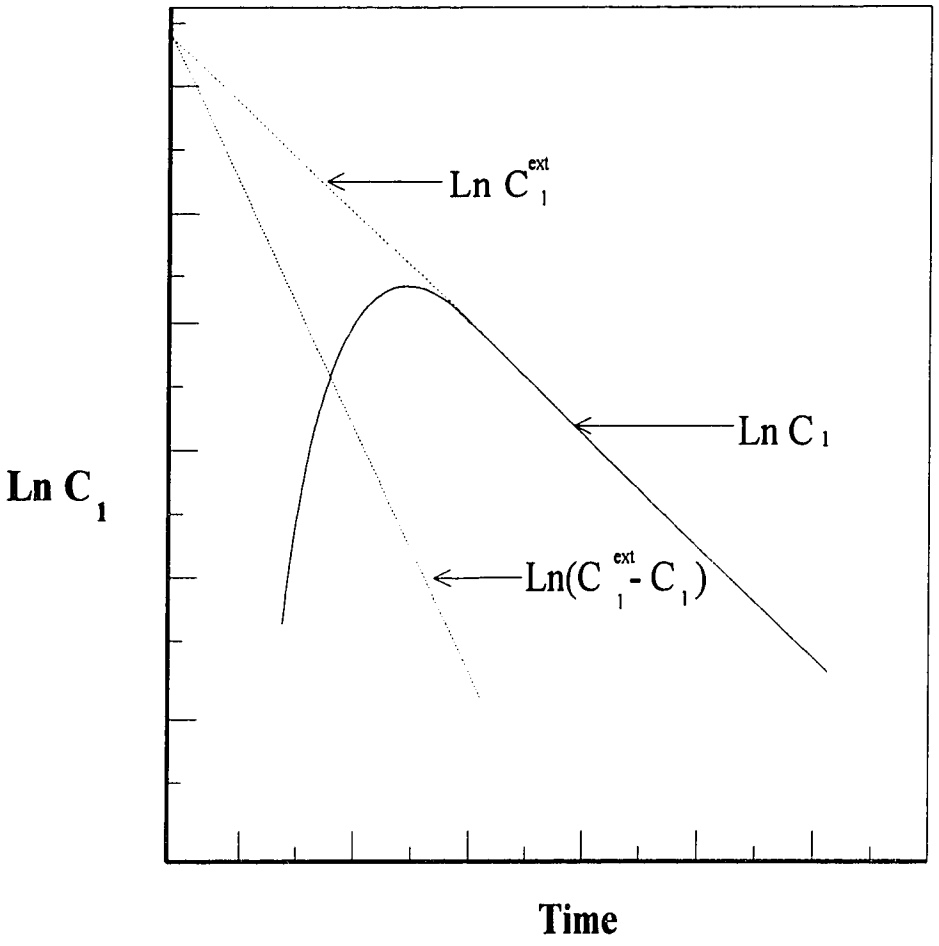


Figure 4 The Method of Residuals (Connors, 1985)

CHAPTER 5

ANAEROBIC TREATMENT PROCESS

The biodegradation of nitrocellulose in anaerobic batch reactors with and without supplemental carbon sources or inducers, such as cellulose, cellobiose, and lactose and, two-stage anaerobic system were investigated in this study. The concept of staged-feed was also conducted to investigate the possibility of biodegradation enhancement of nitrocellulose.

5.1 Effect of Various Enzymatic Inducers

The important concept to test the use of inducer compounds to maintain activity over long periods without the presence of the target compound was pointed out by Grady (1985 and 1986). Babcock and Stenstrom (1993) suggested that an ideal inducer compound would maintain the degradation kinetics and growth characteristics of an enrichment culture without the presence of the enrichment substrate. Sometime, degradative enzyme can be substrate specific. But often it is quiet nonspecific and can be induced by compounds of similar structure or degradation products. This studies try to use this concept to induce the enzyme which can degrade nitrocellulose from different inducers with similar structure or degradation products from target compound.

Three types of enzymatic inducers, namely, cellulose, lactose, and cellobiose, were used in this study. The concentrations of enzymatic inducer were all fixed at 1,000 mg/l and inducer/nitrocellulose ratio was maintained at 10/1. Ten sets of tests were conducted at the same time. Blank one (B-Media) contained only defined media. The data from this blank would give the information of gas production from media itself. Blank two

(B-Culture) contained biomass and defined media. This blank indicates the biodegradation from biomass and media. Three sets of serum bottles were used in experiment, each bottle contains one of the following inducers, i.e., cellulose (C), lactose (L), and cellobiose (CB), respectively. The experiments were conducted in triplicate. These were used as the control groups to evaluate the biogas produced from the bottles containing inducers and nitrocellulose together. Two sets of bottles containing nitrocellulose only, one set had 100 mg/l of NC (NC1) and another set had 1,000 mg/l (NC2). They were also in triplicate. These would provide information on the biodegradation of nitrocellulose without the usage of inducers. These were used for comparison study. Three other bottles containing nitrocellulose and inducers. They are cellulose and nitrocellulose (C-NC), lactose and nitrocellulose (L-NC), and cellobiose and nitrocellulose (CB-NC), respectively. The inducer/NC ratio is fixed at 10/1 in this study.

The results are shown in Figure 5. There were some gases produced in all bottles except in two blanks. It is shown in Figure 5 that there is about 2 days of lag period for lactose and cellobiose, and 3 days for cellulose. Lactose had the highest volume (about 40 ml) of gas production and cellulose had the lowest volume (30 ml). Cellobiose produced less biogas than that from lactose. By comparison the gas produced in bottles with inducers alone and with these had both nitrocellulose and inducers, it can be observed that bottles had inducers alone had more gas produced than these with inducer and nitrocellulose. With the addition of nitrocellulose, lactose and cellobiose were less affected than cellulose. This study shows that nitrocellulose would affect the

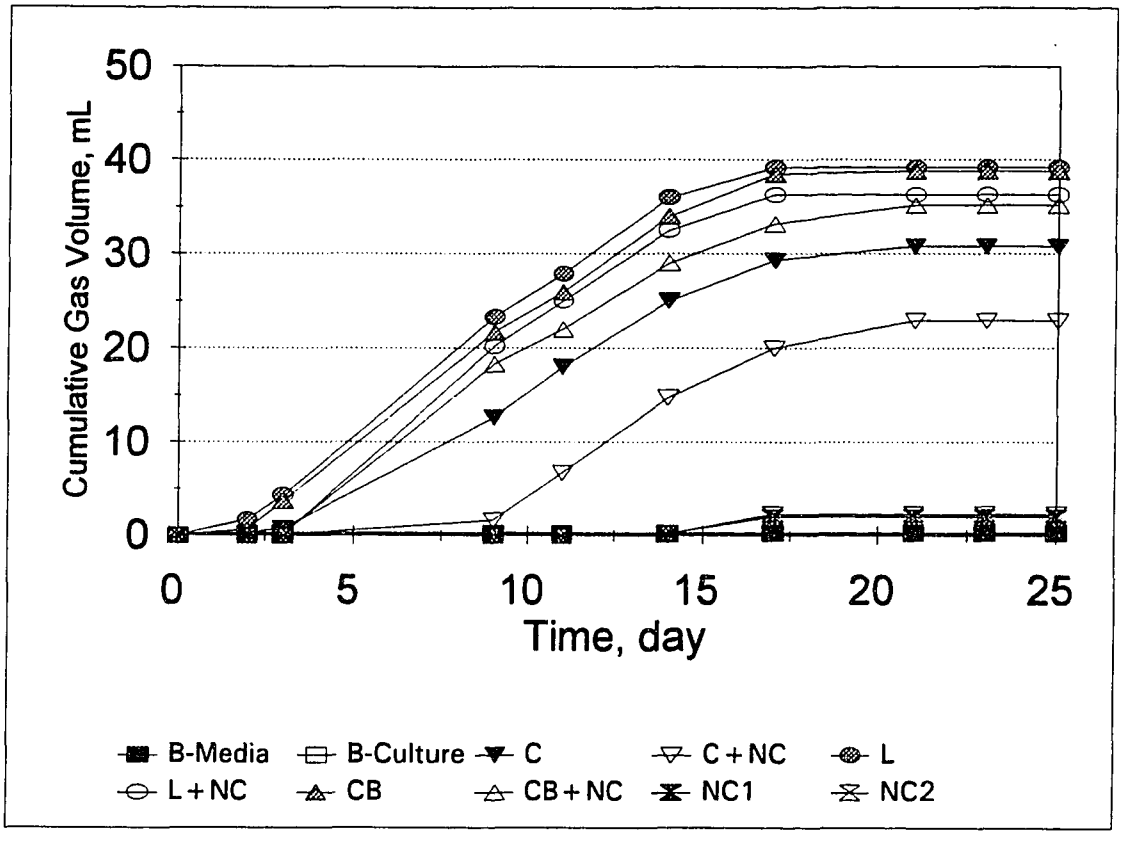


Figure 5 Effect of Various Enzymatic Inducers in Batch Study I

biodegradation of inducers and decrease the gas production. Among all three inducers, cellulose produced least amount of gas. The measured gas productions from this study are compared to the stoichiometric calculated gas production (SGP). The stoichiometric gas production from the utilization of nitrocellulose and cellulose was calculated by using McCarty's approach by assuming that NO_3^- was the main nitrogen source in the medium (McCarty, 1972 and 1969, Duran et al., 1993). The balanced equations for the anaerobic breakdown of each of these substrates are given below;

$C_{12}H_{14}O_{22}N_6$ (Nitrocellulose):

$$C_{12}H_{14}O_{22}N_6 + 6.42H_2O \rightarrow 0.32C_5H_7O_2N + 5.5CH_4 + 3.32CO_2 + 5.68NO_3^- + HCO_3^- + 1.68H^+$$

CH_2O (Cellulose):

$$0.25CH_2O + 0.0089NO_3^- + 0.009H^+ \rightarrow 0.0089C_5H_7O_2N + 0.094CH_4 + 0.112CO_2 + 0.035H_2O$$

Results are shown in Table 4. It is seen in this table that cellobiose has the highest conversion ratio, 57 %, and nitrocellulose has only 5 % of conversion in this study.

Table 4 Comparison of Net Gas Production and SGP
(Effect of Various Enzymatic Inducers Test I)

Desciption	Measured Gas Volume	SGP	Ratio, %
Cellulose	30.5	68.0	44.9
C + NC	22.6	72.0	31.4
Lactose	38.9	72.0	54.0
L + NC	36.0	76.0	47.4
Cellobiose	38.5	68.0	56.6
CB + NC	34.9	72.0	48.5
NC-1	1.7	4.0	42.5
NC-2	2.0	39.5	5.1

unit : mL

After three more months of acclimation, the same experiment was conducted again and results are shown in Figures 6. This results are very similar to the previous experiment. Lactose and cellobiose had almost the same amount of biogas production. However, the addition of nitrocellulose did not affect the biogas production that much, in the presence of these two inducers. Cellulose still produced least amount of biogas among three inducers. The addition of nitrocellulose still affected the biogas production in bottles containing cellulose solution. For bottles containing nitrocellulose only, the samples had also produced less than 7 ml biogas, that is only a little higher than the blank samples.

From the comparison of gas production as an indicator of biotransformation which is shown in Table 5, results are similar to that of Table 4, expect the conversion of cellulose which was dropped from 45 % to 23%, and the conversion of nitrocellulose increased from 5 % to 6 %. Table 5 also shows that nitrocellulose inhibited cellulose degradation in the second test. The percentage gas production reduced from 31.4% to 13.6%.

Table 5 Comparison of Net Gas Production and SGP
(Effect of Various Enzymatic Inducers Test II)

Desciption	**Measured Gas Volume**	**SGP**	**Ratio, %**
Cellulose	15.7	68.0	23.1
C + NC	9.8	72.0	13.6
Lactose	37.9	72.0	52.4
L + NC	39.9	76.0	52.5
Cellobiose	38.3	68.0	56.3
CB + NC	39.5	72.0	54.9
NC	5.3	39.5	6.1

unit : mL

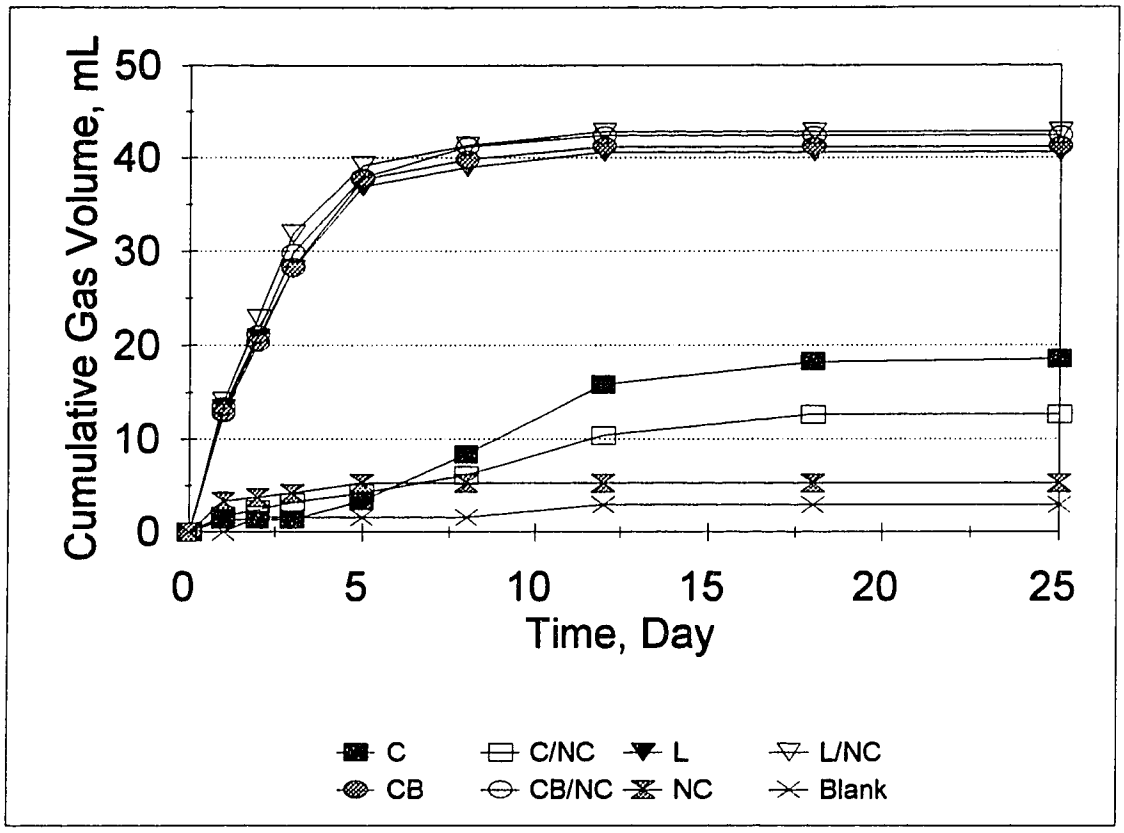


Figure 6 Effect of Various Enzymatic Inducers in Batch Study II
(Inducer/NC = 10/1, Inducer Concentration = 1,000 mg/L)

5.2 Effect of Inducer/Nitrocellulose Ratios

In order to understand the effect of biodegradation caused by different inducer/ nitrocellulose ratios, pre-determined inducer/nitrocellulose ratios were tested. Results are shown from Figures 7 to 9.

In the lactose/nitrocellulose study, the concentration of lactose was fixed at 1,000 mg/L and the amounts of nitrocellulose were changed by the pre-determined inducer/ nitrocellulose ratios. Eight sets of tests were performed. The L/NC ratios were 10/0, 10/1, 5/1, 2/1, 1/1, 1/2, 1/5, and 1/10 as shown in Figure 7. It can be seen in Figure 7 that the lower the lactose/nitrocellulose ratio, the less biogas would accumulate. After 35 days of operation, only these two with ratios of 1/5 and 1/10 had less than 40 ml of biogas accumulation. The biogas produced for other ratios ranged from 40 to 45 ml. The bottle contained with lactose alone still produced more gas than all other bottles in the first 20 days. However these with L/NC ratios of 10/1, 5/1, and 2/1 produced approximately the same amount of gas with control groups after 35 days. Table 6 show that the amount of gas produced were in the range of 31 to 42 milliliter, when the L/NC ratio changed from 10/1 to 1/10 and lactose concentration was remained constant.

Results of comparison of gas production and ratios of conversion are shown in Table 6. The conversion ratios dropped from about 60% to 7% as L/NC ratios changed from 10/0 to 1/10. However, microorganisms were still alive and used lactose as substrate.

For the cellobiose/nitrocellulose study, six sets of CB/NC ratios, 20/1, 10/1, 5/1, 1/1, 1/5, and 1/10, were used in this study. The concentration of cellobiose was fixed at

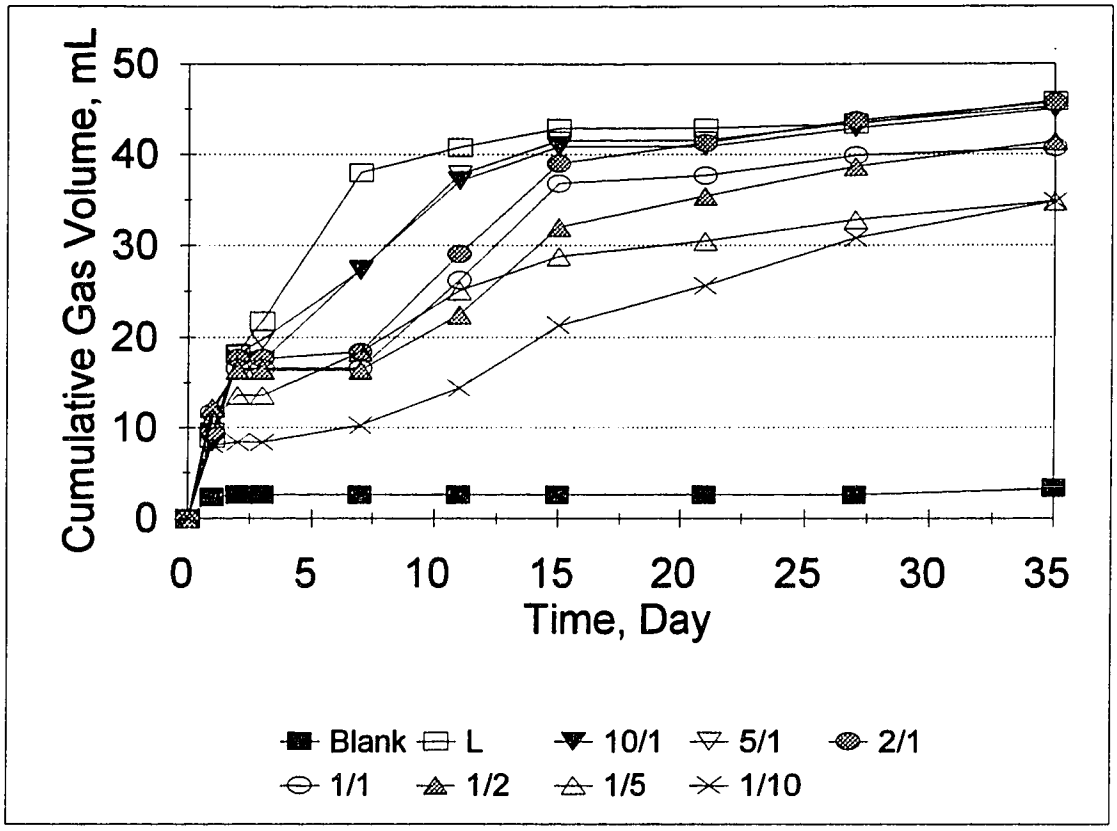


Figure 7 Effect of Various Lactose/Nitrocellulose Ratios in Batch Study
(Lactose Concentration = 1,000 mg/L)

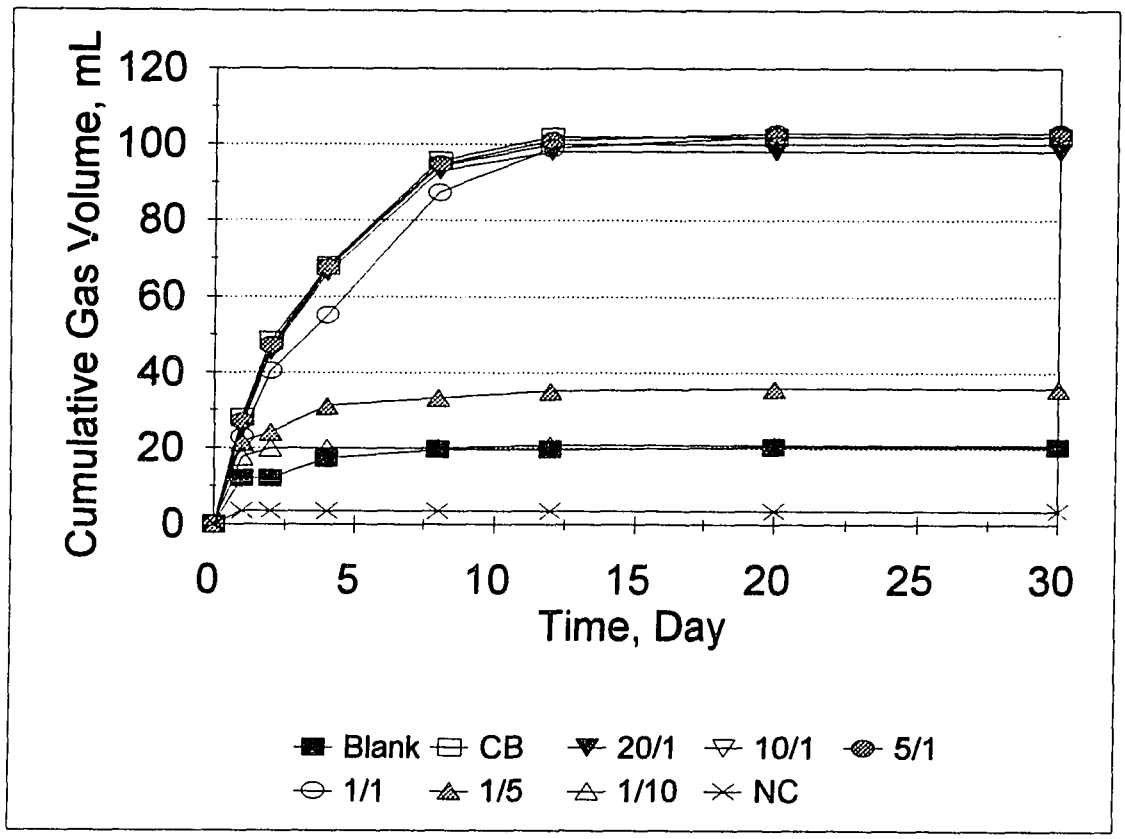


Figure 8 Effect of Various Cellobiose/Nitrocellulose Ratios in Batch Study (Cellobiose Concentration = 2,000 mg/L)

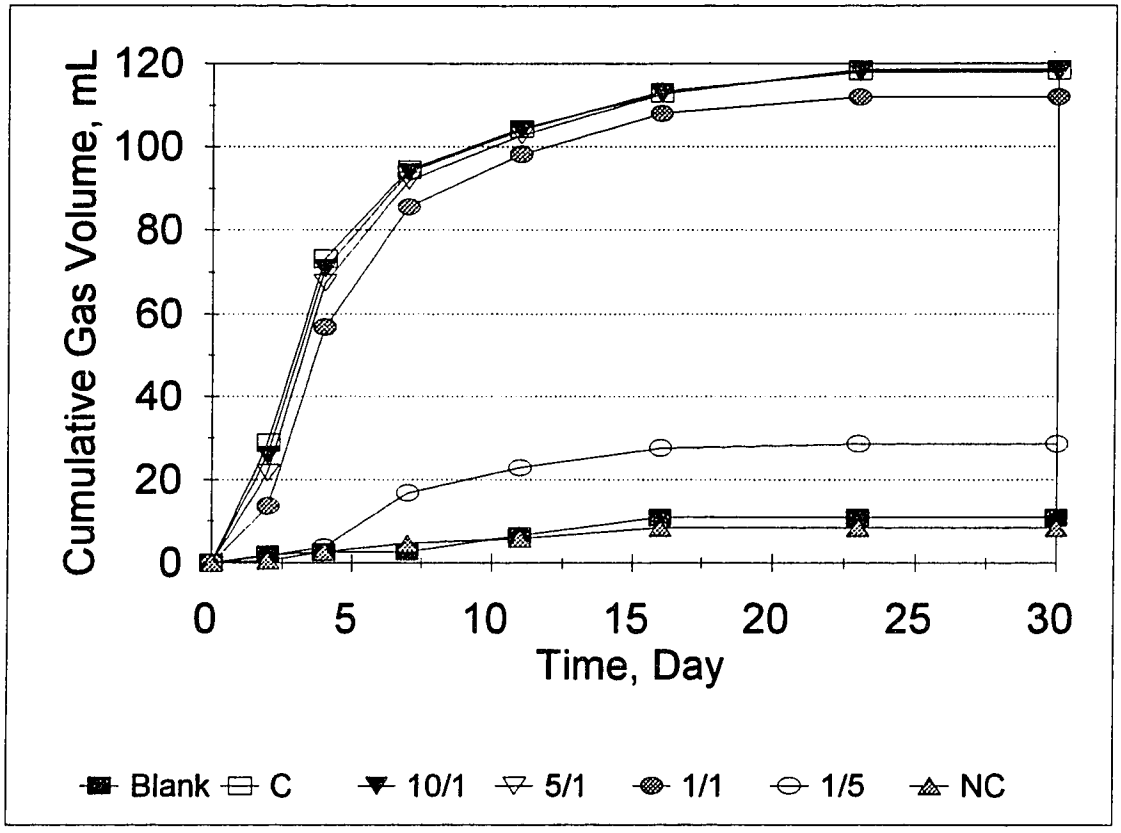


Figure 9 Effect of Various Cellulose/Nitrocellulose Ratios in Batch Study
(Cellulose Concentration = 2,000 mg/L)

Table 6 Comparison of Net Gas Production and SGP
(Effect of Various L/NC Ratios)

Desciption	Measured Gas Volume	SGP	Ratio, %
Lactose	42.6	72.0	59.2
L/NC =10/1	41.7	76.0	54.9
L/NC = 5/1	42.0	78.0	52.5
L/NC = 2/1	42.4	92.0	44.2
L/NC = 1/1	37.4	112.0	32.4
L/NC = 1/2	38.1	152.0	24.4
L/NC = 1/5	31.5	272.0	11.4
L/NC = 1/10	31.5	472.0	6.3

unit : mL

2,000 mg/L and the amounts of nitrocellulose were varied by following the designated inducer/ nitrocellulose ratios. Two sets of blank and control group were carried for comparison study and another set of bottle containing nitrocellulose alone was also used in this study. Figure 8 shows that the biogas production remained the same (about 100 ml) when the CB/NC ratios were higher than 1/1. However, when CB/NC ratio was 5/1 the produced biogas was only one milliliter more than the control group, but that is within the error range. The bottles containing only nitrocellulose produced less biogas than blank. It indicates no nitrocellulose consumption.

Table 7 shows the information of net gas production and SGP (amount of gas measured from test - amount of gas produced from blank). CB/NC ratios of 20/1 and 10/1 have the highest conversion ratio, about 55 %. CB/NC = 1/10 almost had no

Table 7 Comparison of Net Gas Production and SGP (Effect of Various CB/NC Ratios)

Desciption	Measured Gas Volume	SGP	Ratio, %
Cellobiose	81.6	136.0	60.0
CB/NC =20/1	77.7	140.0	55.5
CB/NC = 10/1	79.7	144.0	55.3
CB/NC = 5/1	82.6	176.0	46.9
CB/NC = 1/1	81.7	216.0	37.8
CB/NC = 1/5	15.4	531.0	2.9
CB/NC = 1/10	0.5	926.0	0.05
NC	-	80.0	-

unit : mL

conversion. When CB/NC ratio equals to 1/5, the concentration of volatile organic acids was only about 50 mg/L as acetic acid as compared to 500 mg/L in C/NC study. It seems that microorganisms were inhibited to utilize cellobiose as substrate at high nitrocellulose concentration in microbial hydrolysis. It is seen in Figure 5 that lactose is easier to be used by microorganisms as a substrate than cellobiose and cellulose because of its simple structure. By acclimation, microorganisms can utilize complicate compounds such as cellobiose and cellulose, which can be seen in Table 7. This table shows that at an inducer/nitrocellulose ratio of 10/1, more gas was produced in cellobiose/nitrocellulose (79.7 ml) than lactose/nitrocellulose (41.7ml).

In the cellulose/nitrocellulose study, four sets of C/NC ratios, 10/1, 5/1, 1/1, and 1/5, were used in this study. The concentration of cellulose was kept at 2,000 mg/L and

the amount of nitrocellulose were changed at pre-determined C/NC ratios. Two sets of blank and control group were employed to collect the basic information and another set of bottle containing nitrocellulose was also utilized in this study. Figure 9 shows a similar result as these in the cellobiose study. These with ratios of 10/1, 5/1, and 1/1 had more gas production, but none is more than the control group. The only difference was that when the C/NC ratios were lower than 1/1, more biogas was produced in cellobiose study, approximately 2 ml in the case of inducer/nitrocellulose = 1/5. C/NC ratios higher than 1/1 had the same amount of gas produced, approximate 110 ml.

Table 8 shows the comparison between net gas production and SGP. The ratios are more than 70 % with C/NC ratios higher than 1/1. But none produces more gas than control bottle. However, higher concentrations of volatile organic acids, 300 to 550 mg/L as acetic acid, were found in the bottles with C/NC ratio 1/5. Even for the bottles containing only nitrocellulose, the concentration of volatile organic acids was found to be about 300 mg/L as acetic acid. This shows that some microbial enzymatic hydrolysis did occur during this test. But volatile organic acids could not be utilized by methane-forming bacteria and converted to biogas.

In the inducer/NC tests, a light green-yellow color appeared in the solution containing nitrocellulose, especially in the bottles with low inducer/NC ratios (1/5 and 1/10). Denitration and enzymatic hydrolysis of nitrocellulose might occur in these bottles. The residual sludge of these bottles were dried and extracted by tetrahydrohuran over night. The weight difference of sludge between before and after extraction was used to study the nitrocellulose removal efficiency. The results are shown in Table 9. It is seen in

Table 8 Comparison of Net Gas Production and SGP
(Effect of Various C/NC Ratios)

Desciption	Measured Gas Volume	SGP	Ratio, %
Cellulose	107.5	136.0	79.0
C/NC =10/1	107.1	140.0	74.4
C/NC = 5/1	107.7	156.0	70.9
C/NC = 1/1	101.1	216.0	46.8
C/NC = 1/5	17.7	536.0	3.5
NC	-	80.0	-

unit : mL

Table 9 Nitrocellulos Removal Efficiency in Inducer/NC Study
(by solvent extraction method)

Description	Reduced Weight	Original Weight	Removal Efficiency
L/NC=1/5	339.5	1000	66 %
CB/NC=1/5	212.6	1000	79 %
C/NC=1/5	391.5	1000	61 %
NC	116.6	200	42 %
C/NC=1/1-10D	30.1	200	85 %
C/NC=1/1-15D	23.9	200	88 %
NC-10D	163.7	200	18 %
NC-15D	130	200	35 %

unit : mg

this table that the bottles containing nitrocellulose only had about 40 % nitrocellulose removal and bottles with inducer/NC ratio 1/5 had higher than 60 % removal. This result indicates that nitrocellulose could be converted to other intermediate compounds. But the numbers of these removal efficiency in Table 9 may be overestimated due to the incomplete separation of nitrocellulose and anaerobic sludge by using the extraction of organic solvent that was mentioned in Chapter 4, section 4.1.

