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STUDIES ON THE AEROBIC BIODEGRADATION OF POLYMERS

By

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ABSTRACT

STUDIES ON THE AEROBIC BIODEGRADATION OF POLYMERS

By

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From an environmental perspective, biodegradable polymers offer an attractive alternative to traditional petroleum based nonbiodegradable polymers. In this work, a study was conducted on the aerobic biodegradation of polymers in the presence of municipal sewage sludge. The extent of biodegradation was quantified systematically based on percentage carbon conversion to CO_2. Commercial polymers like PCL, PHB/V and 9-11 acid were found to be potentially biodegradable, whereas PVOH, EVOH and Acrylic coating showed little biodegradation. Focus was directed towards cellulose and starch esters which are potential cost effective biodegradable plastics. Starch propionates of different degrees of substitution (DS) showed a decrease in biodegradability with increasing DS. Two cellulose acetate (CA) products when studied with respect to morphological effects showed appreciable difference in biodegradation between an open honeycomb morphology and a closed, smooth morphology. These results suggest that enzyme accessibility of the glycosidic linkage may be an important factor governing CA biodegradation , and calls for further investigation.

This thesis is dedicated to

my parents, Jimmy, Jessy and James.

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LIST OF ABBREVIATIONS

CA	Cellulose acetate of DS=2.5
C	Polymeric carbon
C/N	Carbon / Nitrogen ratio
DS	Degree of Substitution
EVOH	Ethylene vinyl alcohol
MSW	Municipal Sewage Sludge
NMR	Nuclear Magnetic Resonance
PCL	PolyCaprolactone
PHB/V	Poly hydroxy butyrate/valerate
PVOH	Polyvinyl alcohol
SP	Starch Propionate
SEM	Scanning Electron Microscopy

INTRODUCTION

Today's packaging materials are typically designed for utility and application but with little emphasis on disposability and recyclability. A growing trend is towards material redesign to produce products with increased environmental compatibility. A few examples of national and international inclination towards this trend are listed below [Narayan (1993)].

- Designing parts that can be disassembled and then recycled, reused, or incinerated (SPE ANTEC'92 - Environmental Management Forum).
- German automakers striving to produce recyclable cars - the goal is to have less than 5% of an automobiles volume going into a landfill (R&D Magazine, May '92)
- Legislative trends towards designing for disposability and resource conservation.
- Environmental Management Standards (ISO - SAGE activities; similar to ISO 9000 quality assurance standards)
- International Chamber of Commerce (ICC) charter for the business development " Sustainable Environment"

These trends have brought into focus the concept of "degradable" materials - i.e., materials that are able to disintegrate in the waste stream in an environmentally sound manner. In order to integrate material design with material disposability for degradable materials it is essential to understand their fate in a waste stream. It is

this section of the "cradle to grave" life of a material that provided the thrust for this work.

1.1 OBJECTIVE

The objective of this work was to understand qualitatively and quantitatively on a laboratory scale the aerobic biodegradability of polymeric materials in the presence of municipal sewage sludge. Biodegradation was studied in this work from two perspectives - measurement and materials.

1.1.1 Biodegradation - Measurement perspective

There are various methods of measuring the biodegradation of polymeric materials, some of which have been described in the next chapter along with their advantages and disadvantages. In this work, the aerobic biodegradation of different polymeric materials was measured using an inoculum derived from municipal sewage sludge, based on ASTM-D5209 (several modifications were made to the ASTM method to suit laboratory conditions). A theoretical analysis of the biodegradation process inside this system was carried out. The effects of some of the process parameters on biodegradation were also studied experimentally.

1.1.2 Biodegradation - Materials perspective

The inherent biodegradability of a material in a given environmental framework is related to a number of factors most of which are characteristic of the material itself. Some of these factors are its chemical structure, physical properties, breakdown mechanisms and availability of specific enzyme-producing microorganisms among others. In this work the inherent biodegradability of

different materials was studied and correlated to some of these factors. They provided a better understanding in the design of materials intended to biodegrade under environmental conditions.

Three categories were studied under Materials :-

1) A general category including some commercial polymers and other materials of interest

2) Starch esters

3) Cellulose esters

1.2 SUMMARY OF THE CHAPTERS

This thesis is divided into nine chapters. Chapter 2 provides basic knowledge about waste management infrastructure and biodegradable materials. Chapter 3 describes the different biodegradation measurement techniques in use, their advantages and disadvantages, and a detailed description of the selected process for the measurement of biodegradation. Chapter 4 gives a theoretical analysis of the biodegradation process and an experimental analysis of the effects of some parameters on the measurement process. Chapter 5 presents the results of biodegradation tests carried out on some commercial polymers and other materials of interest. Chapter 6 is an introduction to Chapters 7 and 8, and deals with the structures and characterization techniques which are common to both the starches and celluloses. Chapter 7 presents the effect of varying the extent of chemical modification of starch on its biodegradation. Chapter 8 deals with cellulose acetate having a high degree of substitution and presents the observed effect of morphology on its biodegradation. A theoretical kinetic model for the aerobic

biodegradation of cellulose in this experimental system has also been proposed in this chapter. Conclusions and recommendations for further work are presented in Chapter 9.

BIODEGRADABLE MATERIALS

2.1 WASTE MANAGEMENT INFRASTRUCTURE

Production of solid waste in the United States amounts to approximately 186,400 m. tons per day (68 Million m. tons per year) [EPA (1988)]. The Environmental Protection Agency (EPA), has established a goal of a 75 volume percent reduction in waste over the next eight years [Meister (1994)]. These reductions are supposed to be achieved by a 25% reduction of waste generated at the source, a 25% diversion of waste to recycling, and a 25% reduction of waste by incineration. Degradable materials are one way of reducing waste volume at the source itself. Paper, organic and food wastes comprise approximately 50% of landfill trash, all of which are partially or fully biodegradable [EPA (1988)], (Figure 2.1). However, approximately 20% of this waste mixture comprises of plastics, which are often, and perhaps not always fairly, singled out as the major culprit in ultimate disposal problems.

2.2 PLASTIC WASTE MANAGEMENT

Currently, most products are designed with little or no consideration for their ultimate disposability or recyclability [Narayan, (1992)]. Options to manage our plastic waste, are few and listed as follows [Narayan et al. (1992)]:

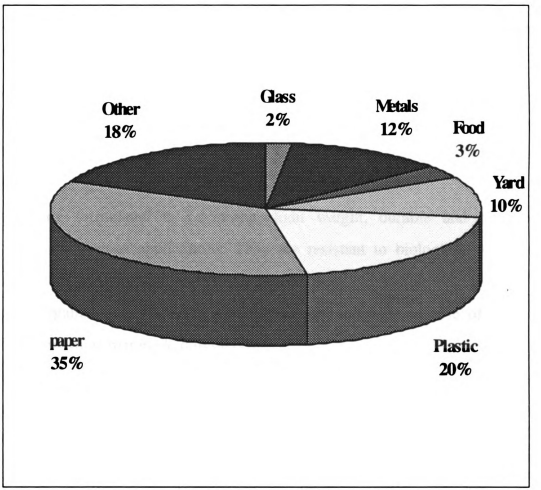


Figure 2.1 - Materials in MSW by volume (Cubic yards)

a) Incineration - It has the benefit of regaining energy from waste, however, it is capital intensive and there are questions about toxic emissions.

b) Recycling and source reduction- It is an accepted viable option, but only where it is technically and economically feasible (i.e. where cleaning, reconverting and reshipping costs do not exceed the virgin resin cost).

c) Landfills- They are a poor choice as a repository of plastic and organic waste and are designed to retard biodegradation by providing little or no moisture with negligible microbial activity.

d) Composting- It is slowly gaining importance as the answer to the plastic waste problem.

Plastics are formulated to be strong, light weight, durable and bioresistant materials for various applications. They are resistant to biological degradation, because,

a) microorganisms do not have polymer-specific enzymes capable of degrading and utilizing most manmade polymers.

b) the hydrophobic character of plastics inhibit enzyme activity

c) the low surface area of plastics with their inherent high molecular weight decreases rate of microbial activity.

The durability and indestructibility of plastics make them the materials of choice for many applications, but this also creates problems when it enters the waste stream. This leads us to the concept of designing and engineering new biodegradable materials; materials that are plastics, i.e. strong, light-weight, easily processed, energy efficient, excellent barrier properties, disposable (mainly for reasons of hygiene and public health), yet break down under appropriate environmental conditions just like it's organic counterpart. Clearly, not all plastics

can and should be made degradable. Single use disposable short-life packaging materials, service ware items, and disposable non-wovens should be targets of new material concepts that allow them to be fully compostable and to be incorporated into the carbon cycle of the eco-system.

2.3 RATIONALE FOR BIODEGRADABLE POLYMERS

Plastics consist of carbon units assembled together to form complex polymeric structures with desirable properties. These carbon units are derived from natural resources and hence should be returned to nature in order to complete nature's own carbon cycle. By the law of conservation of mass we cannot make materials disappear. However we can redistribute them in an ecologically sound manner by nature's ecosystem [Narayan (1989), (1991), (1992)].

Figure 2.2 shows nature's carbon cycle and how biodegradable plastics can fit into this cycle. As shown, green plants fix atmospheric carbon dioxide for their own growth. These plants are then consumed by herbivores, which in turn are consumed by carnivores, which in turn respire to form carbon dioxide and ultimately dead organic matter. This organic matter is decomposed by microorganisms in soil to form carbon dioxide and humic material. The humic material is assimilated by plants and the humic carbon again gets into the carbon cycle. Carbon-based polymers can also enter into this cycle if they can be biodegraded by microorganisms found in waste streams and in the soil. Hence it would be worthwhile to produce disposable plastics that can break down under environmental conditions or in waste disposal systems into products that can be assimilated by nature's ecosystem.

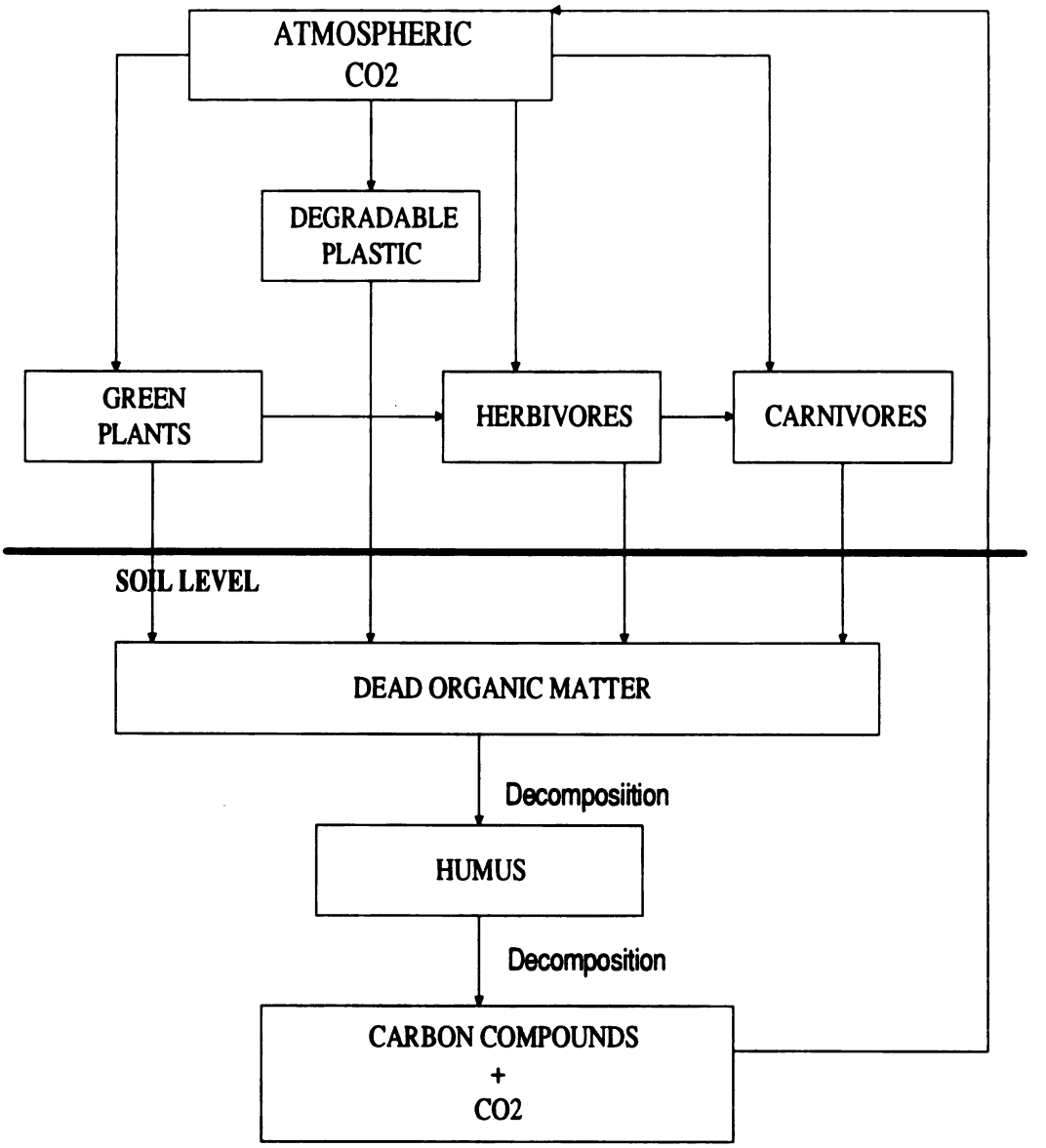


Figure 2.2 - Nature's Carbon Cycle

2.4 BIODEGRADABLE PLASTICS - REGULATORY ACTIONS

As industry began implementing approaches to design environmentally benign products, questions were raised about the practicality, efficacy, and the effects of such products on the environment. This resulted in a number of regulatory actions. A task force of several attorney generals issued recommendations (Green Report I and II) on advertising related to environmentally friendly products [Webster (1991)]. Green Report II states:

> "It may be appropriate to make claims about the 'biodegradability' of a product when that product is disposed of in a waste management facility that is designed to take advantage of biodegradability and the product at issue will safely break down at a sufficiently rapid rate and with enough completeness when disposed of in that system to meet the standards set by any existent state or federal regulations"

The U.S. Federal Trade Commission (FTC) guidelines [FTC (1992)] states:

> "Unqualified degradability claims should be substantiated by evidence that the product will completely break down and return to nature, that is decompose into elements found in nature within a reasonably short period of time after consumers dispose of it in the customary way....."

2.5 BIODEGRADABLE MATERIALS IN THE INDUSTRY

Biodegradable materials found its way into the U.S. industry in the early nineties. Starch-filled polyolefins with less than 10-15% starch were introduced as true

Table 2.1 - Current Status of the US Biodegradables industry

COMPANY	BASE POLYMER	FEEDSTOCK	COST, $/lb	CAPACITY, MM LB/YR
Cargill, Minneapolis, MN	Polylactide (EcoPLA)	Renewable Resources, Corn	1.00 -3.00	10 ('94 scaleup); 250 (mid-1996)
Ecochem, Wilmington, DE	Polylactide copolymers	Renewable Resources, Cheese whey, Corn	< 2.00 proj'd	0.15 ('94 scaleup)
Flexel, Atlanta GA	Cellophane, (Regenerated cellulose)	Renewable resources	2.15	100
Zeneca, (business unit of ICI Americas)	Poly(hydroxybutyrate-co-hydroxyvalerate), (PHBV)	Renewable resources -- carbohydrates (glucose), organic acids	8.00 - 10.00; 4.00 proj'd	0.66, additional capacity slated for '96 is 11 - 22
Novamont, Montedison, Italy	Starch-synthetic polymer blend containing approx. 60% starch	Renewable resources + petrochemical	1.60 - 2.50	50, in Turni, Italy
Novon Products (Warner-Lambert), Morris Plains, NJ*	Thermoplastic starch polymer compounded with 5-25% additives	Renewable resources, Starch	2.00 - 3.00	100
Union Carbide, Danbury, CT	Polycaprolactone (TONE polymer)	Petrochemical	2.7	< 10
Air Products & Chemicals, Allentown, PA	Polyvinyl alcohol (PVOH) & Thermoplastic PVOH alloys (VINEX)	Petrochemical	1.0 -1.25 (PVOH); 2.50-3.00 (VINEX)	150 - 200 (water sol. PVOH); 5 (VINEX)
National Starch & Chemical, Bridgewater, NJ	Low ds starch ester	Renewable resources, Starch	2.00 - 3.00	Not available
MBI/Japan Corn Starch Co., Lansing, MI	Water repellant, thermoplastic modified starches (AMYPOL)	Renewable Resources, Starch	1.0 - 1.50	0.1(pilot scale); 150 slated for early '96
Planet Packaging Technologies, San Diego, CA	Polyethylene oxide blends (Enviroplastic)	Petrochemical	3.00	10
Showa Highpolymer Co., Ltd.	condensation polymer of glycols with aliphatic dicarboxylic acids (BIONELLE)	Petrochemical	approx. 3.00	0.2 (pilot); 7(semi-commercial, end '94)

biodegradable materials [Barenberg et al(1991)], which created a setback to the industry, since it is only the starch which undergoes biodegradation in these materials. Since then, a lot of improvement has taken placed in designing biodegradable materials with different material properties for different applications. The current status of the US biodegradable industry is shown in Table 2.1.

2.6 ENVIRONMENTALLY DEGRADABLE PLASTICS - DEFINITIONS

The following definitions have been obtained from the proceedings of the ASTM subcommittee on environmentally degradable plastics [ASTM, Toronto,(1989)]. They came up with two sets of definitions - one general and broad in scope and the other more specific.

2.6.1 General Definitions

Degradable plastics- Plastic materials that disintegrate under environmental conditions in a reasonable and demonstrable period of time.

Biodegradable plastics- Plastic materials that disintegrate under environmental conditions in a reasonable and demonstrable period of time, where the primary mechanism is through the action of microorganisms such as bacteria, yeast fungi and algae.

Photodegradable plastics- Plastic materials that disintegrate under environmental conditions in a reasonable and demonstrable period of time, where the primary action is through the action of sunlight.

2.6.2 Specific definitions

Degradable plastics- Plastic materials that undergo bond scission in the backbone of a polymer through chemical, biological and/or physical forces in the environment at a rate which is reasonably accelerated, as compared to a control, and which leads to fragmentation or disintegration of the plastics.

Biodegradable plastics- Those degradable plastics where the primary mechanism of degradation is through the action of microorganisms such as bacteria, fungi, algae, yeast.

Photodegradable plastics- Those degradable plastics where the primary mechanism of degradation is through the action of sunlight

ISO 472:1988 (International Standards Organization) introduced a distinction between degradation and deterioration as:

Degradation - A change in the chemical structure of a plastic involving a deleterious change in properties.

Deterioration - A permanent change in the physical properties of a plastic evidenced by impairment of these properties.

MEASUREMENT OF BIODEGRADATION

Biodegradability of a material has become an increasingly desirable property for plastic manufacturers due to plastic waste disposal and recycling problems. Hence, the biodegradation potential of materials have been tested by various techniques ever since interest in biodegradability was generated [Barenberg et al (1990)]. These methods ranged from relatively simple ones like soil burial tests to elaborate ones like the ASTM standards D5338 and D5209. All of them, however give an initial indication of the biodegradability of a material. Some of the measurement techniques used have been discussed briefly here. The pros and cons of these measurement techniques have also been discussed.

3.1 GENERAL METHODS OF MEASUREMENT

3.1.1 FUNGAL TESTS

Plastic resins can be tested for degradation by fungi using specific growth mediums. One such test is the ASTM D1924-63 in which a test specimen is placed on or in a solid agar growth medium that is deficient in carbon. The medium and specimens are inoculated with test microorganisms and incubated for three weeks. Any growth that may occur is dependent on the utilization of a component of the specimen as a carbon source by the organism. If a specimen shows heavy microbial growth and concurrent loss of weight and mechanical properties, it is

considered evidence of biodegradability. Fungal tests have been carried out in the past by various people [Union Carbide, Gilmore (1989), Graves (1994)]. This method helps to determine the effect of specific microorganisms on the test specimen, but does not quantify biodegradation directly. Also, it only accounts for biodegradation in specific cultures and not in a mixed consortia of microorganisms as in a waste environment.

3.1.2 SOIL BURIAL TESTS

This test consists of burying the plastic in moist soil for an extended period of time ranging from 3 to 12 months [Breslin et al.(1993), Union Carbide, Iannotti (1989), Wool (1989)]. The plastic is in the form of tensile bars, films or other molded articles. Mechanical property loss, weight loss, performance properties loss and change in appearance (i.e., evidence of pitting or roughening of the surface indicating bacterial growth), are measured periodically for the buried plastics. This test method is not effective for tracking down end products and reaction mechanisms owing to the diverse nature of the test matrix.

3.1.3 COMPOSTING EXPERIMENTS

In this test plastics are exposed to a simulated compost environment under laboratory conditions. One such test, ASTM D5338 [ASTM D5338 (1992), Snook (1994), Breslin (1993)] closely simulates, (but does not necessarily replicate) large scale composting and is a good measure of the biodegradability of a material. The test environment is a laboratory scale reactor which simulates a self-heating composting system and which uses aeration to control maximum temperature. Plastic exposure occurs in the presence of a synthetic waste undergoing aerobic composting. The test materials are exposed to an inoculum of compost in two liter

vessels through which prehumidified, carbon dioxide free air is passed at a constant flow rate. Carbon dioxide evolved as a result of aerobic biodegradation of the composting samples is trapped in trapping solutions (BaOH or NaOH solutions). The trapping solutions are removed and analyzed periodically for the evolved carbon dioxide which is then correlated to percentage biodegradation of the test material. A blank (compost with no test material) is also run in parallel with the other test bottles to account for the carbon dioxide evolution from the compost. The homogeneity of the compost is an important factor in these experiments, and can lead to large standard deviations between replicates. Also, the diverse nature of the test matrix makes it difficult to track down the end products in the biodegradation of the test material.

3.1.4 ENZYMATIC ASSAYS

Enzyme assays have been carried out by various people in the past [Dijkistra (1989), Angeline (1989), Williams (1989), Allenza (1989)]. In this method, the plastic material is exposed to a specific enzyme(s) solution along with other reactants. The rate and extent of biodegradation are measured by periodic testing for the concentration of degradation products. As in the case of fungal tests, these assays are more specific in their objective, i.e., the focus is degradation by specific enzymes rather than by environmental degradation in a mixed consortia of microorganisms

3.1.5 LIQUID CULTURE EXPERIMENTS

All the biodegradability testing in this work has been done in liquid culture experiments along the general lines of ASTM D5209, which is a standard test method for determination of the aerobic biodegradability of polymeric materials in

presence of municipal sewage sludge. This ASTM protocol was modified so as to obtain optimum conditions for determination of the intrinsic biodegradability of the test material. This test method consists of selection of plastic material for the determination of aerobic biodegradability, obtaining activated sludge from a municipal-waste water treatment plant and preparing inoculum, exposing the plastic material along with nutrients to the aerated inoculum, measuring carbon dioxide evolved as a function of time, and assessing the degree of biodegradability. The percentage theoretical gas production based on measured or calculated carbon content is reported with respect to time from which the degree of biodegradability is assessed. A protein analysis can also be done on the residual solution to complete the carbon balance for the polymer around the system. A summary of the test method is shown in figure 3.1.

This test utilizes the polymeric carbon as the single carbon source, since the carbon present in the inoculum is a small percentage (about 15%) of that in the polymer. This allows for closer tracking of degradation products, and degradation mechanism. There is also improved contact between the test material and the microorganisms since the test solution is under constant agitation. The advantage of this experiment over using an enzyme assay is that it comes closer to biodegradation in the waste environment than pure enzyme cultures. The limitation of this method is that it does not simulate the large scale composting system in terms of temperatures and biodegradation rates in compost and waste piles. The rest of this chapter describes the selected method for the determination of aerobic biodegradability.

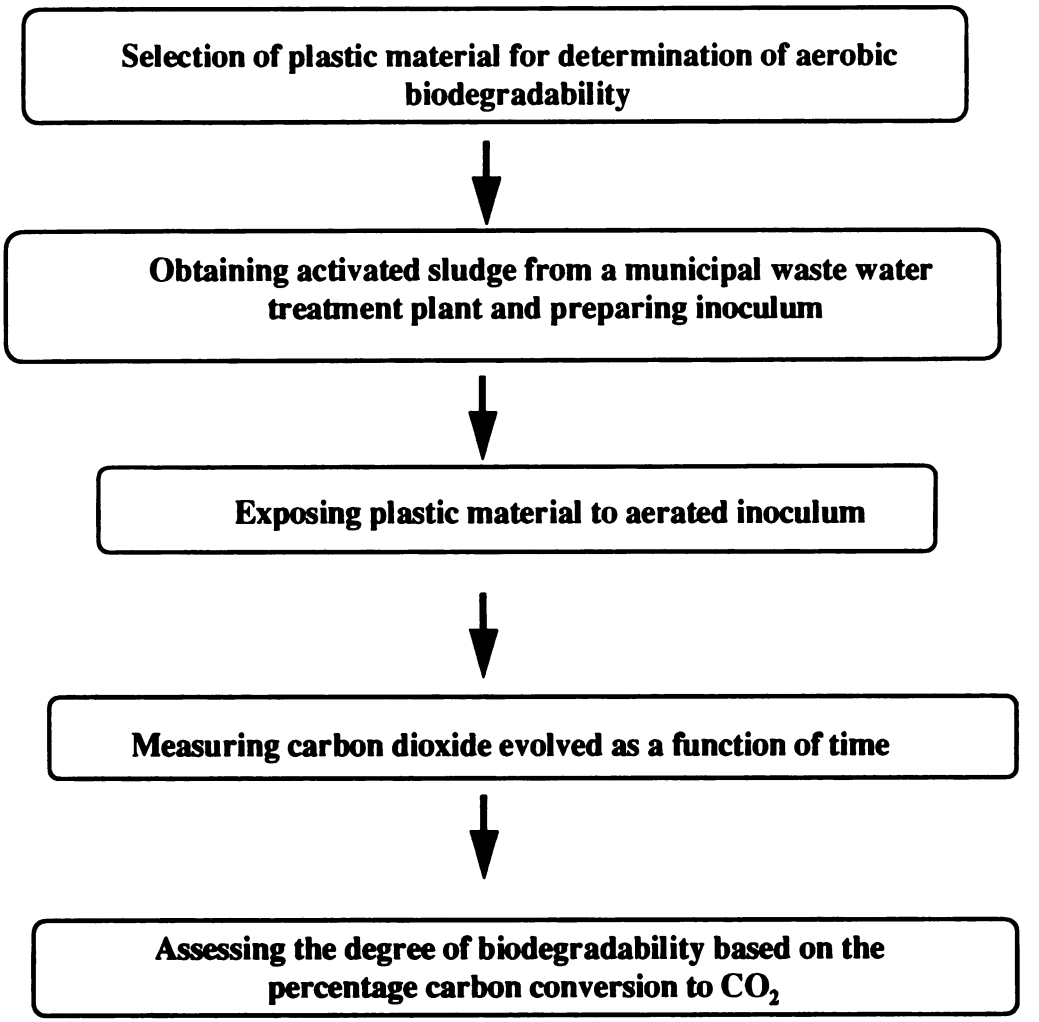


Figure 3.1 - Summary of the test method

3.2 SELECTED MEASUREMENT PROCESS DESCRIPTION

3.2.1 SCOPE

This test method covered the determination of the degree and rate of aerobic biodegradation of synthetic plastic materials (including formulation additives that may be biodegradable) on exposure to activated-sewage sludge inoculum under laboratory conditions. This test determined the intrinsic biodegradability of a material and did not simulate large scale biodegradation in a compost environment.

3.2.2 INOCULUM TEST ORGANISMS

The test inoculum is prepared as specified by ASTM D5209. The source of test organisms was activated sludge freshly sampled from the Waste Water Treatment Plant of East Lansing, Michigan. It is a well-operated municipal sewage treatment plant which receives minimal or no effluent from industry. This sludge is aerated in the laboratory for 4 hours. About 500 ml of the mixed liquor is sampled and homogenized for about 2 min at medium speed in a blender or equivalent high speed mixer. The solution is then allowed to settle for about 30min. If the supernatant still contains high levels of sludge suspended matter at the end of 30min, it is allowed to settle for another 30-40 min. Sufficient volume of the supernatant is decanted to provide a 1-2% inoculum for each test Erlenmeyer flask. Carry-over of sludge is avoided since it would interfere with the measurement of CO_2 evolution during the test. Optionally, counts may be performed on the supernatant fraction to determine microbial numbers. The inoculum should contain 10^6 to 20^6 colony forming units (CFU) per milliliter. It should not be used on the day prepared and may be stored for two weeks at 4°C.

3.2.3 CHN ANALYSIS OF TEST MATERIAL

The test materials[11] were weighed in a Perkin Elmer Autobalance Model AD-4 and tested for their carbon content in the Perkin Elmer 2400 CHN Elemental Analyzer. The PE 2400 CHN uses a combustion method to convert the sample elements to simple gases (CO_2, H_2O and N_2). The sample is first oxidized in a pure oxygen environment; the resulting gases are then controlled to exact conditions of temperature, pressure and volume. Finally, the product gases are separated. Then, under steady state conditions, the gases are measured as a function of thermal conductivity.

Each sample's CHN content was averaged over three runs, and the reproducibility between the runs for C within 1% by wt.

3.2.4 REAGENTS AND TEST SOLUTION

Stock solutions were prepared and added to the experimental test flasks in the following quantities [ASTM D5209]:-

5ml of ammonium sulfate solution (40g/L)

3ml of magnesium sulfate solution (22.5g/L)

3ml of calcium chloride solution (27.5g/L)

6ml of phosphate buffer (Potassium dihydrogen phosphate 8.5g/L, potassium hydrogen phosphate 21.75g/L, sodium hydrogen phosphate 33.4g/L, ammonium chloride 1.7g/L)

12ml of ferric chloride (0.25g/L)

The above stock solutions were added to the flasks along with 30ml of the liquid inoculum. Water was added to the flasks and the solution level brought upto

1500ml. The polymer (test material) of known weight was added to the solution. The carbon to nitrogen ratio maintained in the flask was less than 40:1, based on the amounts of stock solutions used and the amount of carbon in the plastics, which were adjusted accordingly. The test specimens were in the form of fine powders or liquids.

3.2.5 EXPERIMENTAL SETUP

The schematic of the experimental setup is shown in Figure 3.2. It consists of the carbon dioxide scrubbing apparatus, the carbon dioxide production, trapping and measuring apparatus.

3.2.5.1 Carbon dioxide scrubbing and humidifying apparatus -

This apparatus consists of two 10L carboys, one containing about 5L of concentrated NaOH solution and the other containing distilled water. The former is used to scrub the incoming air free of carbon dioxide and the latter is used to restore the air humidity to saturation level at the incubator room temperature. The latter is incorporated to prevent evaporation of water in the flasks. The carboys were connected in series, using vinyl tubing to a pressurized air system and the air purged through the scrubbing solution and humidifier at a constant rate.

3.2.5.2 Carbon dioxide production and trapping apparatus-

This apparatus consists of 2L Erlenmeyer flasks with stoppers, each placed on a magnetic stir plate. Each of these flasks were connected by means of vinyl tubing at the inlet to an inlet air manifold. The outgoing air from the flasks was routed by means of vinyl tubing to two bottles (or tubes) containing NaOH with 5% sulfoorange as the carbondioxide trapping solution. The sulfo orange indicator used has a color change pH range of 11.8 to 10.5.

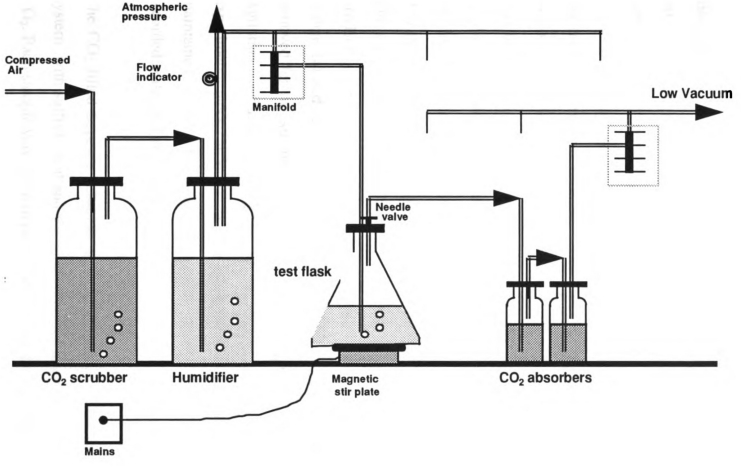
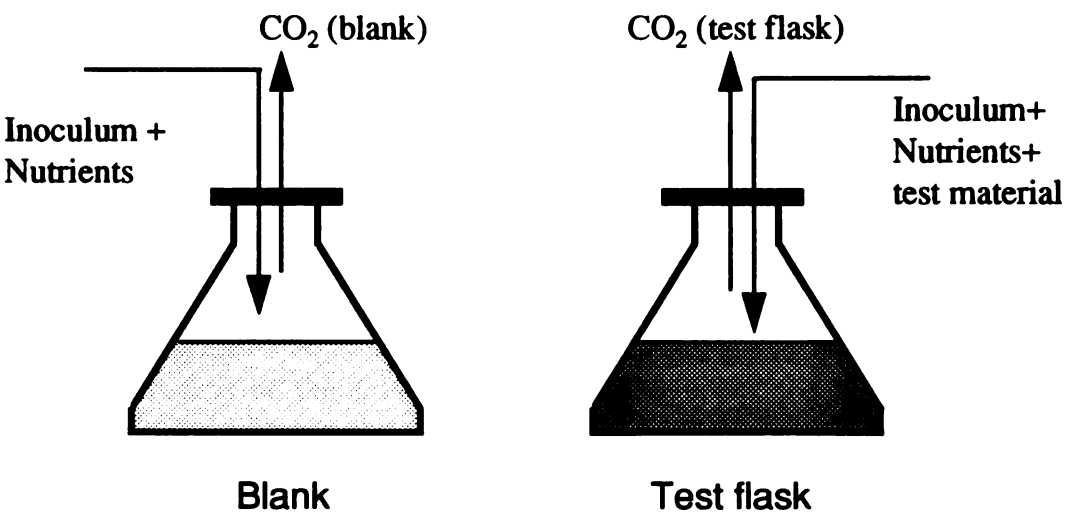


Figure 3.2 - Experimental Setup for Liquid Culture Experiments

3.2.5.3 Experimental Procedure -

The apparatus is set up and the solutions prepared in the Erlenmeyer flasks as described above. The Erlenmeyer flasks are first aerated with carbon dioxide free air for 24 hr. to purge the system of carbondioxide. The test is started by bubbling carbon dioxide free air through the solutions at the rate of 50-100ml per minute (1-2 bubbles per sec) per Erlenmeyer flask. The air flow was maintained by a balance between a pressurized air system connected to the inlet and a low vacuum at the outlet thus preventing the build up of pressure inside the flasks. The air flow rate was maintained at approximately the same rate in all the flasks by adjusting the needle valves fixed in the stoppers of the flasks. The incoming air was scrubbed and humidified prior to entering the flasks through a manifold. The outgoing air was passed through the trapping solutions before going out through a manifold. The carbon dioxide produced in each flask reacts with the trapping solution producing carbonate. After a certain degree of carbon dioxide absorption, the orange colored solution would turn yellow. The CO_2 trapping solutions would be removed for measurement periodically and replaced by fresh trapping solutions. Agitation is maintained throughout the test ensuring good mixing and contact. The experiments were carried out in the incubator room where the temperature was maintained at 28C. The initial test pH is 7+/-1. The pH was remeasured and recorded at the end of the test.

The CO_2 filled trapping solutions were titrated in the Orion 960 Autochemistry system with sulfuric acid and the result obtained in grams of carbon converted to CO_2. Each sample was run in duplicate along with a blank. The CO_2 evolution was calculated by subtracting the blank CO_2 values from the test flask CO_2 values, as shown in figure 3.3.



$$CO_2 \text{ (test material)} = CO_2 \text{ (test flask)} - CO_2 \text{ (blank)}$$

Figure 3.3 - Computation of CO_2 evolution from test flasks

3.2.6 MEASUREMENT OF ABSORBED CO_2

The overall reaction taking place in the flask is:

$$C \text{ (test material)} + O_2 \text{ (air)} = CO_2 + \text{biomass}$$

The reaction taking place during the carbon dioxide trapping is:

$$NaOH + CO_2 = NaHCO_3. \qquad \text{at high pH}$$

$$NaOH + NaHCO_3 = Na_2CO_3 + H_2O \qquad \text{at lower pH}$$

The titration curve [Snoeyink and Jenkins (1980)] for the carbonate system is shown in Figure 3.4.

Total Alkalinity -

In the determination of the total alkalinity a known volume of the solution is titrated with a standard solution solution of strong acid to a pH value in the approximate range of 4 to 5. This endpoint is commonly indicated by the color change of the indicator methyl orange; therefore the total alkalinity is often referred to as the methyl orange alkalinity. The H^+ added is the stoichiometric amount required for the following reactions:

$$H^+ + OH^- = H_2O$$

$$H^+ + HCO_3^- = H_2CO_3$$

$$2H^+ + CO_3^{2-} = H_2CO_3$$

The pH at the true endpoint of the total alkalinity titration should be that of a solution of H_2CO_3 and H_2O. This pH is referred to as $pH(CO_2)$

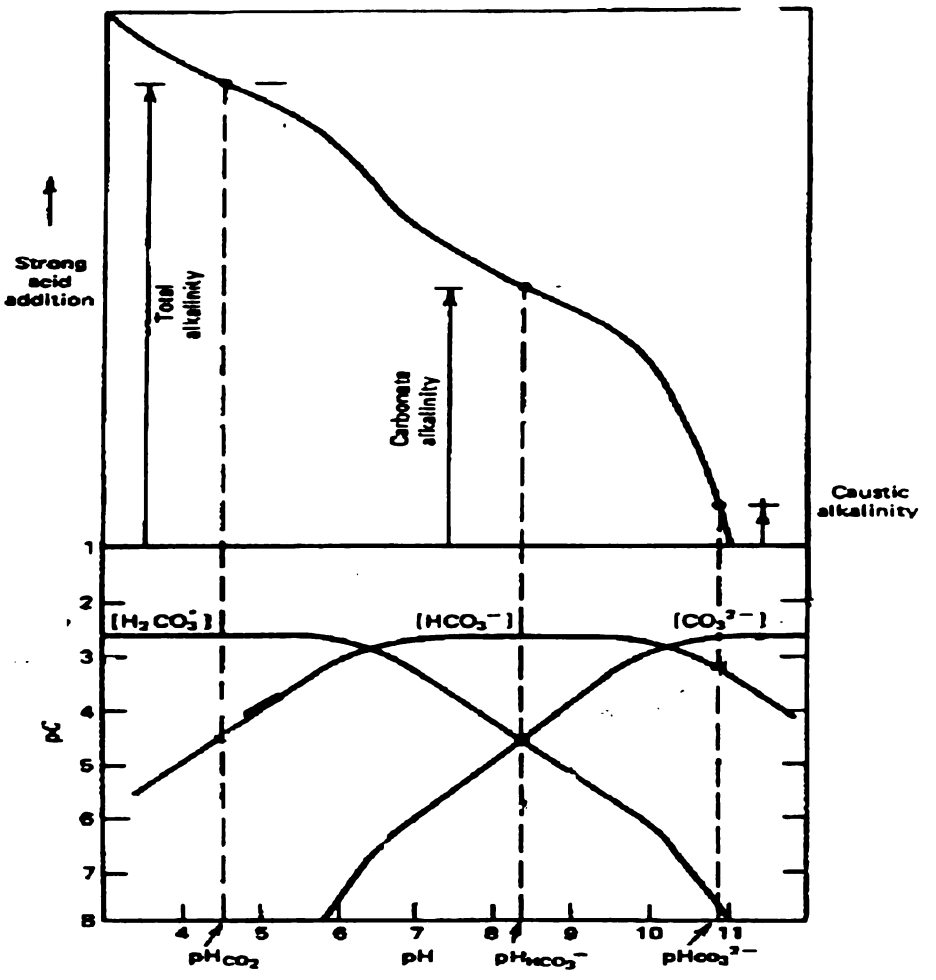


Figure 3.4 - Titration Curve for the carbonate system. The trapping solutions were titrated between pH8.3 and 4.5 with H_2SO_4 to calculate the amount of CO_2 trapped.

Caustic and carbonate Alkalinity -

For a carbonate system we can theoretically identify two more significant pH values that occur during the course of an alkalinity titration. These are the pH(HCO_3^-) and the pH(CO_3^{2-}).The value of pH(HCO_3^-) is about pH 8.3 and is called the carbonate alkalinity, while p(HCO_3^{2-}) is generally between pH 10 and 11 and is called the caustic alkalinity. The former represents the pH of a solution after H^+ has been added to complete the two reactions:

$$H^+ + OH^- = H_2O$$

$$H^+ + CO_3^{2-} = HCO_3^-$$

The latter represents the pH of a solution after H^+ has been added to complete only the following reaction:

$$H^+ + OH^- = H_2O$$

The carbonate alkalinity can be determined by the color change of the indicator phenolphthalein and hence is often referred to as the phenolphthalein alkalinity.

If we make the approximation of complete reaction, then we can assume that by pH 8.3 the reactions

$$OH^- + H^+ = H_2O$$

and $$CO_3^{2-} + H^+ = HCO_3^-$$

are complete, and by about pH 4.3 to 4.7 the reaction

$$HCO_3^- + H^+ = H_2CO_3$$

is complete. We can also deduce that each mole of CO_3^{2-} present will consume one H^+ when the solution is titrated to pH 8.3 and another H^+ as it is titrated from pH 8.3 to 4.5. Thus, the volume of acid used for titration between pH 8.3 to 4.5 can be used to calculate the total alkalinity of the system.

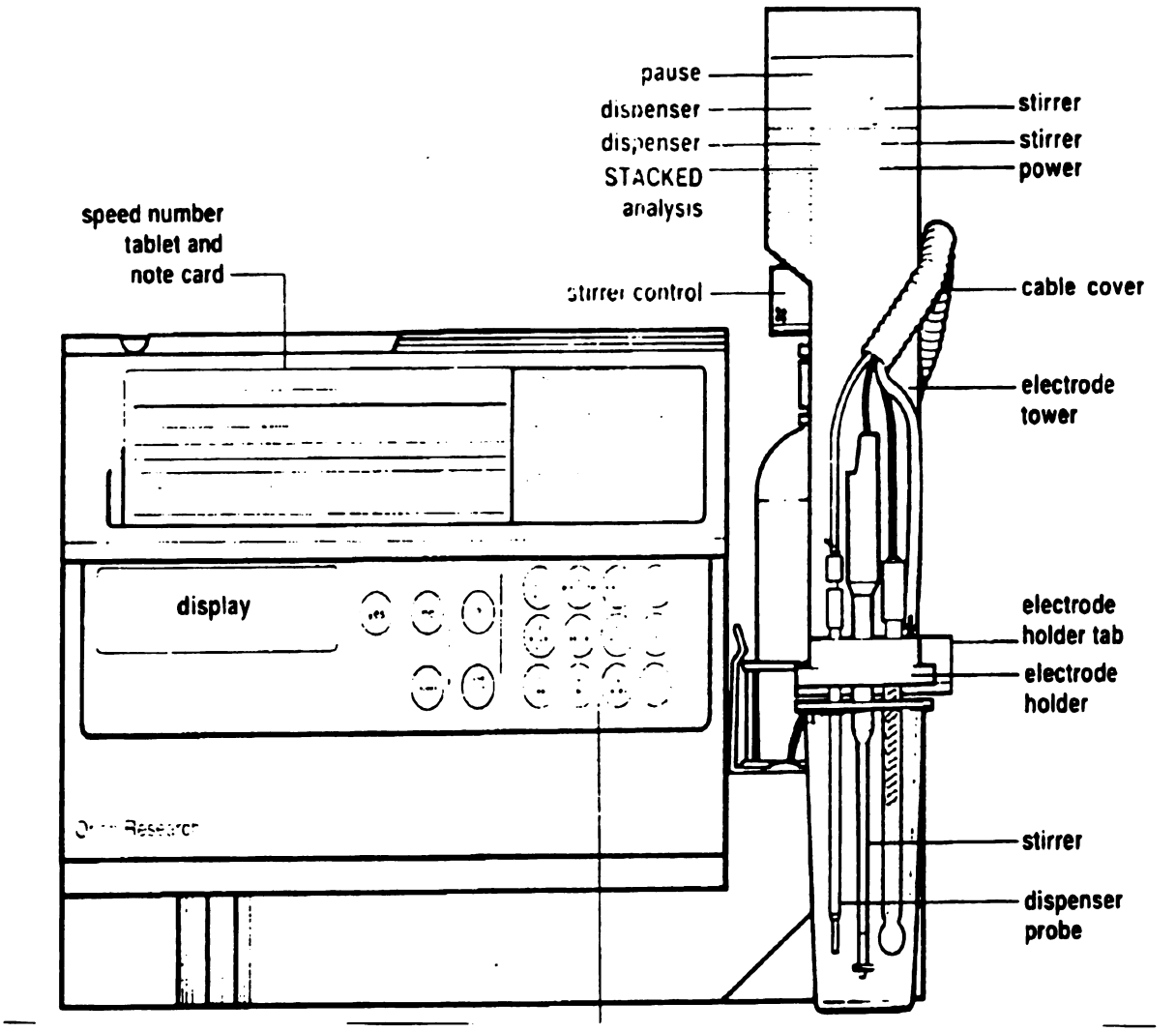


Figure 3.5 - The Orion 960 Autochemistry System

3.2.7 THE AUTOTITRATOR

The Orion 960 Autochemistry System was used to perform all the titrations in this work. The front panel of the autotitrator is shown in figure 3.5. The Orion 960 Autochemistry System consists of the ORION 960 Module and an EA 940 pH/ISE Meter which is placed on top of the module base [Instruction Manual]. The module consists of a microprocessor, which controls the system, an autodispenser, and an electrode tower. The EA 940 provides the display and the keyboard for the entry of information. The technique used for the titration was endpoint analysis based on a preset endpoint. This allowed the titration to proceed to a preset mV or pH value. Titrations were performed as a sequence consisting of three steps.

Step1 - The titration was allowed to proceed to a pH of 12 - 10.5, depending on the starting pH of the CO_2 filled trapping solution. This titration was a constant ml titration in which standard increments of constant volume of titrant was added to the sample to reach the end point.

Step2 - The titration was allowed to proceed to a pH of 8.3 in standard increments of a constant mV. The increment size used ranged from 15 - 30mV depending on the trapping solution.

Step 3 - The titration was allowed to proceed to pH of 4.5 in standard increments of a constant mV. This increment size ranged from 5-15mV depending on the concentration of the trapping solution.

The volume of titrant used for the titration in step 3 was used by the microprocessor to compute the amount of carbon dioxide trapped in the trapping solution and express as mg of carbon. The mV versus ml curve is shown in Figure3.6.

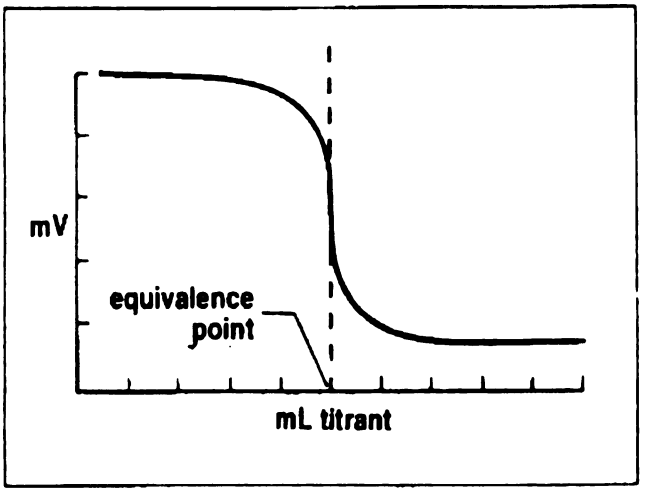


Figure 3.6 - Change in mV vs ml of titrant added for the autotitrator.

A small sample (3 ml-10 ml) of the trapping solution was diluted with water to about 10-15ml and then subjected to titration. The titrant was sulfuric acid with a normality approximately ten times that of the solution being titrated. Since the levels of carbon dioxide evolution varied considerably from flask to flask, it was often necessary to change the normality of the titrant, the amount of sample dilution and the mV or ml step size used in the three steps.

3.2.8 CALCULATIONS

3.2.8.1 Theoretical CO_2 produced

The initial composition of the test material was determined by elemental analysis using a CHN analyzer. This allowed the theoretical quantity of carbon dioxide evolution to be calculated as illustrated in the following manner:

Material is w% carbon.

w/100 * mg of material charged = Y mg carbon charged to the Erlenmeyer flask.

$C + O_2 = CO2$

12 g C yield 44g CO2

Y mg C yield 44/12 * Y mg CO_2

3.2.8.2 Actual CO_2 evolved

The trapping solutions were titrated in two steps, upto pH 8.3 and from pH 8.3 to pH 4.5. In some cases, where the starting pH was very high, the titration was done in three steps, upto pH11.0, from pH11.0 to pH8.3, and from pH8.3 to pH4.5. The volume of acid titrated between pH8.3 to pH4.5 was used by the autotitrator to compute the CO_2 present in the trapping solution in the following manner:

Volume used for titration between pH 8.3 to pH 4.5=V_1

Normality of titrant acid used = N_1

Amount of dissolved C present in the trapping solution in grams

= V_1 * N_1 * 2(moles of HCO_3^-/mole of acid) * 12(g of C/mole)

The CO_2 produced was computed from the above result.

3.2.8.3 Percentage C converted to CO_2

Percentage Carbon converted to CO_2 was calculated as

= (mg C converted to CO_2/ mg C present initially in the test material) * 100

Optionally, the protein content in the final solutions can be determined using protein assay methods.

THE BIODEGRADATION PROCESS

4.1 THE BASIC AEROBIC BIODEGRADATION EQUATION

The basic biodegradation equation for the aerobic biodegradation of any polymer in the presence of a consortia of microorganisms can be generalized as:

$$\text{Polymer} \xrightarrow{\text{microorganisms}} \text{Monomer} \xrightarrow[\text{microorganisms}]{\text{Oxygen,}} CO_2 + \text{humic mass}$$

where, humic mass refers to all the material left behind after the evolution of CO_2. This includes both the cell mass, the undegraded polymer and the smaller monomer units formed during the breakdown process.

4.2 ANALYSIS OF THE BIODEGRADATION PROCESS

4.2.1 The Carbon balance

A material balance can be done for carbon around the aerobic biodegradation system in the following manner:

$$C_{polymer} = C_{CO2} + C_{SOC} + C_{cell\ biomass} + C_{undegraded}$$

where, $C_{polymer}$ = the amount of carbon originally present in the polymer (CHN analysis)

C_{CO2} = the amount of carbon evolved as CO2 during the testing period

C_{SOC} = the SOC (soluble organic carbon) present in solution at the end of the testing period

$C_{undegraded}$ = the amount of carbon present in the residue as undegraded material.

$C_{undegraded}$ can then calculated from the above equation to exactly determine the extent of biodegradation during the testing period. Alternatively, the final solution can be tested for the presence of the original polymeric material, and then tied in with the above equation for verification.

4.2.2 Carbon mineralized to CO_2

The C_{CO2} is often used to determine the extent of biodegradation of the test material. In this work, all the biodegradation data has been reported as the amount of C mineralized to CO_2 and the percentage carbon converted to CO_2 based on original C content. In some cases a protein analysis was done to obtain the C converted to cell biomass.

4.3 FACTORS AFFECTING THE BIODEGRADATION PROCESS

4.3.1 EXPERIMENTAL FACTORS

a) Temperature

The temperature inside the incubator room was maintained at a constant value of 28^0C throughout the test period at a constant value. Microorganisms have different activities at different temperatures. Mesophilic bacteria act at lower

temperatures and thermophilic bacteria act at higher temperatures. In the case of mixed substrates, as is the composting of mixed wastes, the entire consortia of microorganisms in the compost matrix come into play for the breakdown of all the different substrates present. However in the case of a single substrate, as is the case with the liquid culture experiment, the microorganisms and it's temperature range of activity needed for the substrate breakdown is specific. Hence, the composting experiments [ASTM D5338] are taken through a temperature cycle during the course of the experiment, whereas, the liquid culture experiments are carried out at a constant mesophilic temperature. At different temperatures within the mesophilic range, there may be a difference in the rates and also extents of biodegradation. This can be an area of consideration for further study.

b) Aeration rate

The aeration rate also affects the rate and extent of CO_2 production during the aerobic biodegradation test in two ways. Firstly, the air provides the oxygen necessary for the biodegradation process. Hence, insufficient amounts of oxygen can become a limiting factor for the biodegradation equation, and thus govern the levels of CO_2 produced. This would lead to an incorrect measure of the biodegradability of a test material. Hence dissolved oxygen content is measured and kept above the required levels, by maintaining the required air flow rate. Secondly, the rate of air flow through the trapping solution affects the absorption of CO_2 by the NaOH solution. A high flow rate would lead to a lower absorption, whereas a lower flow rate would lead to relatively higher absorption. A balance was struck between the two to satisfy both the requirements. The flow rate was maintained at one to two bubbles per second through the trapping solution.

c) Agitation speed

The speed of agitation of the contents of the experimental flasks also affects the rate of biodegradation. The agitation provided serves to maintain good mixing and contact between the microorganisms and the test material. If there was no mixing, the active surface area of the test material for biodegradation would be greatly decreased, thereby decreasing the rate of biodegradation. Thus diffusion could become a limiting factor for the biodegradation equation instead of kinetics. Hence, agitation levels were kept sufficiently high to ensure that the rates of biodegradation were not diffusion limited. This factor was studied experimentally to determine suitable agitation speeds required for the experiments.

4.3.2 MATERIAL PHYSICAL FACTORS

a) Morphology of the test material

The morphology of the test material affects the rate of biodegradation to a great extent [Buchanan (1992)]. This is because the greater the surface area of the material, the greater number of active sites are exposed to the microorganisms for the breakdown mechanisms. The size of the pores, in case of porous materials, also affects the rate of biodegradation. As is the case with the agitation speeds, the surface morphology of the particles can thus limit the reaction to be mass transfer limited or kinetic limited. Significant surface morphology effects were found during the biodegradation studies on the cellulose acetates

b) Hydrophobicity of the test material

The hydrophobicity of the test material is known to affect it's biodegradation [Weimer (1991)]. If a material is soluble in water (the test medium), the microorganisms have a medium to attach onto the active sites on the test material.

However, there have been some exceptions to this. PHB/V, a copolymer of polyhydroxy butarate and valerate is a hydrophobic polymer, yet undergoes very rapid biodegradation under experimental conditions.

c) Crystallinity of the test material

Biodegradation is also greatly affected by the crystallinity of a material [Weimer (1991)]. Crystallinity decreases the biodegradability of a material. Native cellulose is crystalline in nature and hence insoluble in water. When it is chemically modified to a small extent, it's crystallinity is decreased and it's solubility in water increases, and this makes it more biodegradable.

d) Molecular Weight of the polymer

The molecular weight of the polymer is known to affect the rate of biodegradation to some extent [Fujii et al(1986)]. Smaller chains take lesser time to be assimilated by microorganisms than the longer chains. However, the overall extent of biodegradation is not affected significantly for molecular weight changes in the range of 50,00-100,000. This can also be an area for further study.

4.3.3 MICROBIOLOGICAL FACTORS

There are two factors which would affect the experimental data on the biodegradation rates

a) Size of the microbial inoculum

The size of the inoculum indicates the number of colony forming units (CFU) present in the microbial consortia. If there are less CFUs, it will limit the rate of biodegradation. Hence, it is ensured that there are sufficient microorganisms for

the biodegradation of the entire polymer. As per ASTM D5209, a minimum of 1 - 20 * 10^6 CFUs/ml are specified for the given experiment.

b) Age of the microbial inoculum

Age of the microbial inoculum can affect the rate of biodegradation and the reproducibility of replicates to an appreciable extent [Buchanan (1992)]. When the sewage sludge inoculum is incubated with the test material for some time, it gets acclimatized to the material. In microbiological terminology, the specific enzyme producing microorganisms needed for the degradation of that particular material flourish whereas the other microorganisms start to die. Thus, the resultant inoculum after a certain period of time, is more concentrated in the favorable microorganisms than the original inoculum. This increases the rate of biodegradation to a considerable extent. Also, it increases the reproducibility between the replicates in the experiment. ASTM D5209 prescribes a fresh inoculum, which gives experimental deviations upto ±10% . This inoculum was used in all the experiments.

4.3.4 CHEMICAL COMPOSITION

If all the other conditions are satisfied, i.e., if the reaction is not diffusion limited, oxygen limited and inoculum limited, then the chemical composition of the polymer governs the rate, extent and mechanism of biodegradation. Hence, this is the most important aspect which defines a polymer as biodegradable or not biodegradable. Given the optimum conditions for biodegradation, a polymer can biodegrade only if it's chemical composition and structure is such that it can be broken down and assimilated by microorganisms into smaller end products. This factor has been studied in detail further on in this work.

4.4 LIMITING PARAMETERS IN THE BIODEGRADATION EQUATION

The biodegradation process can be broken down into two basic steps:

4.4.1 Hydrolysis of the polymer to monomer by the hydrolytic enzyme

Polymer + xH_2O ----------> Monomer

This step can be :

a) Diffusion limited

b) Inoculum limited

c) Substrate (polymer) limited

d) Kinetics limited

In this step, the attempt is made to optimize a) and b), so that the substrate is the limiting reactant for the overall reaction and the rate equation is kinetic limited, i.e., the substrate amount limits the total amount of CO_2 produced and the kinetics of depolymerisation dictates the rate equation for this step.

4.4.2 Oxidation of monomer to CO_2

Monomer + O_2 ----------> CO_2 + H_2O

This step can be:

a) Diffusion limited

b) Oxygen limited

c) substrate limited

d) kinetics limited

Of the above mentioned parameters, a) and b) can be controlled, but c) and d) follow after step 1. If the rate of step 1 is more than the rate of step 2, then the

monomer will start to accumulate and the step 2 reaction kinetics will govern the production of CO_2. However if the rate of step 2 is more than the rate of step 1, then the availability of the monomer will govern the rate of CO_2 production.

4.5 APPLICATION TO EXPERIMENTAL RATES OF POLYMER SYSTEMS

For a polymer system, the above mentioned steps and mechanisms result in the development of very complex equations as will be shown later on in the case of cellulose and starch esters. This is because, usually there is more than one hydrolytic enzyme acting during hydrolysis. In the case of starch and cellulose, there are three to four enzymes acting synergistically at different rates to accomplish the process of hydrolysis. When there are chemical modifications involved, other mechanisms other than hydrolysis also come into picture, which make the rate equation more complex. An attempt has been made to explain the rate of CO_2 production mathematically in Chapter 8. Some of the parameters involved in the biodegradation process were studied experimentally and are reported in this chapter.

4.6 EFFECT OF AGITATION SPEEDS

The effect of agitation speed on the rate of biodegradation was studied using Poly capro lactone (PCL) as the substrate.

4.6.1 EXPERIMENTALS

The CHN data on PCL as determined by the Perkin Elmer 2400 Analyser was %C= 63.3, %H = 8.85, %N = 0.087. Cellulose (microcrystalline avicel) was used

as the standard. A blank was also included in the set up which contained only the nutrients and inoculum.

The amount of test material used in each flask was based on 0.8g of carbon and were as follows:

PCL = 1.26 g, C content = 0.8 g

Cellulose = 2.00 g, C content = 0.8 g

The stock solutions used in each flask were as mentioned in chapter 3. 3 ml of ammonium sulfate was added to the solution along with 30 ml of sewage sludge inoculum. The amount of N in the sludge was assumed to be negligible and not taken into account. Water was added to the flasks to bring up the level of solution to 1500ml. The C/N ratio in the solution was calculated to be 25/1. PCL, cellulose and the blank were all run in duplicate. Each flask was placed on a magnetic stir plate which provided the agitation inside the flask. Three different speeds of agitation were maintained They were speeds 3.0, 4.0, 5.0 on the stir plate which corresponded to 700, 825, and 1000 rpm. One set was also run at zero speed.