5.3 Two-Stage Batch Study

Anaerobic treatment of waste can be put into three steps, namely hydrolysis, acidogenesis, and methanogenesis. Microorganisms use extracellulose enzyme to break down large molecules in hydrolysis, convert complex organic compounds into organic acids in acidogenesis, and produce methane from acids in methanogenesis. Usually, acidogenesis and methanogenesis are taken place in one single reactor and the growth conditions must be kept in good balance for both microorganisms (acid formers and methane formers) to survive.

Some researchers suggested that a two-phase anaerobic processes, one for the acid formation and the other for the methane formation, can enhance the degradation of organic substances. It is especially true when the hydrolysis or the organic matter is overall rate limiting process. Since the biodegradation of nitrocellulose was not successful in the previous studies and the biodegradation was limited by hydrolysis step, a set of experiment by using two-stage anaerobic system was conducted for further study.

To simulate the two-stage anaerobic system, the defined media was controlled at pH = 6.0 to maintain the optimal growth condition for acidogenesis. Then, after two or

four days the system was brought back to neutral condition by adding sodium hydroxide to optimize methanogenesis. Two and four days of acidogenesis periods were used in this study as mentioned above. Two blanks, culture blank (B-C) and media blank (B-M), were used to provide the information about the biogas produced not from target compounds. For each testing acidogenesis period, three sets of tests were conducted. One was provided with 2,000 mg/l cellulose only (C-2D and C-4D), one had cellulose/NC ratio of one to one and both had a concentration of 2,000 mg/l (1/1-2D and 1/1-4D), and the last one with 2,000 mg/l nitrocellulose only (NC-2D and NC-4D). Under normal condition, gas should be produced in the methanogenesis stage. However, it was observed in this study, some gas already be produced in the fourth day. It was also found in the study, as shown in Figure 10, that the gas produced in four-day acidogenesis was much more than these for two-day's. There were not much difference of biogas accumulation for two days or four days acidogenesis from the media contained cellulose alone or cellulose and nitrocellulose. However, the production of gas for bottles containing both cellulose and nitrocellulose was not increased, either. Comparing to the earlier cellulose/nitrocellulose study, gases produced for each condition from two-stage study were much less than single phase study. This could be due to the low pH in the first stage. The low pH will inhibit the growth of methanogensis bacteria and also affect the gas production.

Table 10 shows the comparison between net gas production and SGP. There was no improvement on the use of two-stage anaerobic system, even some reduction of gas production was observed. However, the conversion of nitrocellulose only in four-day acidogenesis period was enhanced from 6 % to 12.5 %. In order to obtain more

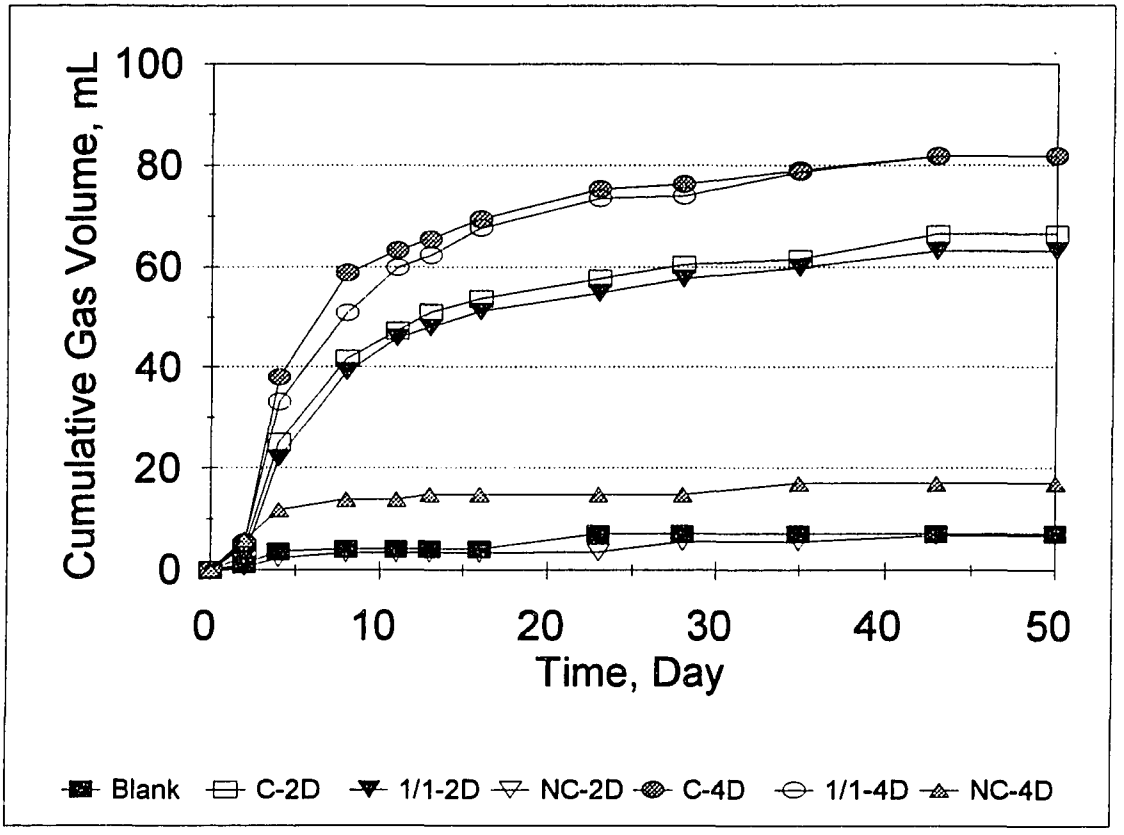


Figure 10 Results of Two-stage Anaerobic Treatment System
(two and four days of acidogenesis period at pH = 6.0)

Table 10 Comparison of Net Gas Production and SGP
(Results of Two-Stage Anaerobic System at pH = 6.0)

Desciption	Measured Gas Volume	SGP	Ratio, %
Cellulose-2D	59.4	136.0	43.9
C/NC-2D	56.0	216.0	25.9
NC-2D	-	80.0	-
Cellulose-4D	74.6	136.0	54.9
C/NC-4D	74.7	216.0	34.6
NC-4D	10.0	80.0	12.5

unit : mL

information on two-stage anaerobic system, another run was conducted in more acidic condition and longer acidogenesis period.

This test was conducted by controlled the mixed liquor at pH = 5.0 to favor the growth of acidogenesis bacteria. Five, ten, and fifteen days of acidogenesis periods were used in this study. The concentrations of cellulose and nitrocellulose were 1,000 mg/L. Results of this study are shown in Figure 11. Comparing to Figure 10, biogas productions in this experiment were less than the previous one. This indicates that the methane forming bacteria may be inhibited under acidic condition for long exposure time. However, the test also shows higher concentration of volatile organic acids (about 550 mg/L as acetic acid) in the solution. Table 11 shows the conversion between net gas production and SGP ratio are much smaller than previous one. This indicates that

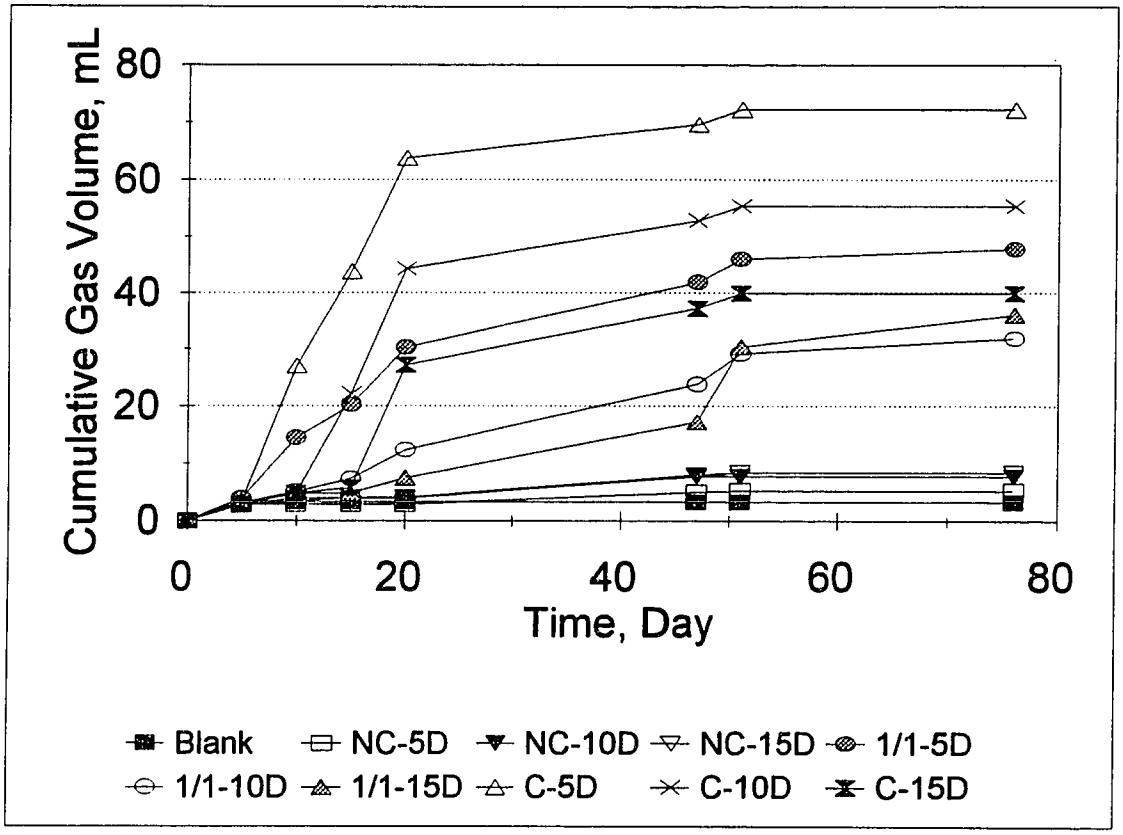


Figure 11 Results of Two-stage Anaerobic Treatment System (five, ten, and fifteen days of acidogenesis period at pH = 5.0)

Table 11 Comparison of Net Gas Production and SGP
(Results of Two-Stage Anaerobic System at pH = 5.0)

Desciption	Measured Gas Volume	SGP	Ratio, %
C-5D	65.5	136.0	48.2
C/NC-5D	41.2	216.0	19.1
NC-5D	2.0	80.0	2.5
C-10D	48.8	136.0	35.9
C/NC-10D	25.3	216.0	11.7
NC-10D	1.2	80.0	1.5
C-15D	33.3	136.0	24.5
C/NC-15D	29.5	216.0	13.7
NC-15D	1.8	80.0	2.3

unit : mL

acidogenesis can be enhanced at lower pH, however, methanogenesis is affected by the low pH. A proper controlled pH and growth environment would be required.

An interesting phenomenon was observed. Three months after the above experiment was conducted, more gas was produced in the serum bottles (about 70 to 90 ml), of which the two-stage anaerobic degradation tests was performed. Then, the microorganisms were transferred to other BMP bottles for further experiment. One bottles contained 2,000 mg/L of cellulose and another 2,000 mg/l of NC. In two other bottles, concentration of cellulose was fixed at 2,000 mg/L and concentrations of nitrocellulose were varied by changing C/NC ratio. The results are shown in Figure 12. Some differences can be seen by comparing Figures 9 and 12. More gas was produced in

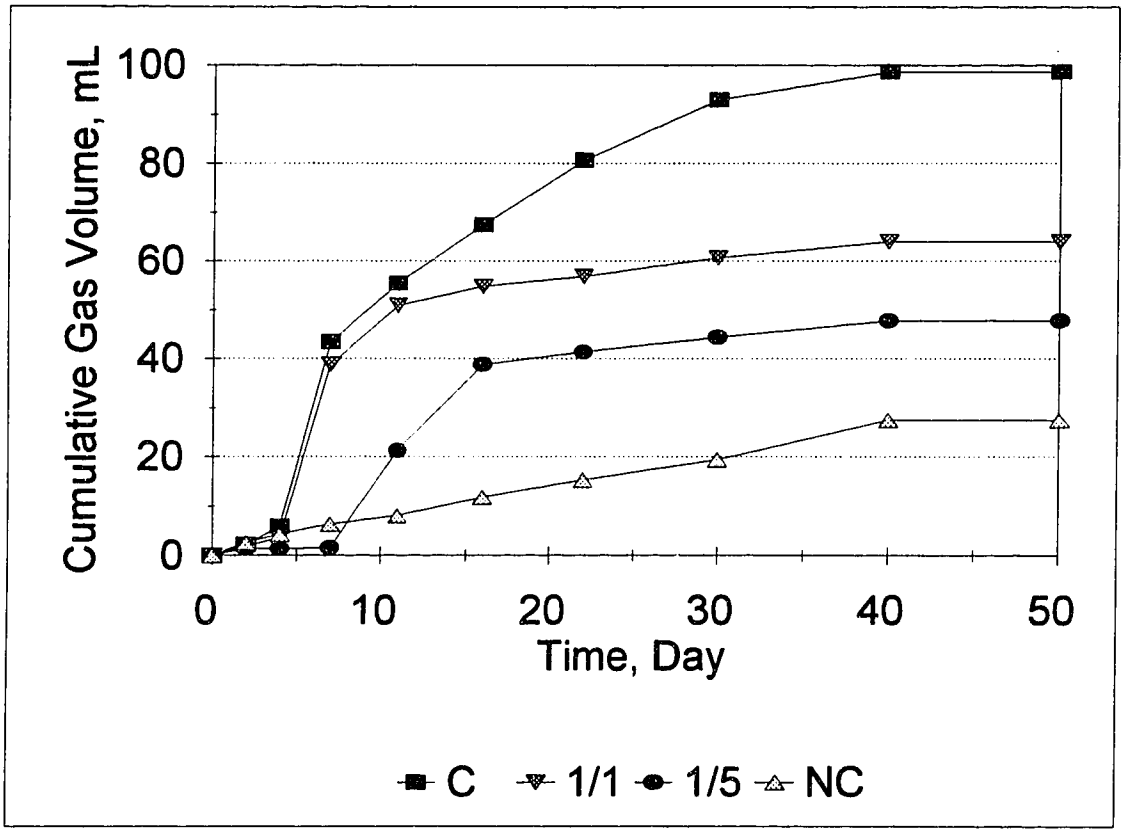


Figure 12 Results of Biodegradation Test with Nitrocellulose and Cellulose by Sludge from Two-stage Anaerobic Treatment System

nitrocellulose in this experiment (25 ml vs. 8 ml). Inhibitions of biodegradation of cellulose caused by addition of nitrocellulose were still observed at both Cellulose/ Nitrocellulose ratios of 1/1 and 1/5. Although this test shows some biogas generation when nitrocellulose was used as the solo carbon source, the amount, however, is only 1/4 that of the cellulose. Some small molecular weight organic acids were detected and large amount of ethanol was presented in the solutions by HPLC analysis. Nitrite and nitrate were also detected by Ion Chromotograph in solutions. Table 12 shows the results of difference between SGP calculation values and net gas productions from this study. This table shows that the conversion of nitrocellulose to gas was about 34.4 % SGP value without any co-substrate. This is better than single stage system. However, after transferring the residual sludge to another set of reactor, more gas production was not observed. This test shows that under certain conditions, microorganisms are able to utilize nitrocellulose as carbon source.

Table 12 Comparison of Net Gas Production and SGP
(Biodegradation of Nitrocellulose and Cellulose)

Desciption	**Measured Gas Volume**	**SGP**	**Ratio**	**Nirate, mg/l**
Cellulose	98.7	136.0	72.6	-
C/NC = 1/1	64.0	216.0	29.6	24.7
C/NC = 1/5	47.7	536.0	14.2	64.3
Nitrocellulose	27.5	80.0	34.4	98.2

unit : mL

5.4 Effect of pH on Biodegradation

The effect of pH on the biosystem was investigated in this part of the study. Five different initial pH values, 6.0, 6.5, 7.0, 7.5, and 8.0, respectively, were used during this part of the study. Two different Cellulose/Nitrocellulose ratios (1/1 and 5/1 by weighted) and two control units (Nitrocellulose only and Cellulose only) were also used in this study. Biogas production, extractable nitrocellulose concentration, nitrate, and nitrite produced were monitored to evaluate the performance of biodegradation. The results are shown in Figures 13 to 17 and Tables 13 to 17.

According to the gas production data, the lower the pH (6.0) the more biogas was produced, except at pH = 7.0 and Cellulose/Nitrocellulose = 1/1. The bottles with pH higher than 7.0 produced only 1/3 or less biogas than these with pH lower than 7.0. It is interesting to note that by comparing gas production with the percentage of nitrocellulose removals in this experiment, it can be seen that the higher the pH the higher removal efficiency. At pH=7.5 and C/NC=1/1 the highest nitrocellulose removal efficiency of 48.91% was observed. In this experiment, nitrocellulose was degraded into the intermediate products which could not be identified at this time, rather than biogas. Based upon the mass balance of nitrogen and the amount of nitrate and nitrite measured by ion chromotograph shown in Tables 13 to 17, the recovery amount of nitrogen was much less than that theoretical calculated value based on nitrogen content of 13.5% in the nitrocellulose. This indicates that some nitrate groups in nitrocellulose either escaped as nitrogen gas or was still attached/bound to the intermediate compounds.

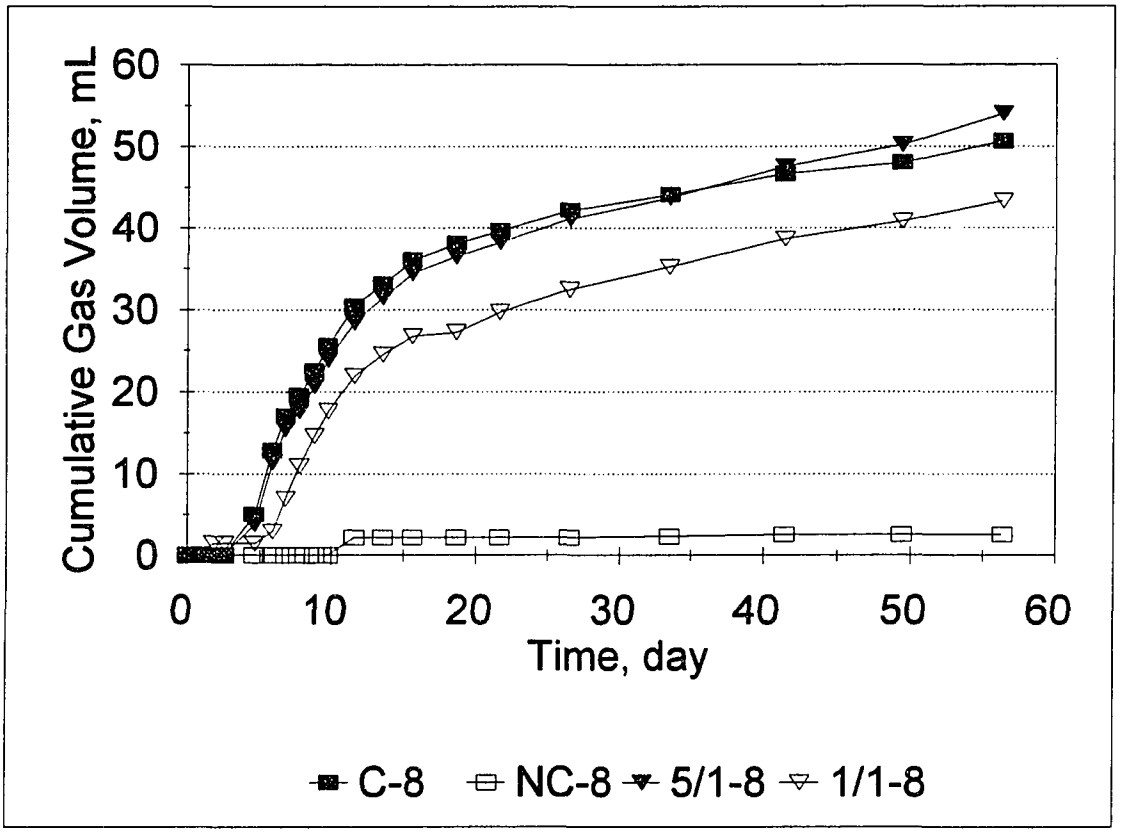


Figure 13 Gas Production during Biodegradation at pH = 8.0

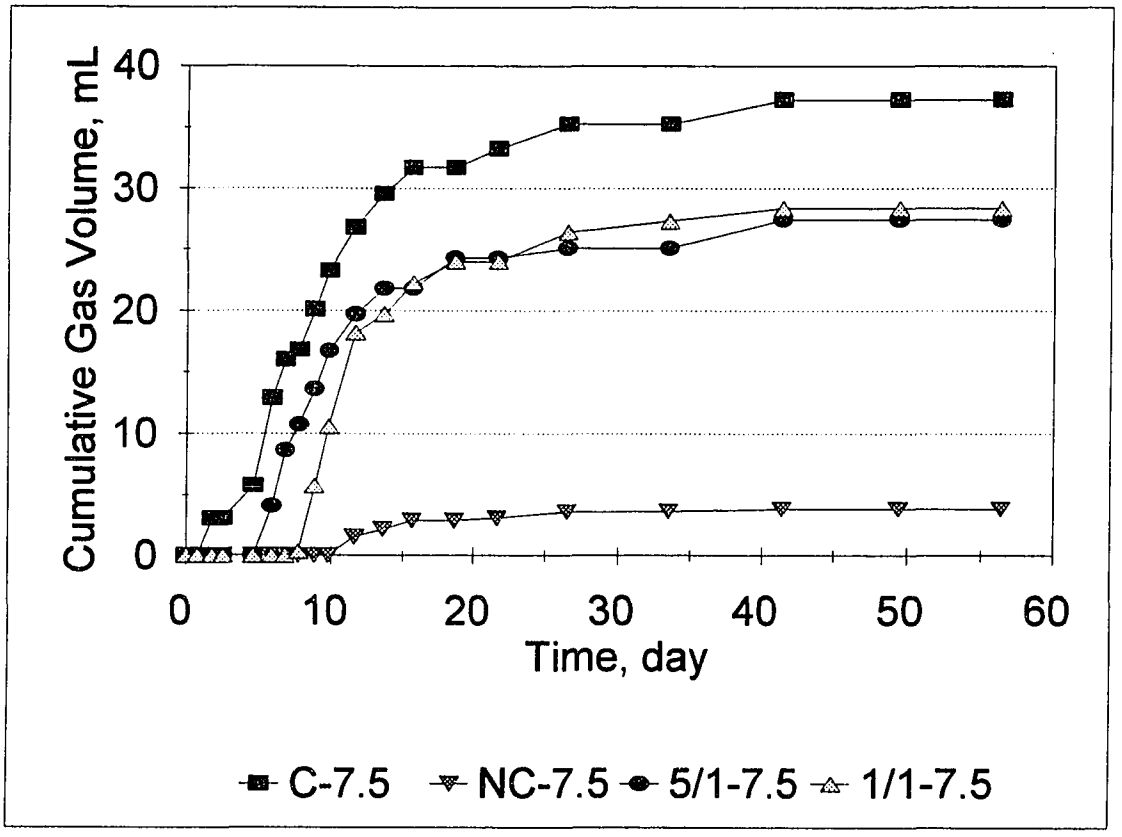


Figure 14 Gas Production during Biodegradation at pH = 7.5

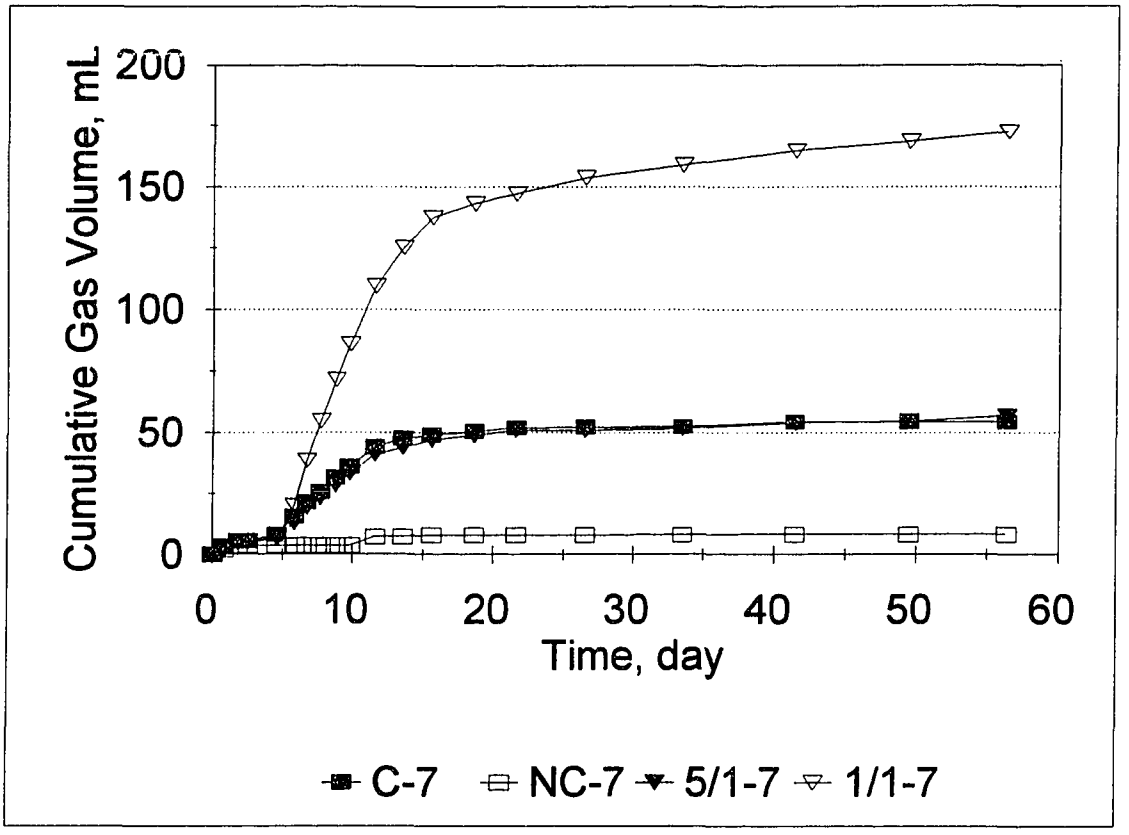


Figure 15 Gas Production during Biodegradation at pH = 7.0

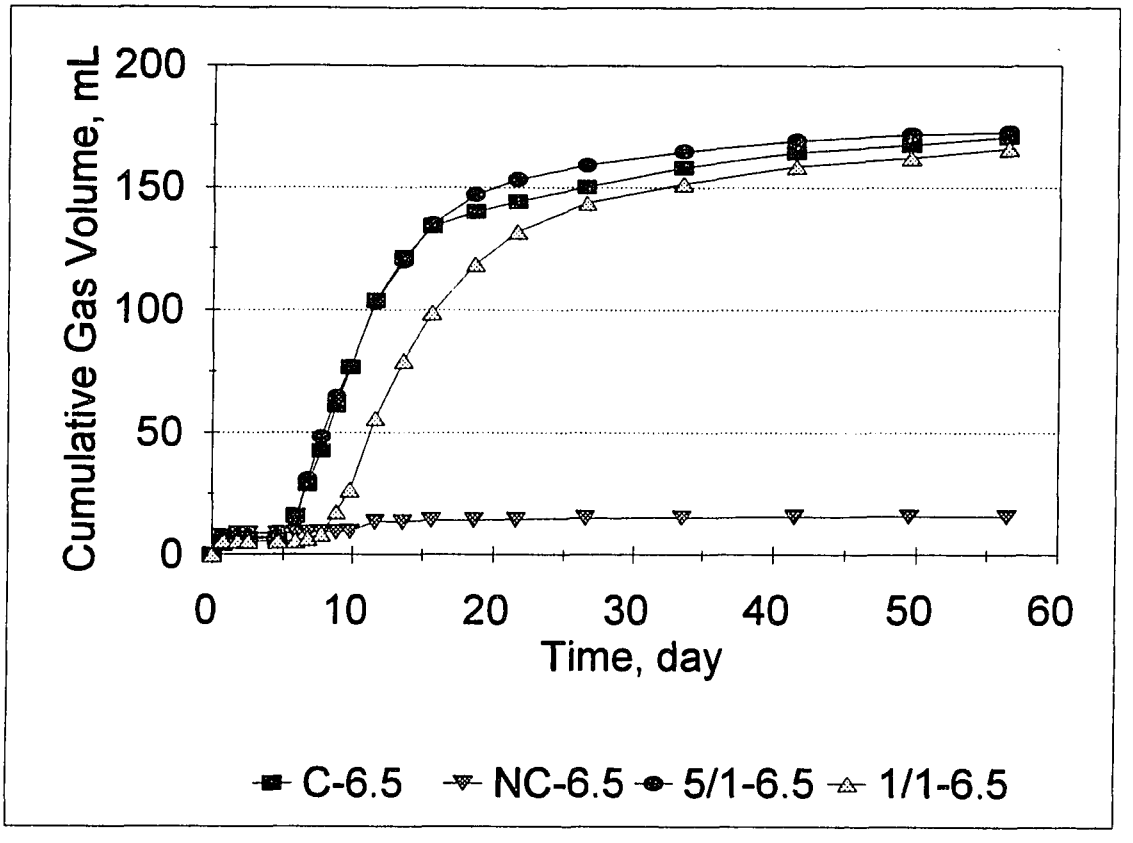


Figure 16 Gas Production during Biodegradation at pH = 6.5

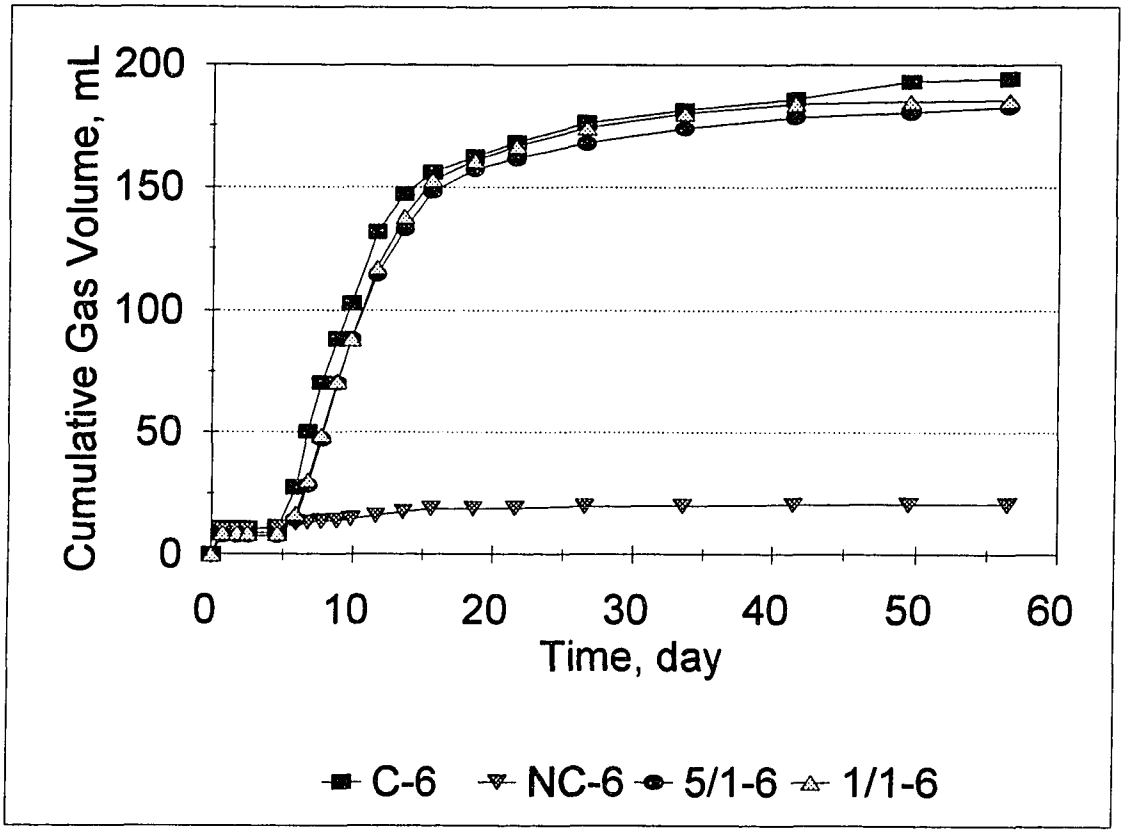


Figure 17 Gas Production during Biodegradation at pH = 6.0

Table 13 Results of Biodegradation at pH = 8.0
(Effect of Various pH on Biodegradation)

Description	Gas Volume, mL	NC Removal, %	Nitrate, mg/L
C only	50.7	-	-
NC only	2.6	46.49	69.41
C/NC=5/1	54.1	32.63	NA
C/NC=1/1	43.4	42.23	73.86

Table 14 Results of Biodegradation at pH = 7.5
(Effect of Various pH on Biodegradation)

Description	Gas Volume, mL	NC Removal, %	Nitrate, mg/L
C only	37.3	-	-
NC only	3.9	41.46	110.49
C/NC=5/1	27.4	37.97	NA
C/NC=1/1	28.4	48.91	28.06

Table 15 Results of Biodegradation at pH = 7.0
(Effect of Various pH on Biodegradation)

Description	Gas Volume, mL	NC Removal, %	Nitrate, mg/L
C only	54.6	-	-
NC only	8.4	35.54	34.66
C/NC=5/1	57.1	14.96	NA
C/NC=1/1	172.6	24.19	12.25

Table 16 Results of Biodegradation at pH = 6.5
(Effect of Various pH on Biodegradation)

Description	Gas Volume, mL	NC Removal, %	Nitrate, mg/L
C only	170.5	-	-
NC only	15.8	29.22	32.72
C/NC=5/1	172.7	5.00	NA
C/NC=1/1	166.0	25.13	93.00

Table 17 Results of Biodegradation at pH = 6.0
(Effect of Various pH on Biodegradation)

Description	Gas Volume, mL	NC Removal, %	Nitrate, mg/L
C only	194.3	-	-
NC only	20.8	23.22	38.58
C/NC=5/1	182.8	-12.26	NA
C/NC=1/1	185.4	17.29	NA

5.5 Sequencing Batch Study

A four-liter flask was used as the bioreactor with a gas collection device. Two sets of identical biosystem were compared. One reactor was first fed with 10 grams of cellulose only and the second reactor was introduced with 10 grams of cellulose and 2 grams of nitrocellulose. After gas productions from both reactors were ceased, mixing was stopped. After the sludge settled, the supernatant was withdrawn from the reactor. Additional substrates were added to the systems and another cycle of treatment was started. The results of gas production in sequencing batch reactor study is shown in Figure 18.