Table 4.1 - Summary of biodegradation results

Agitation speed (rpm)	% PCL-C to CO_2 (33 days)
0	14.10
700	30.89
825	32.71
1000	29.38
Cellulose, 825 rpm	75.06

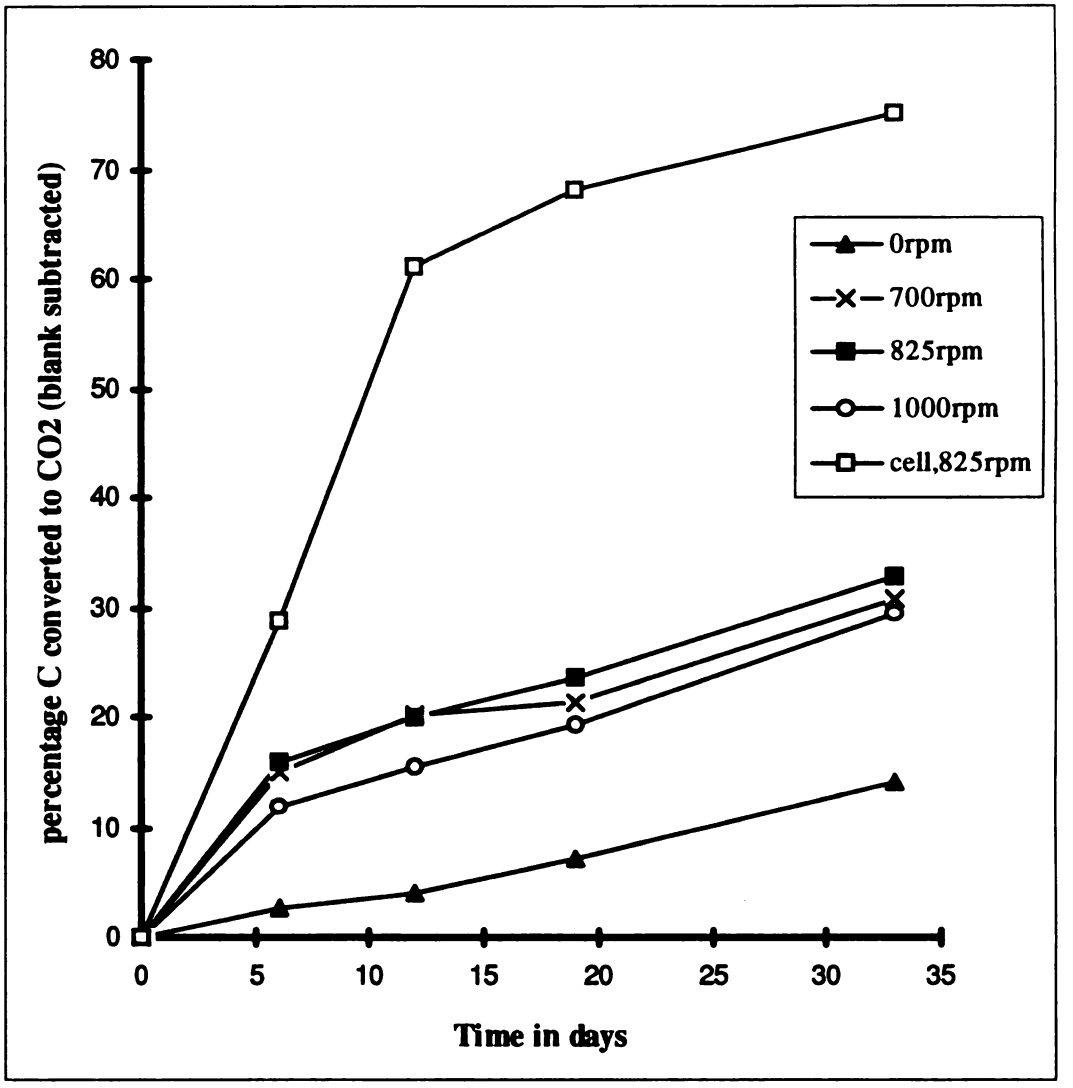


Figure 4.1 - Percentage conversion of PCL carbon to CO_2. Carbon conversion with time at varying agitation rates was plotted for PCLwhich was the substrate in all cases, except in the case of cellulose which was included as a positive control. Each point is an average of triplicate runs.

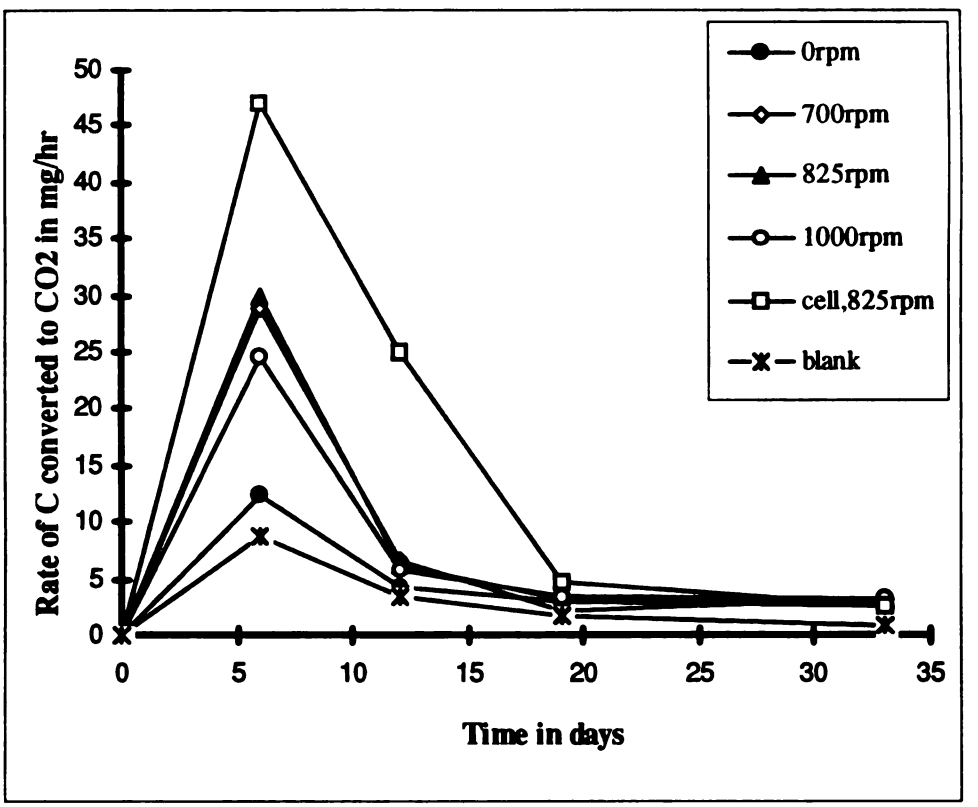


Figure 4.2 - Rate of PCL carbon converted to CO_2 with time. The rate of carbon conversion to CO_2 with time was plotted at different speeds of agitation for PCL which was the substrate in all cases except in the case of cellulose which was the positive control. The rate of C conversion to CO_2 from the blank flask is also included.

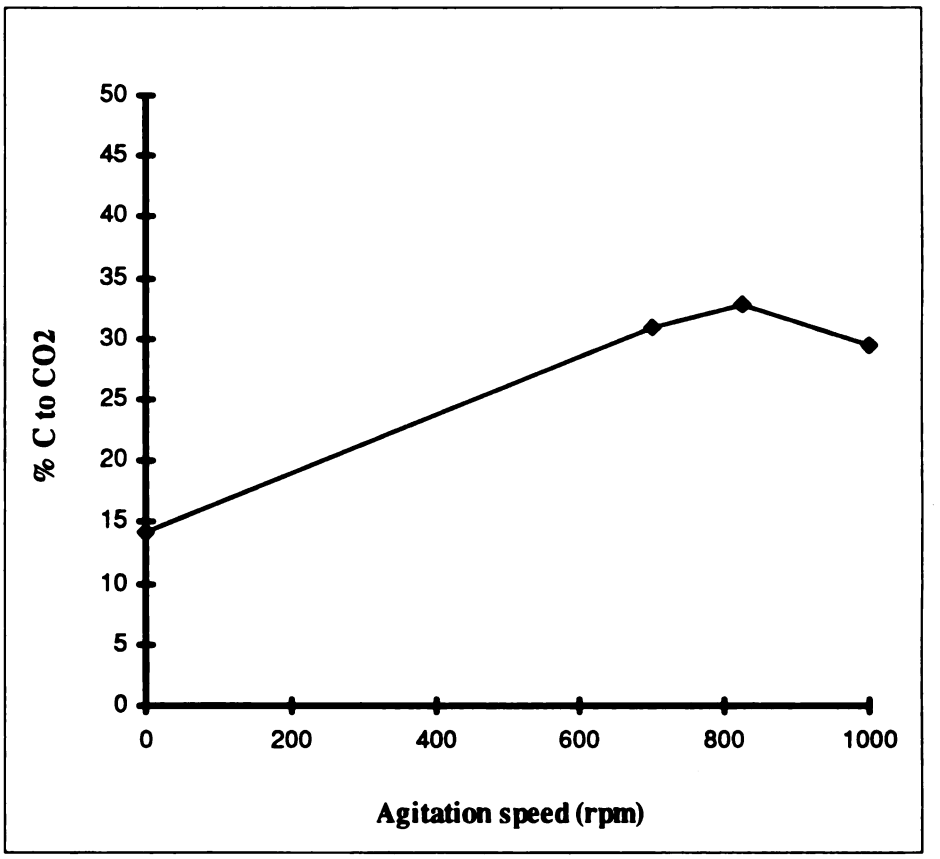


Figure 4.3 - Relation between speed of agitation and percentage C conversion to CO_2. Percentage of PCL C converted to CO_2 in 33 days was plotted for different speeds of agitation.

4.6.2 RESULTS AND DISCUSSION

A summary of the results of the above experiment is presented in Table 4.1. Figure 4.1 shows the rate of C converted to CO_2 with time and Figure 4.2 shows the percentage C converted to CO_2 with time. It can be seen that the standard cellulose reached a CO_2 evolution level of 70% within 30 days. PCL also started degrading rapidly in the first few days and then slowed down to a steady rate after the first week. The relationship between the speed of agitation and the percentage C converted to CO_2 is shown in Figure 4.3. As can be seen the rate of CO_2 production was appreciably lower in the case of zero agitation as compared to the other agitation speeds of 700, 825 and 1000 rpm. There was not much difference between these three speeds in terms of CO_2 evolution. All the experiments in this work were carried out at an agitation speed around 900rpm, thus ensuring that there was adequate mixing between the test materials and the microorganisms and that it was not diffusion limited.

4.7 EFFECT OF ACCLIMATISATION OF MICROBIAL INOCULUM

An attempt was made to study the effect of acclimatisation of the microbial inoculum on biodegradation. Three different samples were considered here, two grades of cellulose acetate of DS 2.5, and a modified starch sample.

4.7.1 MATERIALS TESTED

The three samples were run in triplicate along with a blank and cellulose as the standard. These materials were tested for their CHN contents by the CHN analyser. All the samples were particulate solids between mesh size 80 and 140.

The materials tested were as follows :-

CA-398-3 (Eastman Kodak) - Cellulose Acetate DS 2.5, %C = 48.5, M_n = 30, 000

CA398-30 (Eastman Kodak) - Cellulose Acetate DS 2.5, %C = 48.5, M_n = 50, 000

Modified starch (Michigan Biotechnology Institute) - %C = 49.1

4.7.2 PREPARATION OF INOCULUM

Inoculum1 - This inoculum was prepared as per the procedure mentioned in Chapter 3 by obtaining activated sludge from the waste treatment plant. 50ml of the inoculum was used in each flask for each of the three samples.

Inoculum2 - 1L of Inoculum 1 was centrifuged in an RC5C Sorvall refrigerated Centrifuge and the resultant pellet suspended in 300 ml of water inside a 1L flask along with 2.5 g of cellulose acetate DS 2.4, 1 g of cellulose and same amounts of nutrient solutions used in the experimental flasks. The flask was stoppered and incubated at 28^0C on a shaker for 15 days. An agar medium was prepared in the following manner -

a) 20 g of agar and 15 g of Nutrient Broth 2 were mixed with 1L of water in a 2L flask.

b) The solution was autoclaved at 120^0C for 20 mins.

c) The flask was taken out and allowed to cool slightly before pouring out into petri-dishes.

About 1 ml of the incubated inoculum was plated on the agar medium in each petridish and incubated at 30^0C for 2 days. The colonies formed were then gently scraped off the plates with the help of some water, and the total volume finally made up to 150 ml.

Inoculum 3 - This inoculum was prepared in the same way as Inoculum 2 except for cellulose acetate and cellulose being replaced by starch acetate and starch respectively.

Each of the three samples were inoculated with Inoculum1, the cellulose acetates inoculated with Inoculum 2, and the modified starch inoculated with Inoculum3. The C/N ratio maintained in the flask was approximately 25:1.

4.7.3 RESULTS AND DISCUSSION

No change was observed in the extent of biodegradation with acclimatisation. This may be due to insufficient enrichment of the favorable microorganisms. However, there was an appreciable decrease in the standard deviation between the replicates with acclimatisation. Figures 4.4, 4.5 and 4.6 and Table 4.1 show the difference between the normal inoculum and the acclimatised inoculum with respect to the standard deviation between replicates. This decrease in the standard deviation is more prominent in the modified starch and the CA398-30 samples.

Table 4.2 - Standard deviations between replicates

Sample	Normal inoculum	Acclimatised inoculum
CA 398-3	1.62%	1.11%
CA398-30	9.54%	0.77%
Modified starch	11.37%	1.55%

This uniformity between replicates can be accounted for by the fact that during the incubation of the original inoculum with the substrate, the growth of the substrate

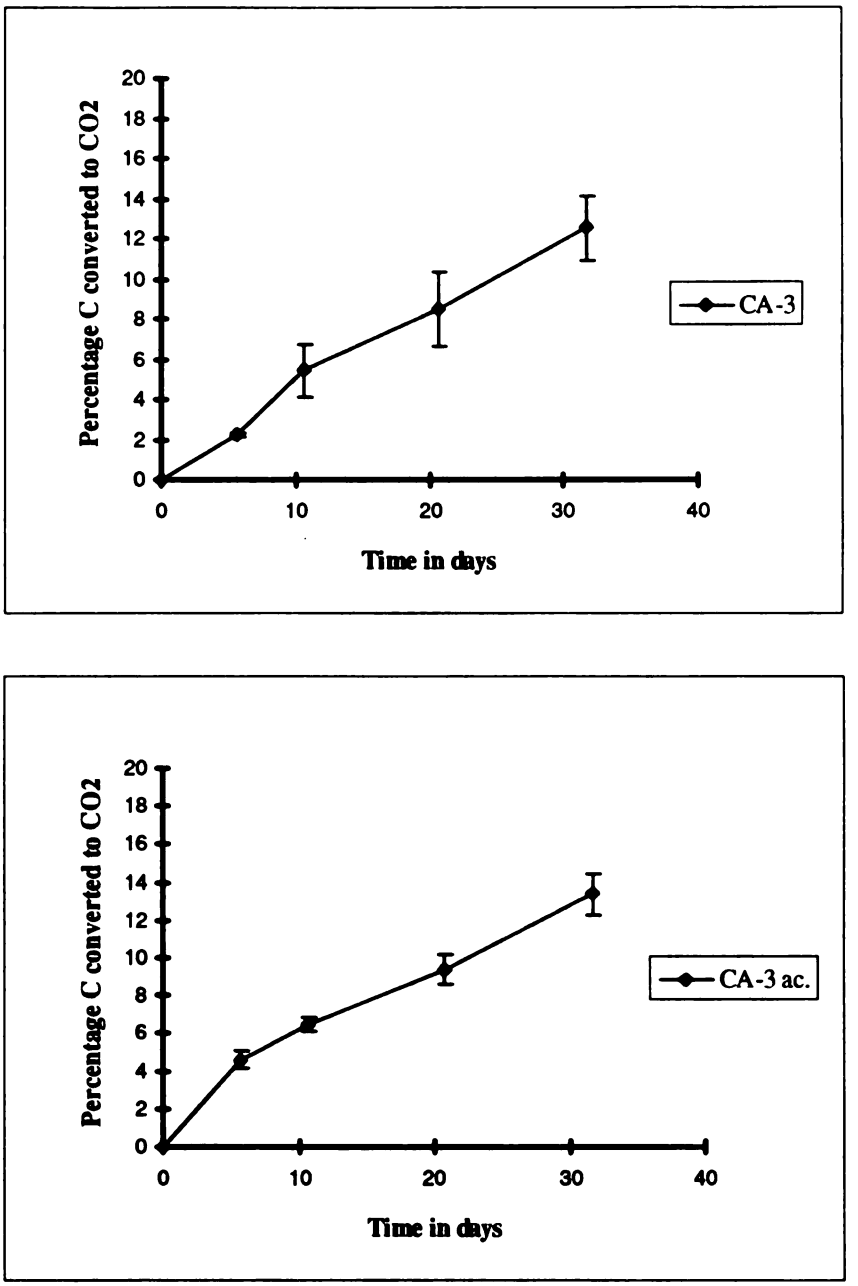
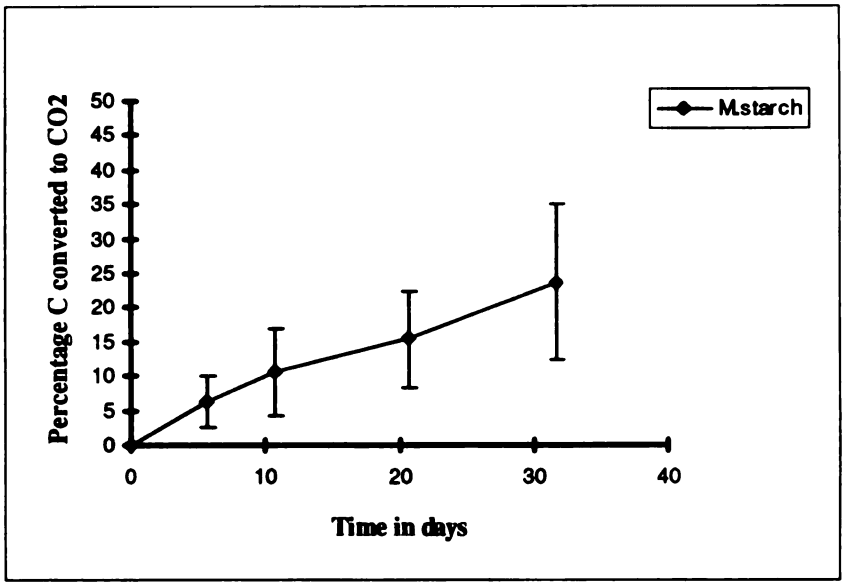


Figure 4.4 - Effect of inoculum acclimatisation on CA-398-3. % C conversion to CO_2 was measured with time using CA39-3 as the substrate for a fresh inoculum (top) and an inoculum that had been acclimatised to the sample for 15 days (bottom). Each point is an average of triplicates.



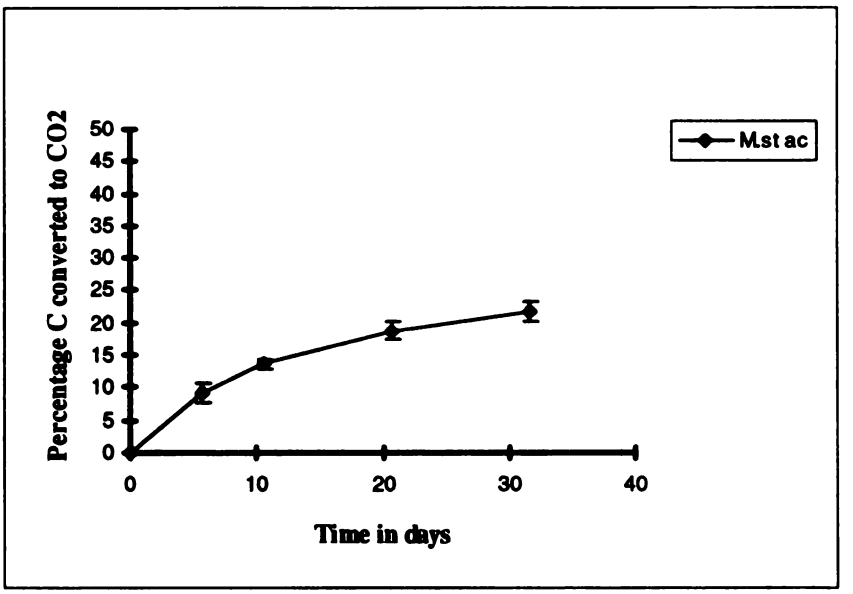


Figure 4.5 - Effect of inoculum acclimatisation on Modified starch. % C converted to CO2 was measured with time using modified starch as the substrate for a fresh inoculum (top) and an inoculum that had been acclimatised to the sample for 15 days (bottom). Each point is an average of trilplicates.

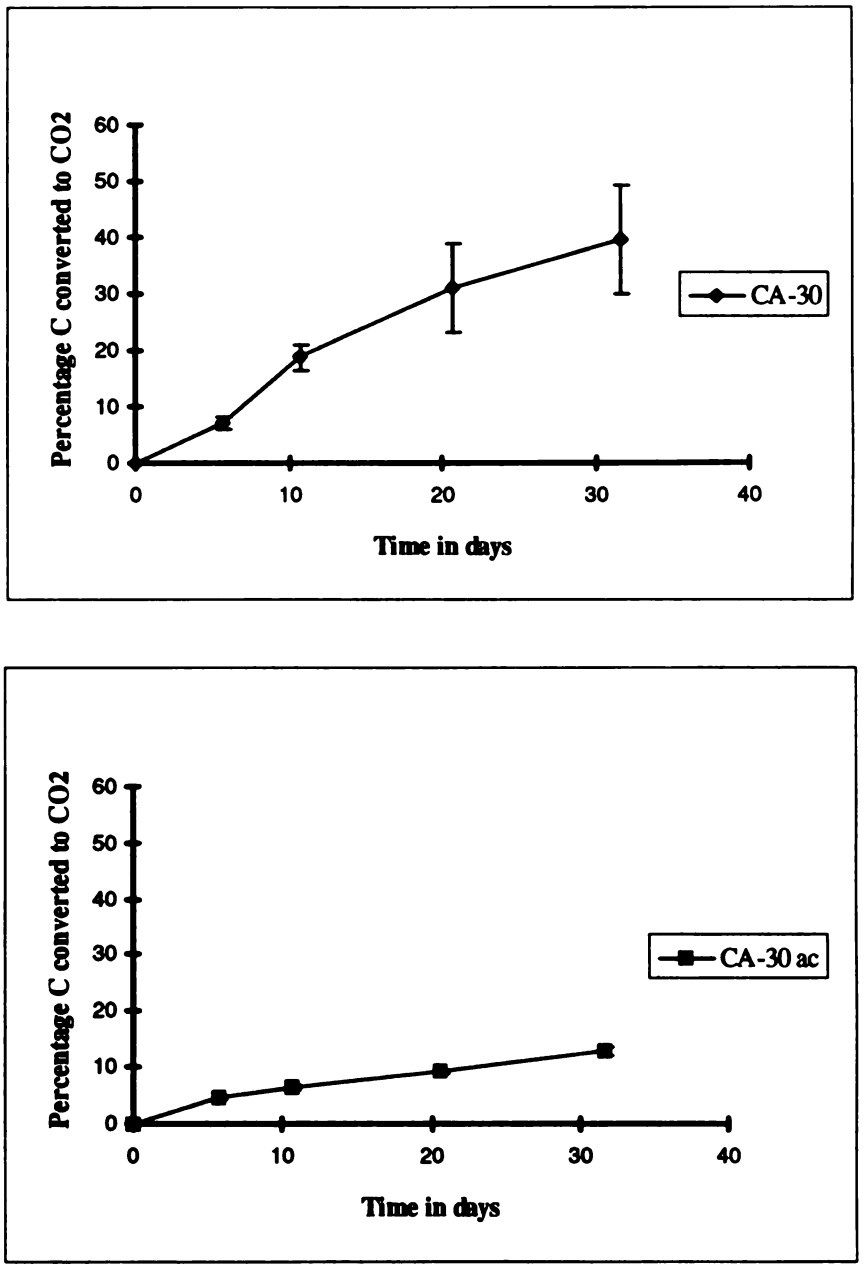


Figure 4.6 - Effect of inoculum acclimatisation on CA 398-30. % C converted to CO2 with time was measured using CA398-30 as the substrate for a fresh inoculum (top) and an inoculum that had been acclimatised to the sample for 15 days (bottom). Each point is an average of triplicate runs.

utilizing microorganisms was favored in comparison to the other microorganisms present, which resulted in a more homogeneous culture for the replicates. Stepwise enrichment of the inoculum to increase the population of the favorable microorganisms is an area which should be explored in detail.

BIODEGRADATION OF COMMERCIAL POLYMERS

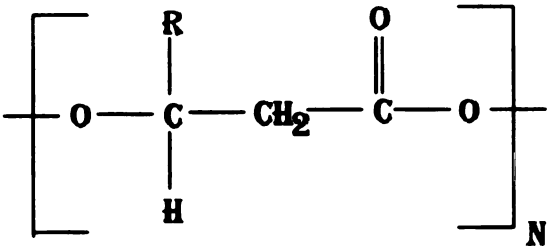
5.1 INTRODUCTION

Because of the growing awareness of environmental issues, most manufacturers of plastics and other short term application materials are looking towards the final fate of the plastics in the waste disposal streams. Increasingly, the need for materials that are environmentally nonpersistent is being recognized as one solution, particularly in those products which cannot be recycled. Most of the common packaging plastics present in the current materials world possess very little or relatively no biodegradability. However, there are some plastics which are biodegradable but with poor mechanical properties. This is because, hydrophobicity is a property of any material that is highly unfavorable from the biodegradability standpoint but highly favorable from the mechanical property standpoint. Biodegradability studies were carried out on some of the polymers available commercially. The results are presented in this chapter.

5.2 POLYMERS TESTED

5.2.1 PHB/V - PHB/V, was obtained from Zeneca Bioproducts. Sold under the trade name BIOPOL by Zeneca, it is a thermoplastic polyester produced from

agricultural feedstocks [Zeneca]. It is a copolymer of polyhydroxy butyrate and valerate, currently produced with the valerate contents ranging from 5 to 20%, either in powder or pellet form. This copolymer can be plasticized, filled, nucleated, etc. much as conventional polymers. They also blend with polymers containing some type of polar group such as PVC, chlorinated polyethylene, polycarbonate and low melt nylons and alloys of these resins. They are not compatible with nonpolar systems as represented by the polyolefins and polystyrene[Galvin, T. J. (1989)]. The chemical structure of PHB/V is shown in Figure 5.1.



R = n-alkyl pendant group of variable chain length

beta-hydroxybutyrate, R=methyl

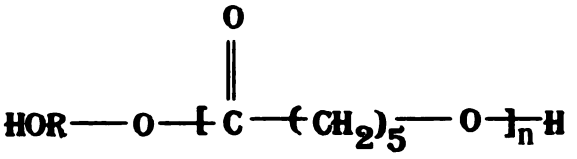
beta-hydroxyvalerate, R=ethyl

Figure 5.1 - Structure of poly(beta-hydroxy alkanoate)

PHB/V has been found to be biodegradable by the action of two extracellular depolymerase enzymes; one enzyme predominantly cleaves dimer from the chain while the other produces predominantly trimers [Gilmore (1990)].

5.2.2 Poly Caprolactone (PCL) - PCL was obtained from Union Carbide. It is a homopolymer of caprolactone, a seven-membered ring compound. PCL resins are miscible or mechanically compatible with many other plastics, like polyethylene, polypropylene, PVC, PET etc. PCL has been shown to be biodegradable by property and weight loss in soil burial tests [Union Carbide].

The polymer can be represented by the structure shown in Figure 5.2



where, R = aliphatic segment

Figure 5.2 - Structure of PCL

Laboratory studies have been reported on the biodegradation pathway of PCL which involves the formation of adipic acid, which undergoes fatty acid degradation. In this process, two carbon atoms are successfully removed as acetyl Coenzyme A, which enters the Citric Acid Cycle and becomes a source of energy for the microorganism [Union Carbide].

5.2.3 Unmodified corn starch (Hylon 7) - This was obtained from National Starch and Chemical Company. It is pure starch containing 70% amylose and 30% amylopectin, and has a molecular weight of around 10,000. It is insoluble in water. The structure of starch is shown in Chapter 6. Starch is a naturally occurring polysaccharide, known to be biodegradable in nature.

5.2.4 PCL/Starch blend - This material was an extruded 50/50 starch/PCL polymer blend. The PCL and starch used were the ones described above. It was prepared at the Composites Centre of Michigan State University.

5.2.5 Polyvinyl alcohol (PVOH) - This sample of PVOH was obtained from Aldrich Chemicals. It was 99+ % hydrolyzed, molecular weight = 85,000 - 140,000. PVOH is a synthetic, water-soluble polymer, produced by the hydrolysis of polyvinyl acetate, since vinyl alcohol exists only in it's isomeric form, acetaldehyde. Vinyl acetate is made by reacting acetic acid and ethylene. Varying the molecular weight and percentage hydrolysis results in a broad range of physical properties. The chemical structure of PVOH is shown in Figure5.3.

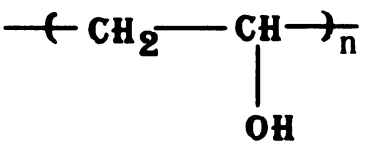


Figure 5.3 - Chemical structure of Poly vinyl alcohol

The proposed mechanism for biodegradation in literature is initial oxidation of the hydroxyl group by an oxidase to a ketone group followed by random cleavage of the polymer chain at β-diketone formations by another hydrolase. This step is followed by the endgroup hydrolysis to acetic acid, which finally produces carbon dioxide and water [Philips (1991)]. However, it has also been reported that increasing the percentage hydrolysis of PVOH decreases it's biodegradation considerably.

5.2.6 Ethylene vinyl alcohol (EVOH) - This sample of EVOH was obtained from EVAL Company of America, Grade LC-F101A. It is a copolymer of ethylene and vinyl alcohol with 32% ethylene content, and a melting point of 183^0C. It is used for making blown films, melt phase forming and blow molding. it has a melt index of 1.6 at 190C [EVAL (1992)].

5.2.7 Glycerol triacetate - This compound was obtained from Aldrich Chemicals. It is a low molecular weight liquid used as a plasticizer. It has a melting point of 3^0C. The structure of glycerol triacetate is shown in Figure 5.4.

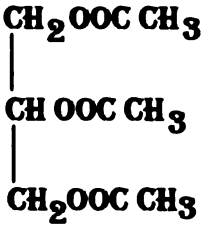


Figure 5.4 - Structure of Glycerol triacetate

5.2.8 Acrylic Coating - This material is sold under the trade name "AquaCoat 584F" by International Blending Corporation (IBC), Minneapolis. It is a thick viscous liquid with a recommended viscosity of 28 to 35 seconds through a number 3 Zahn cup. It is used as a coating material for various paper products and other applications.

5.2.9 9-11 Acid - This product was obtained from Cas Chem and is a derivative of Castor oil. Conversion of dehydrated castor oil by hydrolysis to acid and subsequent distillation yields 9-11 acid, a high purity conjugated fatty acid. In 9-

11 acid, the total linoleic content is 94%, and combined oleic and saturated acids content is less than 10%. This product has properties that can be utilized in a broad list of resin/polymer systems generally directed to coatings, inks and adhesives. They are applicable to resin systems based on 100% solids, solvent based and water based formulations [Cas Chem (1992)]. It is insoluble in water, but soluble in most organic solvents.

5.3 EXPERIMENTALS

The CHN data of the materials tested are given below

Glycerol triacetate - %C = 49.57, %H = 6.13, %N = 0.15

PHB/V powder - %C = 56.79, %H = 7.22, %N = 0.21

PCL powder - %C = 63.3, %H = 8.85, %N = 0.087

Unmodified corn starch (Hylon7) - %C =43.26, %H = 6.52, %N = 0.146

PCL/starch blend - %C = 50.

PVOH - %C = 52.73, %H = 8.79, %N = 0.077

EVOH - %C = 60.45, %H = 10.27, %N = 0.063

AquaCoat 584F - %C = 46.3, %H = 8.59, %N = 1.56

Cellulose was used as the standard in all cases.

5.3.1 Inoculums and test solutions

These materials were run in different sets of experiments conducted in different time frames. The sample concentrations used in the different experimental setups were as follows:-

Experimental Setup #1 -

Aqua Coat 584F = 2.5g, C content = 1.16g

9-11 acid = 2.0g, C content = 1.6g

Cellulose = 4.0g, C content = 1.6g

The C/N ratio in this setup was approximately 35/1, and for the AquaCoat 26/1.

Experimental Setup #2 -

Glycerol triacetate = 3.23g, C content = 1.6g

Cellulose as standard = 4.00g, C content = 1.6g

The C/N ratio in this setup was approximately 35/1.

Experimental Setup #3 -

PHB/V= 2.64g, C content = 1.5g

PCL = 2.36g, C content = 1.5g

Unmodified starch = 3.47g, C content = 1.5g

PVOH = 2.85g, C content = 1.5g

EVOH = 2.48g, C content = 1.5g

Cellulose as standard = 3.76g, C content = 1.5g.

The C/N ratio was calculated to be 33/1.

Experimental setup #4 -

PCL/starch blend = 1.6g, C content = 0.8g

Cellulose as standard = 2.0g, C content = 0.8g

The C/N ratio was calculated to be 25/1.

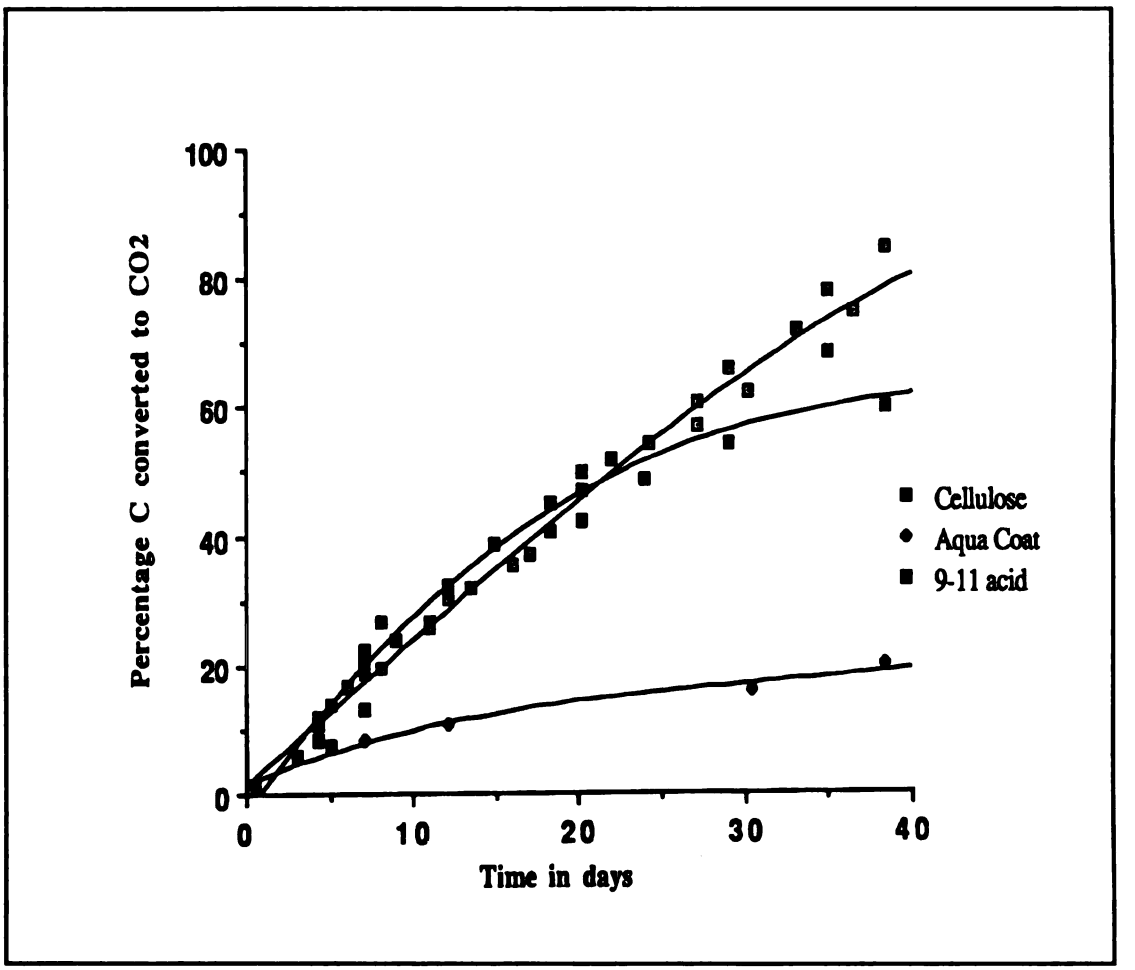


Figure 5.5 - Percentage C converted to CO_2 with time for AquaCoat584F and 9-11acid. Cellulose was used as the positive control. Each line represents an average of duplicate runs. Blank values have not been subtracted in this set of results.

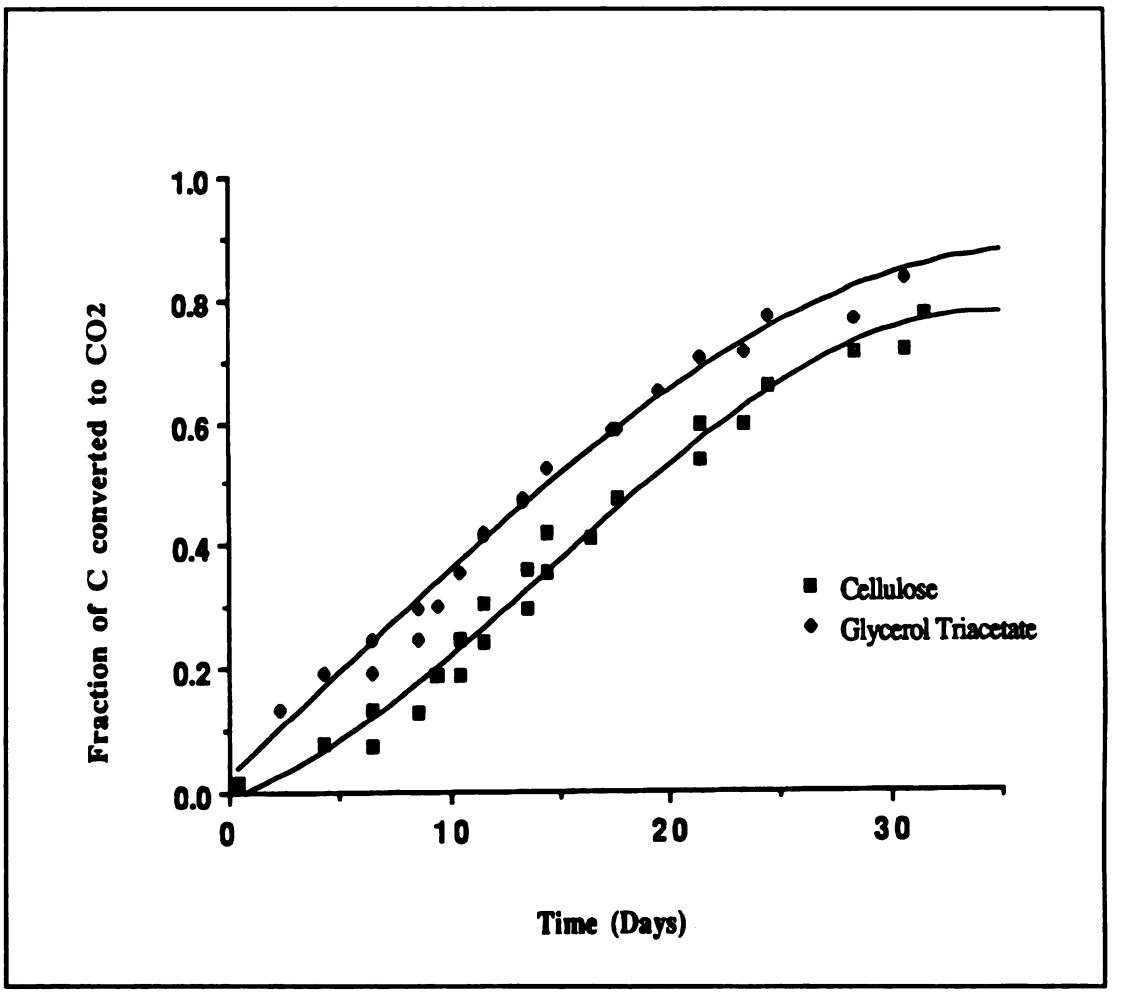


Figure 5.6- Percentage initial C converted to CO_2 with time for Glycerol triacetate. Cellulose was used as the positive control. Each line represents an average of duplicate runs.

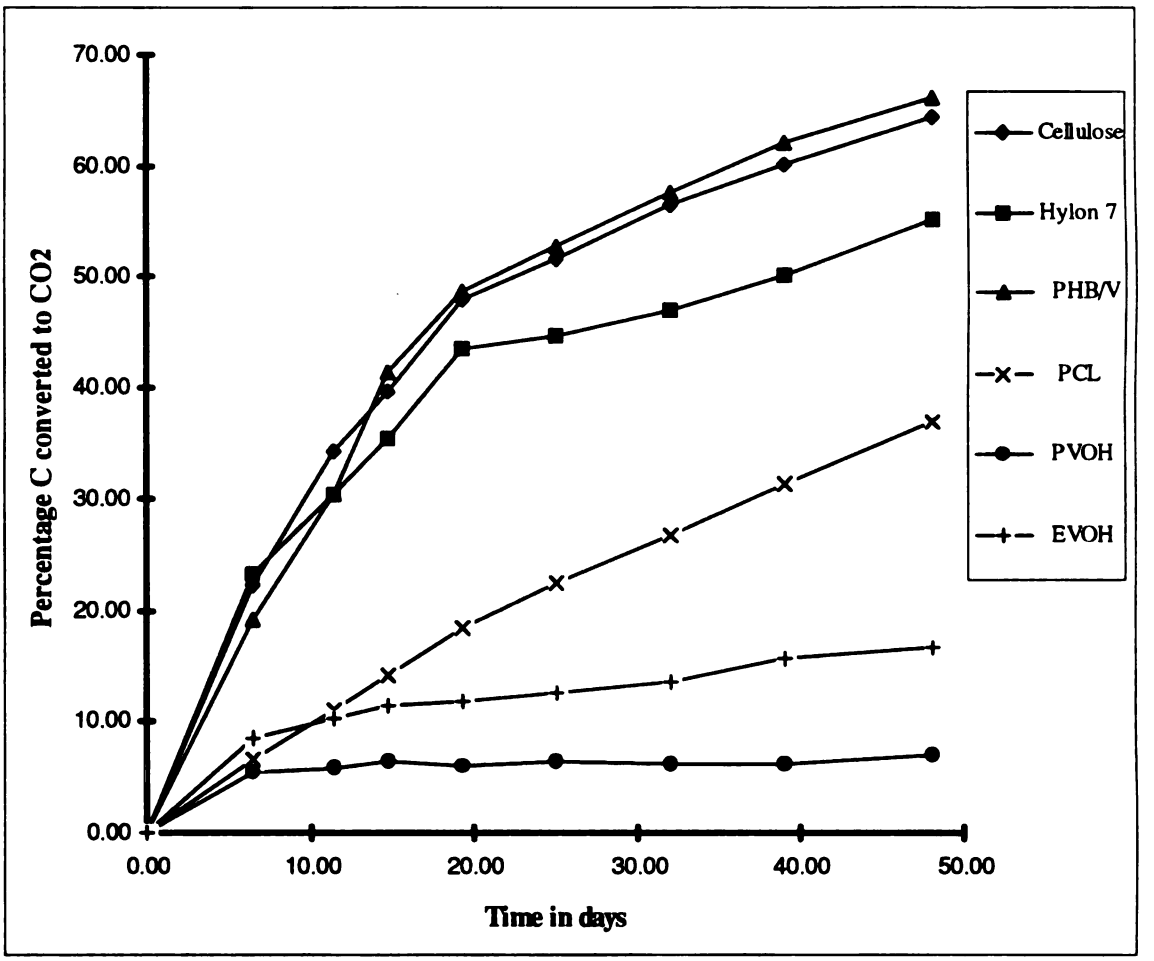


Figure 5.7 - Percentage of initial polymeric C converted to CO_2 with time for various commercial polymers. Cellulose was used as the positive control. Cellulose, Starch, PCL and PHB/V represent the average of triplicate runs; PVOH and EVOH represent the average of duplicate runs.

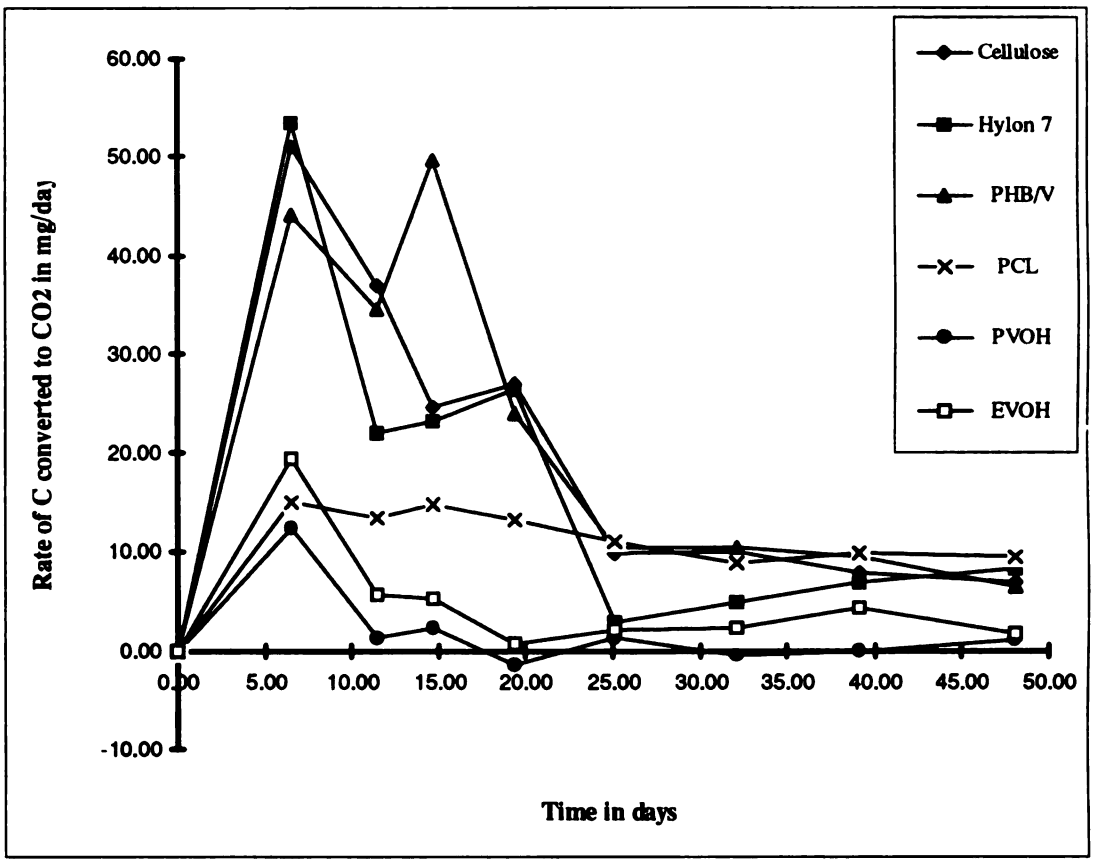
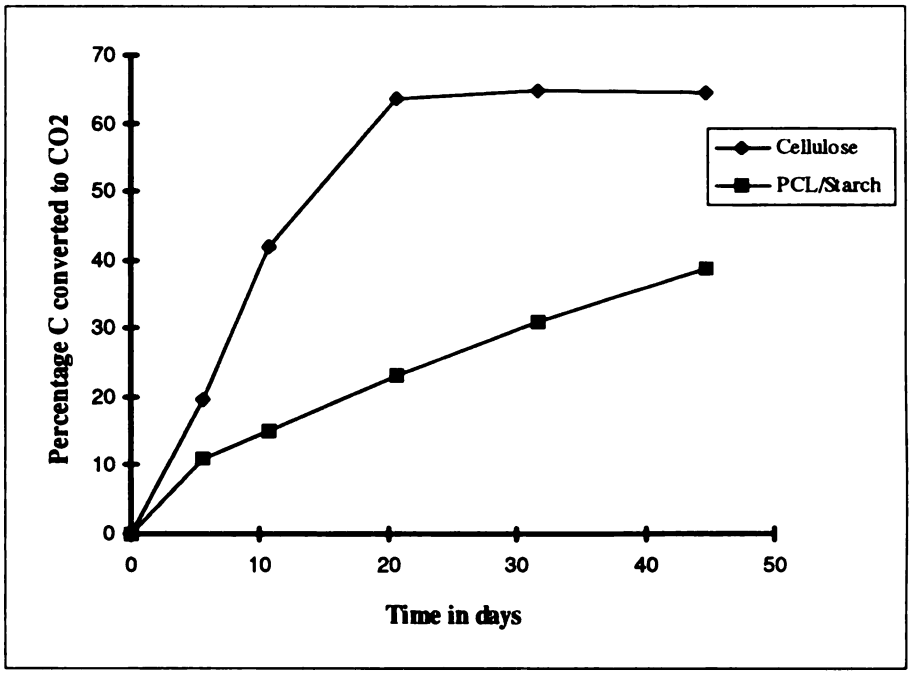


Figure 5.8 - Rate of initial polymeric C conversion to CO_2 with time for various commercial polymers.



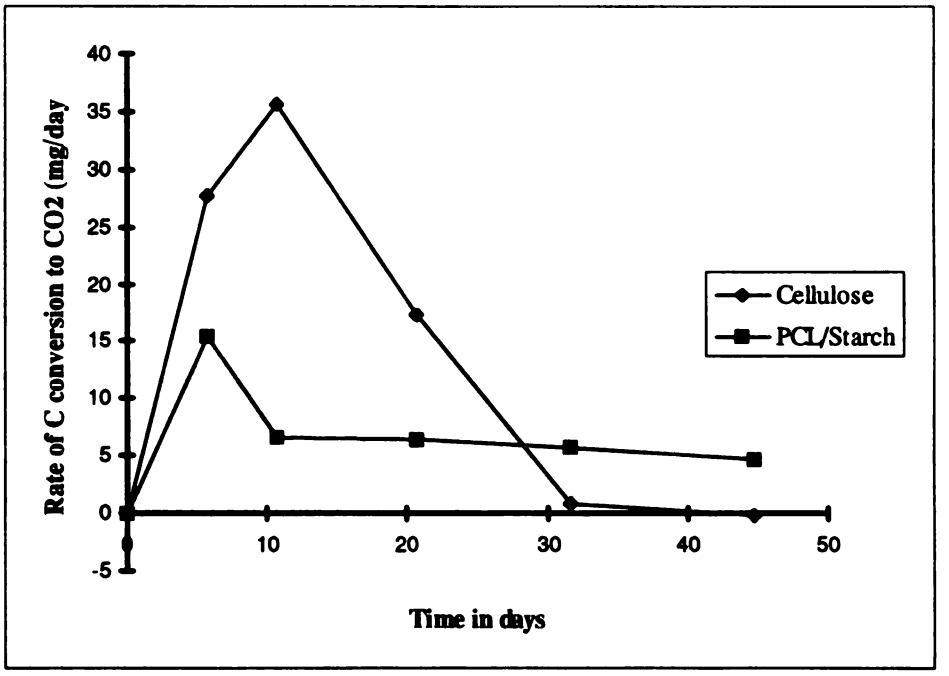


Figure 5.9 - Initial polymeric carbon converted to CO_2 with time for the 50/50 PCL/starch blend. Cellulose is included as the positive control. Each point is an average of duplicate runs.

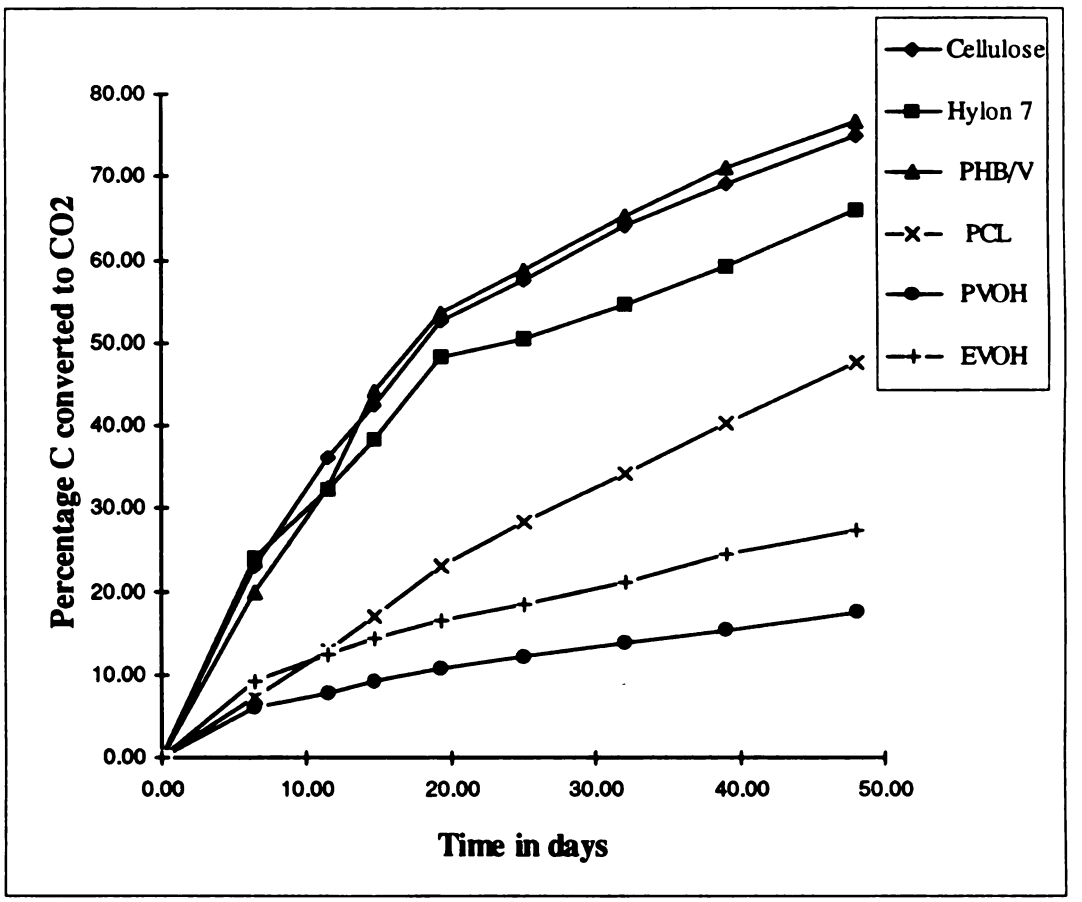


Figure 5.10 - Percentage of initial polymeric C converted to CO_2 with time for various commercial polymers (no blank correction). This graph is a replicate of Figure 5.7 except that no blank correction has been applied here.

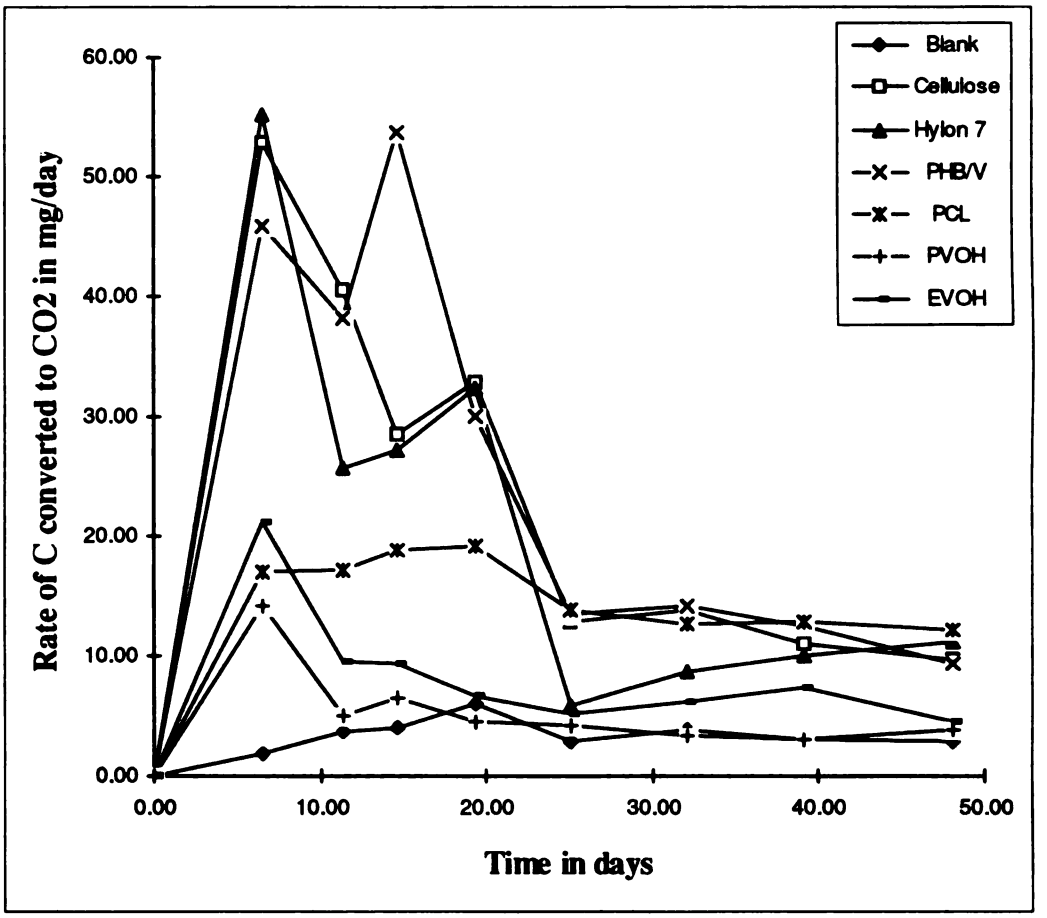
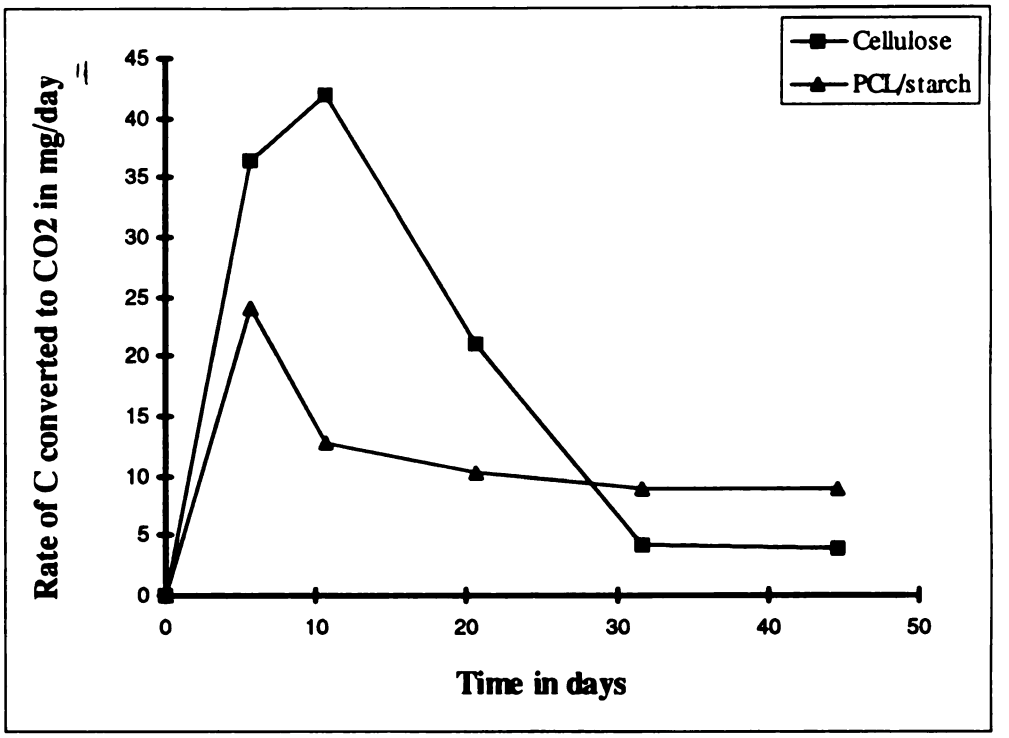


Figure 5.11- Rate of initial polymeric C converted to CO_2 with time for various commercial polymers (no blank correction). This graph is a replicate of Figure 5.8 except that no blank correction has been applied.