In the first cycle of sequencing batch studies, M-1 was fed with 10 grams of cellulose and 2 grams of nitrocellulose and M-2 was fed with 10 grams of cellulose. In the first cycle, the gas production rate (slope of the curve) of M-1 was lower than that of M-2. This means that the addition of nitrocellulose did affect the gas production. In the second cycle, M-1 was fed with 10 grams of cellulose alone and M-2 was fed with 10 grams of cellulose and 2 grams of nitrocellulose. In the second cycle, M-2 showed gas production inhibition, but not for M-1. If nitrocellulose was not hydrolyzed in first cycle, it should have settled and been retained in the M-1 reactor. The Cellulose/Nitrocellulose ratio in the second cycle was the same as in the first cycle for M-1 reactor so it should have exhibited the same inhibition as in the first cycle. But from Figure 18, it can be seen that the inhibition did not occur. This indicates that the nitrocellulose could have been converted to a different compound, however, this compound was not able to further decomposed and utilized by the methane-forming bacteria.

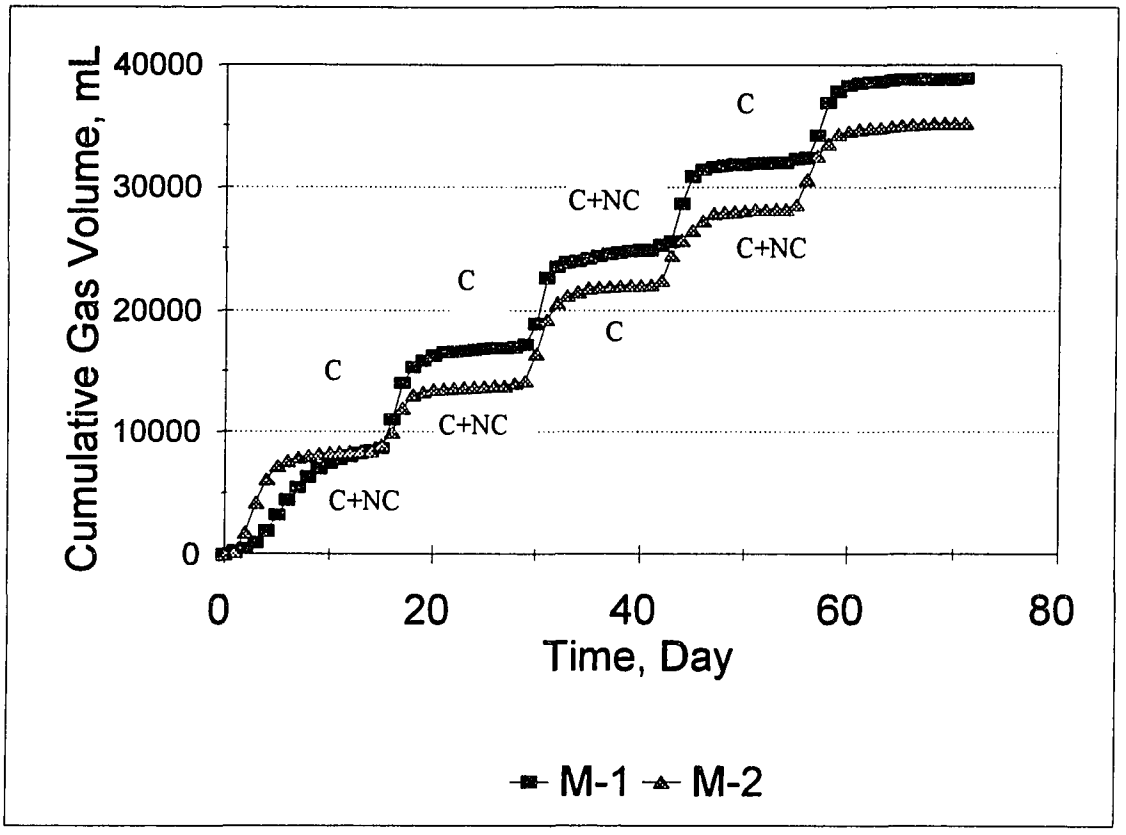


Figure 18 Results of Sequencing Batch Study

5.6 Stage-Feed Anaerobic Study

Two single-stage stage-feed anaerobic reactors (S-1 and S-2) and two identical two-stage stage-feed reactors (T-1 and T-2) were used in this part of the tests. Another reactor (H-1) using horse manure to substitute microorganisms was also studied. The system's hydraulic retention time was controlled at 20 days. Sludge retention time was sustained at about 70 days and pH was controlled at neutral condition by adding sodium bicarbonate as buffer. Cellulose feeding rate was kept at 6 g/L.day and nitrocellulose feeding rate was maintained at 0.6 g/L.day. The results from the stage-feed system are shown in Figures 19 to 21.

It was found in the stage-feed study that the two-stage system produced, sometimes, more gas than single-stage system. For two-stage system, the addition of nitrocellulose did not affect the gas production. In the single-stage systems, the two reactors, fed with cellulose and nitrocellulose, produced almost same amount of gas as the reactor fed with cellulose alone, which was used as the control. Nitrate was found in the effluent and small amount of nitrite was also detected. The results show denitration and hydrolysis occurred during this test.

Figure 20 shows the daily gas production in the two-stage stage-feed system. It can be seen from this figure that the rate of biodegradation was not at a steady state condition, especially for T-1 system. The microbial activities fluctuated. Most of the time, the microorganisms remained in an inactive condition. However, after a period of time (10 to 15 days), there is a peak coming out. The consumption of a large amount of substrate and production of tremendous amount of gas occurred in a very short period of

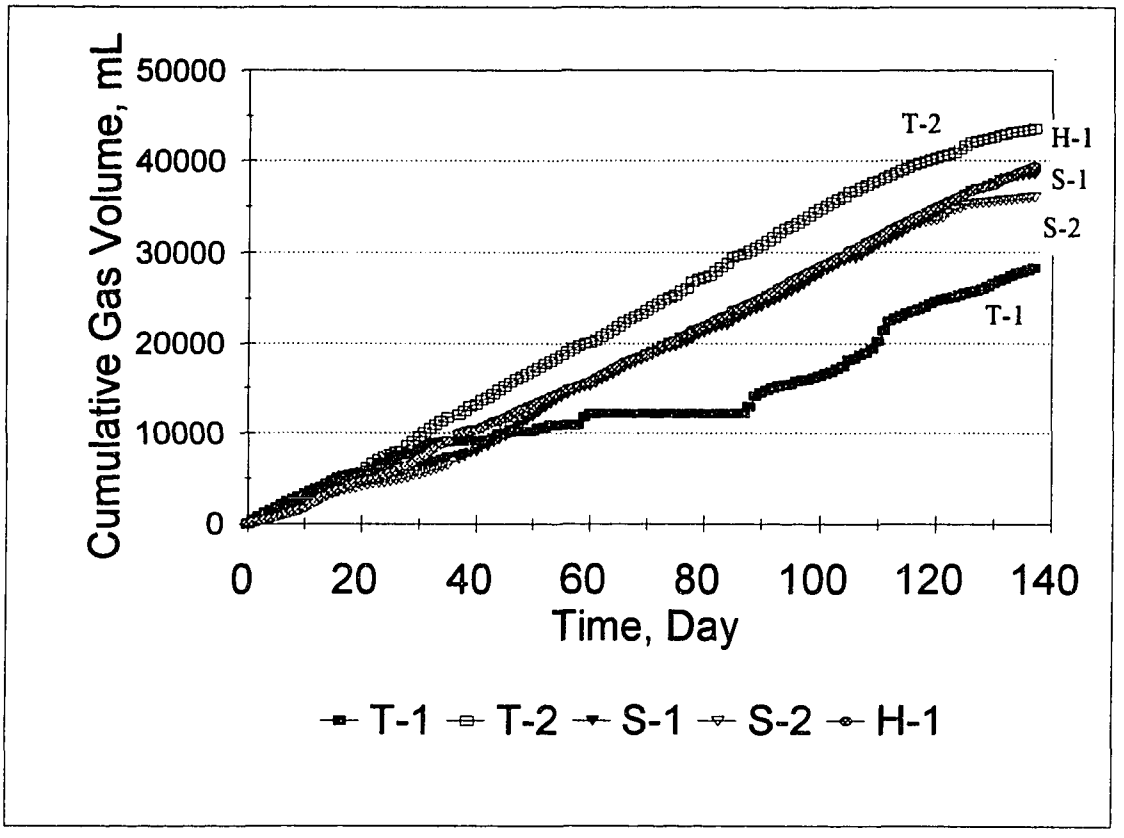


Figure 19 Gas Production in Staged-Feed Reactors

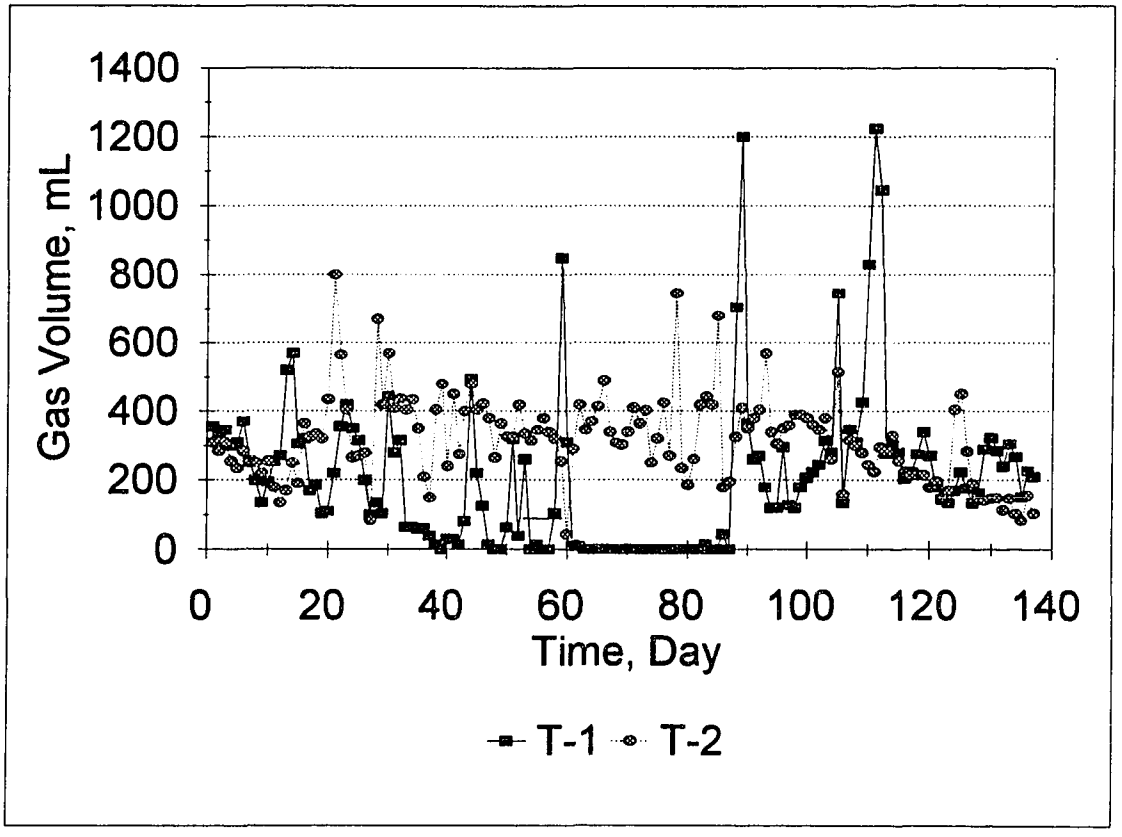


Figure 20 Gas Production in Two-Stage Staged-Feed System

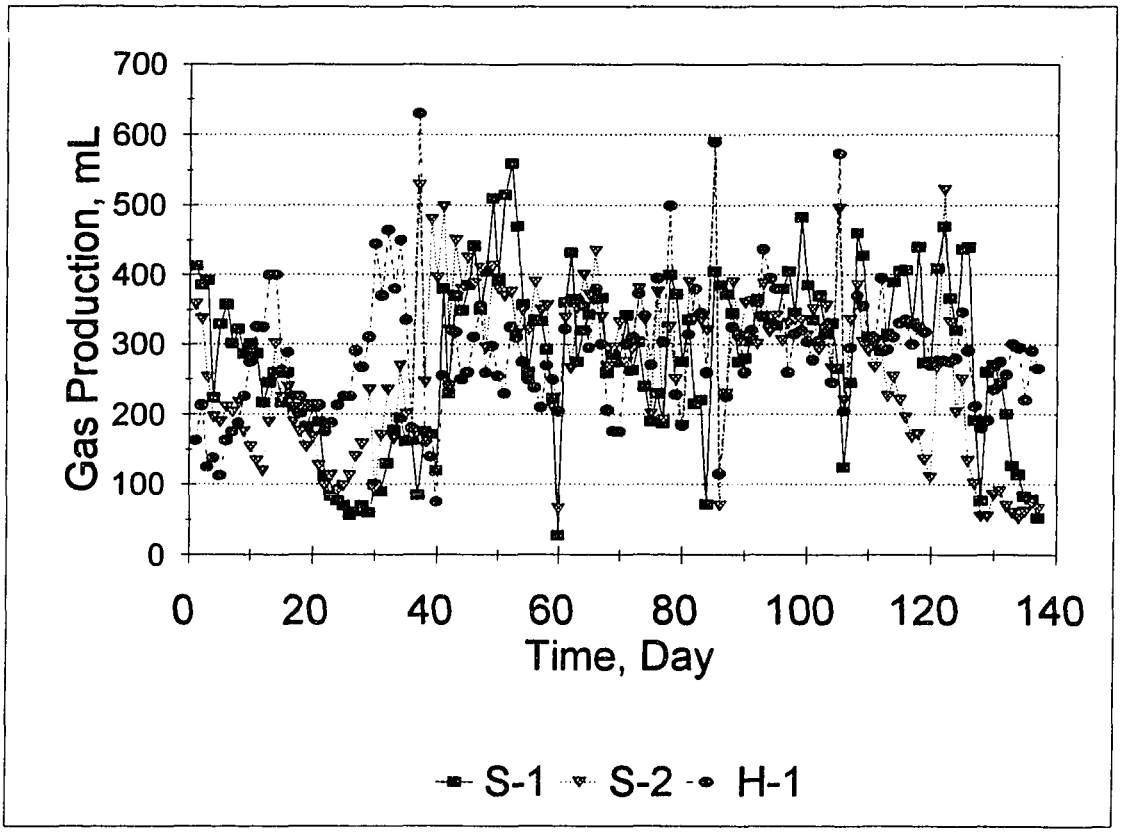


Figure 21 Gas Production in Single-Stage Staged-Feed System

time. The concentration of volatile acid in T-1 was high (greater than 3,000 mg/l). Nitrate and nitrite were also found in the effluent. This shows that microorganisms were still alive and nitrocellulose was converted to simpler compounds and biogas.

Figure 21 shows the performance of the single-stage stage-feed system. It can be seen from this figure that the gas production for all three reactors was approximately the same. The using of horse manure to degrade nitrocellulose is also shown in this figure. It is seen that this reactor did not perform better than the reactors with anaerobic digester microorganisms. The volatile acids produced were in the range of 80 to 200 mg/l, and nitrate and nitrite were also found in the effluent. The system is more stable than the two-stage system, however, the nitrocellulose conversion was lower based on the gas production.

After the experiment, the mixed liquor in the reactors that should contained microorganisms and nitrocellulose was removed from the reactor. It was found that none of the nitrocellulose particles was present in the sludge.

5.7 Nitrocellulose Inhibition Study

In the inhibition study, nitrocellulose was fixed at 1,500 mg/L for each serum bottle and the concentrations of substrate (cellulose) varied from 500 to 2,500 mg/L. The biogas produced from test serum bottles were used as the rate of reaction. The results of this study are shown in Figures 22 and 23. The concentrations of substrate [S] and rates of reaction [V] of anaerobic system with or without the addition of nitrocellulose are shown in Table 18. The plot of 1/V versus 1/S is shown in Figure 24. The two straight lines

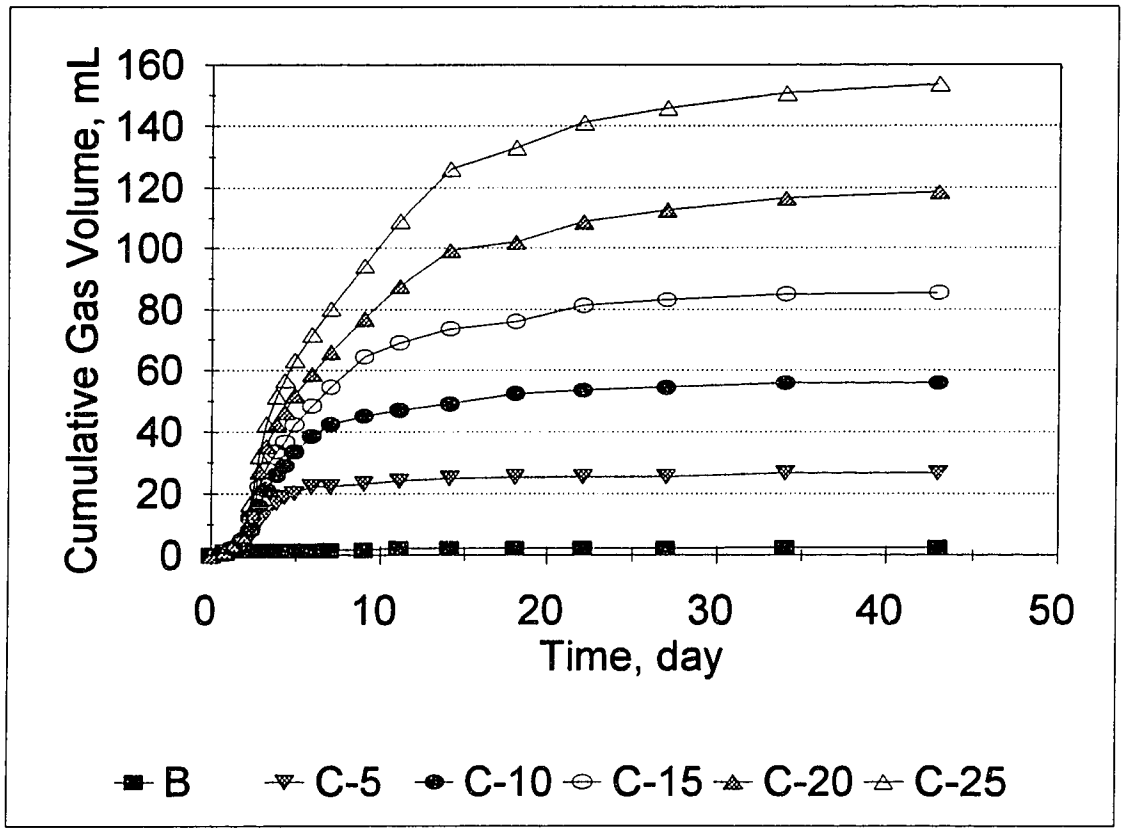


Figure 22 Gas Production in Inhibition Study without Nitrocellulose

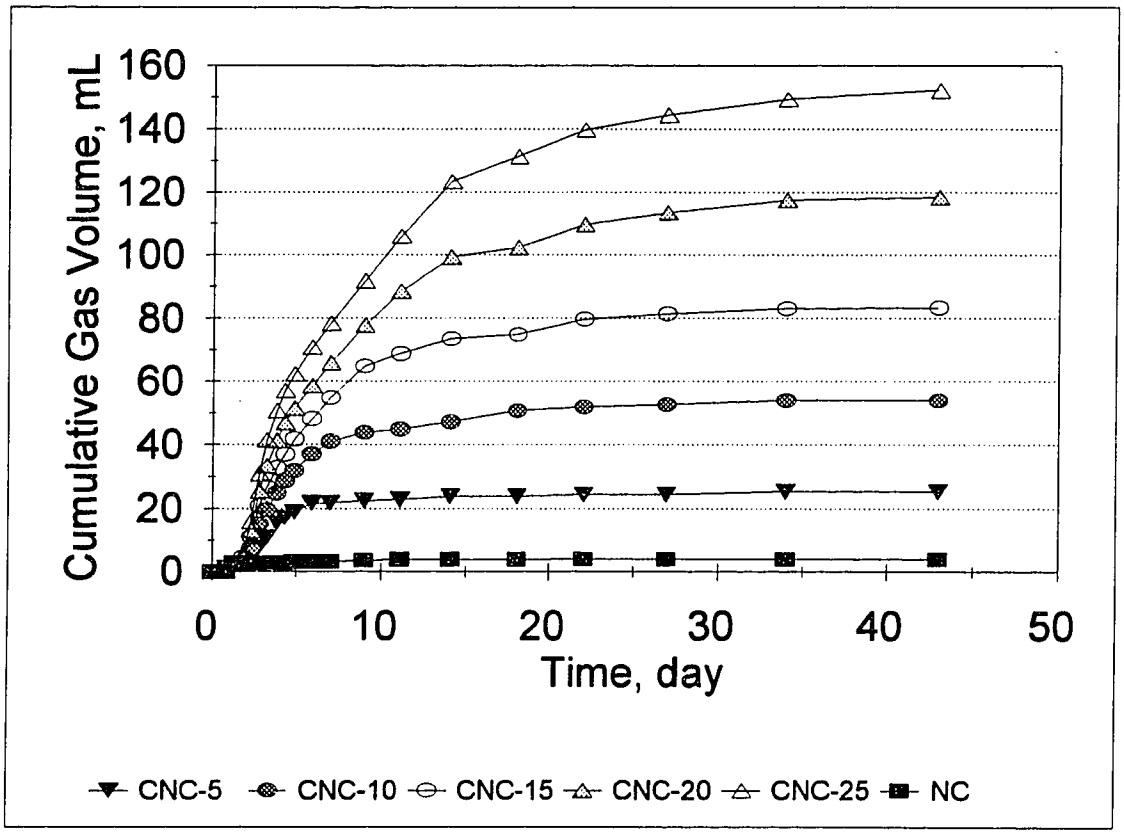


Figure 23 Gas Production in Inhibition Study with Nitrocellulose

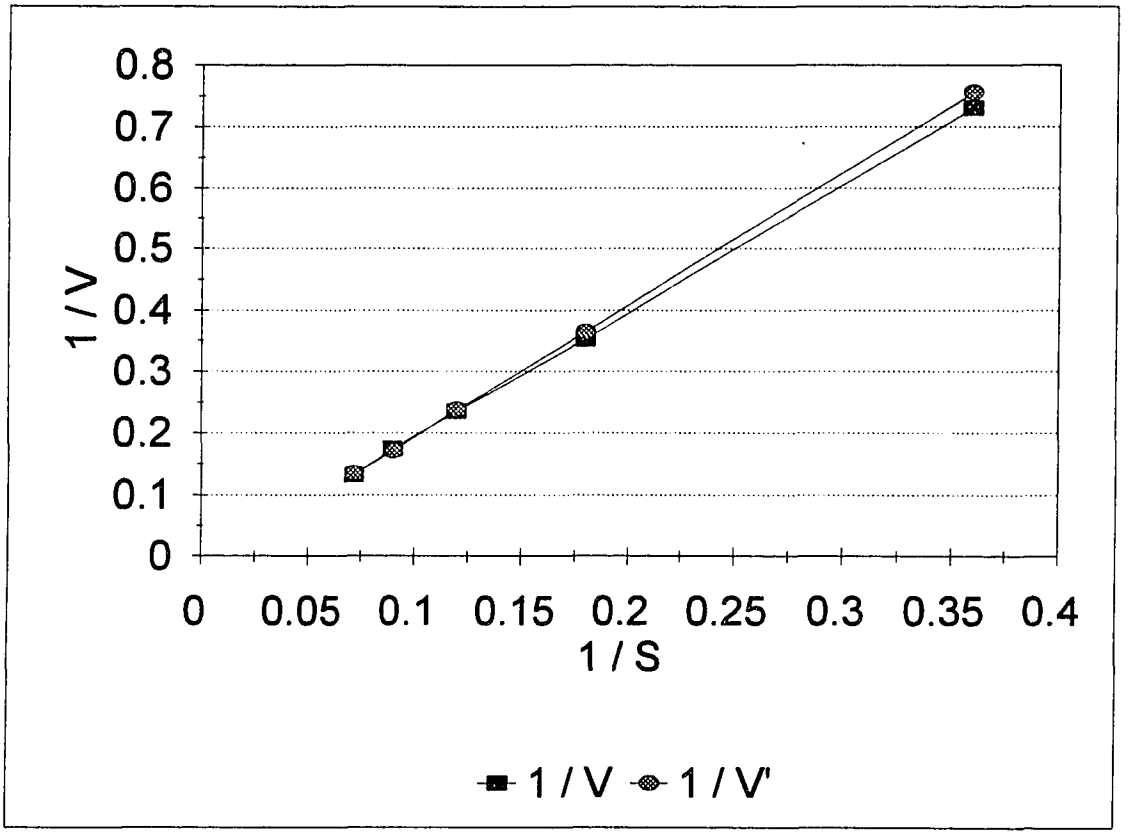


Figure 24 The Lineweaver-Burk Plot in Inhibition Study

almost intercept at 1/V axis. It indicates that the inhibition caused by addition of nitrocellulose behaves like competitive inhibition. For the competitive inhibition, the inhibitory effect can be overcome at higher substrate concentrations. In this study, at higher concentration of cellulose, nitrocellulose did not affect the gas production . The kinetic and inhibition constants derived from modified Michaelis-Menten equation are shown in Table 19.

Table 18 Results of Inhibtion Study of Nitrocellulose

S, x 10^{-3} mole	V, day^{-1}	V', day^{-1}
2.78	1.3672	1.3216
5.56	2.8393	2.7454
8.33	4.2633	4.2098
11.11	5.7337	5.8310
13.89	7.5305	7.4565

Table 19 Kinetic Constants in Inhibition Study

Constant	x 10^{-3}
V_{max}	13.93 M/day
K_M	136.01 M
K_M'	94.84 M
K_i	17.32 M

At the end of this experiment, the samples in each serum bottle were centrifuged and filtered through a 0.45 μm membrane filter to remove the suspended solids. The Soluble Chemical Oxygen Demand (SCOD) was then determined for the filtrate. The results of SCOD are shown in Table 20. Comparing the gas production between the samples which contained cellulose and those with or without the presence of nitrocellulose, it can be seen that the same amount of gas production was observed for various concentrations of cellulose. However, at higher cellulose concentration (CNC-25, 2500 mg/L), the test bottles with cellulose and nitrocellulose had higher SCOD. This indicates that more soluble organic source was released in the test bottles with cellulose and nitrocellulose. This may be caused by the dissociation of nitrocellulose in the biological system. However, the soluble organic source did not further convert to methane and carbon dioxide.

Table 20 Results of Soluble Chemical Oxygen Demand (SCOD) in Inhibition Study

Sample	pH	Gas, ml	SCOD, mg/l	Sample	pH	Gas, ml	SCOD, mg/l
Blank	7.67	2.5	65.3 ± 8.5	**NC**	7.61	4.1	53.6 ± 7.7
C-25	7.00	153.8	53.6 ± 7.7	**CNC-25**	7.04	152.3	114.1 ± 24.4
C-20	6.98	118.6	60.2 ± 17.8	**CNC-20**	7.01	118.6	39.8 ± 16.4
C-15	7.10	85.4	71.2 ± 6.0	**CNC-15**	7.11	83.4	65.3 ± 3.4
C-10	7.19	55.9	52.1 ± 4.3	**CNC-10**	7.27	54.0	57.2 ± 1.5
C-5	7.33	26.8	45.0 ± 1.5	**CNC-5**	7.35	25.5	81.2 ± 8.8

5.8 Effects of pH and Cellulose Particle Size on NC Biodegradation

Cellulose with three different particle sizes, Sigma 20 (average 20 μm), Sigma 50 (average 50 μm), and Sigma 100, were used in this part of the study. Five different pH values, 4.5, 5.0, 6.0, 7.0, and 8.0, were used before seeding to study pH effect. Gas production and SCOD were employed as monitoring parameters for biological system. The results of gas production are shown in Figures 25 to 32. The data of SCOD and pH changes are presented in Table 21. Results of this study show that the optimal final pH for gas production ranged from 6.4 to 6.3. Type 20 and 50 celluloses with nitrocellulose produced higher SCOD than those with cellulose only. This indicates that nitrocellulose may be co-degraded by anaerobes with these two types of cellulose.

5.9 Effectiveness of Biodegradation

From all the studies conducted, it is obvious that the measurement of gas production is not a good indicator for nitrocellulose degradation. For high inducer/NC ratio, high gas production or high conversion ratios can be observed as in Tables 6 to 9. However, once the ratio drops, it is difficult to tell if nitrocellulose change or not.

Low gas production does not mean substrate was not changed. It only means the final gas product was not formed. This is especially true for nitrocellulose. It has been observed over and over again in the batch study, stage-feed study, and sequential batch study that intermediate compounds such as organic acids and nitrates were detected. Unfortunately, some other intermediate compounds were not able to be identified.