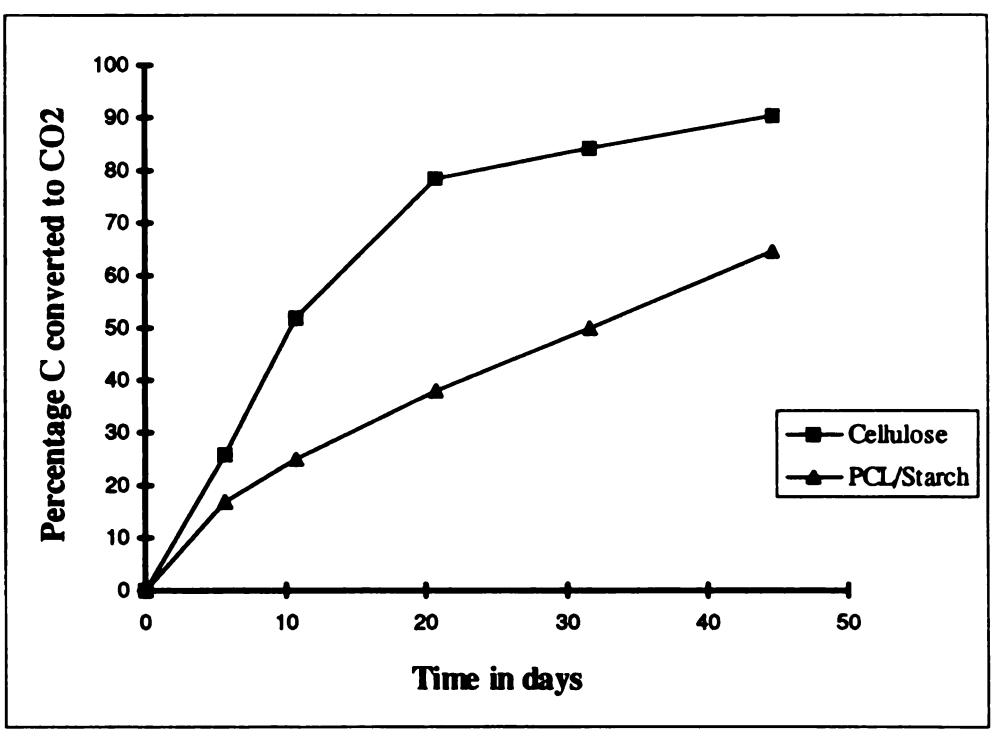


Figure 5.12 - Initial polymeric carbon converted to CO_2 with time for the 50/50 PCL/starch blend (no blank correction). This graph is a replicate of Figure 5.9 except that no blank correction has been applied here.

5.3.2 RESULTS AND DISCUSSION

Figures 5.5 through 5.9 show the %C converted to CO_2 and rate of CO_2 produced with time for all materials tested. Figures 5.10 through 5.12 show the results of Experimental setups #3 and #4 without the blank correction. Table 5.1 gives a summary of the biodegradation results as well as the standard deviations between replicates.

Table 5.1 - Summary of biodegradation results for all setups

	Material	%C to CO_2	St. deviation
Setup #1	Cellulose	77.8*	-
38 days	Aqua Coat 584F	15.0*	-
	9-11 acid	51.1*	-
Setup #2	Cellulose	70*	-
30 days	Glycerol triacetate	78*	-
	Cellulose	60.14	7.3%
	PHB/V	62.14	6.5%
Setup #3	PCL	31.26	4.1%
39 days	Starch	50.17	7.1%
	PVOH	6.19	0.9%
	EVOH	15.61	9.9%
Setup #4	Cellulose	64.55	4.2%
44 days	PCL/Starch blend	38.69	5.6%

* Blank values not subtracted

The first and second experimental setups were conducted during the early part of this work. In these two setups, each tube containing the trapping solutions were removed only when the solution turned yellow. This resulted in more data points for the faster degrading materials than the slower ones. Hence, the CO_2 evolution curves have been obtained by curvefitting data points as shown. Also, this method made it difficult to subtract the blank values from the test flask values.

AquaCoat 584F showed a very low rate and extent of biodegradation. This is important because the biodegradability of the coating affects the biodegradability of coated paperboard and other coated articles by creating a non permeable barrier to the degrading microorganisms. Cellulose, the positive standard reached a CO_2 evolution of 78% in 38 days. The 9-11 acid also showed substantial biodegradation within 38 days showing it's potential for further biodegradation. In the second setup, Glycerol triacetate underwent rapid degradation to CO_2 within the first few days. Cellulose reached a CO_2 evolution of 70% in 30 days.

In all the remaining setups, the trapping solutions were removed for titration periodically, all at the same time, irrespective of the levels of CO_2 generation. This allowed for averaging of the duplicate or triplicate runs, subtracting blank values and for calculating the standard deviations between the replicates. PHB/V underwent rapid biodegradation within the first few days at the same rate as cellulose. PCL on the other hand had a slower, but almost constant rate throughout the time frame of the experiment. Starch also underwent rapid biodegradation to about 50% of CO_2 evolution within thirty days. PVOH was recalcitrant to biodegradation throughout the test period. It is interesting to note that EVOH showed a slightly higher percentage biodegradation than PVOH (although it also

had a higher standard deviation). The biodegradability of PVOH, well established in literature, is also very sensitive to the percent hydrolysis. Increasing the percent hydrolysis of PVOH increases it's crystallinity, and reduces it's solubility [Philips, J.,(1991)]. Crystallinity affects biodegradability adversely. The sample of PVOH used in this case, was 99+% hydrolyzed, which accounts for the low extent of biodegradation achieved in this experiment. EVOH, on the other hand was a copolymer of PVOH and ethylene, where the latter has been established as highly recalcitrant to biodegradation in literature. However, the presence of ethylene in the copolymer decreases it's crystallinity, thus rendering the vinyl alcohol segments in the chain to be more susceptible to enzymatic attack. In the last setup, the PCL/starch blend showed a steady biodegradation rate throughout the time frame of the experiment. In this case, since the blend was in the form of small extruded pieces, the surface area of the pieces might have dictated the rate of biodegradation.

CELLULOSE AND STARCH ESTERS

6.1 BACKGROUND

The complex structure of cellulose and cellulosic materials introduces considerable problems to a researcher attempting to measure it's biodegradation. The rate and extent of cellulose biodegradation by microorganisms and their enzymes is dependent in part on various physical and chemical parameters. Moreover, because cellulose is a substrate of complex structure, a number of substrate related factors are major determinants of biodegradability [Weimer (1991)]. Two general types of substrates have been used to measure cellulose biodegradation. The first group includes relatively unaltered natural substrates such as pure crystalline cellulose; the second includes modified cellulose substrates whose biodegradation occurs more rapidly (e.g., substituted celluloses) or is more easily observed (e.g., dyed celluloses).

Cellulose derivatives have proved to be more complicated in it's kinetics of biodegradation than unmodified cellulose. Considerable work has been done on cellulose derivatives like cellulose acetate and carboxy methyl cellulose. However, most of the work so far done in this field has been focused on derivatives with a low degree of substitution of the chemically modifying group; only recently has

some detailed work been done on derivatives with a higher degree of substitution of the modifying group.

Similar work has been done on starch and starch derivatives, though to a lesser extent. There are some basic differences between the structure of starch and cellulose, but their behavior in a consortia of microorganisms is essentially the same. In this work, cellulose and starch esters were studied in detail, the results of which have been reported in the next two chapters.

In order to study the effects of chemical or physical modifications on the biodegradation of these esters, it is essential to characterize them properly. Some fundamental points and characterization techniques which were common to both the esters have been discussed in this chapter.

6.2 THEORETICAL

6.2.1 STRUCTURE OF STARCH

Starch is the reserve food in plants, and it's granules are large enough to be seen in many plant cells when examined through a microscope [Bailey and Ollis (1986)]. Starch consists of two fractions: amylose and amylopectin. Both are homopolymers of -D- glucopyranosyl units, except that amylose is a linear polymer and amylopectin is a branched polymer.

Amylose - Amylose is the straight chain polymer of glucose subunits with molecular weight ranging from several thousand to half a million. Amylose

typically comprises about 20 percent of starch. It's chains are formed by the presence of α-1,4-glycosidic bonds as shown in the Figure 6.1.

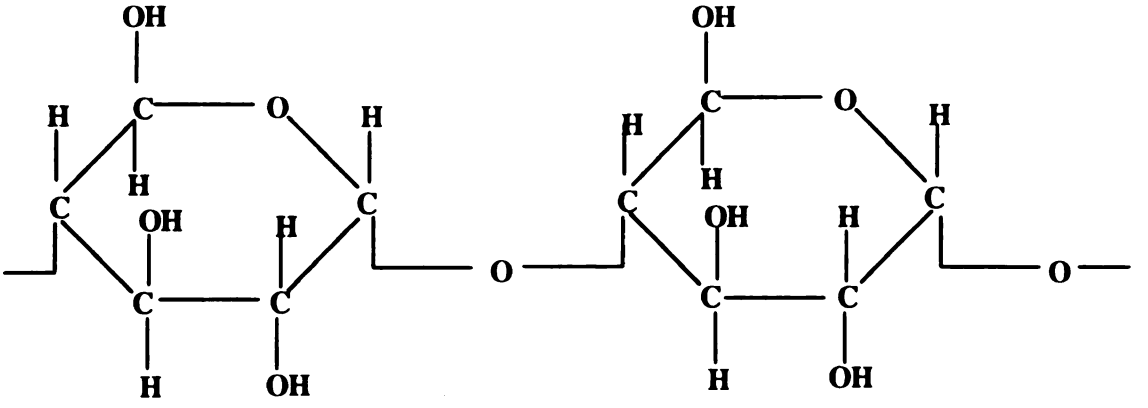


Figure 6.1 - Structure of unmodified starch (Amylose fraction)

Amylopectin - While the amylose fraction of starch consists of straight chain, water-insoluble polymers, the bulk of starch is amylopectin. The structure of amylopectin is shown in Figure 6.2.

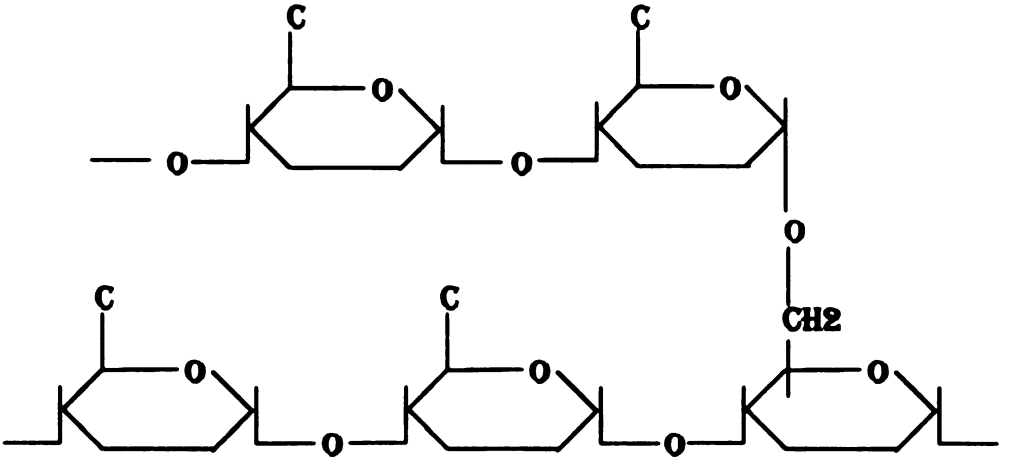


Figure 6.2- Structure of unmodified starch (Amylopectin)

Amylopectin is distinguished by a substantial amount of branching. Branches occur from the ends of the amylose segments averaging 25 glucose units in length. Such structures arise when condensation occurs between the glycosidic -OH on one chain and the 6 carbon on another glucose. The Amylopectin is formed by crosslinking between the linear chains by the 1-6 linkages.

6.2.2 STRUCTURE OF CELLULOSE

Cellulose, a major structural component of all plant cells is the most abundant organic compound on earth. Although the glycosidic linkage in cellulose occurs between the 1 and 4 carbons of successive glucose units, the subunits are bonded differently than in starch. The structure of cellulose is shown in figure 6.3.

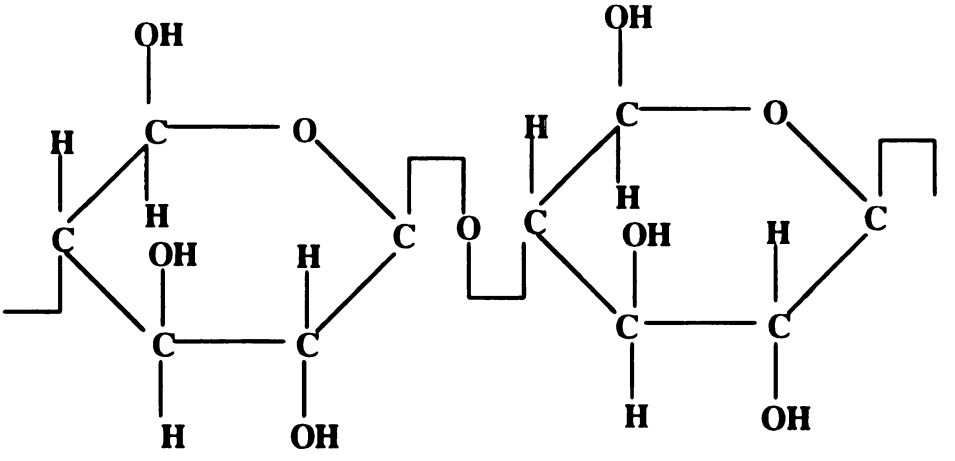


Figure 6.3 - Structure of cellulose

The difference in structure between starch and cellulose is significant. While many microorganisms, plants and animals possess the enzymes necessary to break down the β1,4- glycosidic bonds of starch, very few living creatures can hydrolyze the

β1,4 bonds of cellulose [Bailey and Ollis(1986)]. One of the common products of enzymatic cellulose hydrolysis is cellobiose, a dimer of two glucose units joined by a β-1,4-glycosidic linkage.

6.2.3 STRUCTURE OF CELLULOSE AND STARCH ESTERS

The cellulose and starch esters are produced by esterification. There are three OH groups on the monomer unit . They are at the C2, C3, and C6 positions as shown in Figure 6.4. Out of these three the C6-OH is the most reactive OH group, and the C2 and C3-OHs are less reactive. Hence during a substitution reaction, the C6-OH is first substituted, and then the C2 and C3-OHs. The three OH groups on a monomer unit of the polysaccharide are shown in Figure 6.4.

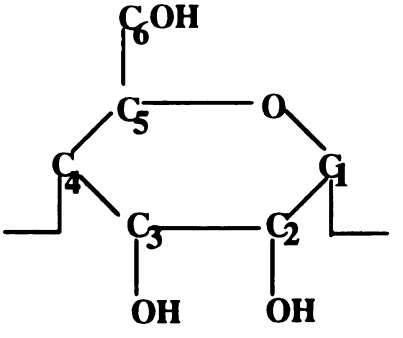


Figure 6.4- Position of OH groups on the monomer unit of cellulose/starch.

The substitution of the acetyl groups on the monomer unit results in the structure shown in Figure 6.5 . This figure shows substitution at all three OH groups of the monomer unit.

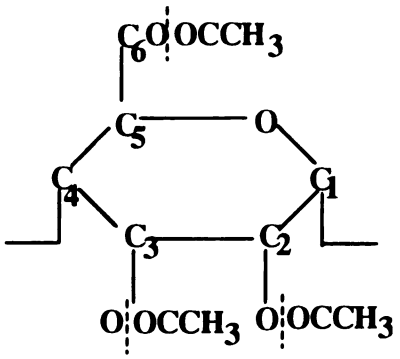


Figure 6.5 - Structure of a triacetate monomer unit of cellulose or starch

6.3 EXPERIMENTAL CHARACTERIZATION OF CELLULOSE AND STARCH ESTERS

Characterization of the esters was done based on two factors

a) Degree of substitution

b) Position of substitution

The former was done with the help of 1H NMR and the latter with the help of 13C NMR.

6.3.1 DEGREE OF SUBSTITUTION

The properties of cellulose and starch acetates (propionates, etc.) are a function of the acetyl content, the type of starch, the size and shape of it's molecular components, and the method of pretreatment. Measurement of the acetyl content is a prime method for characterizing these acetates [Whistler (1972)].

Acetyl content

The percentage acetyl content of the acetate is defined as

$$\% \text{ Acteyl} = \frac{(CH_3CO)_{m.w} * DS}{(\text{Acetate})_{m.w}} * 100$$

In the above expression,

DS = degree of substitution of the acetate.

(CH_3CO)m.w.= 43

(acetate)m.w. = (CH_7O_5)m.w. + $(H_3 - DS)$m.w. + $((CH_3CO)$m.w * DS)

= 159 + 3 - DS + 43DS

= 162 + 42 DS

Degree of substitution

The definition of the DS of the acetate follows from the above expression and is given as;

$$\text{Degree of Sustitution (D.S)} = \frac{162 * \% \text{ Acetyl}}{4300 - (42 * \% \text{ Acetyl})}$$

The DS is the number of OH groups out of the three OH groups on a monomer unit that is substituted by the acetyl group.

In the case of propionate, the (CH_3CO)mw is replaced by (CH_3CH_2CO)mw = 57 and the calculations carried out as before.

6.3.1.1 DETERMINATION OF DS BY THE METHOD OF TITRATION

The DS of cellulose and starch acetates is usually determined by the conventional titration method [Whistler (1972)]. The sample is ground, dried for 2hr at 105 +/-

3°C and cooled in a desicator. 1g of the sample is transferred to a 250 ml. flask, and 50ml. of a 75% ethanol solution in distilled water is added. The loosely stoppered flask is agitated, warmed to 50°C, held at that temperature for 30mins., and cooled; 40ml of 0.5N NaOH is added while swirling. The flask is stoppered, allowed to stand for 72hr. with occasional stirring. The excess alkali is back titrated with standard 0.5N HCl using phenolphthalein as indicator. The solution is allowed to stand for 2hr., so that any additional alkali leaching from the solution can be titrated. A blank is titrated at the same time, substituting the original starch/cellulose for the sample. The % acetyl is calculated as shown

$$\% \text{ Acetyl} = \frac{[\text{ml.(Blank)} - \text{ml.(Sample)}] * \text{normality of the acid} * 0.043 * 100}{\text{Sample wt., g. (dry basis)}}$$

The titration method yields experimental error which results in appreciable deviations between replicates. It is also a time consuming method. A new method was evolved in this work which determined the DS by NMR. This method reduced experimental errors to a great extent and also saved a lot of time and effort. This method has been described in detail.

6.3.1.2 DETERMINATION OF DS BY PROTON NMR

Nuclear Magnetic Resonance is a highly dependable and less time consuming process as compared to the traditional titration method, and is a viable alternative for the determination of the DS of these esters. The NMR method has been used by Buchanan et. al. [Buchanan (1992)] for the determination of the DS of cellulose acetates with excellent accuracy. However, it has been used very little for the

starch esters. NMR was used to determine all the DS values used in this work for both the starch and cellulose esters.

Sample preparation

a) About 50-100 mg of the modified starch or cellulose was weighed accurately in a small sample bottle. DMSOd6 absorbs moisture rapidly, hence care was taken to avoid exposure to atmospheric air during the sample preparation.

b) About 1-1.5 ml of DMSOd6 was added to the sample with the help of a pipette. The bottle was covered with a cap and placed on a stir cum hot plate. The stirrer was turned on and the temperature raised to 70-80^{0}C, so that the sample dissolved quickly (the modified starches are not readily soluble in DMSOd6, and heating the solution decreases the dissolving time). In order to prevent moisture from accumulating in the bottle, the air inside the bottle was flushed with nitrogen during the heating process.

c) After half an hour (or until the solution became clear), the heat was turned off. 1-2 drops of TFA/DMSOd6 (50/50 vol./vol. mixture) was added to the solution very carefully with the help of a pipette. The solution was allowed to stir for a few seconds more and then taken off the stirrer.

d) The solution was filtered and transferred from the bottle into a clean NMR tube with the help of a pipette and some quartz wool. (This is essential in the case of the starch acetates and propionates, since the higher DS starch acetates are not very soluble and hence remain as small particles in the solution which spoils the spectrum). The height of the solution in the NMR tube was about 5 cm.

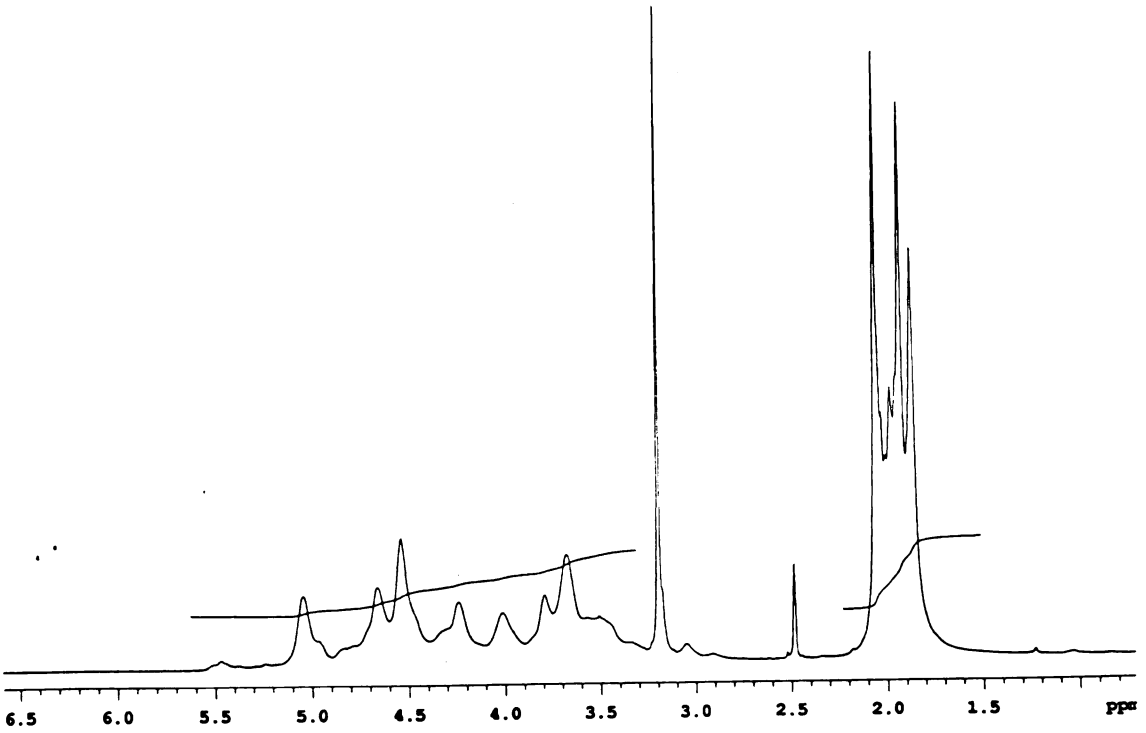


Figure 6.6 - Proton NMR of Cellulose acetate without TFA

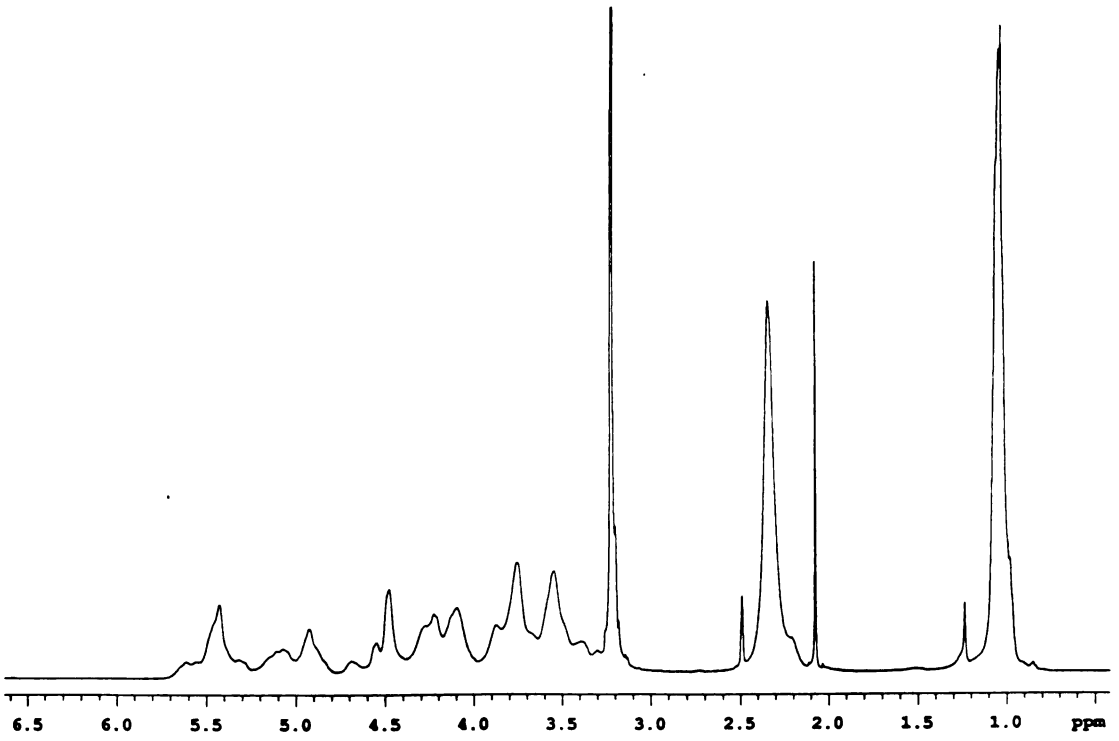


Figure 6.7 - Proton NMR of Starch propionate without TFA

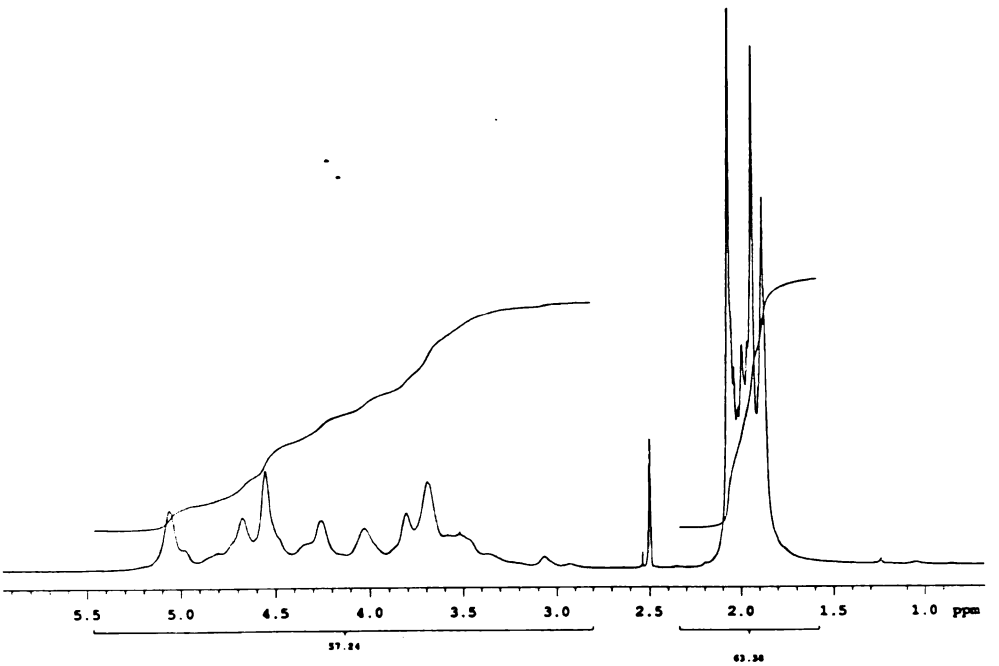


Figure 6.8 - Proton NMR of CA with TFA

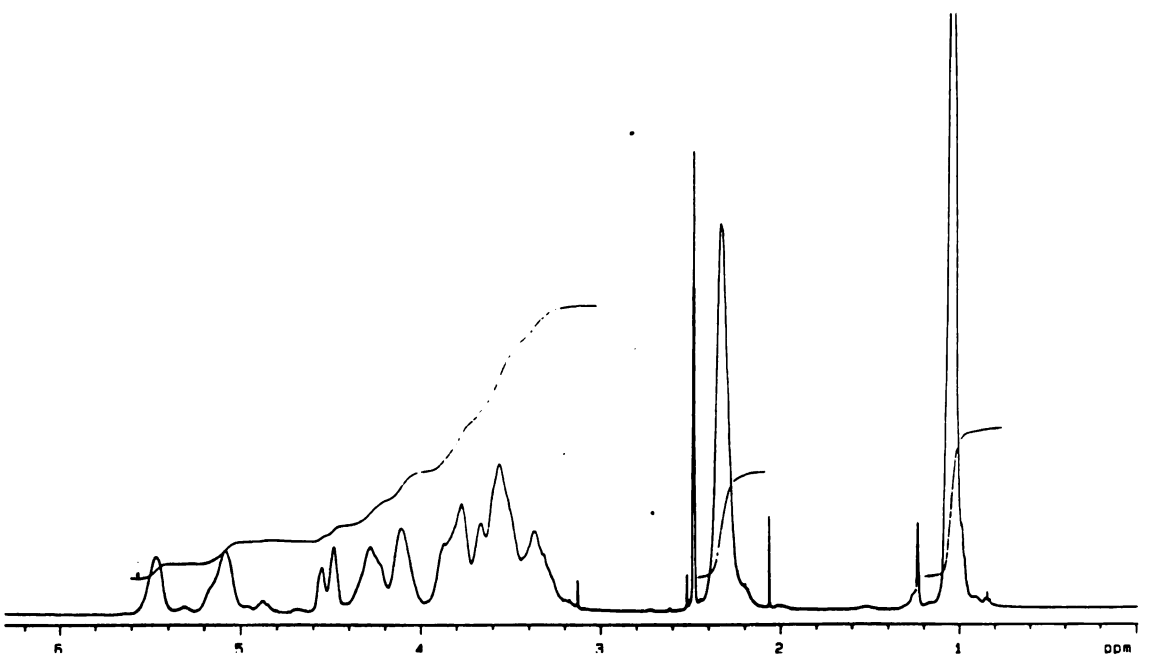


Figure 6.9 - Proton NMR of Starch propionate with TFA

Proton NMR spectra

In order to calculate the DS of these esters, it is essential to get a good spectrum. During the early part of this work, TFA was not used, hence some problems were encountered due to the presence of water peaks. The gradual improvements in the spectra with a few modifications are presented here. DMSOd6 absorbs moisture rapidly, hence care was taken to avoid exposure to atmospheric air during the sample preparation. Cellulose acetate of known DS was used as a standard to determine the accuracy of this method. The proton NMR spectrum of cellulose acetate DS 2.5 without TFA in Figure 6.6 shows the methyl peak and the ring protons. As can be seen, there is a very tall peak in the region of the ring protons. The spectrum of starch propionate without TFA in Figure 6.7 shows the methyl and methylene peaks and the ring protons. Here also there is very tall peak in the region of the ring protons. This tall peak is due to the water impurity in the sample which makes it impossible to compute the correct peak area of the ring protons. This problem was taken care of by adding 1-2 drops of TFA (Tri-flouro-acetic acid) [Buchanan (1992)] which removed the water hydroxyls and the ring hydroxyls from the region of interest. The resultant spectra were as shown in Figures 6.8 and 6.9.

Initial spectra obtained at room temperature did not provide very good shimming in the instrument. This was due to the viscosity of the sample which was high owing to high sample concentrations in the solution. Hence, the spectra were obtained at 60-80C to get better shimming. Phasing of the spectra was very important in the integrating the peak areas for accurate determination of the DS of the sample. Inaccurately phased spectrums can give inaccurate peak areas and DS values.

Calculation of DS from proton NMR

The chemical structure of starch is as shown in Figure 6.1. There are 5 protons and 2 hydroxyl groups in the ring, two methylene protons and one hydroxyl on the sixth carbon. The ring protons, methylene protons of C6 and the hydroxyls are spread out between 2.8 and 5.5 ppm approximately, whereas the acid group protons are at 2.0 or 1.0 ppm depending upon the acid group, i.e., acetic or propionic. When TFA is added to the sample, it removes the hydroxyls of the ring away from the ring proton region. Thus the area under the peaks occurring between 2.8 and 5.6 ppm are due to 5 ring protons and 2 C6 protons. The acid methyl peak is due to 3*DS number of protons.

Thus,

$$\frac{\text{Area under the acid methyl peak}}{\text{Area under the ring and methylene protons peak}} = \frac{3 * DS}{7}$$

The DS was thus calculated by integrating the peak areas.

6.3.2 POSITION OF SUBSTITUTION

The carbon atoms present in the acetate and propionates and their positions within the molecule with respect to other atoms was investigated by C13 NMR. An attempt was made to determine the positional substitution of the acetyl groups, i.e., whether they are substituted in the C2-OH, C3-OH or C6-OH positions. All the C13 NMR was carried out on the Varian 500mHz instrument using a 10mm. probe. The concentrations used were in the range of 8-10% wt/vol. (100mg/ml approx.) of the sample. The solvent used was Di-methyl-sulfoxide-d6 (DMSOd6).

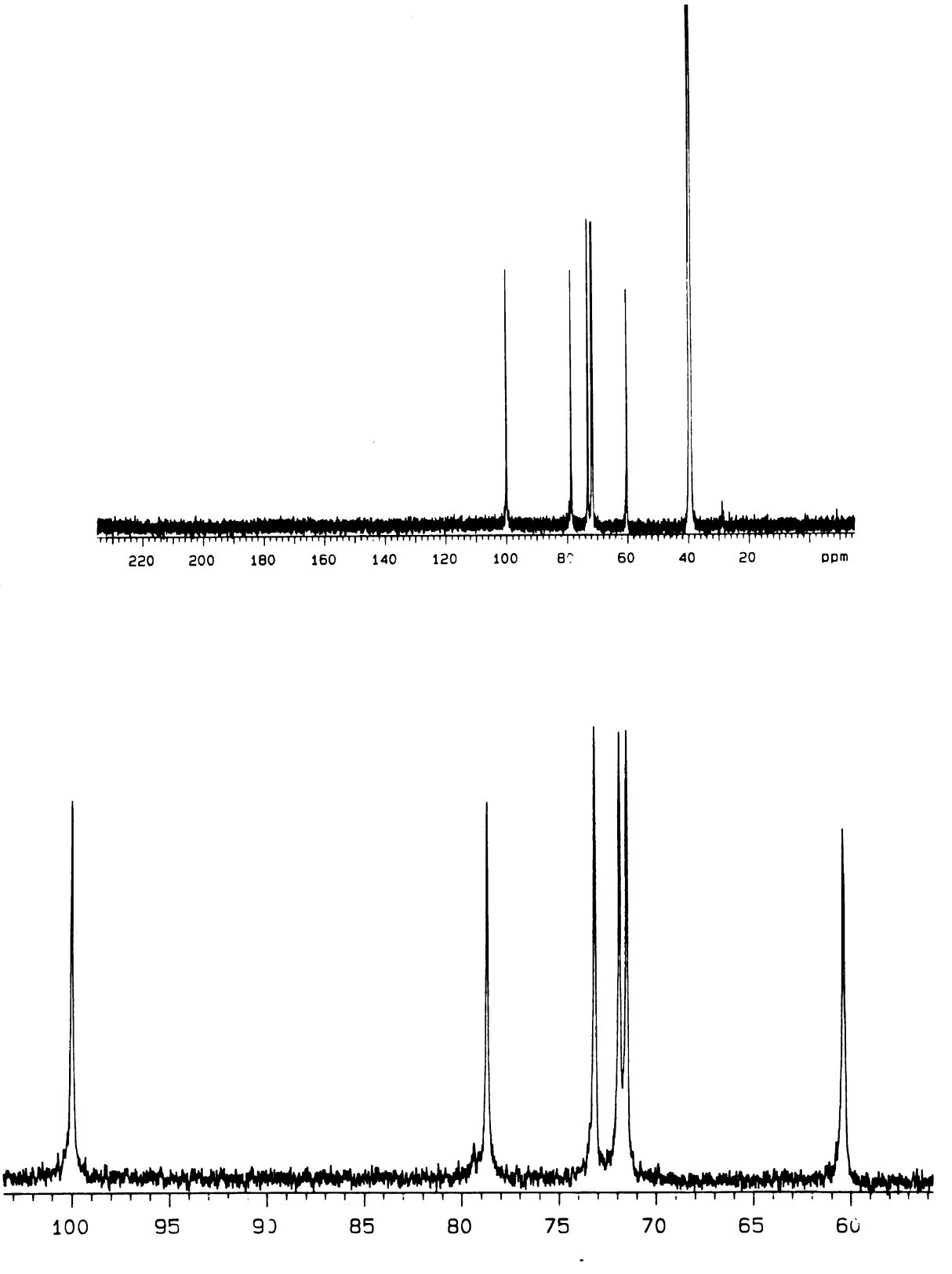


Figure 6.10 - C13 NMR of unmodified Starch

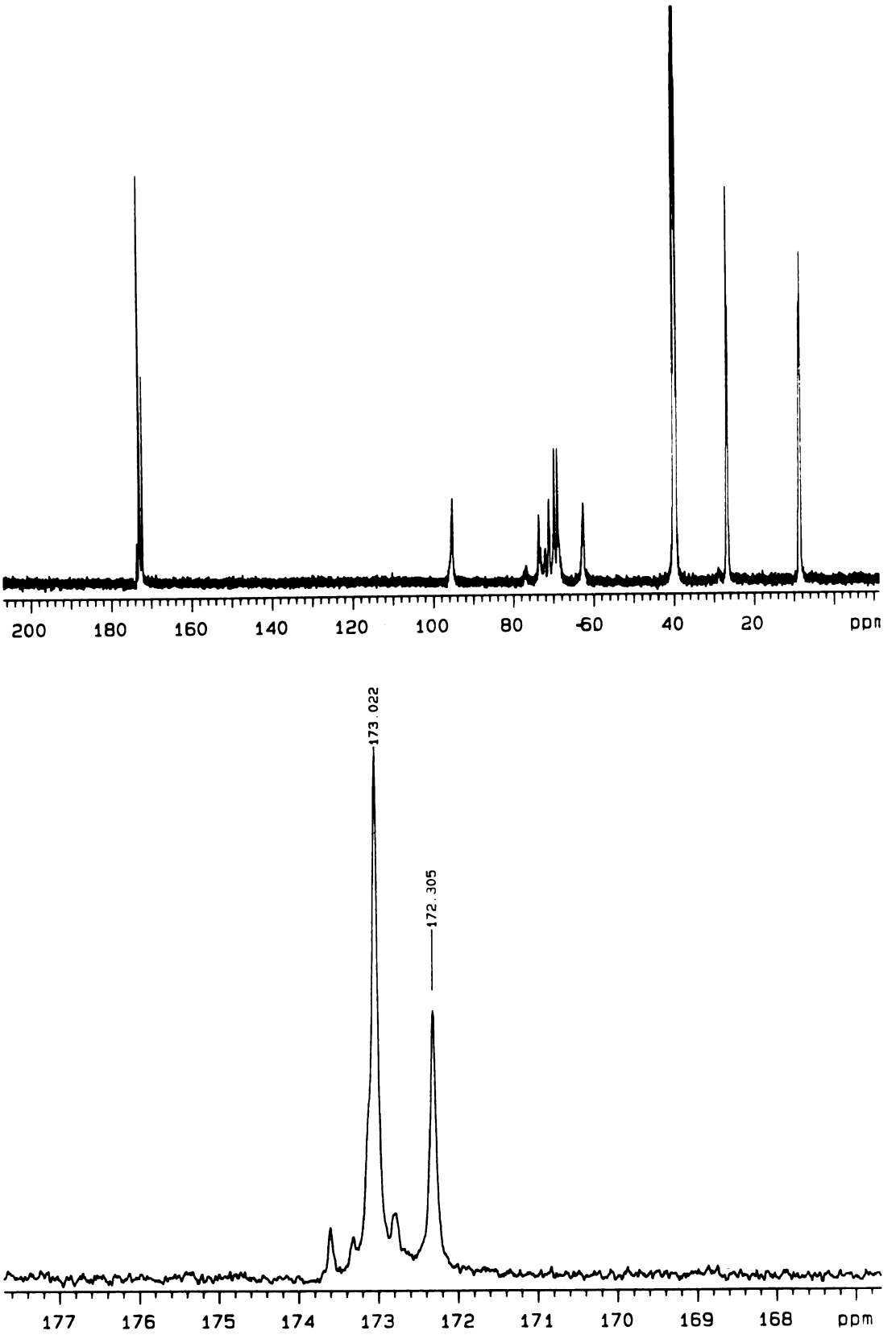


Figure 6.11 - C13 NMR of starch propionate of DS 3.0

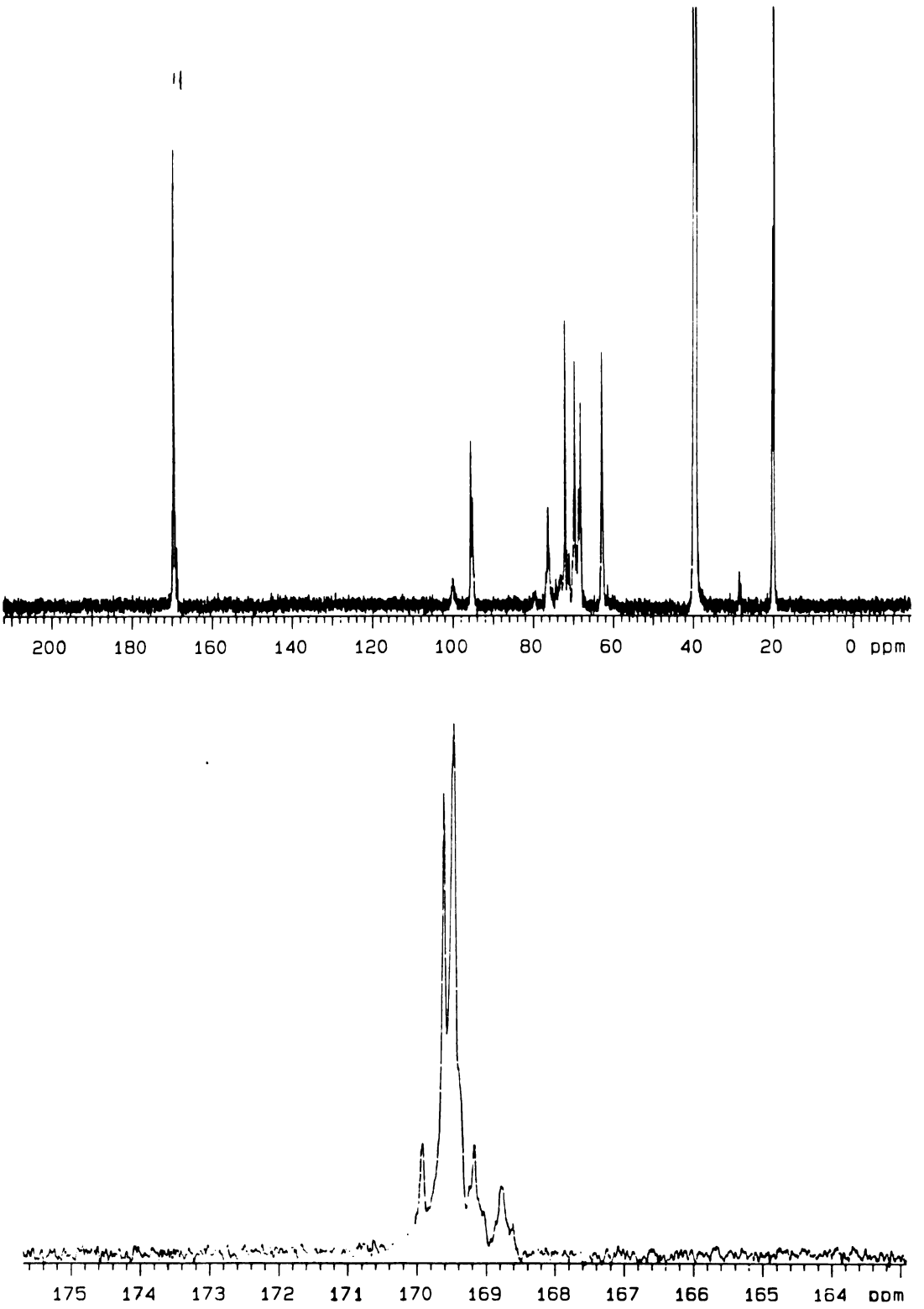


Figure 6.12 - C13 NMR of starch acetate DS2.0

Figure 6.10 shows the C13 NMR of unmodified starch. The 6 peaks represent the 6 carbon atoms in the starch structure. Based on their neighboring atoms, each of these peaks were identified as C1, C4, C5, C2, C3 and C6 from left to right of the spectrum. C13 NMR cannot be used for quantitative analysis because of an effect called the Nuclear Overhaus Effect (NOE). Hence these peaks in the C13 spectrum cannot be integrated for calculation of DS or extent of positional substitution.

Figure 6.11 shows the C13 spectrum of starch propionate of DS 3.0. This spectrum had the same peaks as that of the unmodified starch except for two distinct peaks occurring at a ppm of 170-175 and two more at approximately 8ppm and 26ppm. The former are the carbonyl peaks, and the latter are the methyl/methylene signals from the acetyl/propyl group substituted in the starch. The leftmost peak had a height and integrated area almost twice that of the second one. This is because the C2 and C3 carbons have very similar molecular surrounding, as a result of which the carbonyl peaks from the acetyl groups substituted at these carbons have approximately the same chemical shift. The C6 carbon has a slightly different environment and so has a carbonyl peak slightly shifted from the other two carbonyl peaks. Figure 6.12 shows the C13 spectrum of starch acetate of DS 2.0. The difference between the acetate and propionate showed up in an additional methylene signal and a shift in the carbonyl peak further down for the propionate.

The C6-OH group is theoretically the most reactive and thus the more easily substitutable one than the other two OH groups. So, when the starch is partially acetylated, this difference should show up on the C13 NMR spectrum. Figures 6.13 and 6.14 show the carbonyl peaks of starch propionate with a DS of 1.0 and 1.7 respectively. In the case of the cellulose acetates, the cellulose is first

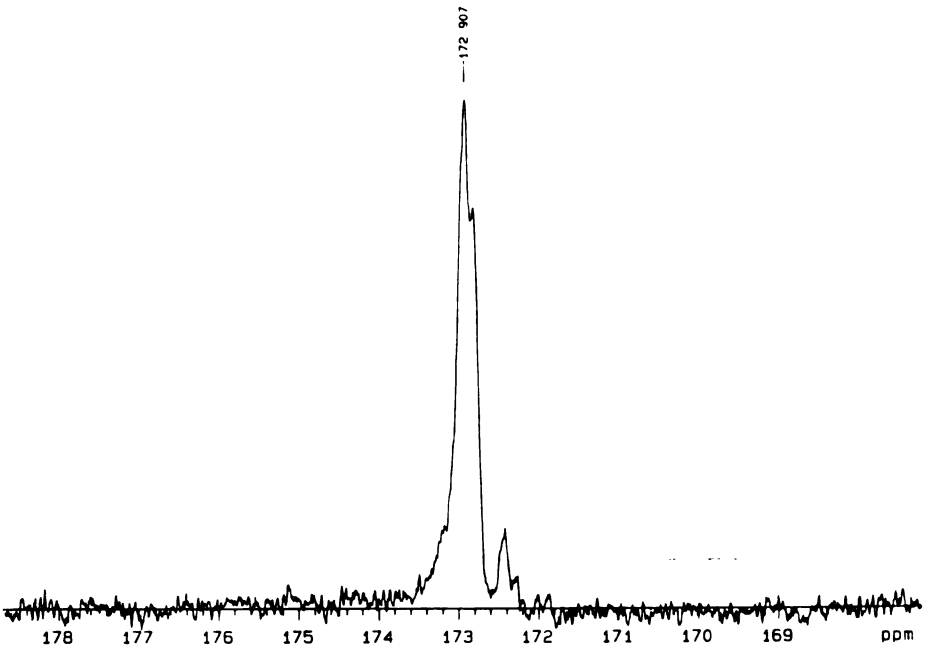


Figure 6.13 - C13 NMR of starch propionate DS1.0 (Carbonyl peaks)

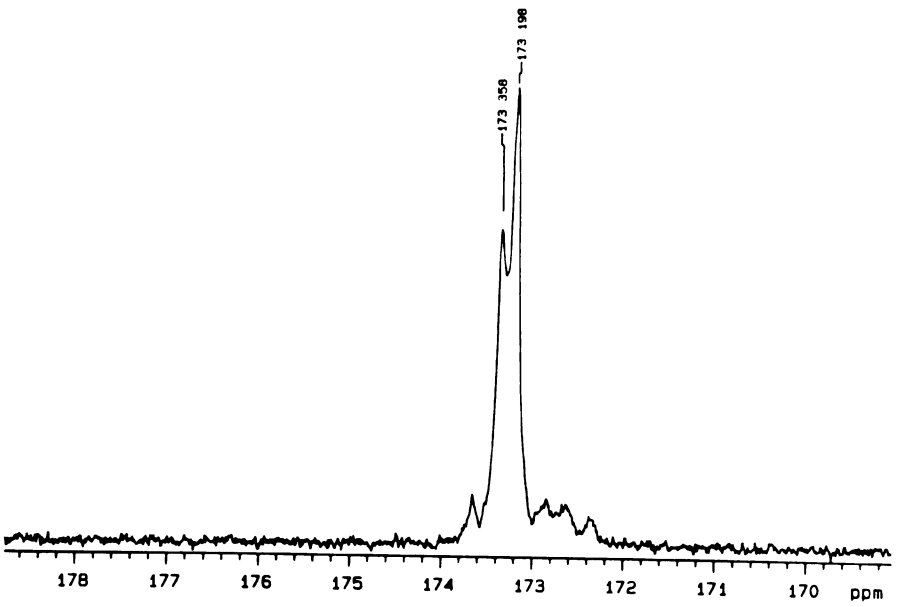


Figure 6.14 - C13 NMR of starch propionate DS1.7 (Carbonyl peaks)

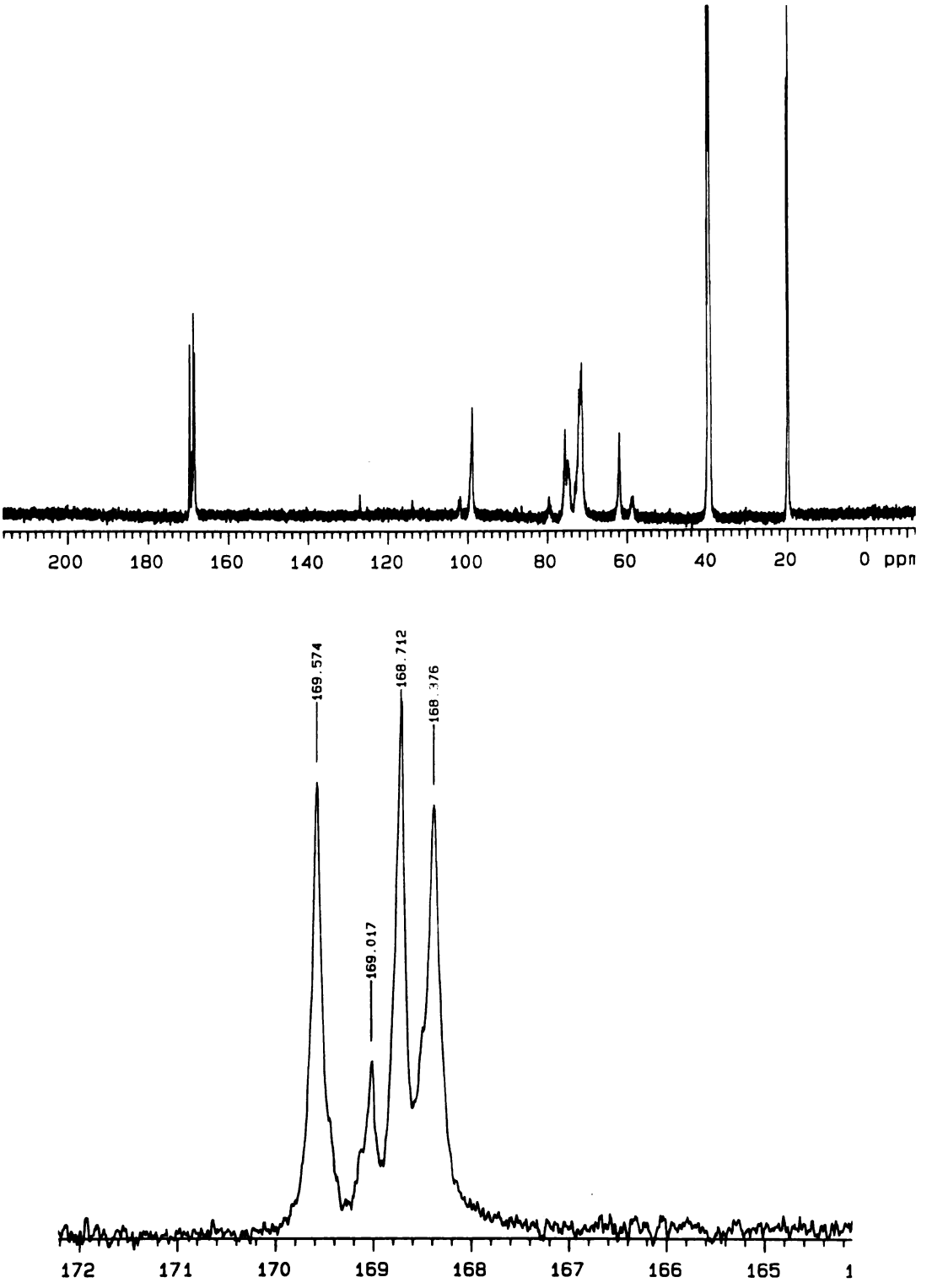


Figure 6.15 - C13 NMR of cellulose acetate DS 2.5

acetylated completely and then back hydrolyzed to give the desired DS. So, in this case, most of the carbonyls should occur at the C2, C3 positions. Figure 6.15 shows the spectrum of cellulose acetate of DS 2.5. A comparison between the cellulose acetate DS 2.5 and starch acetate DS 2.0 (Figure 6.12) shows a distinct difference in the position of the carbonyl hyperfinely split peaks on the spectrum. Detailed interpretation of the spectrum was not done in this work.

6.4 CORRELATION OF BIODEGRADABILITY WITH POSITION AND DEGREE OF SUBSTITUTION

It has been mentioned in literature that biodegradability is affected by the position of substitution. According to Bhattacharjee et al. [Bhattacharjee (1971)], work done on Carboxy-methyl cellulose showed that chain scission takes place most readily between two adjacent unsubstituted residues but, nevertheless, can occur at a residue having a 6- or a 2- substituted algycone. For e.g. a derivative with a DS of 2.0, and having 2- and 3- substituted would show a retarded rate of degradation as compared to a derivative with a DS of 2.0, but with 6- and 2-(or 3-) substituted. Although, it was intended to explore this area further in this work, it could not be done. This is an important area for further research, since it relates back directly to the method of synthesis of the derivatives, i.e., whether they are stoichiometrically derivatised to the desired DS or completely derivatised and then back hydrolyzed to the desired DS.

The degree of substitution affects the rate of biodegradation of the starch and cellulose esters to a major extent. It was found that the higher the degree of substitution, the lower is it's rate of biodegradation. This area has been studied in detail in Chapter 7.

STARCH ESTERS

7.1 BACKGROUND

Starch is a natural polymer which is readily biodegraded in both aerobic and anaerobic environments to form smaller end products. Unfortunately it cannot be used as it is for the production of plastic materials owing to it's high moisture sensitivity and poor thermal processibility. Modification of natural polymers through the acetylation or propionation of the available hydroxyl groups permits the formation of appropriate film-forming plastic copolymers. However, the technical difficulties associated with the development of a biodegradable product are numerous. Environmentally non persistent materials are required to have both stability during the useful lifetime of the product and performance characteristics that the public has grown to expect while being rapidly degraded in biologically active environments, such as waste water treatment or composting facilities. These product requirements often seem mutually exclusive [Buchanan et al. (1992)].

The biodegradation of modified starches and celluloses of DS>1 is a hotly debated field. Very little experimental work has been done in this area. Biodegradation of these materials has been studied at very low DS levels within the solubility range of the material, but not at high DS levels. The relationship between the degree of substitution of starch esters and it's biodegradability has been studied a little

previously, but under different environmental conditions. Rivard et al. did some work on various modified natural polymers and their fate in an anaerobic environment. In their work, cellulose, starch, and xylan were substituted with acetate to various degrees, and the effect of this modification on the anaerobic biodegradation assessed through a biochemical methane potential (BMP) protocol. Significant reduction in anaerobic biodegradability resulted with all polymers at substitution levels of between 1.2-1.7. In all the three cases, biodegradability was reduced to about 10% after a substitution level of 1.7 was reached. Similar work was done by Bhattacharjee et al. [Bhattacharjee (1971)] on cellulose ethers. He reported that cellulose ethers with a DS greater than 1.0 generally do not biodegrade while those with a DS of less than 1.0 will degrade due to attack of microorganisms at the unsubstituted residues of the polymer.

In this work, starch propionates of different DS were synthesized and tested for their aerobic biodegradabilities in the presence of municipal sewage sludge as per the procedure described in Chapter 3. The results are reported in this chapter.

7.2 SYNTHESIS OF THE MODIFIED STARCHES

The chemically modified starches were synthesized at Michigan Biotechnology Institute by the Biomaterials group. Starch propionates of different DS were synthesized by the following procedure [Methods in Carbohydrate Chemistry, V].

The glass reactor was assembled as shown in Figure 7.1. 25g of the Hylon VII starch sample was weighed out and mixed with 100 ml of DMSO in a 250 ml Erlenmeyer flask on a stir plate to make a fine paste.