Another interesting observation was found after the data collection stage. During the data analysis period, two reactors were kept in the temperature control chamber with

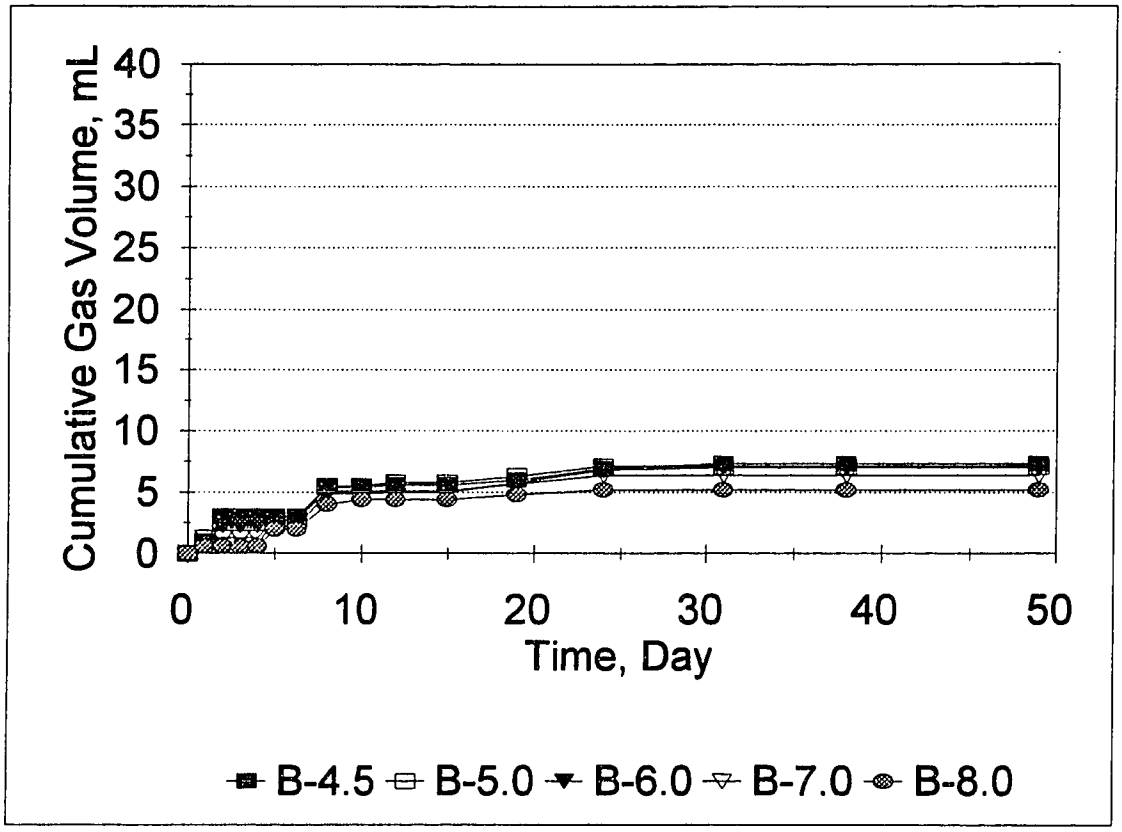


Figure 25 Gas Production in Culture Blank (sludge only) at Various pH (Effect of Particle Size Study)

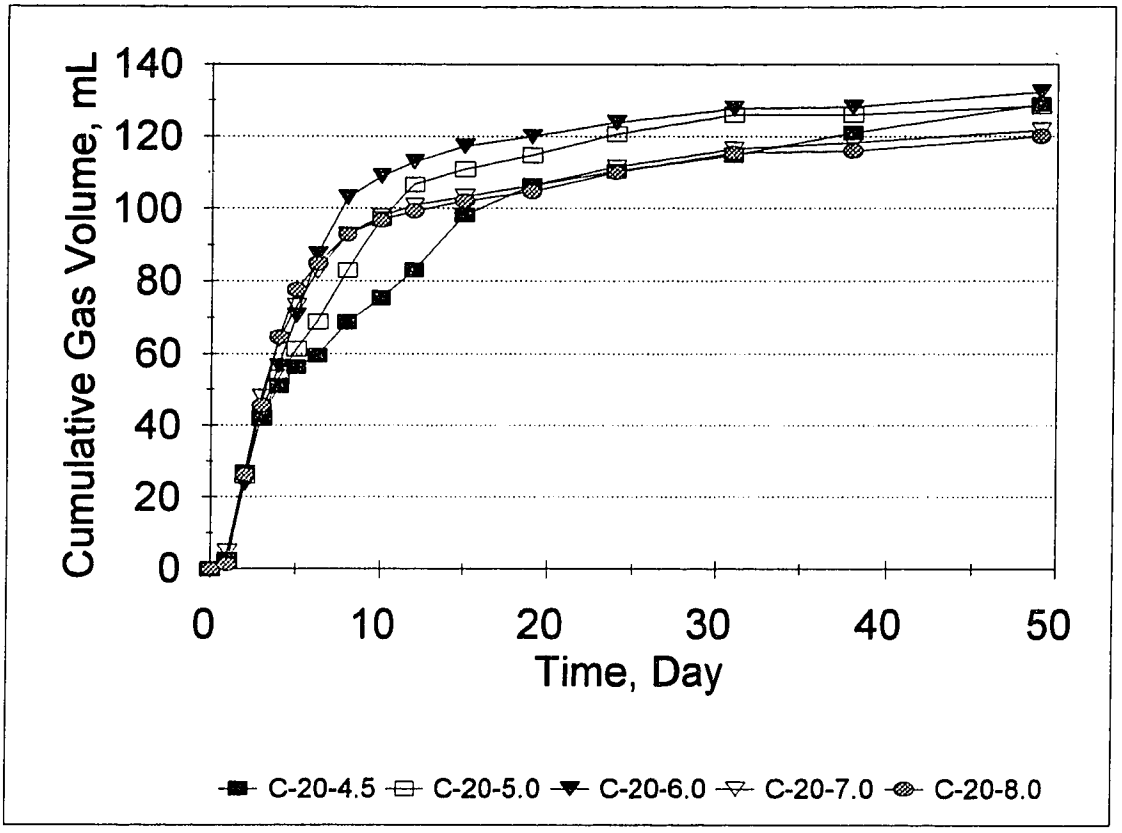


Figure 26 Gas Produced in Type 20 Cellulose at Various pH
(Effect of Particle Size Study)

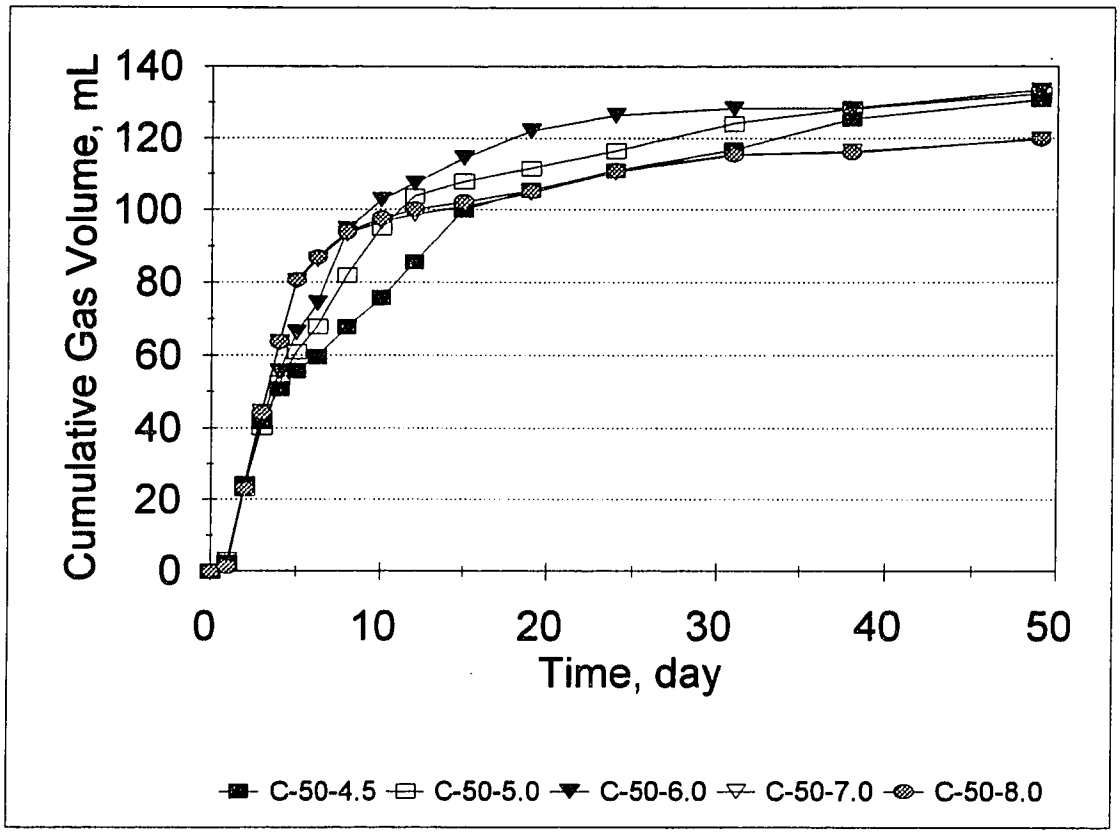


Figure 27 Gas Produced in Type 50 Cellulose at Various pH
(Effect of Particle Size Study)

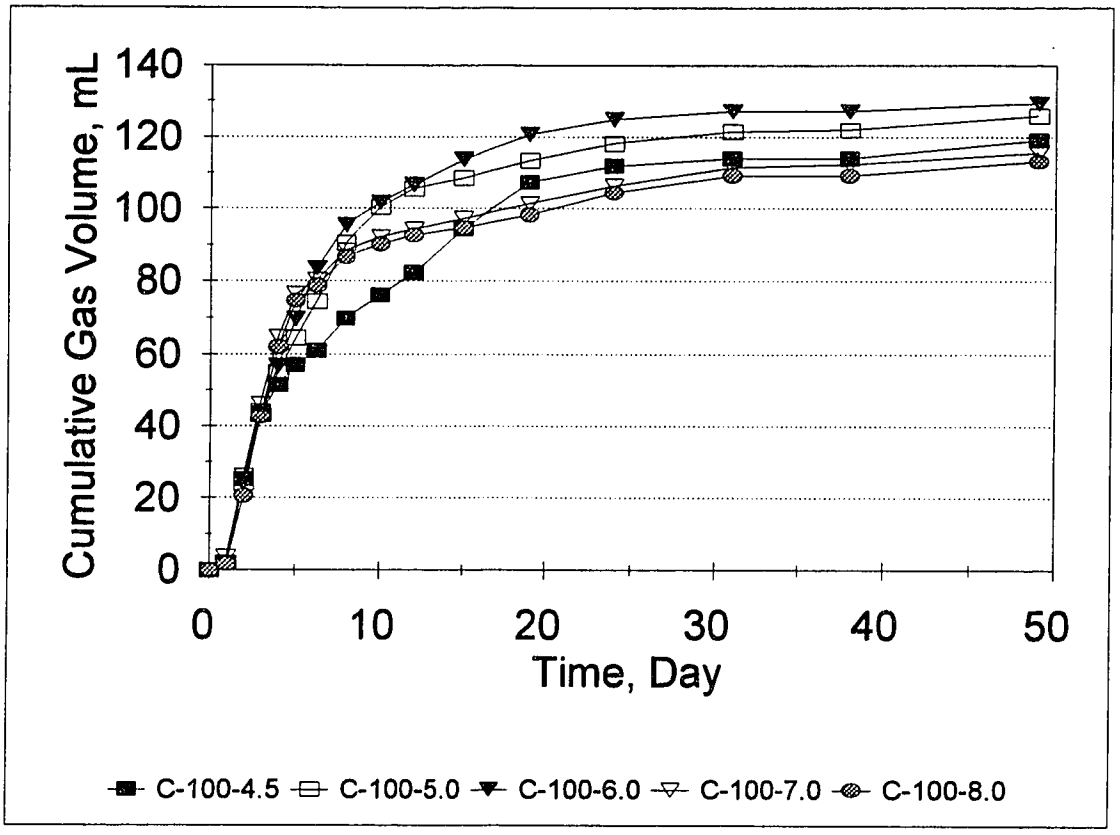


Figure 28 Gas Produced in Type 100 Cellulose at Various pH
(Effect of Particle Size Study)

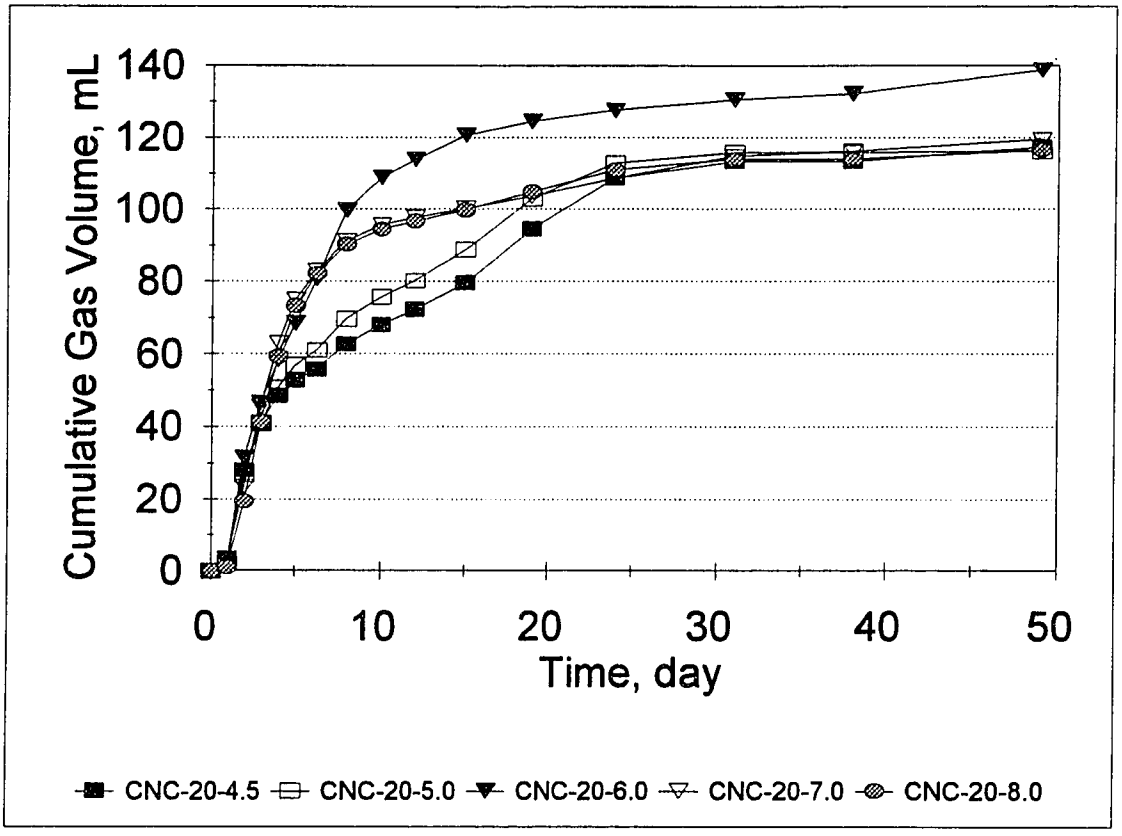


Figure 29 Gas Produced in Nitrocellulose and Type 20 Cellulose at Various pH (Effect of Particle Size Study)

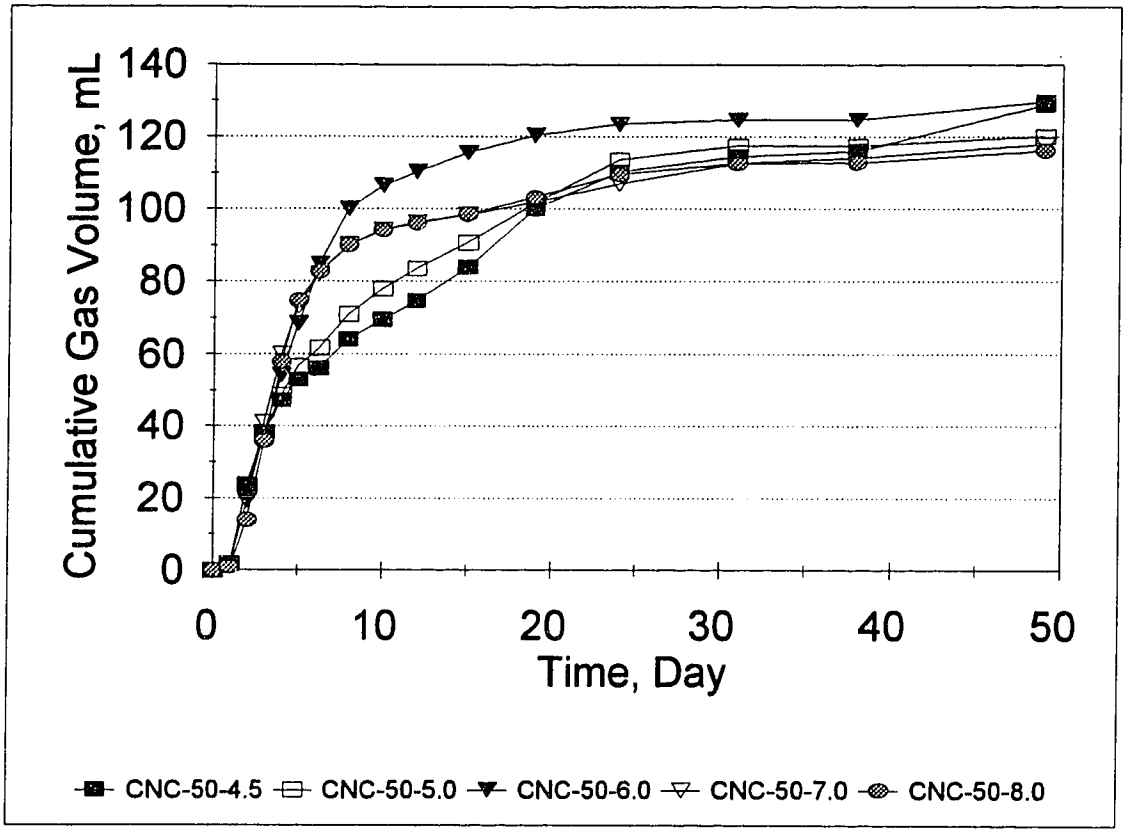


Figure 30 Gas Produced in Nitrocellulose and Type 50 Cellulose at Various pH (Effect of Particle Size Study)

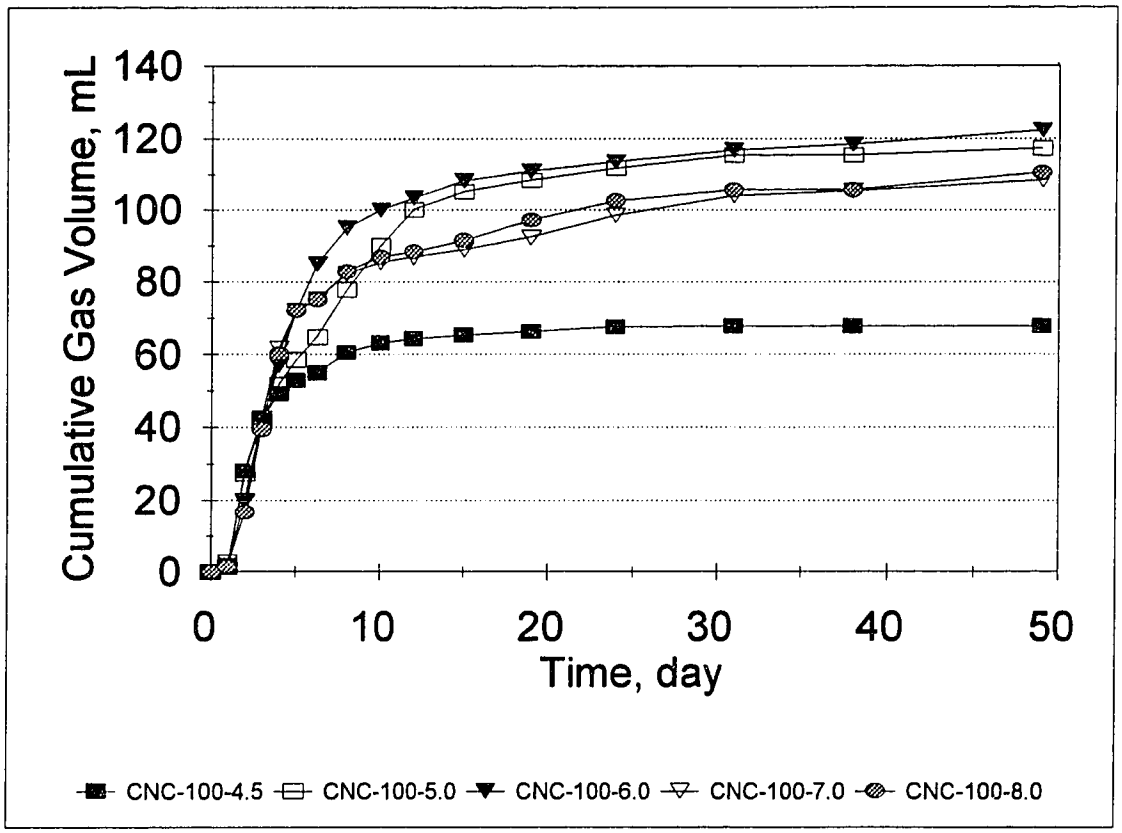


Figure 31 Gas Produced in Nitrocellulose and Type 100 Cellulose at Various pH (Effect of Particle Size Study)

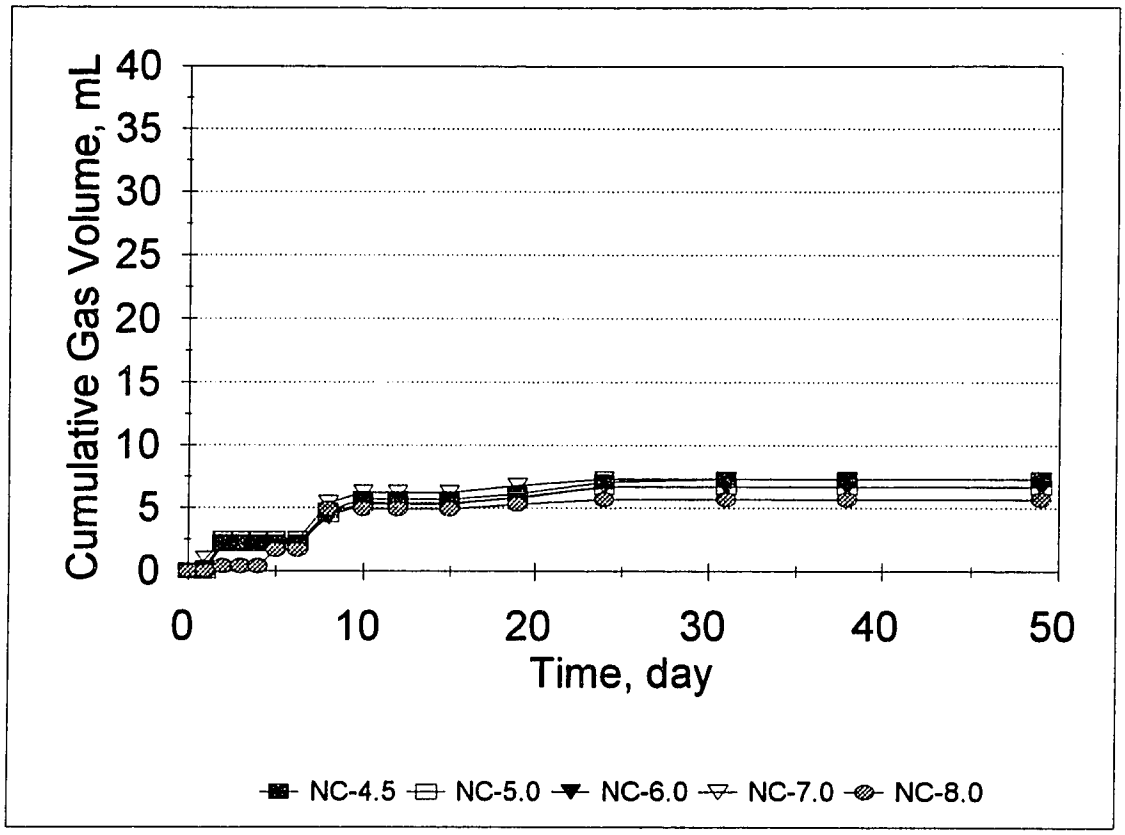


Figure 32 Gas Produced in Nitrocellulose Only at Various pH
(Effect of Particle Size Study)

Table 21 Soluble Chemical Oxygen Demand in Effects of Cellulose Particle Size Study

Initial pH	**8.0**	**7.0**	**6.0**	**5.0**	**4.5**
	Blank				
Final pH	7.59	7.10	6.58	6.51	6.50
Gas, ml	5.2	6.4	7.0	7.1	7.3
SCOD, mg/l	136 ± 3	96 ± 19	107 ± 30	122 ± 2	126 ± 6
	C-100				
Final pH	6.88	6.83	6.40	6.35	6.31
Gas, ml	113.3	115.6	129.4	126.0	119.3
SCOD, mg/l	207 ± 6	321 ± 7	369 ± 2	432 ± 3	419 ± 2
	C-50				
Fianl pH	6.87	6.76	6.35	6.38	6.33
Gas, ml	119.9	119.7	133.5	132.5	130.8
SCOD, mg/l	179 ± 2	274 ± 3	282 ± 2	333 ± 13	289 ± 3
	C-20				
Final pH	6.85	6.67	6.33	6.29	6.26
Gas, ml	120.2	121.8	132.3	128.5	129
SCOD,mg/l	218 ± 18	276 ± 2	352 ± 4	322 ± 2	280 ± 13
	CNC-100				
Final pH	6.88	6.77	6.35	6.31	5.84
Gas, ml	110.6	108.5	122.4	117.4	67.8
SCOD, mg/l	234 ± 3	294 ± 2	379 ± 8	375 ± 3	1076 ± 42
	CNC-50				
Final pH	6.88	6.77	6.34	6.31	6.31
Gas, ml	116.3	118.0	129.7	120.2	129.0
SCOD, mg/l	231 ± 12	370 ± 11	389 ± 2	507 + 2	354 ± 6
	CNC-20				
Final pH	6.97	6.83	6.35	6.24	6.21
Gas, ml	116.7	119.5	138.8	116.2	117.4
SCOD, mg/l	225 ± 6	264 ± 2	307 ± 6	538 ± 3	542 ± 16
	NC				
Final pH	7.59	7.11	6.57	6.47	6.49
Gas, ml	5.8	7.3	6.7	6.7	7.3
SOCD, mg/l	103 ± 21	114 ± 3	128 ± 9	121 ± 7	128 ± 3

no additional substrate addition. After four months, gas production was not increased. The reactors were emptied and it was found that all nitrocellulose and most bacteria disappeared. This is a further evidence that nitrocellulose can be degraded biologically. Therefore, a system with anaerobic digestion with addition of enzyme inducer will be useful to desentilize nitrocellulose.

CHAPTER 6

HYDROCHLORIC ACID HYDROLYSIS OF NITROCELLULOSE

In the previous study on hydrolysis of nitrocellulose using diluted acid, it was found that better hydrolysis can be only obtained at higher temperatures and pressures (Hsieh and Tai, 1994). From previous study, results show that from single stage hydrolysis process yielded about 75% glucose conversion and from two stage hydrolysis process converted about 78% of nitrocellulose to glucose at 70 °C. From this study show that the single stage hydrolysis is as good as the two stage hydrolysis. However, extremely care must be taken to avoid glucose destruction. Therefore, this part of the study was focused on using concentrated hydrochloric acid at intermediate temperature and ambient pressure to hydrolyze waste nitrocellulose in single stage. In this study, 0.4 gram of nitrocellulose and various amount of concentrated hydrochloric acid (with a concentration of about 38%) were added to the media tubes with predetermined acid/solid ratios. These tubes were put into a water bath controlled at designated temperatures (50 to 90 degree Celsius). Tubes were then removed from the water bath at various intervals, quenched in ice water, and analyzed for glucose contents.

6.1 Effect of Reaction Temperatures

Five different temperatures, 50, 60, 70, 80, and 90 degree Celsius, were used to evaluate the temperature effect on nitrocellulose hydrolysis. During the experiment, it was found that the hydrolysis reaction was so slow at 50 °C, it was impractical to calculate the

activated energy at this temperature, therefore, it was removed from analysis. A typical result of nitrocellulose hydrolysis at four testing temperatures with a acid/solid (A/S) ratio of 6 ml/ 0.4 gram is shown in Figure 33. Figure 33 shows that hydrolysis reaction does follow Arrhenius equation, which indicates the higher temperature the faster the reaction. This figure also shows that at 90 °C, it took about 9 minutes to convert nitrocellulose to the maximally produce glucose. But at 60 °C, approximately 63 minutes were needed to reach the maximum glucose level for the same A/S ratio.

It is also shown in Figure 33 that although different time of periods were required to reach the maximum production at different reaction temperatures, the maximum glucose production from nitrocellulose hydrolysis was almost the same as long as the A/S ratio remained the same. In this case, 0.9 mmole glucose. This experiment indicates that the maximum amount of glucose that can be produced from acid hydrolysis of nitrocellulose is controlled more by the A/S ratio rather than temperature. The temperature only affects the rate of hydrolysis reaction.

Four different temperature, 90, 80, 70, and 60 degree Celsius, and six Acid/Solid ratios, 2 ml/0.4 g, 4 ml/0.4 g, 6 ml 0.4 g, 8 ml /0.4 g, 10 ml 0.4 g, and 12 ml /0.4 g were used in the kinetics study. The method to estimate the rate constants for acid hydrolysis has been mentioned previously. The rate constants for acid hydrolysis of nitrocellulose at different temperatures and A/S ratios are listed in Tables 22 and 23. Based on Arrhenius Equation (Eq. 3), there is a linear relationship between the natural logarithm of rate constants and the reciprocal of reaction temperature in °K. Therefore, the plots of ln K verse 1/T were used to calculate the activated energy and Arrhenium frequency factor.

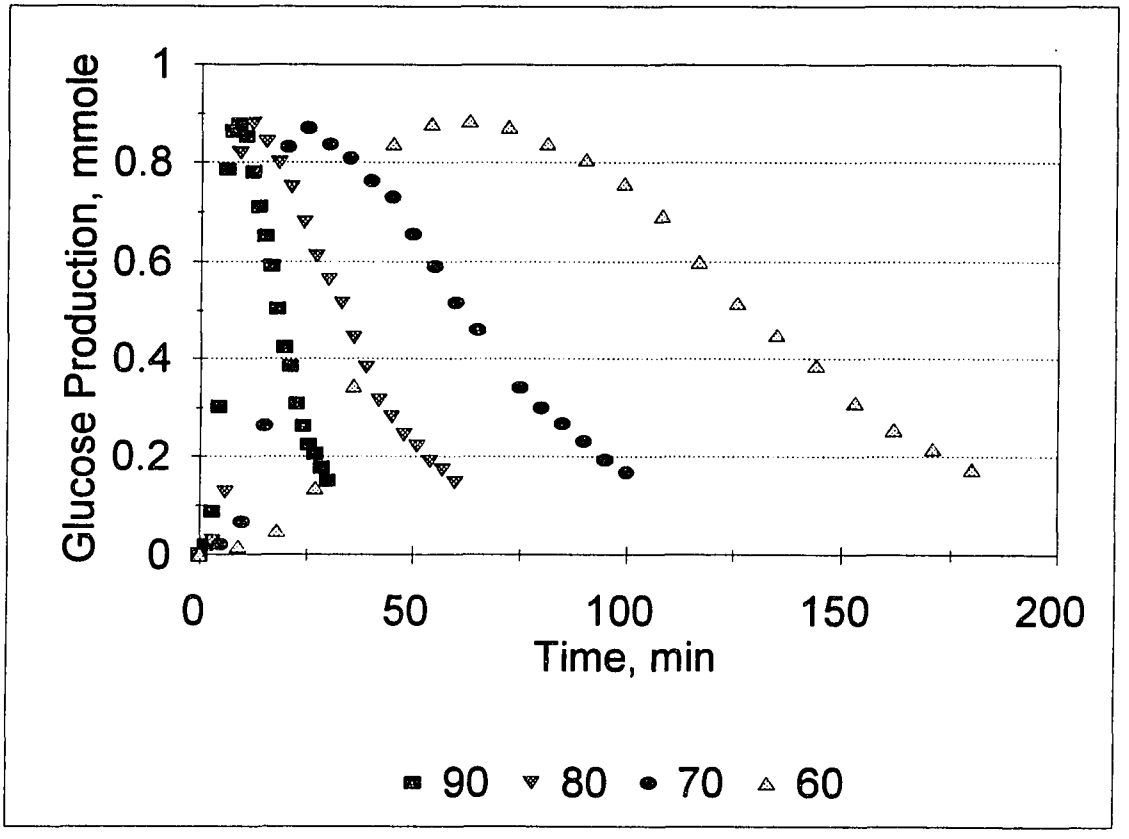


Figure 33 Results of Acid Hydrolysis (A/S = 6 ml/0.4 g) at Various Temperatures

Table 22 Rate Constants of Nitrocellulose Hydrolysis (K_1) at Various Temperatures

Temp, °C	K-2*	K-4*	K-6*	K-8*	K-10*	K-12*
90	0.74439	0.79586	0.84082	0.88874	0.91456	0.93951
80	0.39593	0.42746	0.44677	0.47495	0.48554	0.49537
70	0.22962	0.24766	0.26509	0.27411	0.28367	0.28959
60	0.10874	0.11642	0.12287	0.12796	0.12951	0.13573

* number represents the amount of acid(ml) in A/S ratio

Table 23 Rate Constants of Glucose Degradation (K_2)at Various Temperatures

Temp, °C	K-2*	K-4*	K-6*	K-8*	K-10*	K-12*
90	0.08409	0.08765	0.09261	0.09466	0.09613	0.09716
80	0.04035	0.04184	0.04304	0.04471	0.04577	0.04679
70	0.02647	0.02798	0.02874	0.02953	0.03018	0.03089
60	0.01617	0.01723	0.01885	0.01831	0.02089	0.01881

* number represents the amount of acid(ml) in A/S ratio

Results are shown in Figures 34 and 35. Both figures show that each line in the plot of ln K verse 1/T has similar slope, which means the amount of activated energy required for each part of the reaction is also similar. Therefore, an average value of the slopes of these lines (six different A/S ratios) is calculated and this average value used represent the activation energy of acid hydrolysis. Base on this result, the activation energies required to hydrolyze nitrocellulose to glucose and then decomposed glucose to small molecular weight organic acids are 15,233 Kcal/mole and 12,568 Kcal/mole, respectively. Arrhenius frequency factor for these two reactions are 1.2650×10^9 and 2.8475×10^6, respectively. The rate constants can be expressed by Arrhenium equation as follows:

$$K_1 = 1.2650 \times 10^9 \exp(-15{,}233 / RT), \text{ and}$$

$$K_2 = 2.8475 \times 10^6 \exp(-12{,}568 / RT)$$

where K_1 = rate constant of hydrolysis of nitrocellulose, min^{-1}

K_2 = rate constant of degradation of glucose, min^{-1}

Another set of experiments was conducted at ambient temperature (about 20 °C) for a period of 5 days. From this study, it was found that hydrolysis did occur at room temperature and atmospheric pressure with concentrated hydrochloric acid. But the hydrolysis reaction was much slower than at intermediate temperatures. It took about 5 days to completely dissolve the nitrocellulose and convert it to glucose. However, the quantity of glucose formed was much lower than the value obtained at intermediate temperature. It was thought that the rate of nitrocellulose hydrolysis and glucose degradation should be of the same order of magnitude at ambient temperatures, unlike what observed at higher temperatures, where the rate of nitrocellulose hydrolysis was

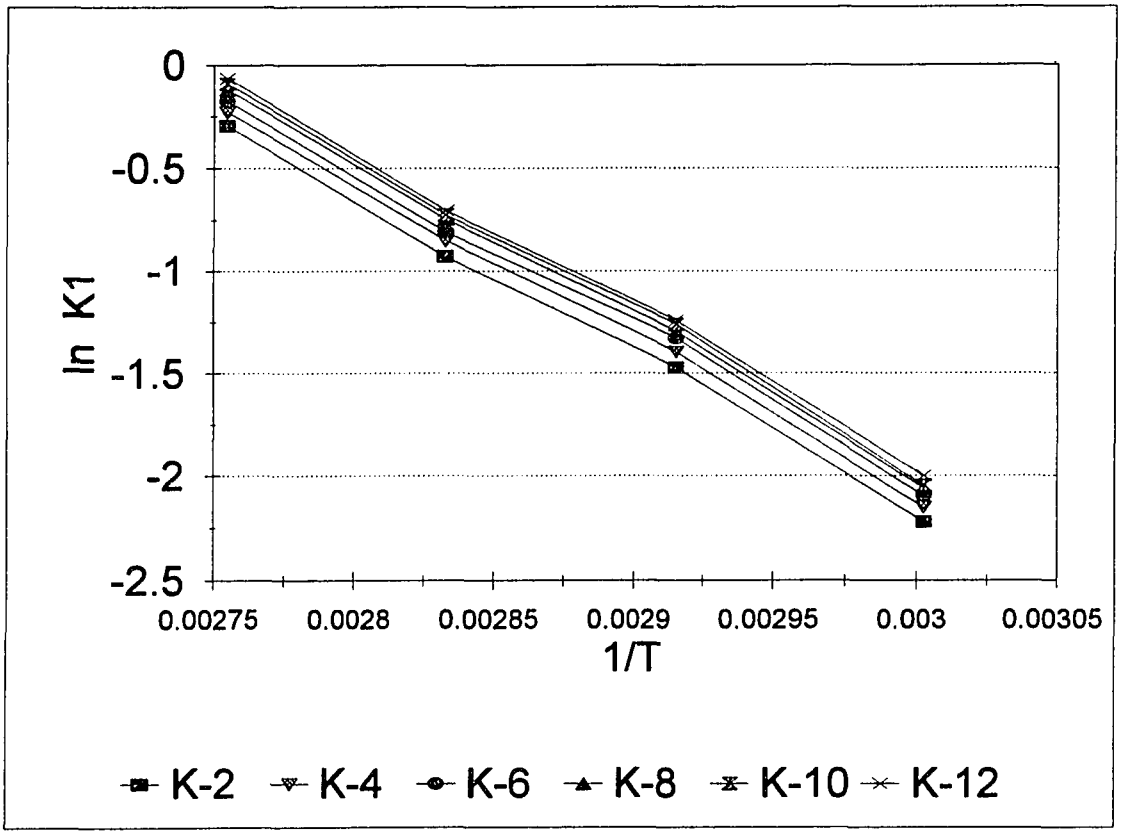


Figure 34 The Activated Energy of Nitrocellulose Hydrolysis at Various A/S Ratios

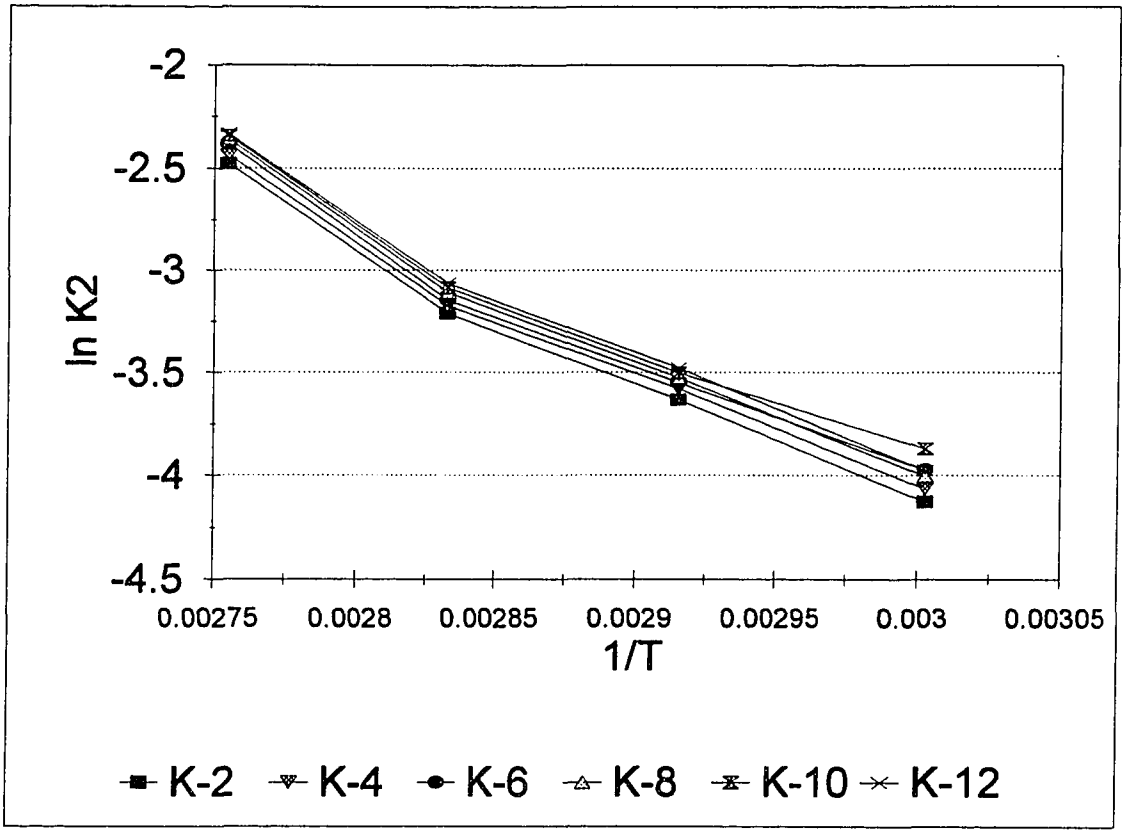


Figure 35 The Activated Energy of Glucose Degradation at Various A/S Ratios

much faster than glucose degradation. During this experiment, all the glucose produced from nitrocellulose hydrolysis was degraded into small molecular weight organic acids at almost the same rate.

Another study using diluted hydrochloric acid at room temperature was also conducted but the reaction rate for acid hydrolysis was too slow to be observed within a reasonable reaction time. This study shows that of the two most important factors affecting acid hydrolysis, acid concentration and temperature, acid concentration has more influence.

6.2 Effect of Acid/Solid Ratio on Acid Hydrolysis

The effect of Acid/Solid ratios on acid hydrolysis was investigated in this part of study. Six different hydrochloric acid (ml) / nitrocellulose (g) ratios, 2/0.4, 4/0.4, 6/0.4, 8/0.4, 10/0.4, and 12/0.4, were studied The experiments were used to evaluate the performance of hydrolysis and glucose degradation. The data which generated in this study, including glucose production in mmole, glucose concentration in mM, and glucose yield in % verse reaction time, are shown in Appendices A to C.

Saeman (1945) has shown that cellulose hydrolysis and glucose degradation can be modeled as first-order reactions. Figure 36 shows that the plot of natural logarithm of glucose concentration verse reaction time curve was very similar to what Saeman observed in his work. Nitrocellulose hydrolysis can, therefore, be modeled as a first-order reaction. All results showed a similar pattern for the hydrolysis study. The glucose production first increased, reached a maximum value, then slowly decreased. As previously indicated, the maximum concentration of glucose produced depends on

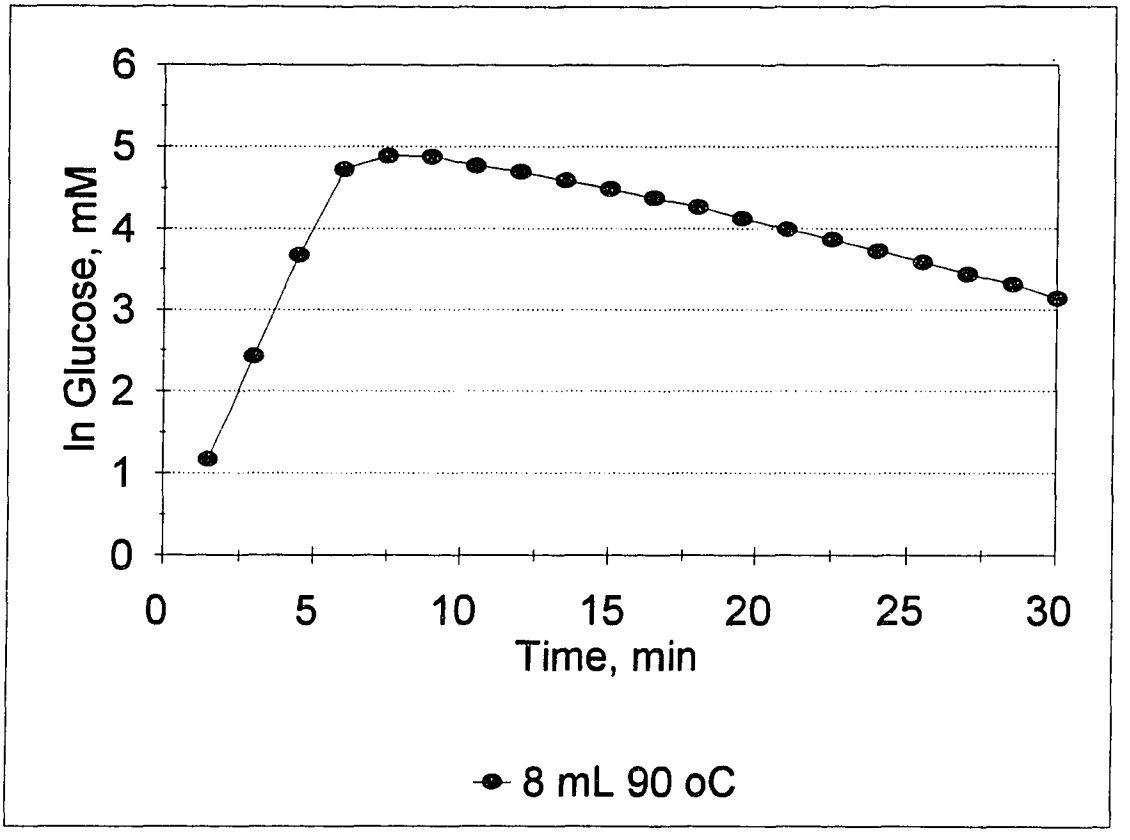


Figure 36 The Plot of Natural Logarithm of Glucose Concentration verse Reaction Time (A/S ratio = 8 ml /0.4 g, at 90 °C)

reaction temperature and A/S ratio. For the curve obtained (Figure 36), in the first stage, where the glucose production increases, can be called the stage of nitrocellulose hydrolysis; and the second stage, where the glucose concentration decreases, can be called glucose degradation.

This study tried to determine the relation between rate of nitrocellulose hydrolysis and Acid/Solid Ratio. Plots of rate constants verse A/S ratios were conducted at different scales. It was found that the only plot that could express these two parameters with a linear relationship was the plot of natural logarithm of rate constants for nitrocellulose hydrolysis and glucose degradation verse natural logarithm of Acid/Solid ratios. These plots are shown in Figures 37 and 38. These two figures show that even though each test was conducted at different temperatures each plot had a similar slope. The average slope of nitrocellulose hydrolysis is 0.1286 ± 0.0052 and the average slope of glucose degradation is 0.084 ± 0.0008. The results indicate that the more hydrochloric acid added to the reaction the faster the nitrocellulose would degrade, and the less glucose would remain in solution. It also indicates that acid/solid ratio will affect the reaction rate of nitrocellulose hydrolysis more than glucose degradation.

6.3 Effect of Acid Concentration

Different hydrochloric acid concentrations (38, 30.4, 25.3, 21.7, and 19 %) were reacted with 0.4 gram nitrocellulose at 80 and 90 degree Celsius. The experimental procedure was similar to the approach for the study of the effect of Acid/Solid ratio study. A natural logarithm plot of rate constant verse acid concentration (%) was employed to evaluate the effect of acid concentration on hydrolysis of nitrocellulose and glucose degradation. The

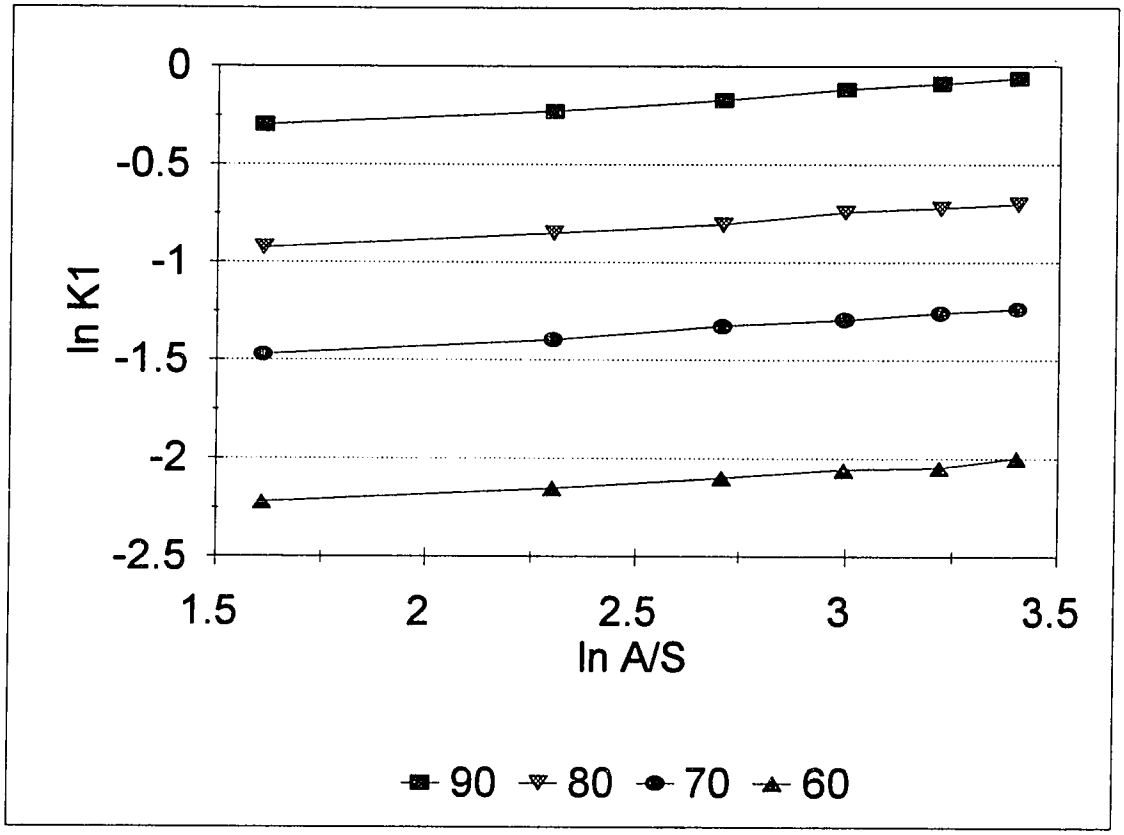


Figure 37 The Relationship between ln K_1 and ln A/S Ratio at Various Temperatures

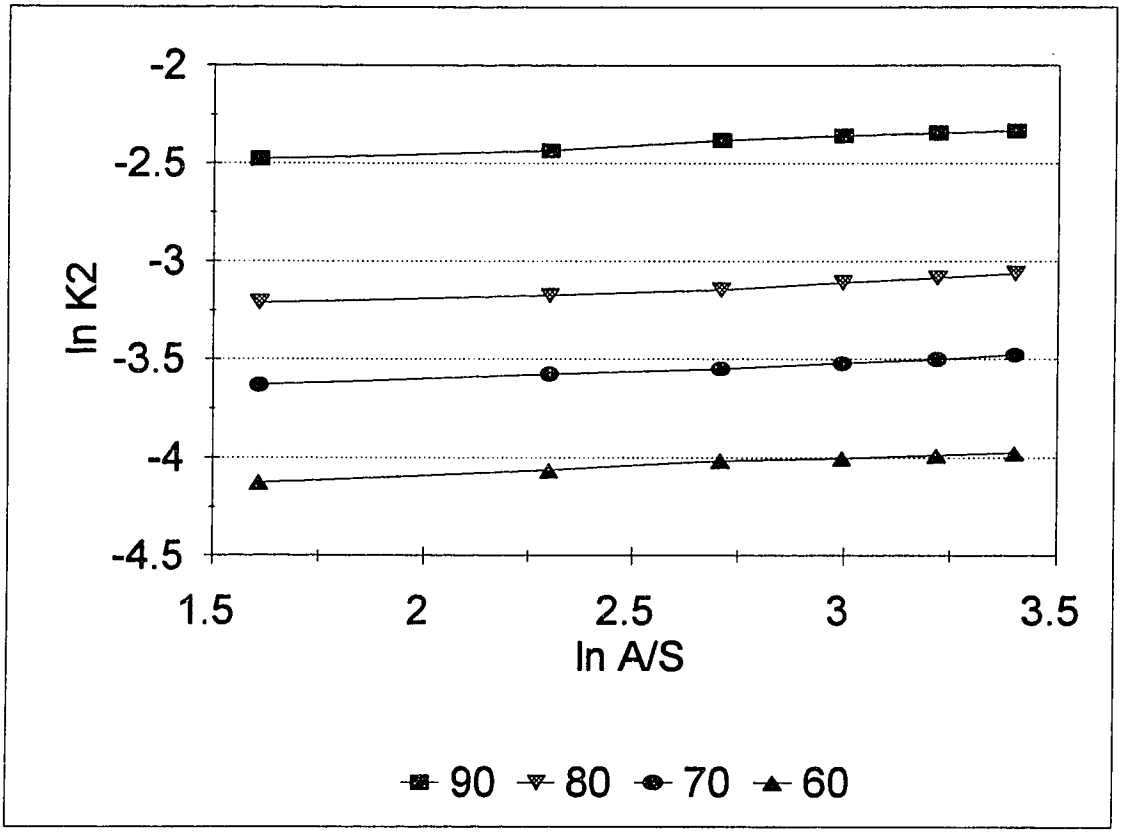


Figure 38 The Relationship between ln K_2 and ln A/S Ratio at Various Temperatures

results are shown in Figures 39 and 40. A linear relationship is shown to exist between the natural logarithm of rate constant and acid concentration for both nitrocellulose hydrolysis and glucose degradation. The slope of ln K_1 verse ln A is 1.8183 ± 0.0103 and the slope of ln K_2 verse ln A is 0.5436 ± 0.0093. This test shows that the higher the acid concentration, the faster the reaction. The results also indicate that acid concentration affects the reaction rate of hydrolysis process more than that of glucose degradation.

By combining all the parameters studied together, the reaction of nitrocellulose hydrolysis and glucose degradation can be expressed as a function of acid concentration, acid/solid ratio, and temperature. The complete reaction of nitrocellulose hydrolysis and glucose degradation can be expressed by the following kinetic models :

$$K_1 = 1.0841 \pm 0.0729 \times 10^6 (A)^{1.81831 \pm 0.0103} (A/S)^{0.1286 \pm 0.0052} \exp(-15{,}233 \pm 89/RT)$$

$$K_2 = 5.5082 \pm 0.2901 \times 10^5 (A)^{0.5436 \pm 0.0093} (A/S)^{0.0844 \pm 0.0008} \exp(-12{,}568 \pm 319/RT)$$

where K_1 = rate constant of hydrolysis of nitrocellulose, min^{-1}

K_2 = rate constant of degradation of glucose, min^{-1}

A = Acid Concentration, %

(A/S) = Acid/Solid Ratio, ml/g

T = Absolute Temperature, °K, and

R = Universal Gas Constant, 1,987 g-cal/(g-mole)(°K)

This equation shows that higher acid concentration, acid/solid ratio, and/or temperature will have faster reaction for both hydrolysis and degradation reaction. However, these two equations also indicate that the three parameters have more stronger effect on nitrocellulose hydrolysis than on glucose degradation.

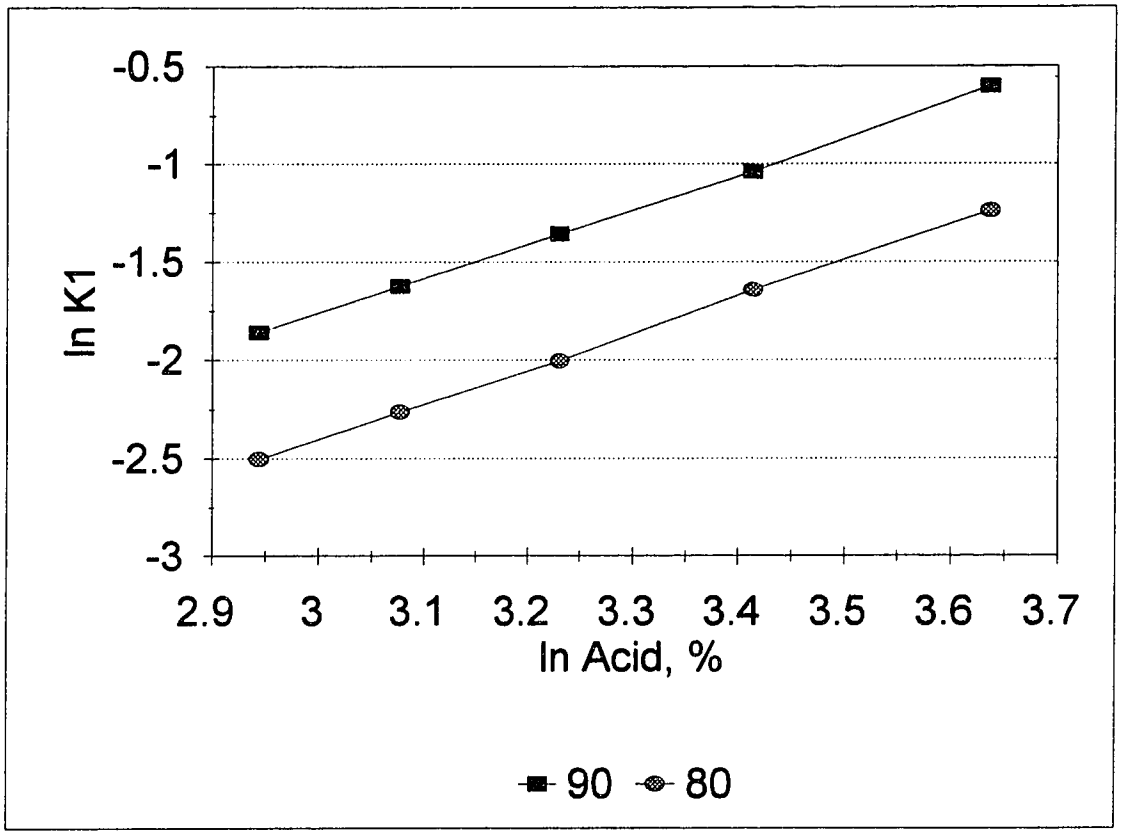


Figure 39 Effect of Acid Concentration on Nitrocellulose Hydrolysis

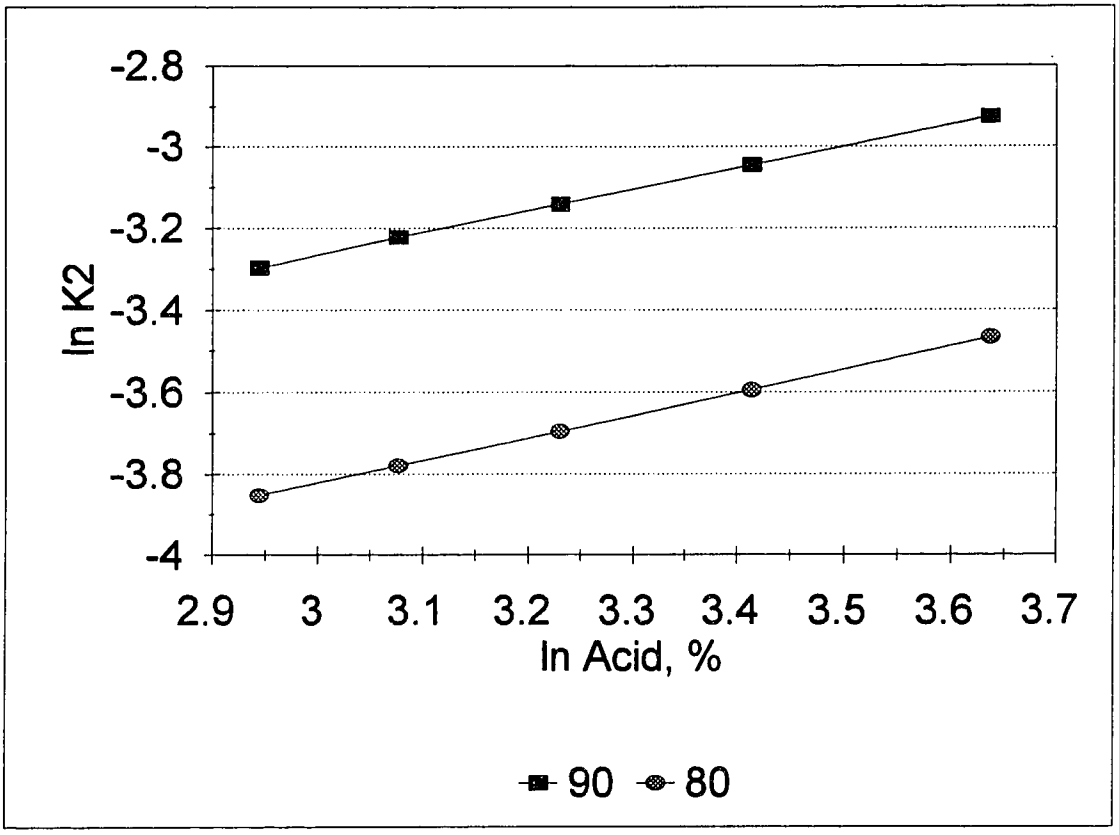


Figure 40 Effect of Acid Concentration on Glucose Degradation

6.4 Glucose Conversion

Glucose is the dominant end product for acid hydrolysis of cellulosic materials. The glucose produced at various acid/solid ratios were determined. Glucose Yield, Y, is defined as :

$$Y = \frac{\text{Total Glucose Produced}}{\text{Total Potential Glucose}} \times 100\%$$

The total potential glucose is a theoretical value which is obtained by calculation based on a nitrogen content in nitrocellulose of 13.5 %.

Experiments at several temperatures were performed and the results were similar. The glucose yields during nitrocellulose hydrolysis with different A/S ratios in 90 °C is shown in Figure 41. The glucose yield for each tested A/S ratios at other temperatures are shown in Appendix C. Figure 41 shows that the glucose yield from nitrocellulose hydrolysis is affected by A/S ratios. The higher A/S ratios the stronger the acid to hydrolyze nitrocellulose to glucose and the higher the glucose yield. It has mentioned earlier that the reaction temperature does not affect the glucose yield that much but it does influence the rate of the reaction. The maximum glucose yields decreased from 85 percent for an A/S ratio of 12/0.4 to 38 percent for an A/S ratio of 2/0.4. This confirms the observation stated earlier that the glucose yield is related to both reaction rate of nitrocellulose hydrolysis and the glucose degradation. The higher the nitrocellulose hydrolysis rate, the more glucose remained in the solution.