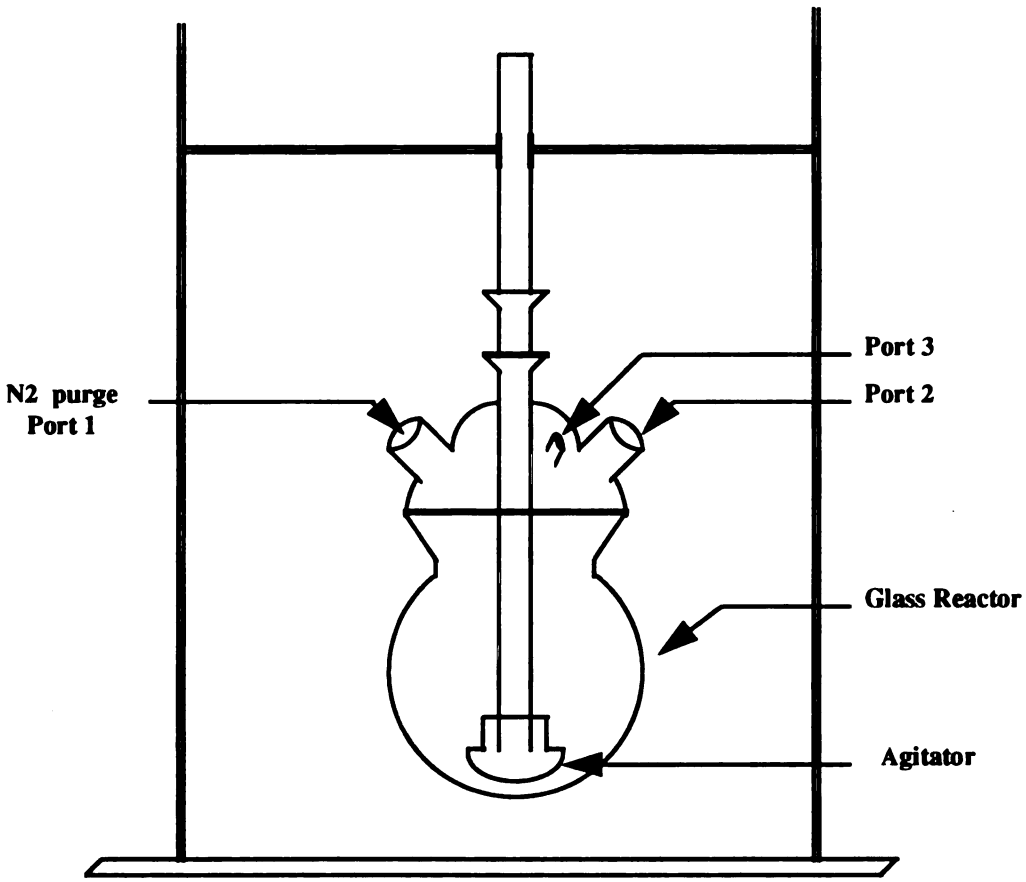


Figure 7.1 - Glass Reactor Assembly for synthesis of modified starches

Care was taken while using the hygroscopic chemicals to keep moisture absorption to a minimum while being transferred to the reaction vessel. The slurry was added to the vessel through port 1 using a short stemmed funnel. The agitator was turned at medium speed to attain good mixing without spattering the liquid. The Erlenmeyer flask was rinsed out with another 100 ml of DMSO which was added to the reaction vessel through the same port. Using the silicone oil bath, the vessel was heated to 110^0C, and the temperature inside the vessel was kept below 90^0C. At the end of the heating period, the solution was a clear translucent liquid, yellow liquid. The vessel was cooled to room temperature using a water bath. A solution of DMAP (Dimethyl amino pyridine) and 50 ml of DMSO was prepared. Amounts of catalyst, sodium bicarbonate and the anhydride were dictated by the moles of starch and the degree of substitution. The catalyst was 4 mol% of the anhydride. the sodium bicarbonate was added using a powder funnel through port 1. The moles of sodium bicarbonate added was equal to the anhydride. DMAP solution was added through the same port using the same funnel as the bicarbonate. The solution was allowed to stir until there were no clumps of sodium bicarbonate left. A solution of propionic/ acetic anhydride was prepared in 100 ml of DMSO and added using a dropper funnel at the rate of 1-2 drops per second. After addition of the anhydride, the reaction was allowed to proceed for 15 min. The product was precipitated in water, blended and filtered. The product was washed five times or until the pH of the solution was the same as the original wash water. The product was filtered and then allowed to dry in a vacuum oven.

Starch propionates of different degrees of substitution were prepared and tested for their biodegradabilities under a given set of experimental conditions.

7.3 BIODEGRADATION OF STARCH PROPIONATES WITH VARYING DS

7.3.1 Materials tested

The CHN data of these materials were determined using the Perkin Elmer 2400 CHN Elemental analyzer. The CHN data on the materials tested are given below.

Sample1 - Starch propionate, DS = 1.0 (sample AG-2-14-92), %C = 47.13, %H = 6.57, %N=0.14.

Sample2 - Starch propionate, DS = 1.5 (sample AG-10-23-92, 12L scale-up), %C = 50.15, %H = 6.45, % N = 0.17.

Sample 3 - Starch propionate, DS = 1.7 (sample ADL 9-30-92, 1st 10 gallon scale-up), %C = 51.22, %H = 6.62, %N = 0.15.

Sample 4 - Starch Propionate, DS = 2.7 (sample AG-10-21-92), %C = 53.93, %H = 6.50, %N = 0.14

Sample 5 - Cellulose (Microcrystalline Avicel) used as a positive standard. Aldrich Chemical Company, 20 micron particulate solid, %C = 39.98, %H = 18.37, %N = 0.076.

The DS of all the starch propionates were determined through proton NMR. The NMR spectra of these materials are presented in Figures 7.2 -7.5

7.3.2 Inoculum and test solution

The test materials were of known weight based on their carbon contents. The C/N ratio maintained in each flask was 36:1. This was maintained by adjusting the amounts of stock solutions used.

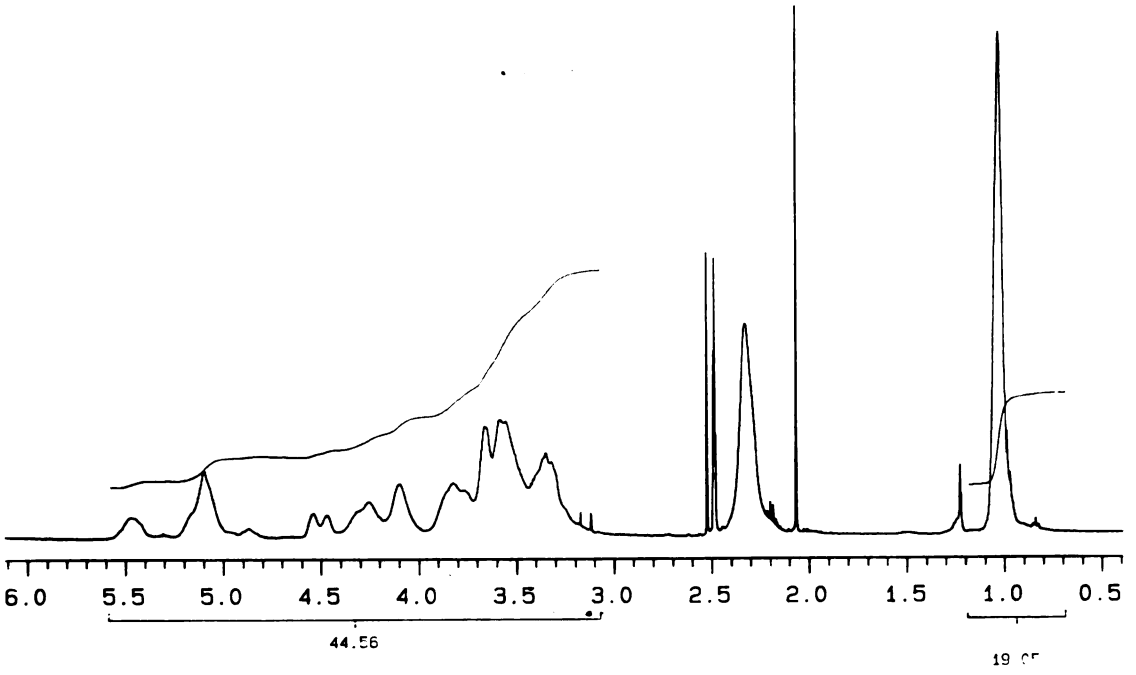


Figure 7.2 - Proton NMR of Starch propionate DS = 1.0

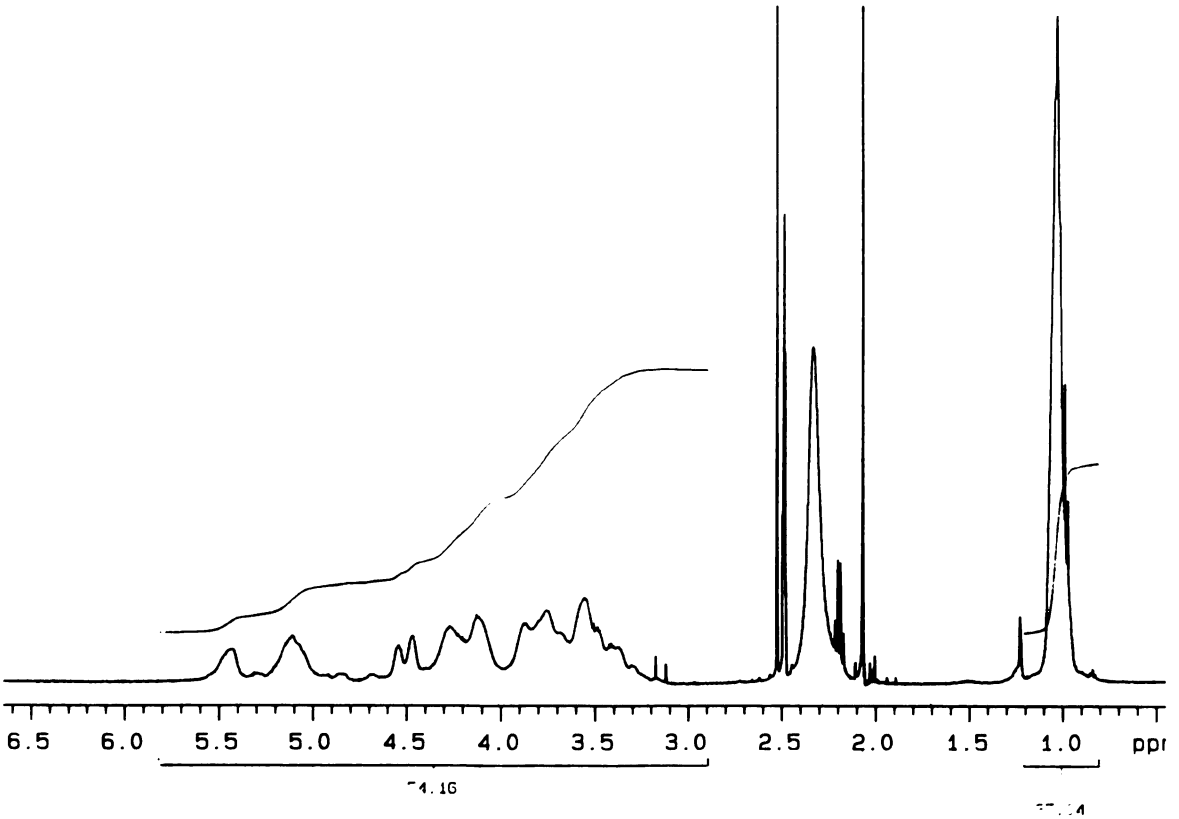


Figure 7.3 - Proton NMR of Starch propionate DS = 1.5

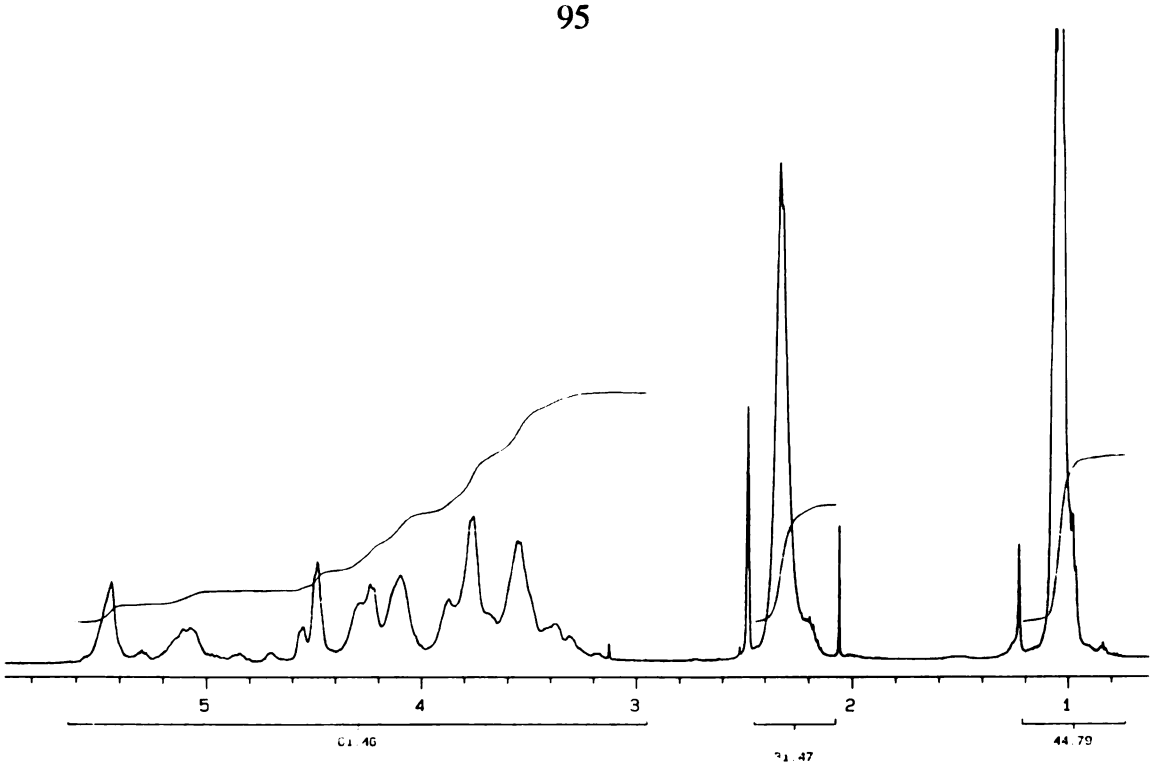


Figure 7.4 - Proton NMR of Starch Propionate DS = 1.7

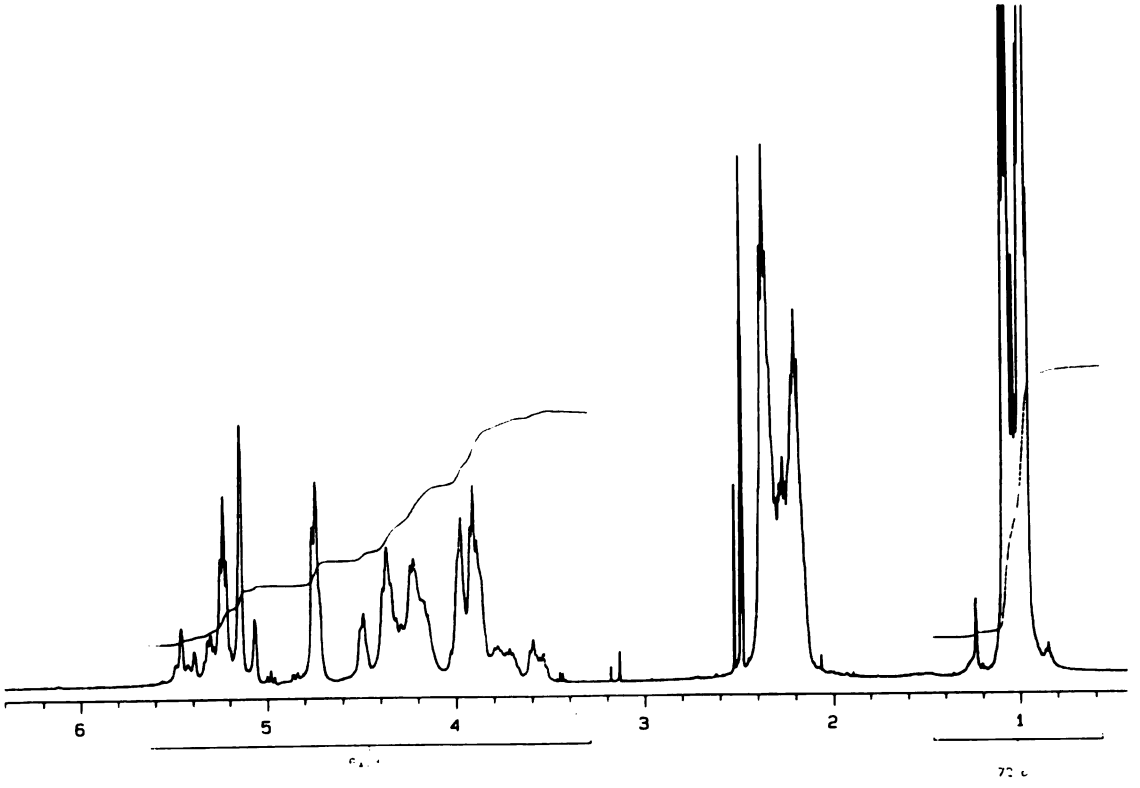


Figure 7.5 - Proton NMR of Starch Propionate DS = 2.76

The amounts of test materials used were as follows :-

Sample 1 = 3.39 g, C content = 1.6 g

Sample 2 = 3.19 g, C content = 1.6 g

Sample 3 = 3.12 g, C content = 1.6 g

Sample 4 = 2.97 g, C content = 1.6 g

Cellulose = 4.00 g, C content = 1.6 g

The stock solutions used in each flask were as mentioned in chapter 3, except that 5 ml of ammonium sulfate was added. 30 ml of the sludge inoculum was used. Water was added in the flasks and the level brought up to about 1500 ml. The N in the sludge was negligible and not taken into account. The total N in the solution was calculated as 0.0451 g.

The C/N ratio in the solution thus worked out to be 35.5.

7.3.3 Results and Discussion

The experiment was carried out in the manner described earlier. Figures 7.6 and 7.7 show the percentage carbon converted to CO_2 with time for all the samples tested. From the two figures, it can be seen that the standard, cellulose reached a CO_2 evolution of 77% in a period of 40 days. It was found that for all the materials the rate of CO_2 evolution reached a maximum in the first 5-10 days. The test was run for a period of 50 days. The results are shown in Table 7.1. The modified starches took off to a slow start, and the extent of biodegradation in the given time frame decreased with increasing degree of substitution. The experimental correlation of the DS and extent of biodegradation is shown in Figure 7.8. Starch propionate of DS 1.0 reached a biodegradation extent of 35%, whereas the DS 3.0 reached a biodegradation extent of only 7%. Figure 7.9 shows the experimental correlation of DS and percentage biodegradation relative to

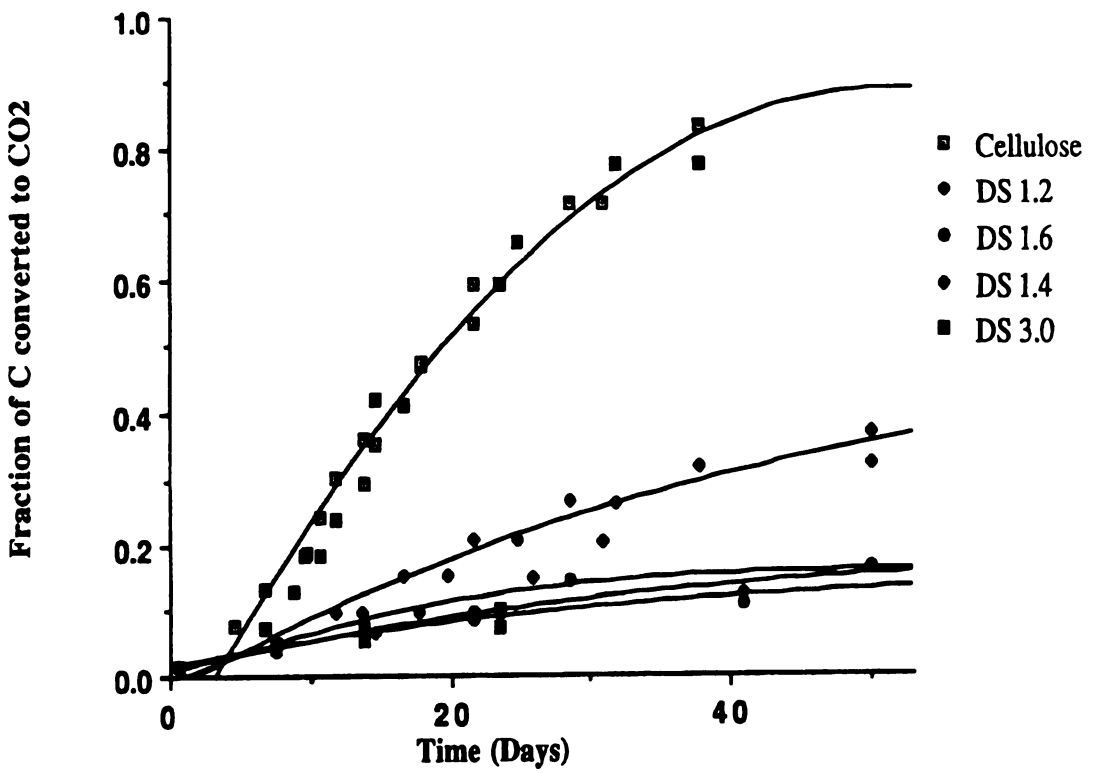


Figure 7.6 - Percentage of initial polymeric C converted to CO_2 with time for starch propionates of varying DS. Cellulose is included as the positive control. Each curve represents an average of duplicate results. No blank correction has been applied to the above data.

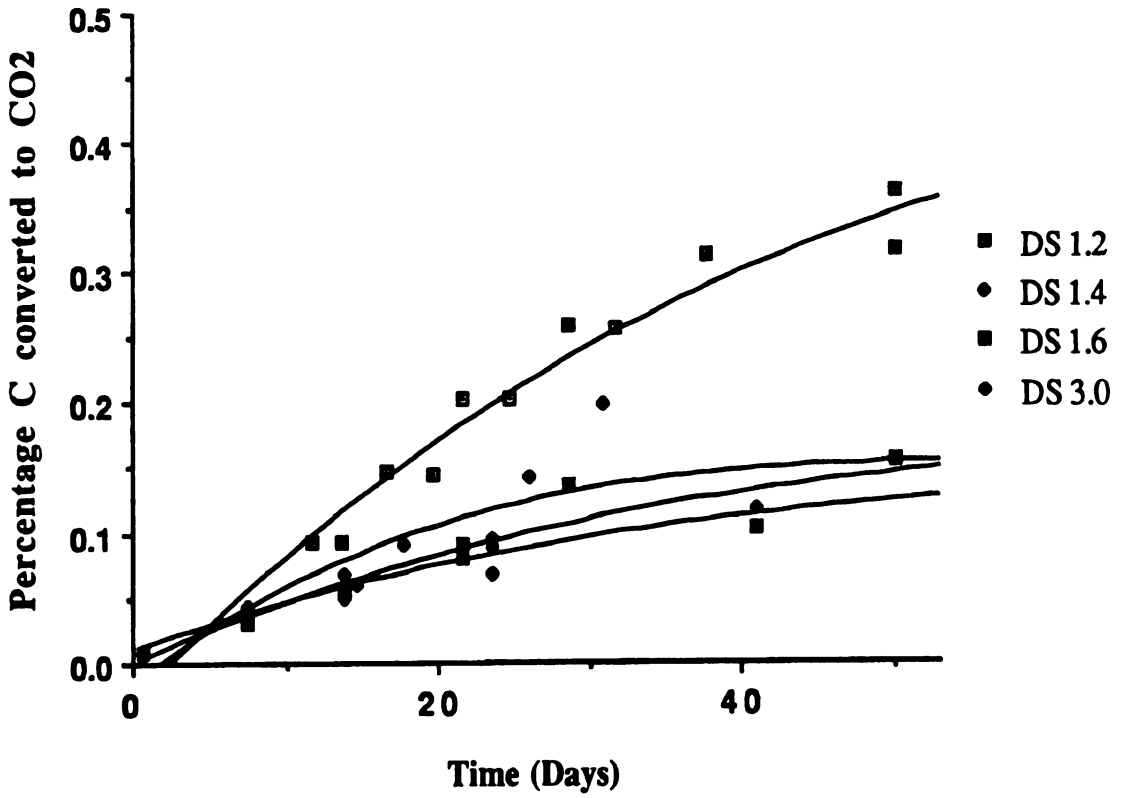


Figure 7.7 - Percentage of initial C converted to CO_2 with time for starch propionates of varying DS (Cellulose excluded). This figure is a replicate of Figure 7.6 with the cellulose curve excluded from the graph.

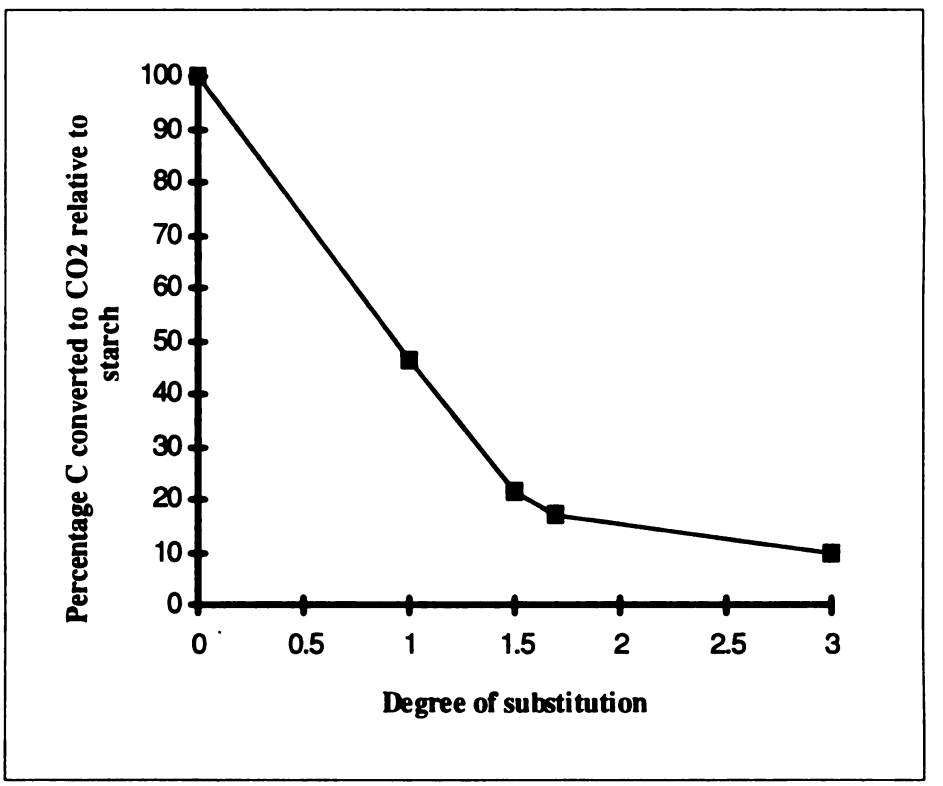


Figure 7.8 - Relation between biodegradation and degree of substitution. Percentage of polymeric carbon converted to CO_2 at the end of 48 days was plotted for starch propionates with different degrees of substitution relative to percentage C conversion to CO_2 for unmodifiedstarch (DS=0) which was scaled to 100%.

unmodified starch (unmodified starch was run in the next set of experiments, where the standard cellulose had the same rates as this set).

Table 7.1 - Summary of biodegradation results

Materials	%C converted to CO_2
Cellulose	78%
SP -DS 1.0	32.5%
SP-DS 1.5	15%
SP-DS 1.7	12%
SP-DS 3.0	7%
Unmodified corn Starch (Chapter 5)	70%

SP - Starch propionate

7.3.3 Conclusions

These results clearly show that in an aerobic environment, the biodegradability of starch propionates decreases with increasing DS. The decrease in the extent of biodegradation with increasing chemical modification in other environments have been given different explanations. All of them however relate to the accessibility of the starch or cellulose backbone (the 1-4 linkage) by the degrading enzymes. This inhibition may be either a result of the increased hydrophobic nature of the modified polymers, which reduces wetting and thus enzyme/microbe surface contact, or the stearic hindrance presented by the modifying groups on the polymer, which reduce enzymatic activity. Accessibility of the polymer backbone to the enzymes has also been related to morphology and the porosity in literature. These factors have been investigated experimentally in Chapter 8.

7.3.4 Limitations of the above data

The experiment conducted on the starch propionates was one of the first biodegradability experiments carried out in this work. Hence, it had some drawbacks which are listed below.

a) A large shaker was used for all the test flasks instead of individual stir plates for each of these flasks. The large shaker did not provide adequate mixing between the materials and the inoculum. Glass beads were added to the solutions to improve the mixing. This was achieved but still not to a satisfactory level.

b) Some problems were encountered in the air flow system which affected the air flow rates. This was because of a lot of back pressure inside the flasks. The flasks were sealed with tygon tapes to prevent them from popping. There was a positive pressure at the inlet and the outlet of the trapping solutions was exposed to air. This created some problems in the flow lines, as a result of which the air flow rates were uneven.

CELLULOSE AND CELLULOSE ACETATES

8.1 BACKGROUND

The technical difficulties associated with designing biodegradable polymers are extremely complex. Biodegradable polymers must be both cost effective and have acceptable performance characteristics, typical of common synthetic polymers and, at the same time, they must be non-persistent in the environment [Buchanan et al.(1993)]. Viewed from current technology, these requirements are often mutually exclusive. Based on this view point, cellulose esters offer a very attractive potential in terms of material properties and environmental persistence, since it is derived from a natural polymer, cellulose, which is readily biodegraded in the environment.

Cellulose is readily biodegraded in both aerobic and anaerobic environments to yield smaller end products which are assimilated into the nature's carbon cycle. However, cellulose is not readily processable, since it's melting temperature lies above it's decomposition temperature [Buchanan et al (1992)]. This is a common problem with unmodified naturally occurring polymers. The biodegradability of cellulose esters has been a widely debated topic. Derivatisation of cellulose improves it's thermal stability with lower melting temperatures, but increases it's

resistance to biodegradation. In order to understand the mechanism of biodegradation of cellulose acetate, it is essential to understand it's structure.

8.2 STRUCTURE OF CELLULOSE ACETATE

The structure of cellulose and cellulose acetate is shown in Figure 8.1. The cellulose acetate polymer is basically a random copolymer of mono, di, triacetate and unsubstituted monomer units on the polymer backbone. In the case of cellulose acetate DS=2.4, the di and tri substituted units predominate in the polymer. It has been found that substitution at the 2- and 3- positions affect the chain scission at the glycosidic bond. Cellulose acetate will be referred to as CA in this chapter.