Other than glucose, citric and formic acids constituted a major part of organic acids from the hydrolysis process of nitrocellulose by using High Performance Liquid

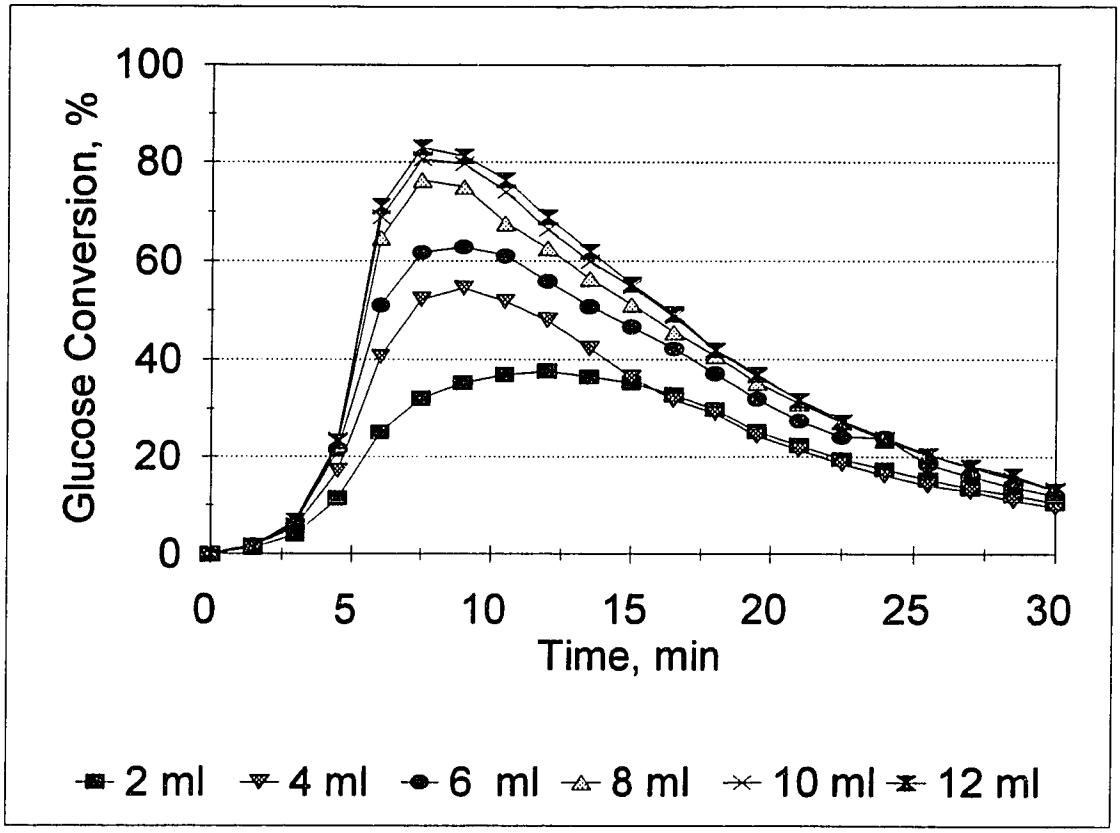


Figure 41 Glucose Yield in Nitrocellulose Hydrolysis with Various A/S Ratios

Chromotograph (HPLC) analysis. Small amounts of oxalic, malic, pyruvic, succinic, glycolic, and adipic acids were also detected in the hydrolyzate.

6.5 Change of Acid Concentration During Acid Hydrolysis

The acid concentration was measured by titration with sodium hydroxide. The test monitored the hydrogen ion change during hydrolysis process. The change of acid concentration in the aqueous phase is shown in Figure 42. It shows that there is an initial decrease of acid concentration followed by an increase of concentration to its original value and then a further gradual increase. If a small amount of substrate (higher A/S ratios) were used in the test, the pH drop was small and the acid returned to its original concentration and stopped at that value. However, when a large amount of substrate (lower A/S ratios) were used, the final acid concentration would be higher than the original concentration as it is shown in Figure 42. The decrease of acid concentration during the early stage might be due to the adsorption of acid on solids and the increase of concentration during the later stage could be due to the desorption of hydrogen ions resulting from the decreasing solids content. This observation was also reported in Ullal's (1984) work.

6.6 Nitrogen Balance

In order to determine the change of nitrogen forms during acid hydrolysis, 0.4 gram of nitrocellulose with different amounts of hydrochloric acid was put into media tubes and sealed with a teflon liner air tight cap. These tubes were then put into a water bath controlled at 60 and 80 degree Celsius. Tubes were removed from the water bath at

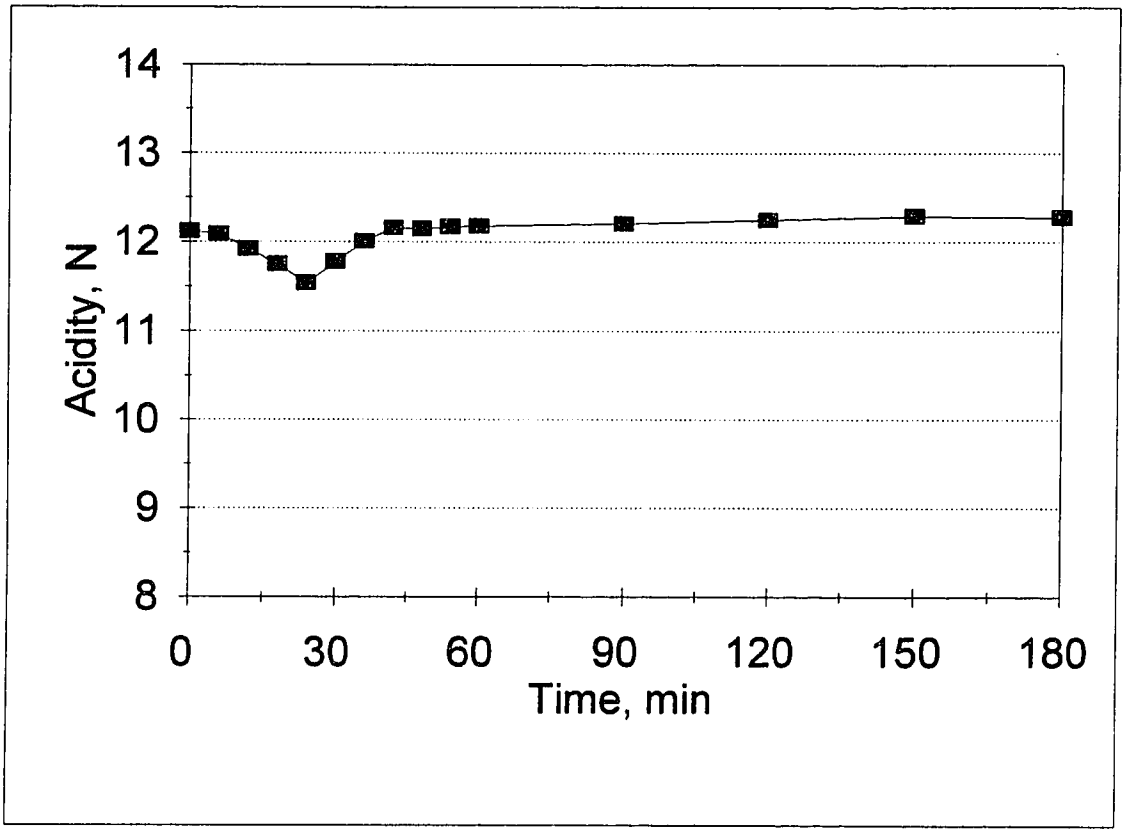


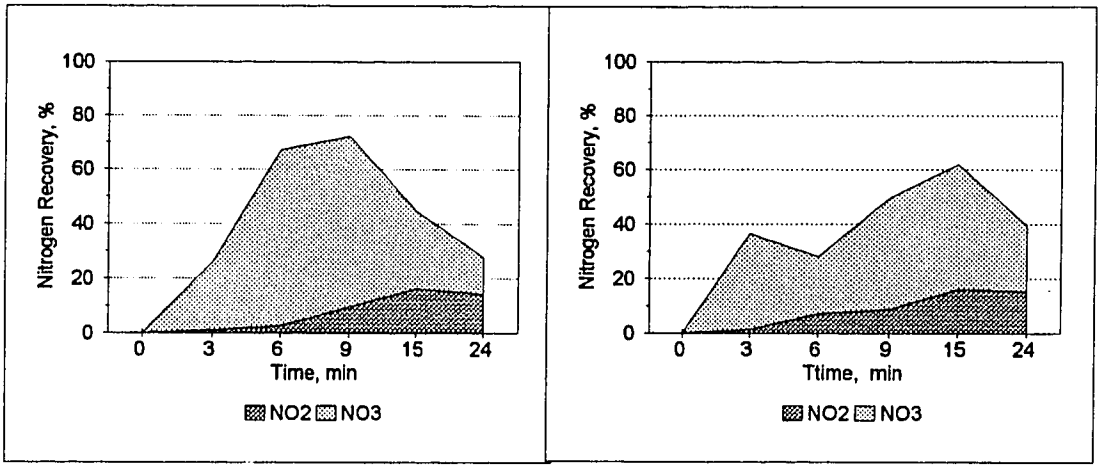
Figure 42 Changes of Acid Concentration during Acid Hydrolysis

various intervals and quenched in ice water, and 10 ml of 10 N sodium hydroxide solution was injected into tubes. The tubes were shaken and the contents were analyzed for nitrate and nitrite content by Ion Chromatography. During the process of hydrolysis, first a light yellow color was observed in the tube, and then it turned to reddish brown , at same time nitrocellulose disappeared gradually, finally all solids were gone and the color had changed to brown or dark brown. The nitrogen dioxide is the only reddish brown gas among all different nitrogenous gases. The gas produced in hydrolysis could be the nitrogen dioxide. In order to confirm this, sodium hydroxide solution was injected into tube to react with nitrogen dioxide. When caustic solution was injected into tube, the reddish brown color disappeared. Nitrogen dioxide reacts with hydroxide ion to form nitrate and nitrite. Nitrogen dioxide also can react with water to form nitrous and nitric acids. The reactions can be expressed as the following equations :

$$2NO_2 + 2\ OH^- \rightarrow NO_3^- + NO_2^- + H_2O$$

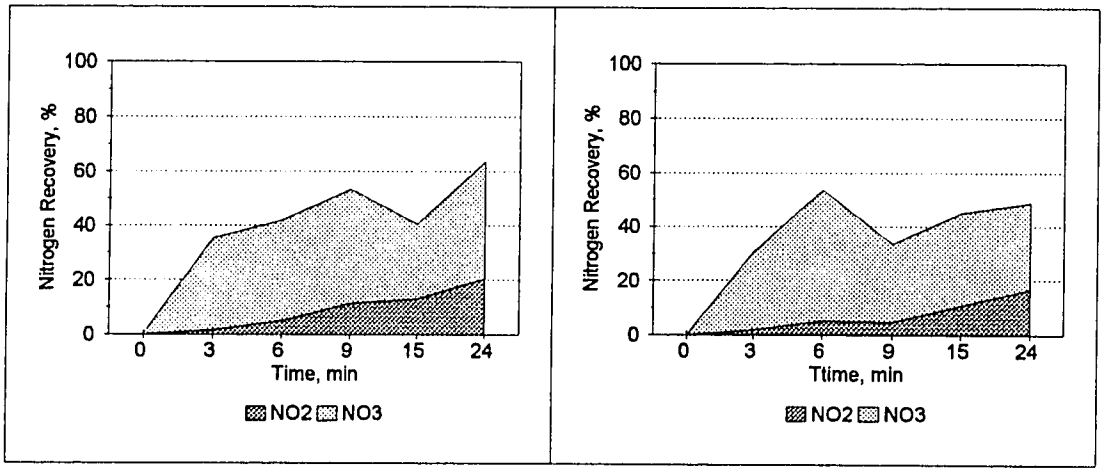
$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$$

All nitrogen balance calculation of tested nitrocellulose were based on a nitrogen content of approximately 13.5 %. The nitrogen recovery results are presented from Figures 43 to 44. From the results of the nitrogen recovery study, it was found that no consistent pattern could be obtained. Generally, the concentration of nitrite increased slowly. However, the concentration of nitrate increased to a peak concentration, although some tests showed a concentration drop before the peak concentration. After that the nitrate concentration was either dropped or remained the same in solution. At 60 °C, the maximum nitrogen recovery was about 85% at A/S ratio = 10 ml / 0.4 g, and about 50%



A/S = 10 ml/ 0.4 g

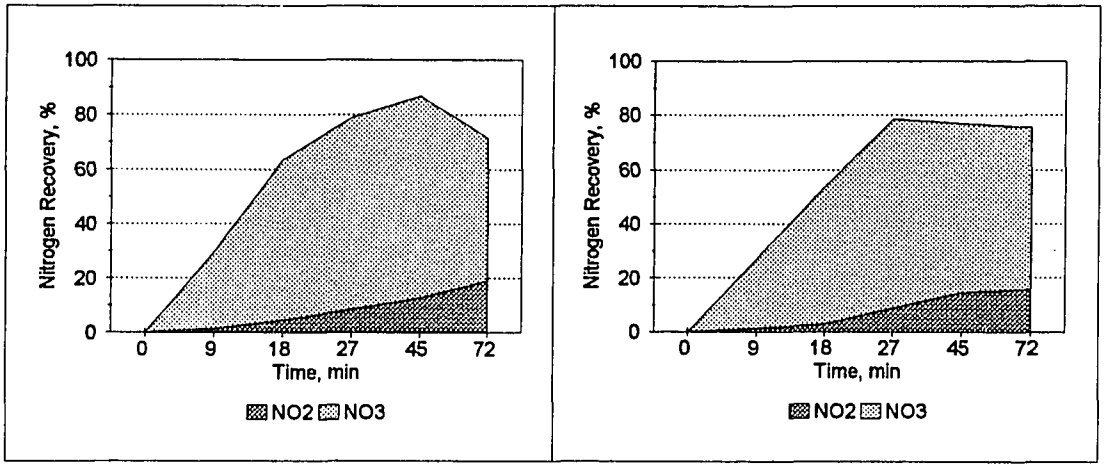
A/S = 8 ml/ 0.4 g



A/S = 6 ml/ 0.4 g

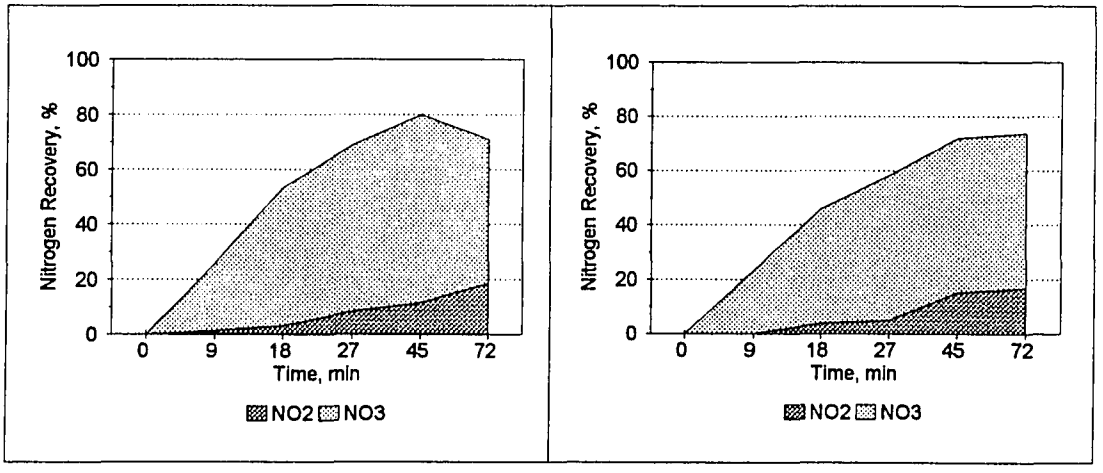
A/S = 4 ml/ 0.4 g

Figure 43 Nitrogen Recovery in Nitrocellulose Hydrolysis at 80 °C



A/S = 10 ml/ 0.4 g

A/S = 8 ml/ 0.4 g



A/S = 6 ml/ 0.4 g

A/S = 4 ml/ 0.4 g

Figure 44 Nitrogen Recovery in Nitrocellulose Hydrolysis at 60 °C

nitrogen recovery was obtained at 80 °C and A/S ratio = 4 ml / 0.4 g. This experiment indicates that more nitrogen can be recovered at lower reaction temperature and higher A/S ratio based on the measurement of nitrate and nitrite by ion chromatograph in caustic solution.

Most of the nitrogen recovery in this experiment was measured in the nitrate form and only small amount of nitrite was detected. This could be due to the unstable nature of nitrite. Ammonia was also detected from the solution, but comparing to the concentration of nitrate and nitrite, ammonia was negligible. When the supply of oxygen is limited part of the nitric oxide is converted to nitrogen trioxide and the rest of nitric oxide can also react with nitrogen dioxide to form nitrogen trioxide.

$$4NO + O_2 \rightarrow 2N_2O_3$$

$$NO + NO_2 \leftrightarrow N_2O_3$$

This could be the reason why only 85% of nitrogen was recovered in the form of nitrate and nitrite. The nitrogen that was not found in the solution might have escapes from tube during the injection of caustic solution. Another explanation may be due to the HNO_3 produced from hydrolysis of nitrocellulose that was further reduced to NO, N_2O, and N_2. In this situation, nitrogen can not be detected in either nitrate or nitrite from Ion Chromatograph analysis.

CHAPTER 7

PROPOSED NITROCELLULOSE TREATMENT METHOD

This study has shown that biodegradation of nitrocellulose is not an economically and technically attractive alternative. The study also showed that it is possible to break down nitrocellulose through acid hydrolysis. However, the use of acid hydrolysis has one drawback, the expense of the strong acid. Fortunately, the recovery of acid is an existing technology (Goldstein and Easter, 1992), which can be used for this treatment method. Therefore, a schematic flow diagram as shown in Figure 45 is recommended for treatment of nitrocellulose. The nitrocellulose is first treated with strong acid and broken down to glucose by acid hydrolysis. Electrodialysis can be used to recover the acid. The glucose produced during hydrolysis will be converted to ethanol or other useful products by fermentation after neutralization. Again, the utilization of fermentation for glucose has been widely used in industry, and it can be adapted without problems. More discussion about this technology are presented below.

7.1 Acid Separation and Recovery

7.1.1 Hydrochloric Acid Stripper and Absorption

The hydrolyzate solution leaving the reactor still contains all the hydrochloric acid originally added to the reactor. The acid must be separated from the sugars, not only to permit fermentation, but also to reduce processing costs by recovering and recycling the hydrochloric acid. The volatility of hydrochloric acid gas allows it to be stripped from the hydrolyzate at reduced pressure. With pure water, this product can be carried all the way

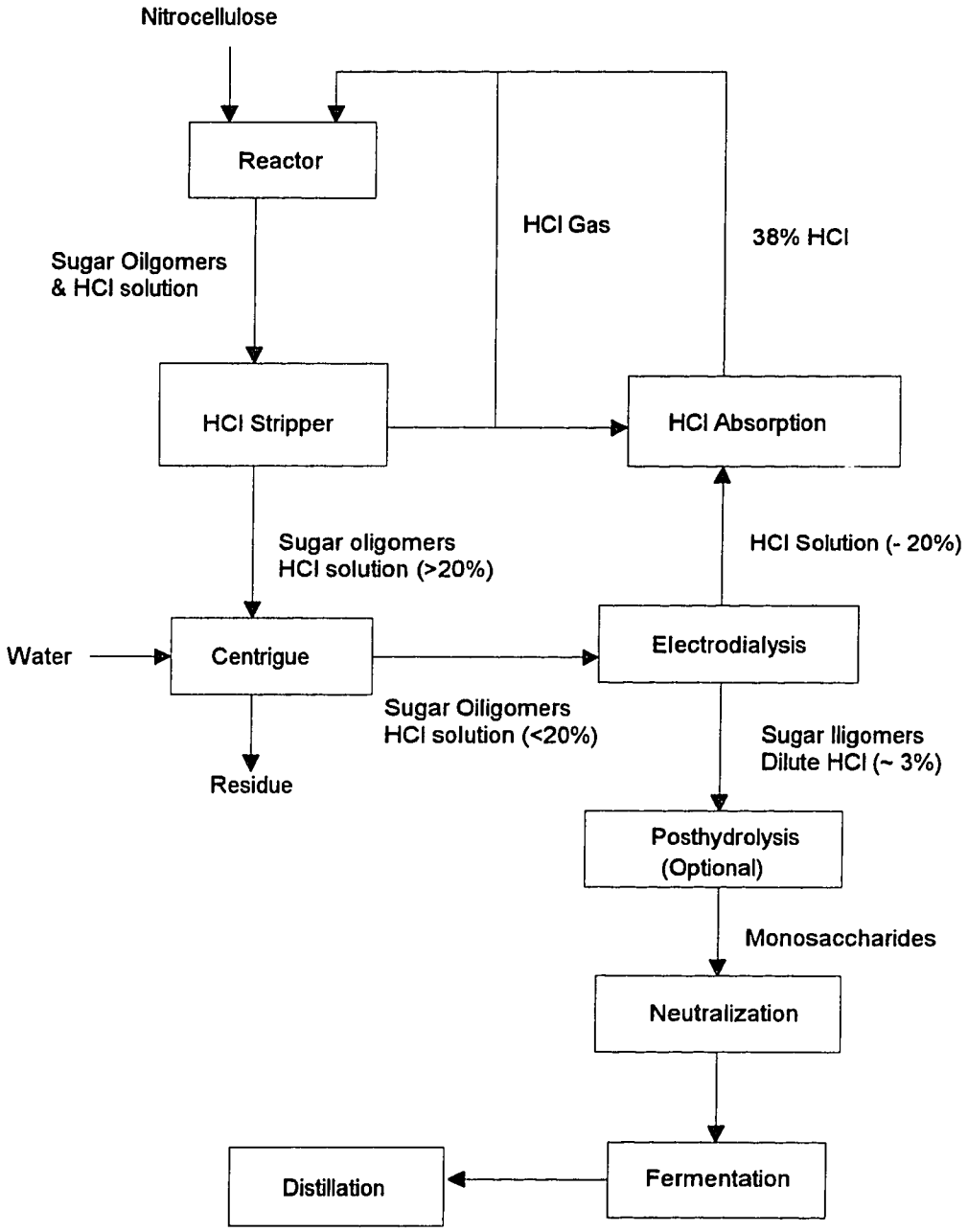


Figure 45 Proposed Schematic Flow of Acid Hydrolysis of Nitrocellulose

to the azeotrope at 20.2% hydrochloric acid. However, the hydrochloric acid also binds to the sugars in hydrolyzate as well as water, leading to a reduction in hydrochloric acid volatility and an upward shift in the azeotropic composition in addition to that which occurs at the reduced pressure. However, hydrochloric acid volatility can be compensated by increasing the temperature in the stripper, but it cannot exceed the temperature limit adopted for the reactor. Higher temperatures would also cause degradation of the sugars. In stripping process, 78% of the original acid can be stripped as a 48.6% solution (Goldstein, 1992).

The hydrochloric acid stripped from the hydrolyzate would be recycled. Most of the acid recovered by the electrodialysis unit would enter an absorption system, as an approximately 20% hydrochloric acid solution. The remaining hydrochloric acid gas would be compressed sufficiently to bring the concentration of the solution that leaves the absorber, up to 45% before being reintroduced into the reactor while maintaining this concentration at the moderate temperature in the reactor.

7.1.2 Electrodialysis

Hydrochloric acid and water form a maximum boiling azeotrope and breaking this barrier can be difficult and costly. During World War II, the Germans operated concentrated wood hydrolysis plants and their experiences in hydrochloric acid recovery have been reported. The methods they used include combinations of atmospheric, vacuum, and extractive distillation, as well as evaporation using a mineral oil or steam as a heat transfer medium. Spray drying by direct contact with a stream of hot air has also been tried.

Nguyen et al. (1981) designed a two-stage system where the first recovery stage utilizes vacuum distillation and the final stage extractive distillation with calcium chloride. Forster et al. obtained a US. patent in 1980 for an organic solvent extraction technique. This technique covers the C5-C9 alcohols including the primary, secondary, and tertiary isomers. The first recovery step involves removing hydrochloric acid from the hydrolyzate by continuous, countercurrent extraction. In the second stage, hydrochloric acid is recovered by distillation.

Hydrochloric acid recovery from wood hydrolyzates can also be accomplished by electrodialysis using synthetic polymer membranes. An electrodialysis cell is constructed from an electrolytic cell by placing a cathodic membrane adjacent to the cathode and an anodic membrane next to the anode. When an applied electromotive force causes hydrogen ions to migrate toward the cathode and chloride ions to the anode, the interior compartment solution loses hydrochloric acid while the external compartment solutions gain hydrochloric acid. The applied electromotive force causes the migration of ionic components, while the concentration difference creates transport by diffusion and osmosis. Both the osmotic and the electromotive process transports hydrochloric acid against its concentration gradient.

Urano et al. (1984) investigated the acidic wastewater which is released from the iron and steel industry and demonstrated the acids (sulfuric and hydrochloric acids) can be efficiently concentrated by electrodialysis. An apparatus for the electrodialysis used in this study is shown in Figure 46, and the properties of ion-exchange membranes of Selemion CMV and AAV (Asahi Glass Co. Ltd.) are shown in Table 24.

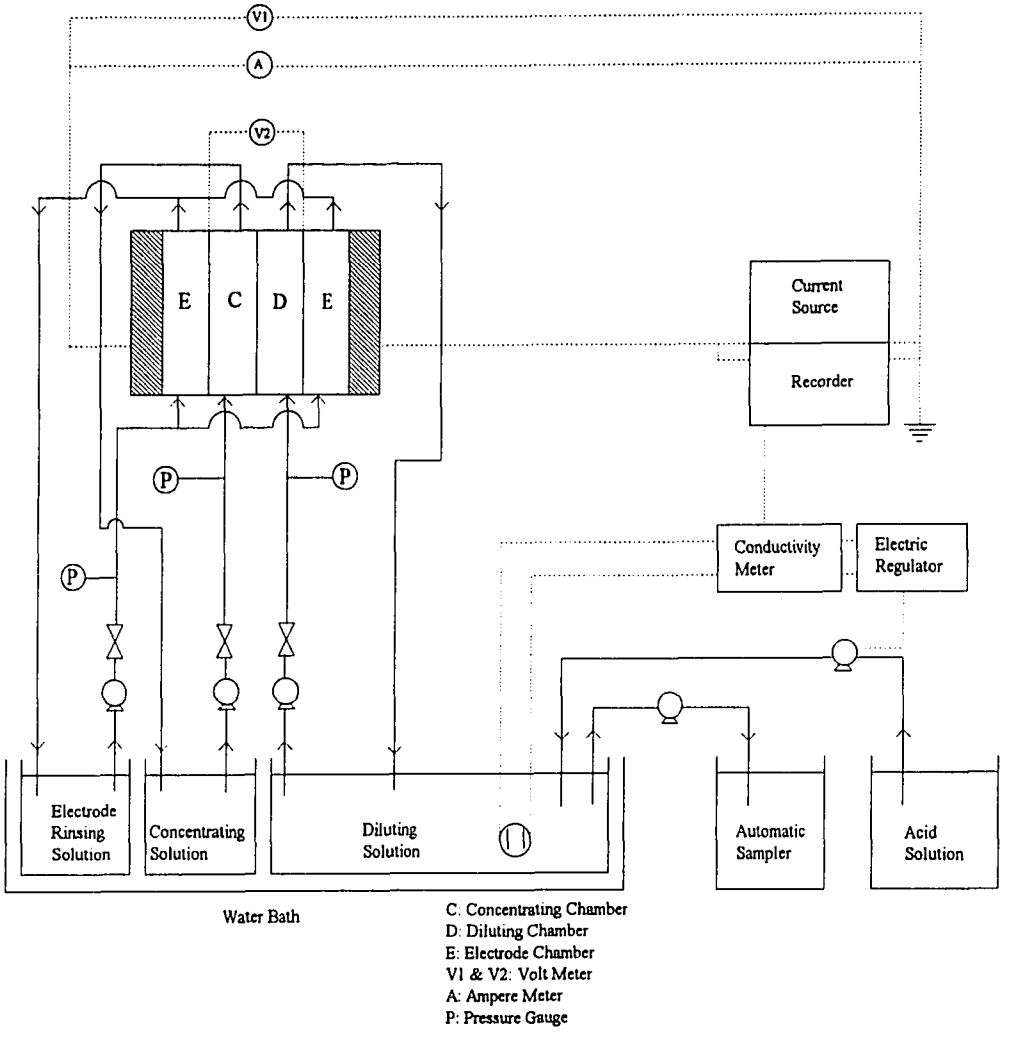


Figure 46 Experimental Apparatus for Electrodialysis (Urano et al., 1984)

Table 24 Properties of Ion-Exchange Membranes (Urano et al., 1984)

	Anion-Exchange Membrane	**Cation-Exchange Membrane**
Commercial name	Selemion AAV	Selemion CMV
Thickness, cm	1.3×10^{-2}	1.3×10^{-2}
Ion-exchange capacity, equiv/g-dry membrane	7.7×10^{-4}	2.0×10^{-3}
Transport number	> 0.90 for Cl^-	>0.91 for H^+
Weight of dry membrane, g/cm^2-membrane	1.3×10^{-2}	1.4×10^{-2}

More than 40% current efficiencies was reported by Huang and Juang (1986) in sulfuric acid-glucose-xylose mixture from dilute sulfuric acid hydrolysis by electrodialysis process. The ion-exchange membranes, Selemion CMV and Selemion AMV, used in this study were manufactured by Asahi Glass Co. of Japan. They are homogeneous membranes, and their properties are listed in Table 25.

Goldstein's works (1989 and 1992) showed the technical feasibility of using membrane technology to separate hydrochloric acid from sugars in cellulose hydrolyzates. Two membrane systems were chosen for their ability to withstand exposure to 20% hydrochloric acid and 60% sulfuric acid in this study. The membraned stack was procured from Ionics Co. containing 20 type 103-QZL-386 anion-exchange membranes and 20 type 61-CZL-386 cation-exchange membrane. The properties of membranes are listed in Table 26. They indicated that the permeability of disaccharides was less than 1% of the acids

Table 25 Specifications of Ion-Exchange Membranes (Huang and Juang, 1986)

	Selemion CMV	**Selemion AMV**
Type	high acidic ion-exchange membrane	high basic ion-exchange membrane
Base material	Tevilon cloth (PVC)	Tevilon cloth (PVC)
Thickness, mm	0.12-0.15	0.11-0.14
Effective electrical resistance, Ω/cm^2	190-230	280-320
Transport number	0.91-0.93	0.94-0.96
Burst strength, kg/cm^2	6-8	4-7

Table 26 Properties of Ion-Exchange Membrane (Goldstein et al, 1989)

	Anion-transfer membrane	**Cation-transfer membrane**
Commercial name	103-QZL-386	61-CZL-386
Reinforcing fabric	Modacrylic	Modacrylic
Weight, mg/cm^2	15.3	14.0
Thickness, mm	0.63	0.6
Burst strength, kg/cm^2	10.8	8.0
Capacity, meq/dry gram resin	2.1	2.7

permeability and acid flux in diffusion dialysis was only 6% of acid flux at optimum current density in electrodialysis. Ideally, the separation of hydrochloric acid from the sugar in the hydrolyzates by electrodialysis should provide a maximum yield of recovered acid at maximum concentration with minimum power consumption using minimum membrane area. Their experimental results made it obvious that these conditions cannot be met simultaneously. At the highest current efficiencies and, thus, the minimum membrane area, the final acid concentration in the concentration was too low. At the highest final acid concentrations, the percentage of acid transferred fell off, and power consumption and membrane area were high. As hydrochloric acid passed through the membranes, water was also transferred by osmotic forces. As the volume of acid and water transferred to the recovery stream increased, the volume of the hydrolyzate stream decreased. The sugars were retained in the hydrolyzate at concentrations up to 60%. The hydrochloric acid concentration of the hydrolyzate at the end of electrodialysis was about 3%, based on acid and water alone. This acid can be neutralized with base before fermentation.

7.2 Ethanol Fermentation and Purification

The microbial conversion of agricultural substrates into ethanol is an ancient practice that certainly predates the science of microbiology, the chemistry of the distillation process, and the engineering of ethanol fermentation plants. Pasteur's research with French wines in the 1860s defined the basic concepts of the fermentation process and commercial interests in beer, wine, and hard liquor production promoted continual advances in the understanding of the biochemistry of ethanol fermentation.

7.2.1 Effect of Microorganisms

When microorganisms are grown on sugars in the presence of oxygen, they obtain cellular material and energy by oxidizing these organic compounds. As a result of this oxidation, carbon dioxide and water are produced as metabolic waste products. The excess electrons from the oxidation of sugars are carried by an electron transport system to oxygen, the final electron acceptor, and water is formed. Certain microorganisms are able to grow on sugars in the absence of oxygen, utilizing sugars as electron acceptors instead of oxygen. During this anaerobic growth, sugars are oxidized and excess electrons are transferred to organic acceptor molecules and ethanol is produced as a waste product of the fermentation process instead of water. Microorganisms responsible for ethanol production are facultative, i.e. they can grow with or without oxygen. If air is allowed to enter the fermentation process in sufficient quantities, then microbial metabolism will switch from an anaerobic, ethanol-producing process, to the more efficient aerobic process (Krebs cycle), and no further ethanol will be produced. The previously produced ethanol may actually be utilized (glycolytic pathway) and oxidized to carbon dioxide and cell material. Thus, microbes produce ethanol when growth conditions do not support oxidative metabolic process, thereby requiring these facultative microorganisms to employ a less efficient pathway which produces ethanol as a metabolic waste product.

Although numerous microorganisms are capable of producing ethanol, not all are suitable for industrial processes. Also, no one culture is ideal for efficient conversion or high attenuation of all substrates. Yeast cultures, (in particular *Saccharomyces* sp.) have been most extensively examined. Various species of *Saccharomyces* are used for ethanol

production processes because they are very efficient in converting sugars into ethanol and are not as strongly inhibited by high ethanol concentrations as are other microbes. Theoretically, one mole of glucose can produce two moles of ethanol (511 Kg of ethanol from 1000 Kg of glucose). The yeast ethanolic fermentation is the most efficient pathway for ethanol production, but it is not the only pathway leading to ethanol accumulation. The other pathways and involved microorganisms are listed in Table 27. Recently, bacterial cultures of *Bacillus* and *Clostridium* species have been explored for high-temperature ethanol fermentation processes. *Bacillus* and *Clostridium* are able to grow as thermophilic microorganisms and may therefore reduce the cost of the fermentation and distillation processes. However, the yield of ethanol by bacterial cultures is not as high as in yeast fermentations.

Conventional ethanol fermentations are usually conducted as batch processes where the reactor is charged with substrate, the microbial inoculum is added, and the process allowed to run to completion, about 4-10 days (Munnecke, 1981). The fermentation tank can be mechanically agitated by impellers to decrease diffusion limitations, or the natural agitation created by escaping carbon dioxide may be sufficient. In batch processes, the sugar is added batchwise at decreasing intervals to the growing culture, or continuously at an increasing rate as the microbial population expands. After the fermentation is complete, the cells are removed before distillation. The same type of fermenter used in batch processes can also be used with slight modification for continuous-flow operation. Here, the sugar and nutrient medium are continuously added to the reactor, and the effluent, which contains ethanol and cell material is continuously treated for cell separation and product recovery. Since the concentration of sugar in the

Table 27 Anaerobic Metabolism of Pyruvate (Brandt, 1981)

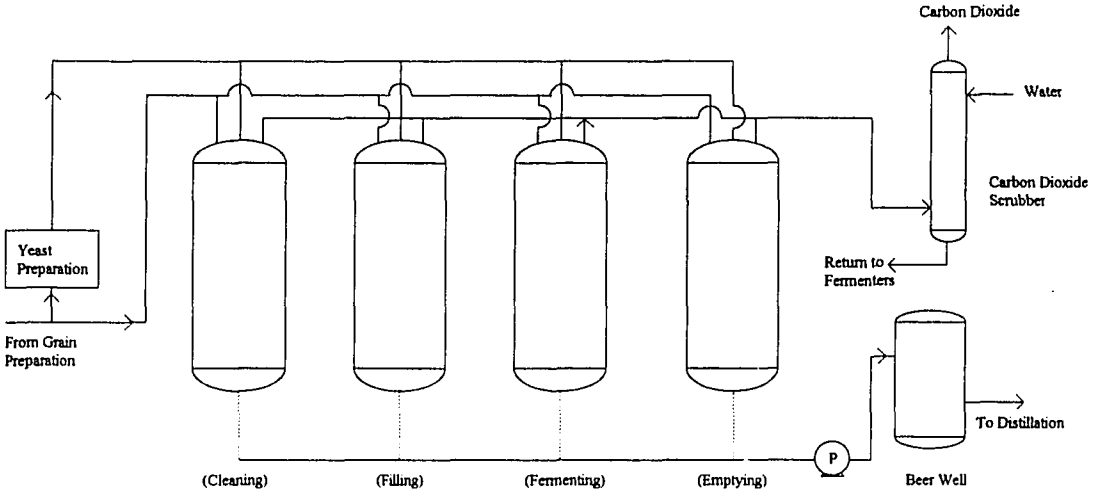
Type of fermentation	**End products**	**Microorganisms**
Ethanolic	Ethanol Carbon dioxide	Yeast *Zymomonas*
Mixed acid	Lactic, Formic, and Acetic acids Carbon dioxide Hydrogen Ethanol	*Clostridium* and many enteric bacteria
Butanediol	As in mixed acid plus 2,3-butanediol	Bacillus and other bacteria
Acetone/butanol	Acetic acid Butyric acid Ethanol and Butanol Acetone Isopropanol Carbon dioxide Hydrogen	*Clostridium*
Homolactic	Lactic acid	*Lactobacillus* *Streptococcus*

fermenter remains close to zero, there is no direct problem of high sugar concentrations adversely affecting cellular growth or ethanol production. The rate of sugar addition has to be regulated so that inhibitory levels of ethanol do not occur and cause decreased growth rate. The continuous fermenter for best efficiency should be operated near, but below, the maximal cellular growth rates. The processes for both batch and continuous fermentation of ethanol are shown in Figure 47.

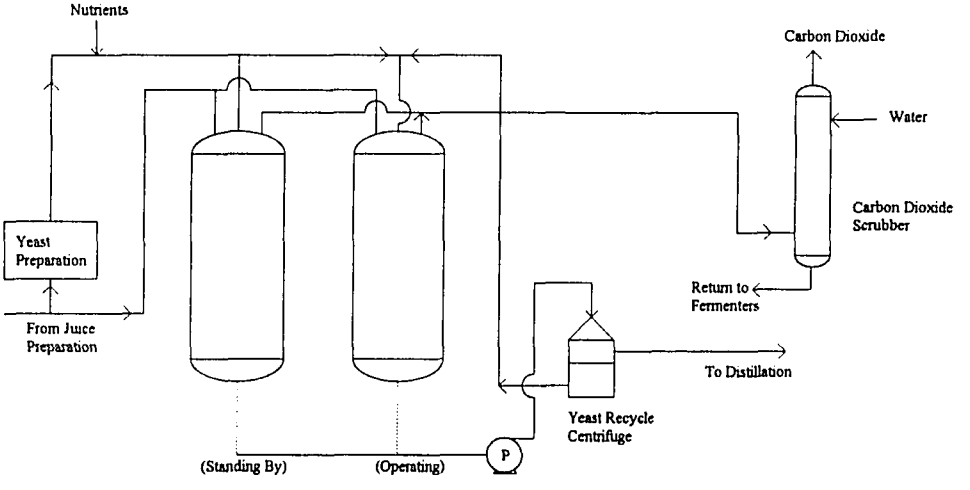
A modification of the continuous fermentation process involves conducting the fermentation under a vacuum. Operating under vacuum, ethanol can be continuously removed from the broth as it is produced and its inhibitory effects on cell growth are reduced. This modification allows for higher rates of ethanol production per liter of fermentation broth and creates a condensate containing a higher ethanol concentration for better distillation efficiency. Since ethanol production is not dependent on cellular growth, nongrowing cells can be immobilized in gels and placed into continuous-flow reactor. By maintaining nongrowth conditions, glucose conversion to ethanol can reach to above 95%. Another advantage of this process is that high cell densities are maintained, even higher than with cell recycling methods, and it does not require costly continual cell centrifugation and recycling. The efficiency of ethanol production by immobilized cells on a gram dry weight basis is reported to range upward from 80% in comparison to the productivity of free cell suspensions.

7.2.2 Ethanol Recovery

Ethanol recovery has been traditionally accomplished by distillation. A train of towers operating in series is employed, each accomplishing one or two separation of the ethanol



Batch Fermentation Processe



Continuous Fermentation Process

Figure 47 Processes for Batch and Continuous Fermentation of Ethanol (Brandt, 1981)

from components of the fermentation broth. The first tower (so called stillage separation) is designed to strip all ethanol from the broth and to increase the ethanol concentration in the overhead. The solids in the broth will be removed from bottom of the stream. The distillation sequence after the beer still will vary with the type of ethanol product. Potable ethanol requires refining to the specifications for the product in which it is used. Industrial ethanol requires removal of impurities, including fusel oils, which are byproducts of the fermentation. In addition, anhydrous industrial ethanol requires that an entrainer be added to break the water-ethanol azeotrope in a separate tower. The ethanol and entrainer are then separated in another tower. The processes for distillation of various ethanol products are shown in Figure 48 (Brandt, 1981). Anhydrous industrial ethanol requires at least four distillations in a standard design. To produce 99.5% fuel ethanol from the fermentation broth, a conventional distillation process consumes a great deal of energy. New concentration and dehydration technologies were studied at the Research Association for Petroleum Alternatives Development (RAPAD) program such as heat pump distillation, azeotropic distillation, supercritical fluid extraction and per-vaporization methods (Miyakawa, 1986).

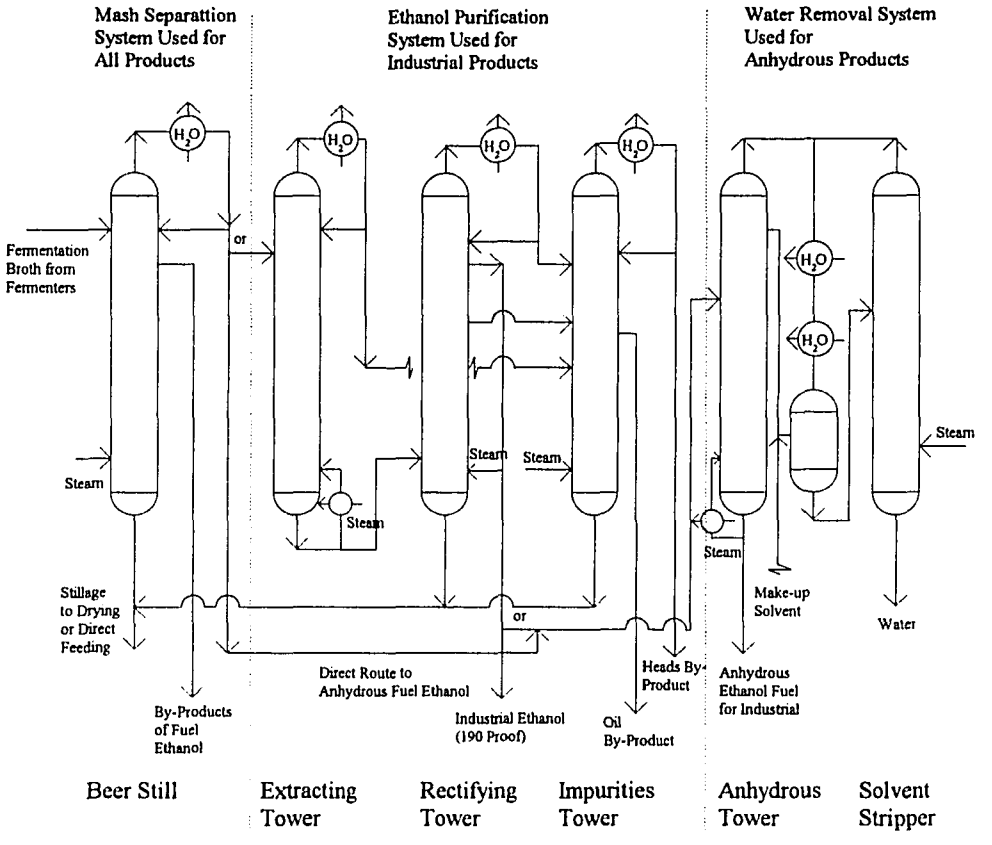


Figure 48 Processes for Distillation of Various Ethanol Products (Brandt, 1981)

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

This investigation has demonstrated the potential for utilization of acid hydrolysis and anaerobic digestion to treat munitions-grade nitrocellulose. Based on experimental results, the following conclusions and recommendations are made :

8.1 Anaerobic Treatment Process

1. The study showed that biodegradation of nitrocellulose through conventional anaerobic digestion is difficult, if it is the sole carbon source in the wastewater.
2. Experiments showed that nitrocellulose degradative enzyme could be induced by any of the three inducers added, i.e. lactose, cellobiose, and cellulose. Although gas production was low in the study, formation of the intermediate compounds, such as volatile organic acids indicate that partial biodegradation of nitrocellulose was achieved.
3. The experiment indicated that two-stage anaerobic system was not an effective enhancement for the decomposition of nitrocellulose. The stage-feed system has better conversion.
4. Nitrocellulose adversely affects the biodegradation of cellulose and decreases the gas production at inducer/nitrocellulose ratios lower than 1/1.
5. The experiments showed that nitrate was released from nitrocellulose by the hydrolysis of the nitrocellulose nitrate ester group which could be enhanced by the anaerobic microorganisms.

6. It can be concluded from this study that higher gas production was observed with a decrease in particle size distribution of the cellulose substrate. With the addition of Type 20 and Type 50 cellulose, a 48.9% of nitrocellulose conversion could be obtained.
7. Competitive inhibition was observed in the anaerobic biodegradation of nitrocellulose. The inhibitory effect can be overcome at higher cellulose concentrations. The kinetics and inhibition constants of nitrocellulose on cellulose biodegradation are listed as follows:

$$V_{max} = 13.93 \text{ mM/day},$$

$$K_m = 136.01 \text{ mM},$$

$$K_m' = 94.84 \text{ mM, and}$$

$$K_i = 17.32 \text{ mM}.$$

8. Since nitrocellulose can be converted to intermediate compounds and not the final gas product within the reasonable operation time. The development of analytical method to analyze nitrocellulose and the intermediate compounds are necessary.

8.2 Hydrochloric Acid Hydrolysis of Nitrocellulose

1. Acid hydrolysis at moderate temperatures showed good promise for treatment of nitrocellulose. Over 60 percent of the nitrocellulose was converted to glucose in the hydrolysis process under optimal conditions.
2. Acid hydrolysis of nitrocellulose was related to acid concentration, the ratio of acid to nitrocellulose, temperature and time. At 90 °C, the hydrolysis reaction needed only about 9 minutes to reach maximum glucose yield (about 85%). The hydrolysis

reaction took approximately 63 minutes to reach maximum glucose yield at 60 °C. Temperature only affected the rate of reaction, it did not influence the maximum glucose yield.

3. From the kinetic study of nitrocellulose hydrolysis and glucose degradation, the reaction rates were found to be in the following equations, respectively :

$$K_1 = 1.0841 \times 10^6 A^{1.8183} (A/S)^{0.1286} \exp(-15{,}233/RT)$$

$$K_2 = 5.5082 \times 10^5 A^{0.5436} (A/S)^{0.0844} \exp(-12{,}568/RT)$$

4. It was found that nitrogen was released as NO and NO_2 during the hydrolysis process. However the undesirable NO and NO_2 can be easily converted into nitrate and nitrite by passing them through a caustic solution scrubber.
5. Other than glucose, citric and formic acids constituted a major part of organic acids from the hydrolysis process. Small amounts of oxalic, malic, pyruvic, succinic, glycollic, and adipic acids were also detected in the hydrolyzate.
6. Treatment of nitrocellulose with an acid hydrolysis process, followed by a hydrochloric acid recovery system and an ethanol fermentation system is proposed to convert nitrocellulose waste into useful end products.

APPENDIX A

GLUCOSE PRODUCTION IN NITROCELLULOSE HYDROLYSIS AT VARIOUS TEMPERATURES

In this appendix, data of glucose production that generated from acid hydrolysis of nitrocellulose at various temperature were presented in the unit of mmole.

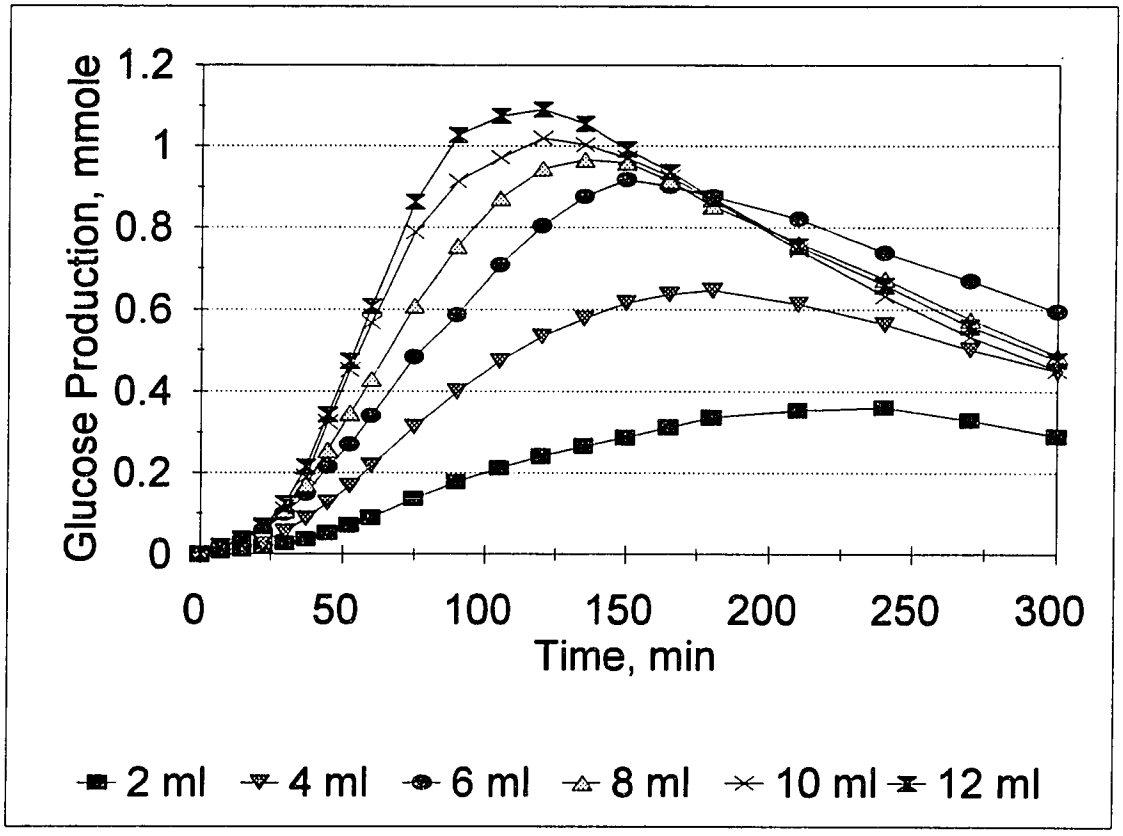


Figure A-1 Glucose Production in Nitrocellulose Hydrolysis at 50 °C

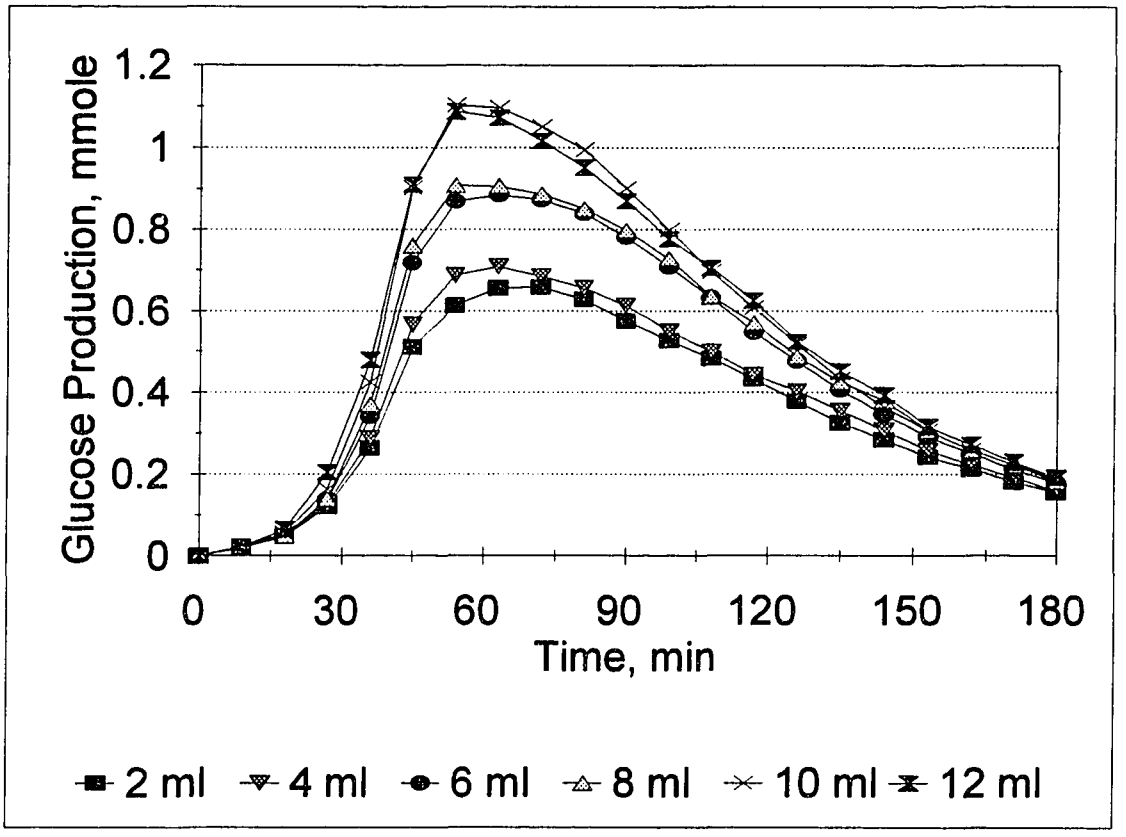


Figure A-2 Glucose Production in Nitrocellulose Hydrolysis at 60 °C

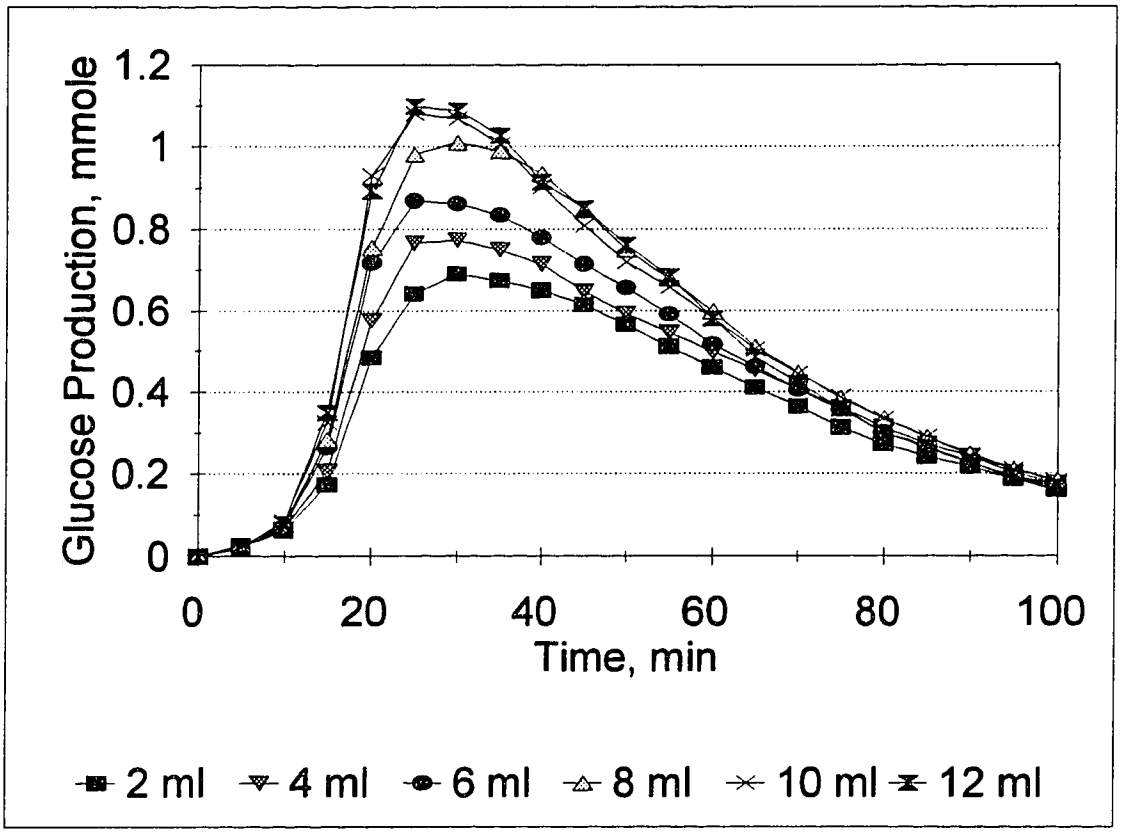


Figure A-3 Glucose Production in Nitrocellulose Hydrolysis at 70 °C

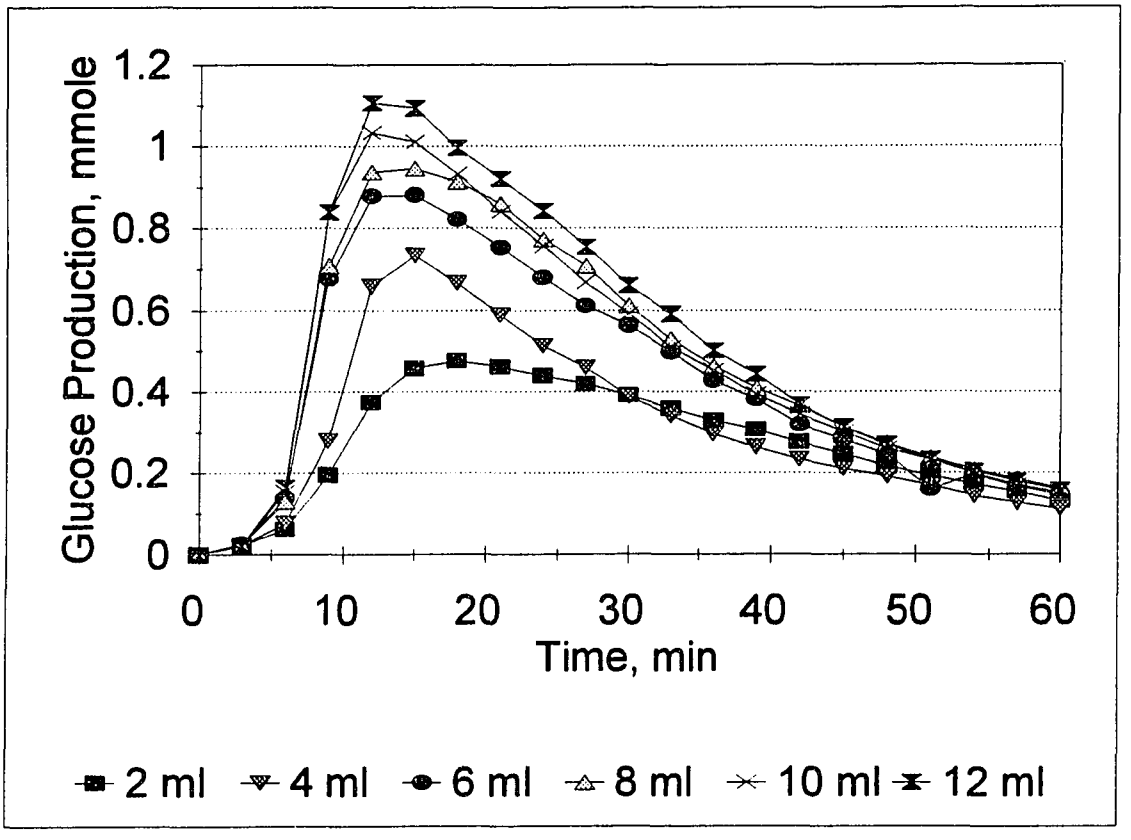


Figure A-4 Glucose Production in Nitrocellulose Hydrolysis at 80 °C

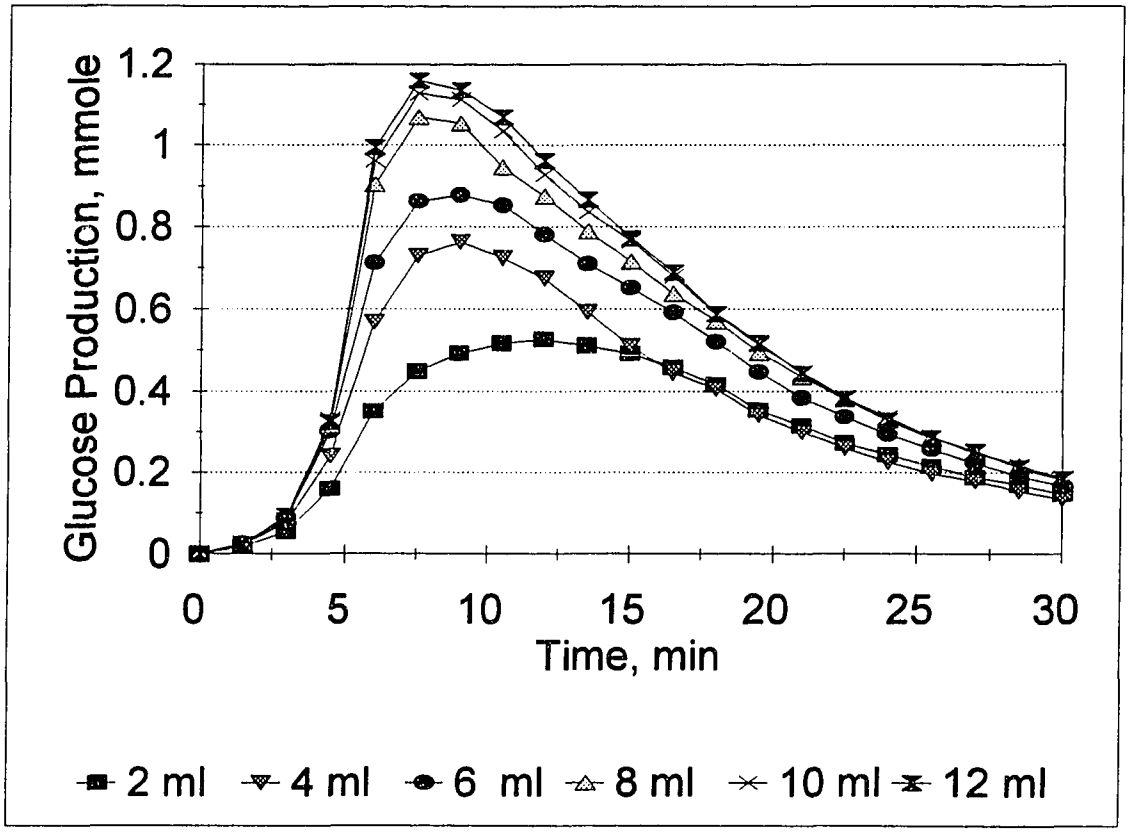


Figure A-5 Glucose Production in Nitrocellulose Hydrolysis at 90 °C

APPENDIX B

GLUCOSE CONCENTRATION IN NITROCELLULOSE HYDROLYSIS AT VARIOUS TEMPERATURES

In this appendix, data of glucose concentration that generated from acid hydrolysis of nitrocellulose at various temperatures were presented in the unit of mM.

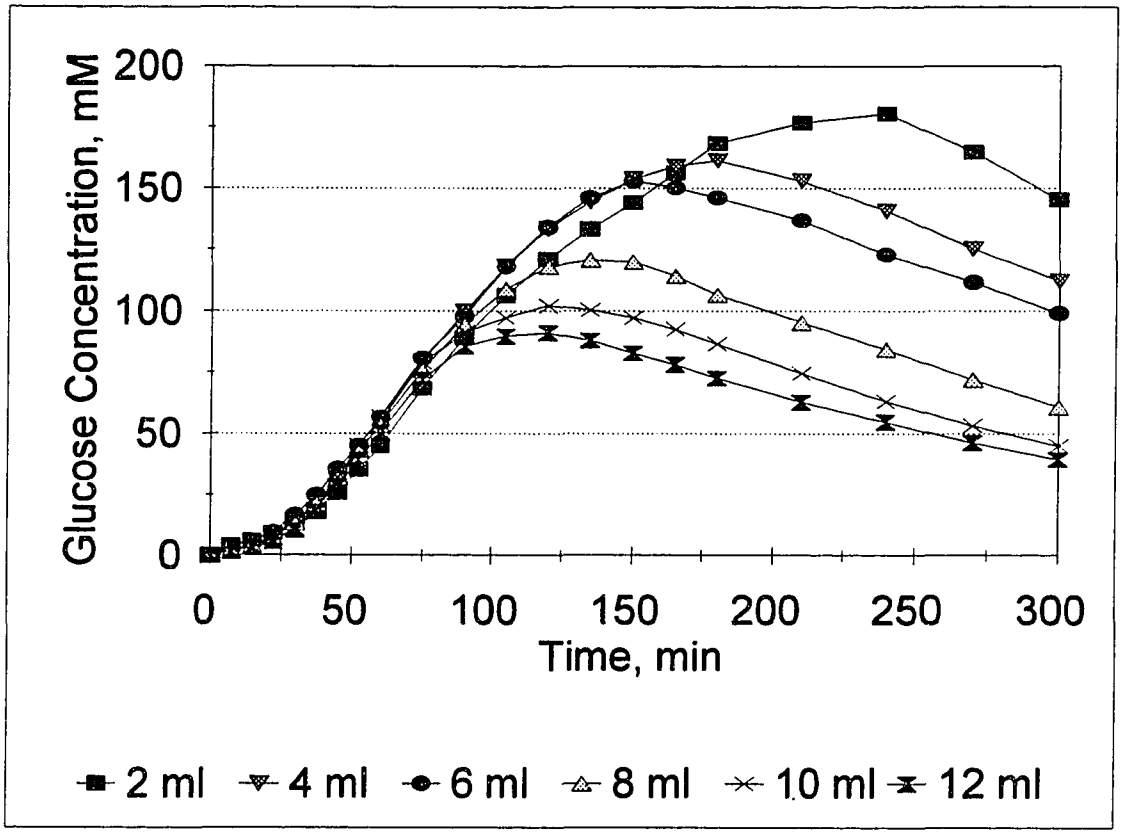


Figure B-1 Glucose Concentration in Nitrocellulose Hydrolysis at 50 °C

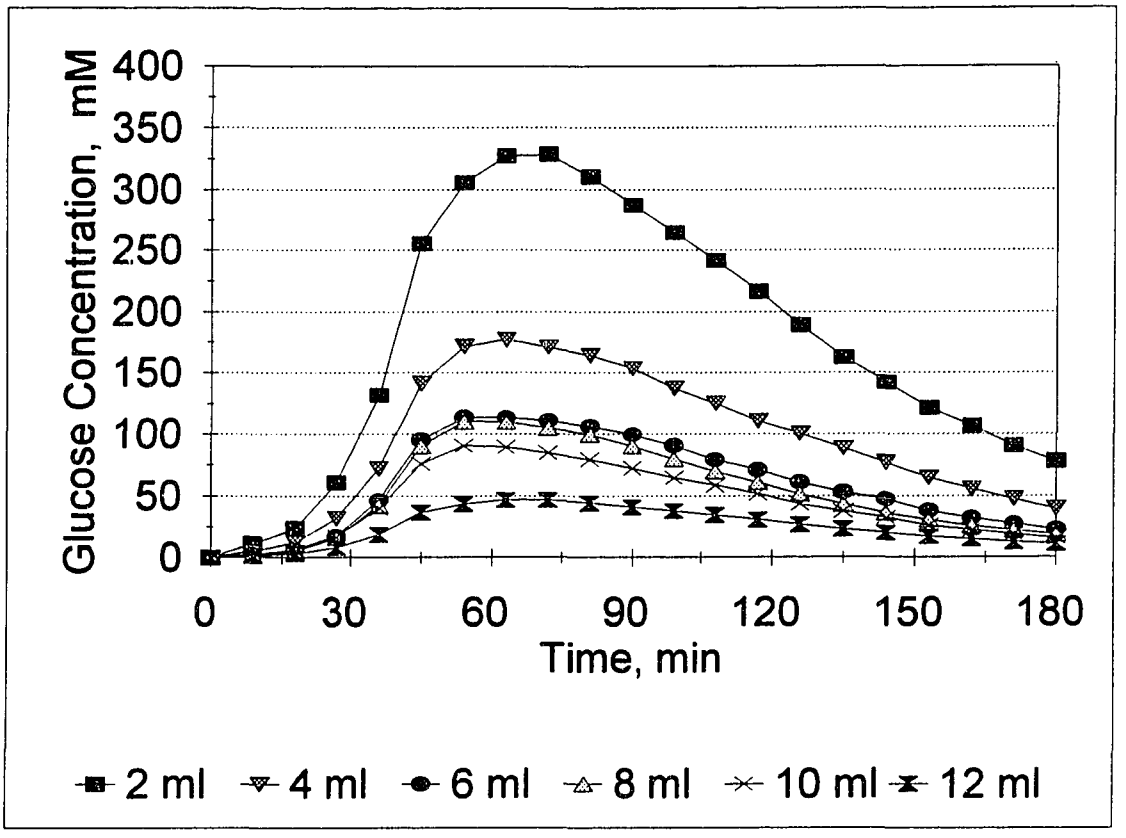


Figure B-2 Glucose Concentration in Nitrocellulose Hydrolysis at 60 °C

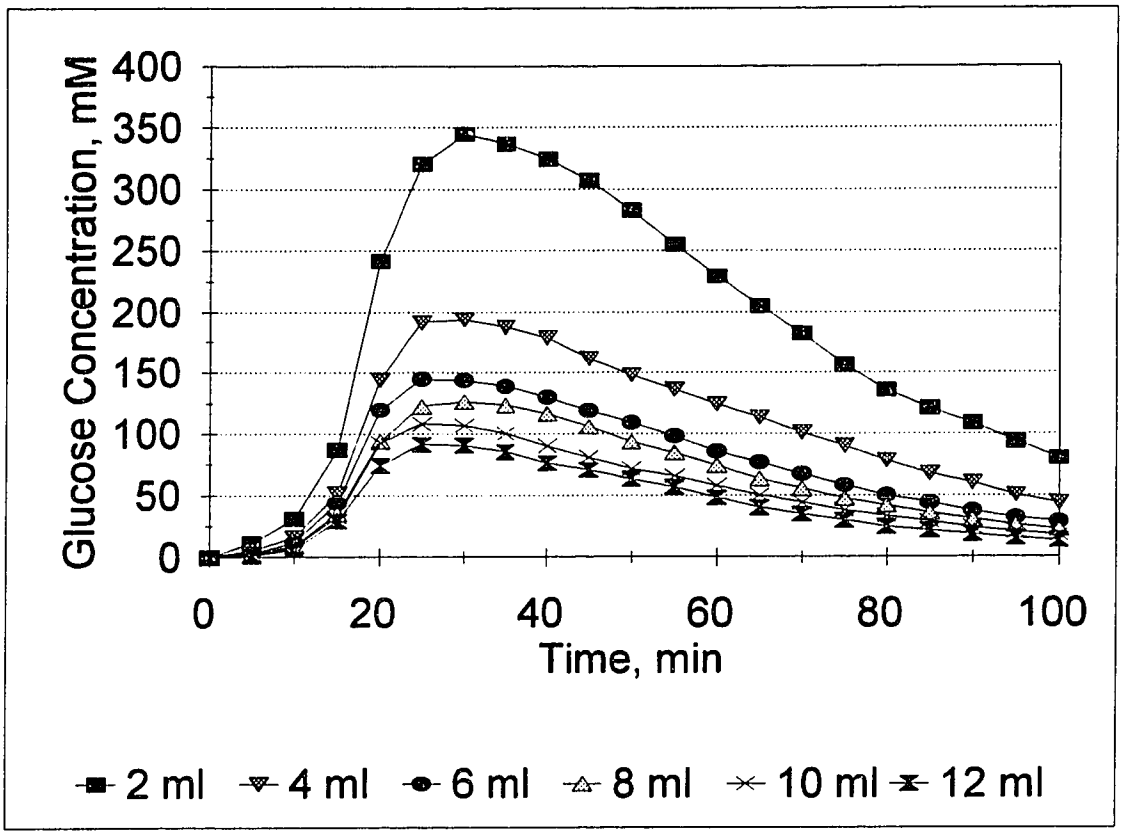


Figure B-3 Glucose Concentration in Nitrocellulose Hydrolysis at 70 °C

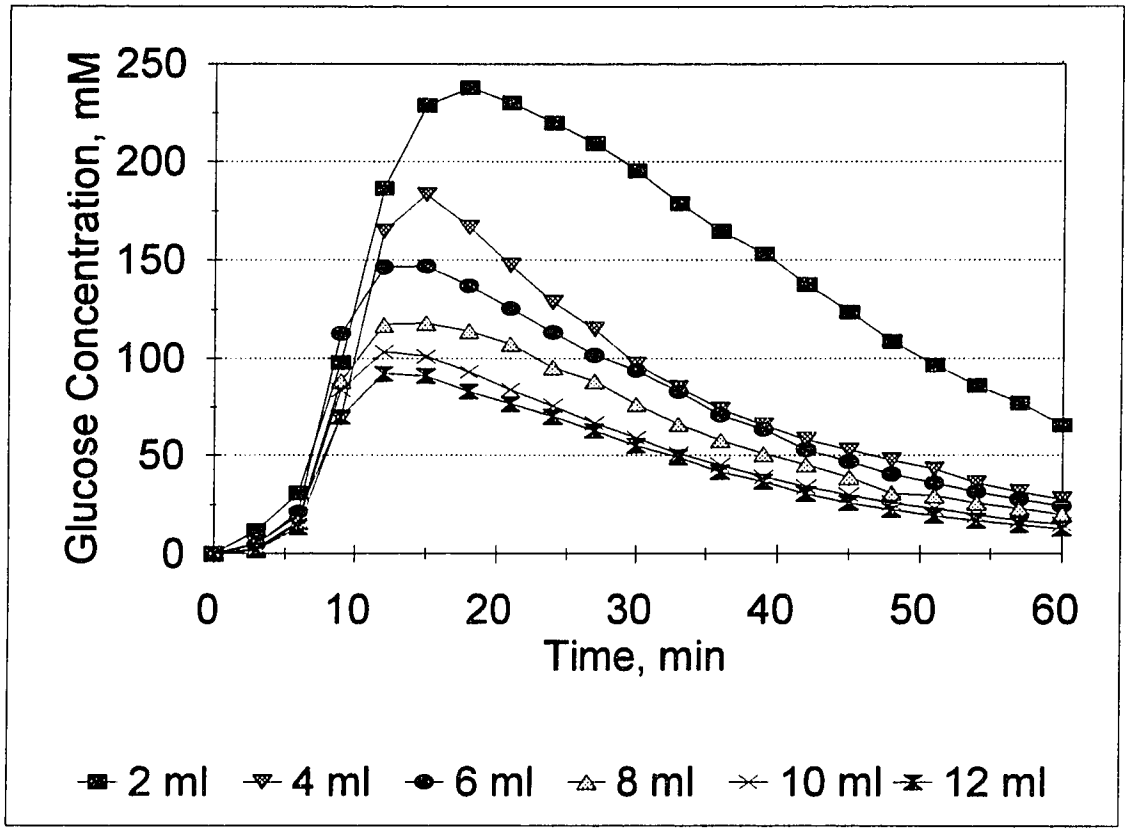


Figure B-4 Glucose Concentration in Nitrocellulose Hydrolysis at 80 °C

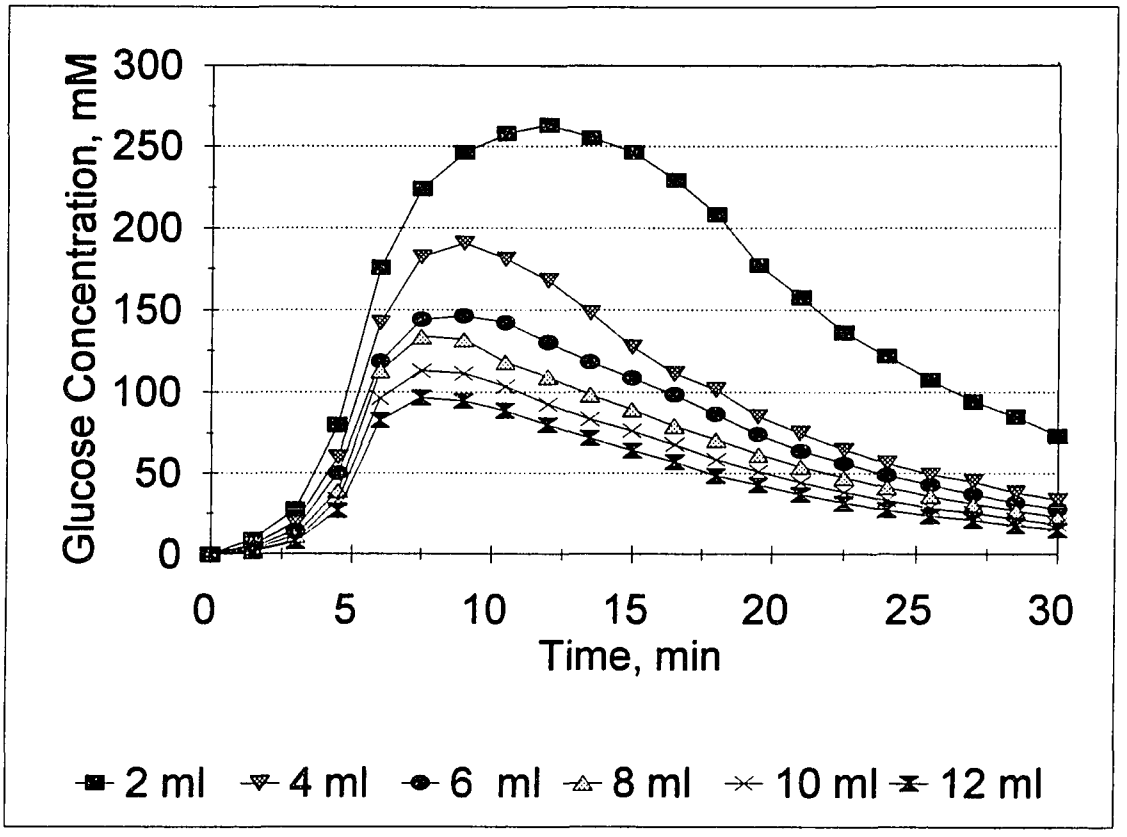


Figure B-5 Glucose Concentration in Nitrocellulose Hydrolysis at 90 °C

APPENDIX C

GLUCOSE CONVERSION IN NITROCELLULOSE HYDROLYSIS AT VARIOUS TEMPERATURES

In this appendix, data of glucose conversion that generated from acid hydrolysis of nitrocellulose at various temperatures were presented in the unit of %.

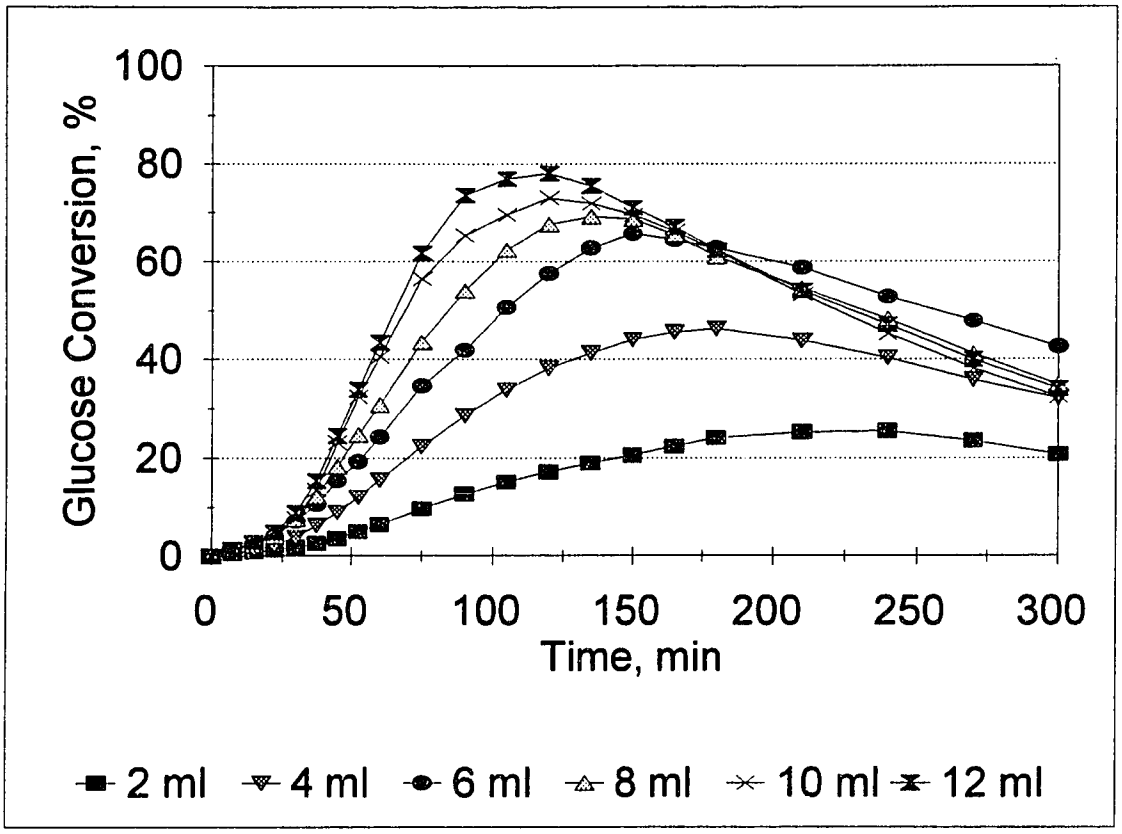


Figure C-1 Glucose Conversion in Nitrocellulose Hydrolysis at 50 °C

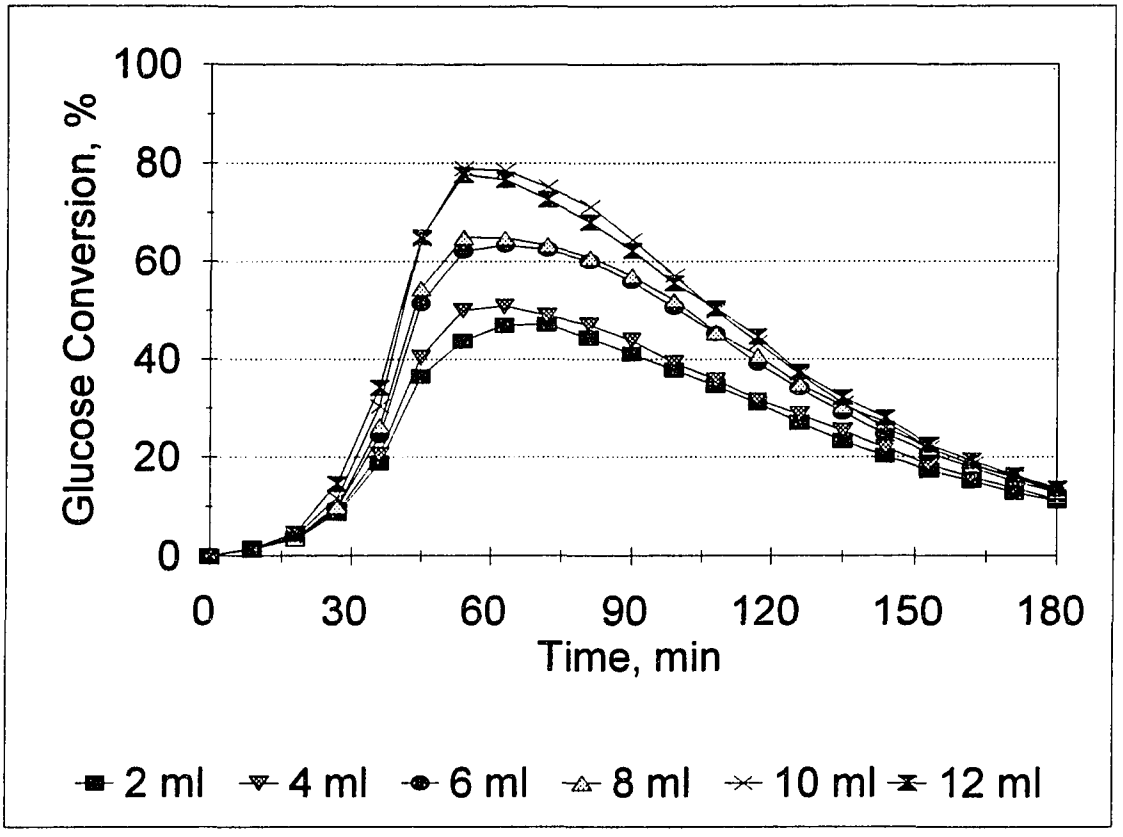


Figure C-2 Glucose Conversion in Nitrocellulose Hydrolysis at 60 °C

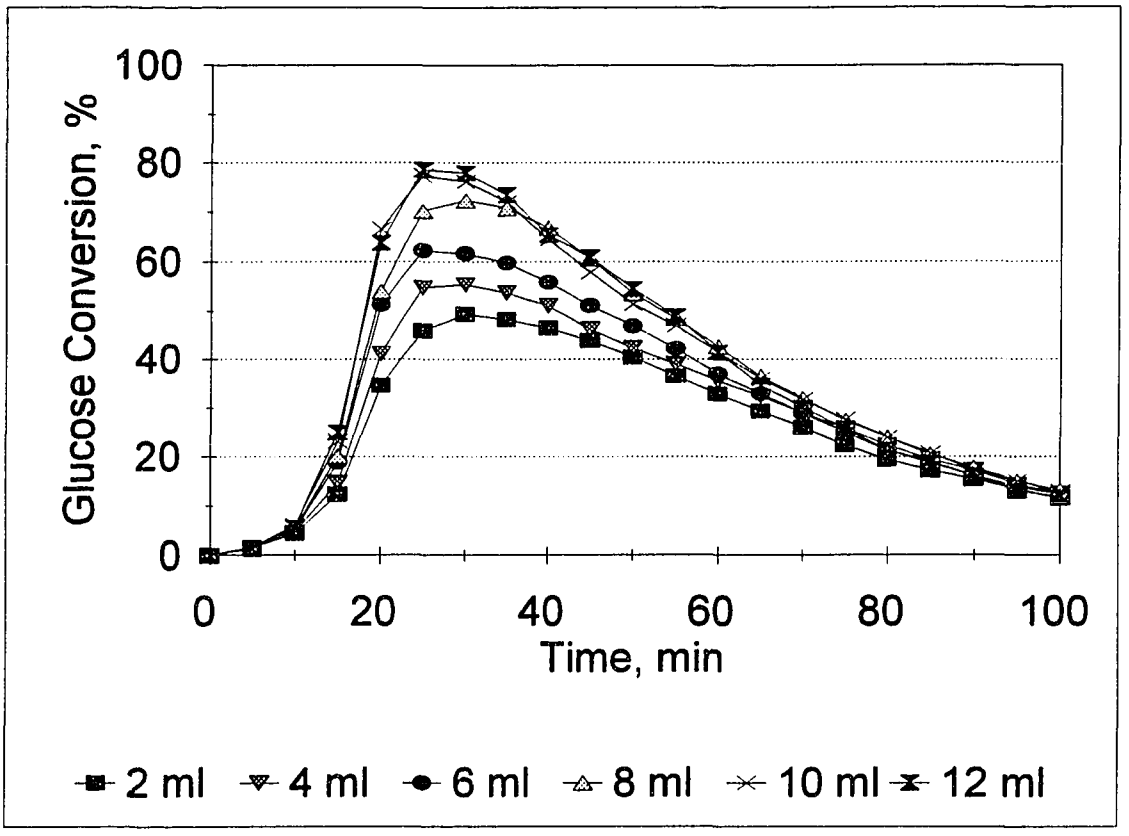


Figure C-3 Glucose Conversion in Nitrocellulose Hydrolysis at 70 °C

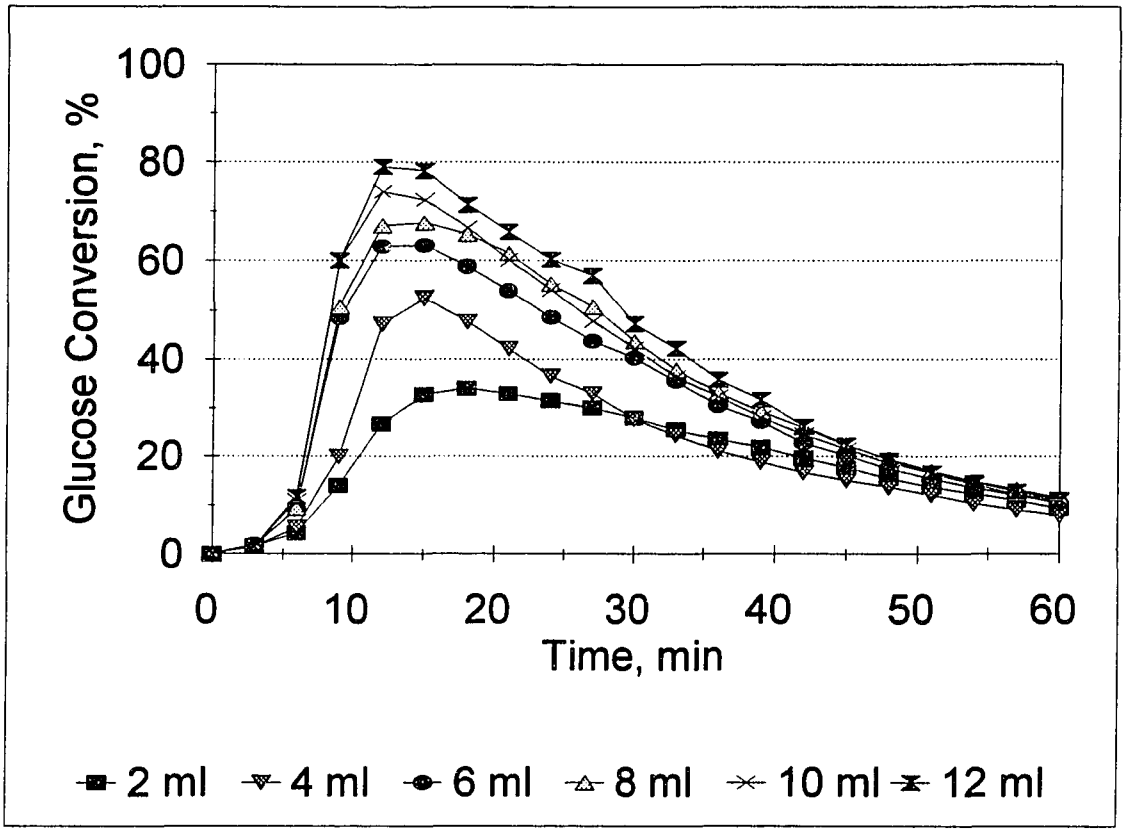


Figure C-4 Glucose Conversion in Nitrocellulose Hydrolysis at 80 °C

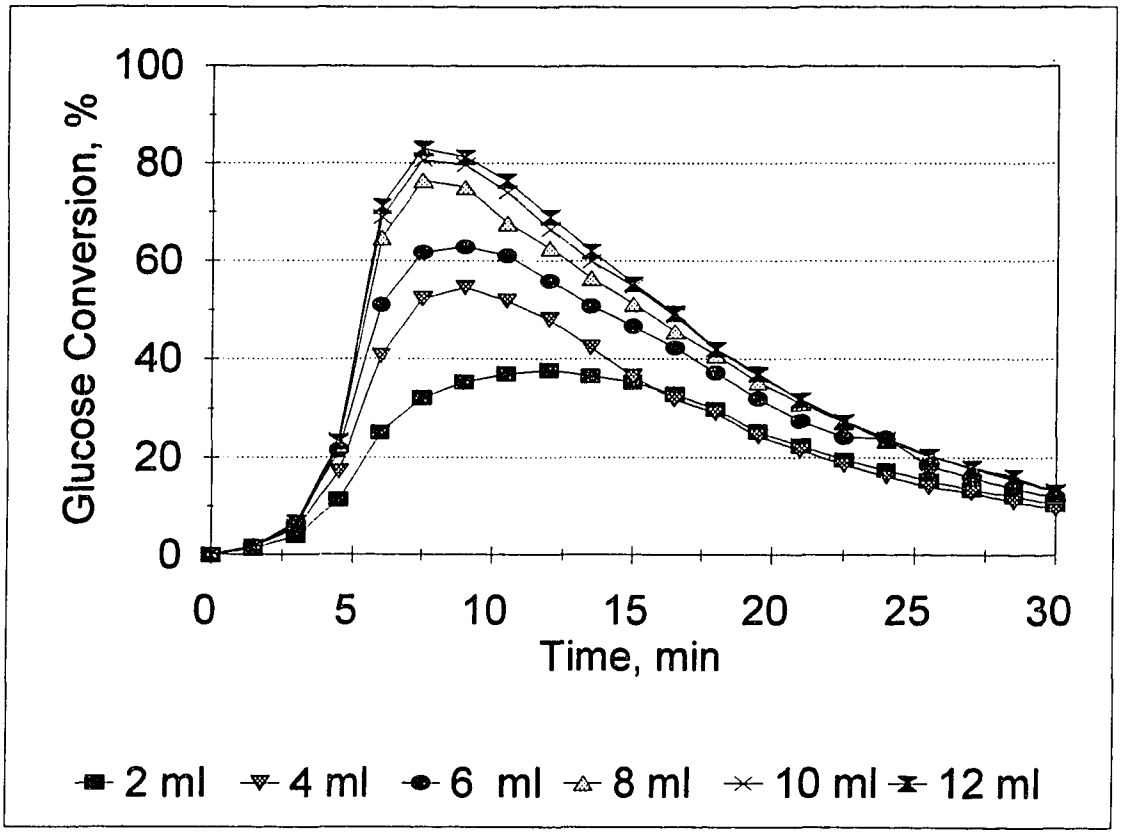


Figure C-5 Glucose Conversion in Nitrocellulose Hydrolysis at 90 °C

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