8.3 C_X - C_1 MECHANISM OF CELLULOSE BIODEGRADATION

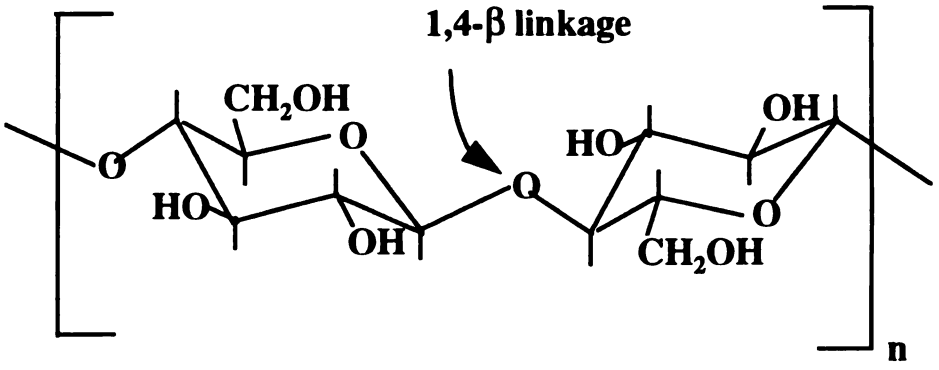
The enzymatic hydrolysis of cellulose is complex owing to three major factors [Focher et al (1991)] -

- Heterogeneity of the system
- Dual nature of the insoluble substrate (crystalline and amorphous)
- component multiplicity of the enzymatic system

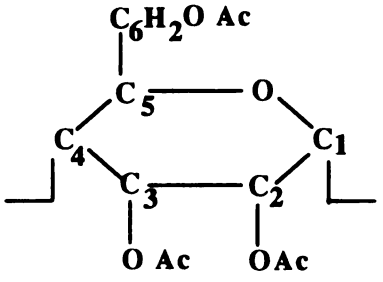
The mechanism of enzymatic hydrolysis of cellulose has been explained in principle, in terms of the sequential action of three different enzyme complexes [Shimada and Takahashi (1991)] which are -

- C_x, Endo-cellulase, catalyses random hydrolysis of β-1,4 glycosidic linkages of inner amorphous regions of a cellulose chain in a random manner

Structure of Cellulose



Structure of cellulose triacetate monomer



Structure of cellulose acetate of DS 2.4
(acetyl content = 40.2)

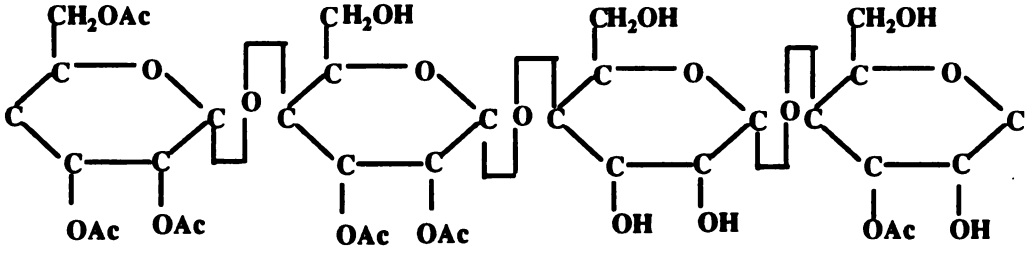


Figure 8.1 - Structure of Cellulose acetate-random copolymer of monoacetate, diacetate, triacetate and unsubstituted units

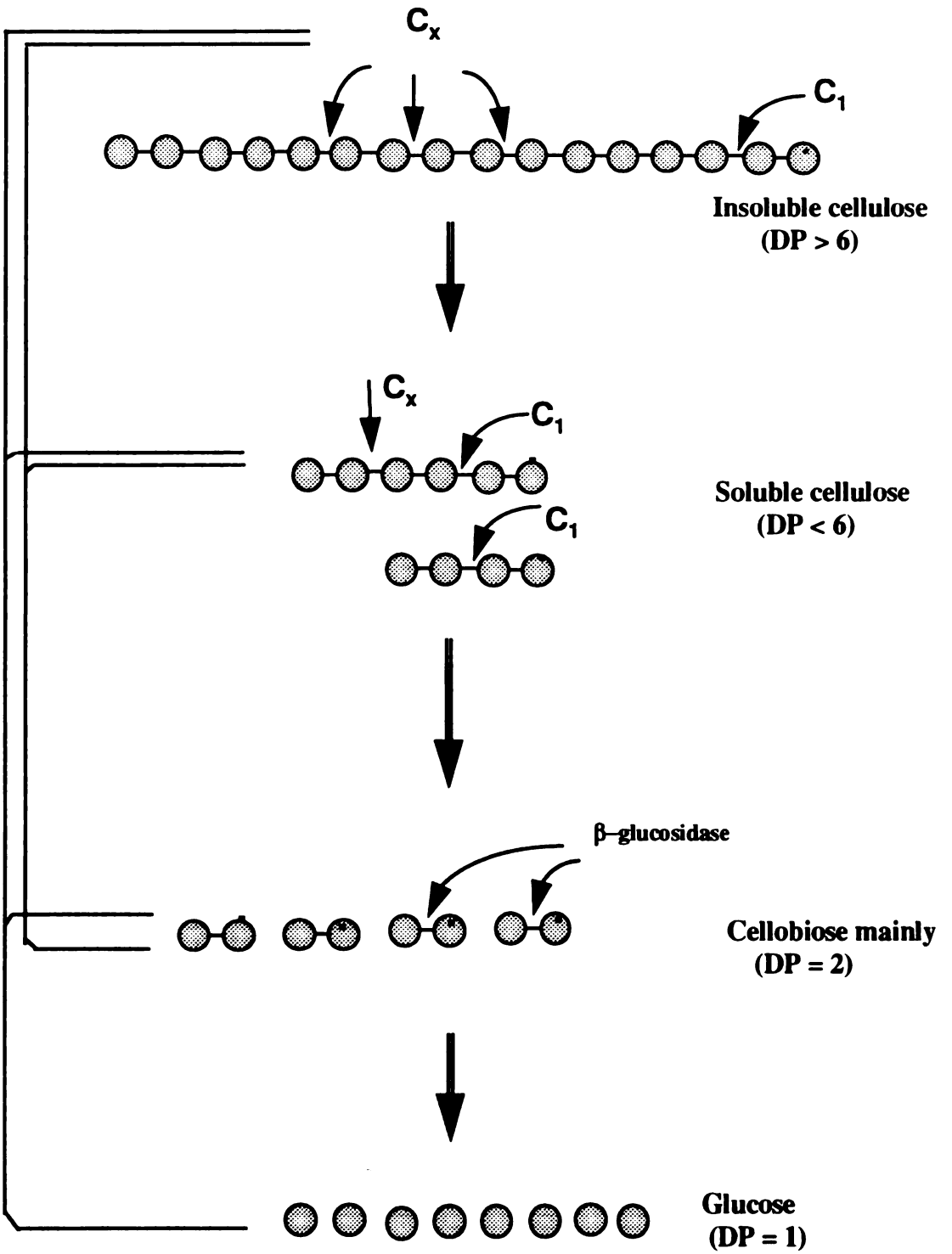


Figure 8.2 - C_x - C_1 mechanism of enzymatic hydrolysis

- C_1, Exo-cellulase, catalyses the endwise hydrolysis of β-1,4 glycosidic linkages, in principle splitting off cellobiose product from the non reducing end of the crystalline cellulose chain.
- β-glucosidase catalyses the hydrolysis of the β-glycosidic linkage of cellobiose units.

The enzymatic hydrolysis of cellulose takes place by the C_X - C_1 mechanism as shown in Figure 8.2, which involves the synergistic action of the three hydrolases.

8.4 CA BIODEGRADATION LITERATURE

Some work has been done on the enzymatic degradation of cellulose ethers, and it has been seen that the degree of substitution is the most important factor in the biological degradation of cellulose ethers. Similar work has also been done on some cellulose esters yielding similar results. It was found that when the DS of the cellulose derivative increased to above zero, the biodegradation rates decreased rapidly, to the extent that there was almost no biodegradation reported for DS of 1.0. Carboxy methyl cellulose was studied extensively for it's biodegradation by Bhattacharjee et al. (1971), and it was found that since the unsubstituted residues are the main focal point of attack, there needs to be at least two unsubstituted residues for hydrolysis (chain scission) to occur. In related studies on hydroxyethyl cellulose [Wirick et al. (1968)], it was found that chain scission takes place readily between two unsubstituted residues, but nevertheless can occur at a residue having a 6- or 2- substituted aglycone. Rivard et al. conducted studies on the anaerobic bioconversion of cellulose acetates to methane, and reported that the biodegradability of the acetates decreases with increasing DS and shows

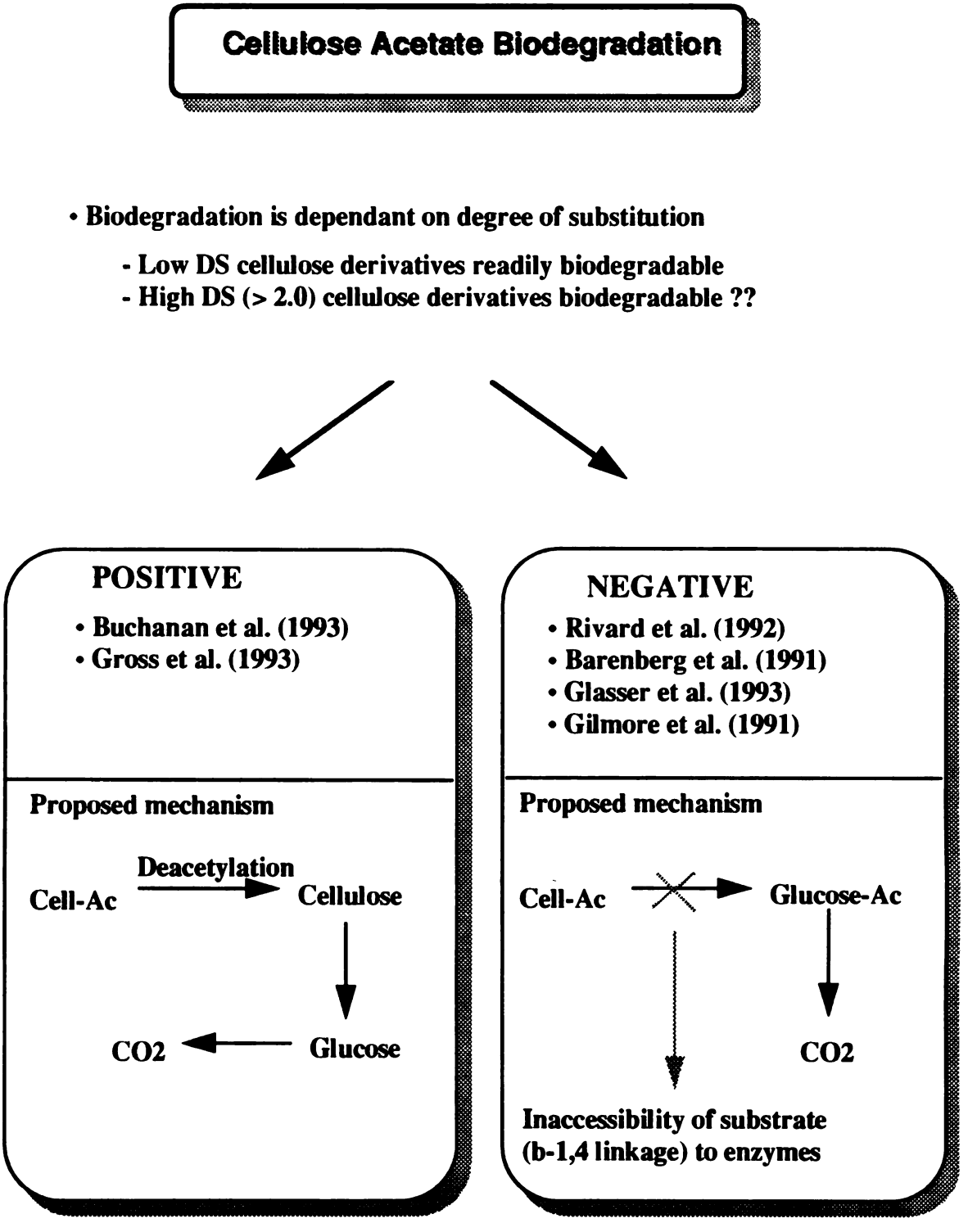


Figure 8.3 - Summary of the conflicting reports on CA biodegradation

negligible biodegradation after a DS of 1.5-1.7. Similar results were reported by Weimer et al. Some studies were also conducted on blends of high DS cellulose acetates and propionates with PHB/V [Gilmore et al (1993)]. It was shown that both environmental and enzymatic assays on the blend films showed a strong inhibitory effect of cellulose acetate butyrate or cellulose acetate propionate on PHB/V degradation: the weight loss of the films never exceeded 10% when the cellulose ester content was 50% or higher. In contrast to the above results, Buchanan et al.., conducted studies on cellulose acetates and propionates and showed that DS of as high as 2.5 in the case of acetates and 1.7 in the case of propionates underwent rapid biodegradation in an aerobic enriched environment. The conflicting reports and proposed mechanisms on CA biodegradability are summarized in Figure 8.3.

This chapter deals with the biodegradation of cellulose acetate of DS 2.5 obtained from two different sources - Eastman Kodak and Hoechst Celanese.

8.5 CA BIODEGRADATION :- EXPERIMENTAL

8.5.1 Materials tested

Two samples from different sources were tested for their biodegradability. The molecular weights of the samples were obtained from Eastman Chemical Co. The DS of these materials were determined by proton NMR on a VXR 500. The carbon contents of the material were obtained through a Perkin Elmer 2400 CHN Elemental analyzer. The two samples of cellulose acetate were CA398-30 and JLF68.

CA398-30 :- This sample was obtained from Eastman Chemical Co., and had a molecular weight of around 30,000, and a DS of 2.5 as determined by proton NMR (Figure 8.4). It was sieved to obtain a fraction between mesh size 120(.007 inches and mesh size 140(.003 inches). It had a bulk density of 80 lb/cu.ft.. It had a %C=48.59, %N=0.023, %H=5.89.

JLF68 :- This sample was obtained from Hoechst Celanese. It was a plastics grade product with a molecular weight of around 30,000, and a DS of 2.49 (acetyl value of 55.5%) as determined by proton NMR (Figure 8.5). It was sieved to obtain a fraction between mesh size 120 (0.007 inches) and mesh size 140 (0.003 inches). it had a packed bulk density of 26.0 lb/cu.ft. It had a %C=48.04, %N=0.007, %H=5.88.

A summary of the material properties is given in Table 8.1

Table 8.1 - Material Properties of the CA samples

	CA 398-30	**JLF68**
SOURCE	Eastman Kodak	Hoechst Celanese
(M_n)*	58,000	62,000
(M_w)*	161,000	188,000
Polydispersity	2.76	3.04
C content	48.59%	48.04%
DS	2.5	2.5

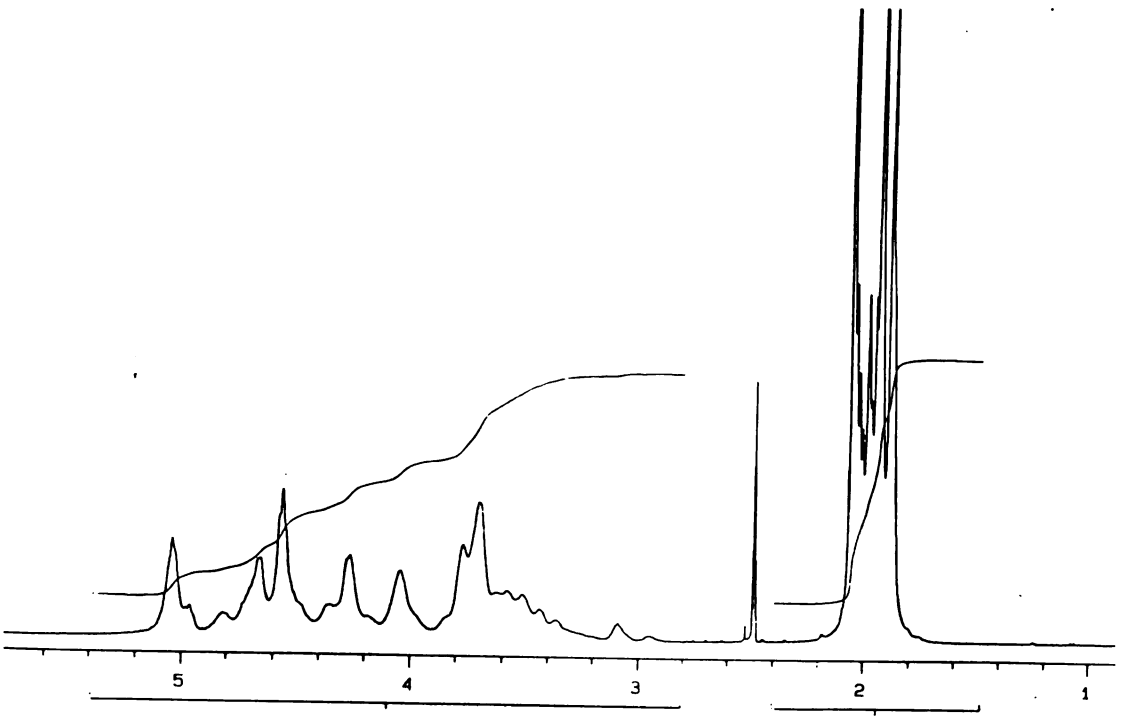


Figure 8.4 - Proton NMR of CA-398-30

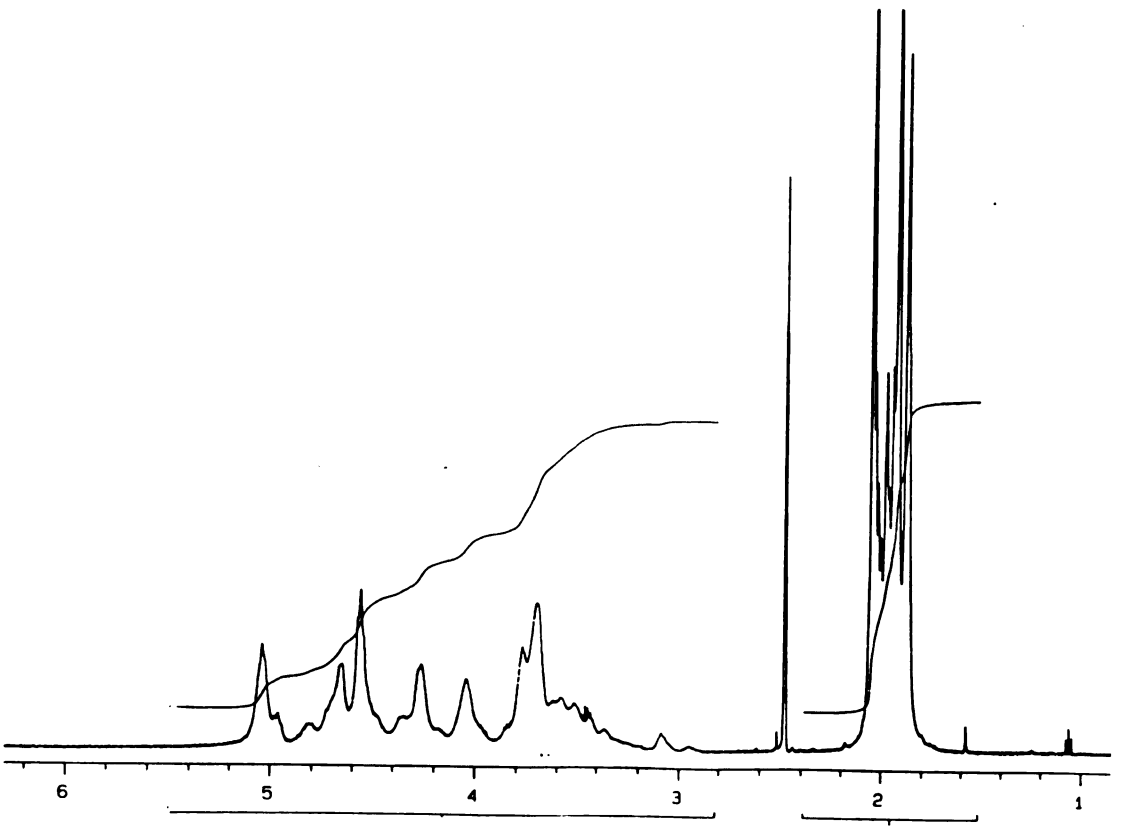


Figure 8.5 - Proton NMR of JLF-68

8.5.2 Inoculum and Test solution

The test materials were of known weight based on their carbon contents.

CA398-30 = 3.09 g, carbon content = 1.5 g

JLF68 = 3.12 g, carbon content = 1.5 g

Cellulose = 3.76 g, carbon content = 1.5 g

The stock solution used in each flask were as mentioned in chapter 3 except that 5 ml of ammonium sulfate was added. 30 ml of the sludge inoculum was added and water was added to the flask upto a level of 1500 ml. The C/N ratio maintained was approximately 33/1.

8.5.3 Results

The biodegradation results of this experiment are shown in Figures 8.6 and 8.7. Figure 8.8 shows the same results but with no blank correction. This experiment was run for 40 days, during which time, the % C conversion to CO_2 was 0.48 for CA398-30 and 61.17 for JLF68. The standard cellulose reached a CO_2 evolution level of at the end of 40 days. As can be seen there was a considerable difference between the two CAs, which was surprising since they were essentially the same material with the same properties (Table 8.1). When the samples were examined under the scanning electron microscope, it was found that the materials had a very different morphology. Bulk Density data from the supplier's specifications also showed some difference in values. The differences between the two samples are summarized in Table 8.2.

8.5.3.1 Scanning electron Microscopy - Figures 8.9 and 8.10 show the SEM micrographs of the two CA samples taken by a JEOL 35CF Scanning Electron Microscope. The powders were first mounted on a platinum stub and carbon paint was applied at the edges of the stub to improve conductivity. The samples were

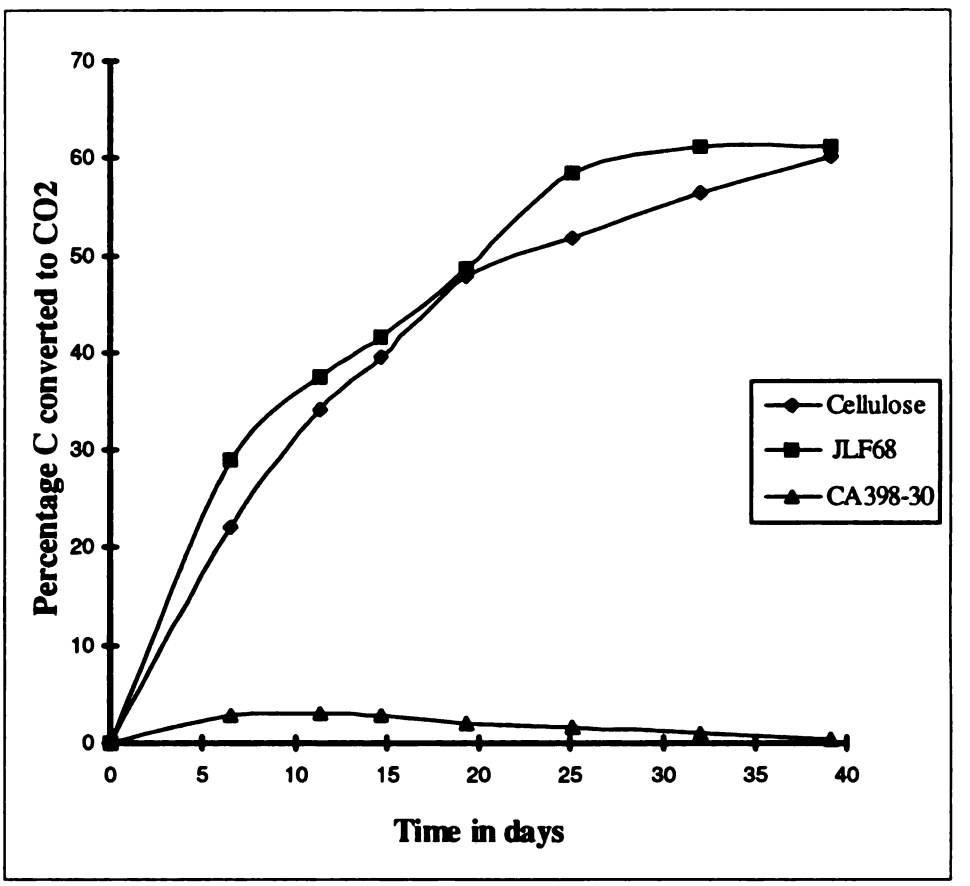


Figure 8.6 - Percentage of initial polymericC converted to CO_2 with time. Percentage C conversion to CO_2 for two CA products of DS 2.5 was measured with time. Cellulose was included as a positive control. Each curve represents the average of duplicate runs.

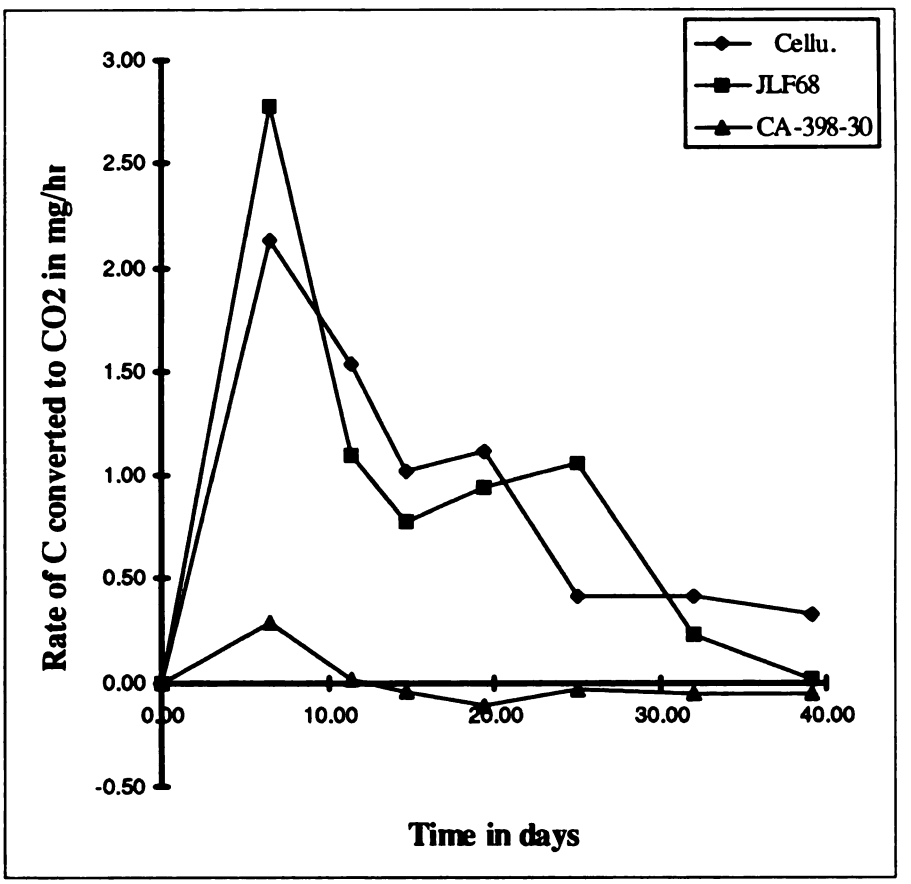


Figure 8.7 - Rate of initial polymericC conversion to CO_2 with time. Rate of carbon conversion to CO2 for two CA products of DS 2.5 was measured with time.

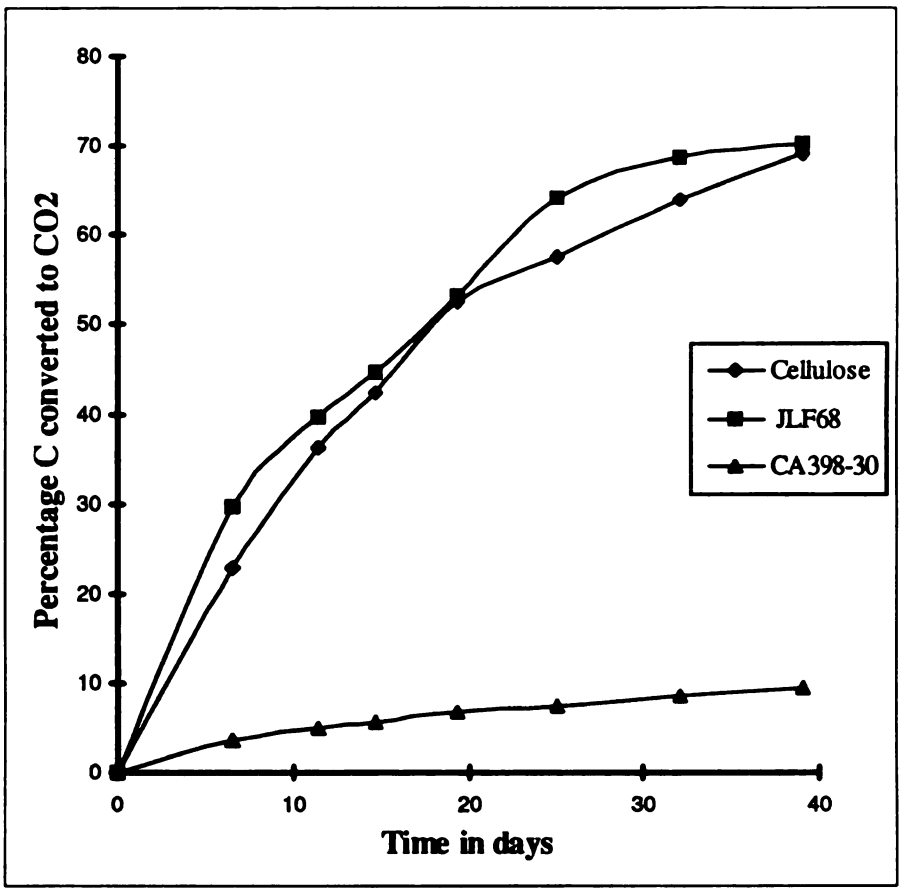


Figure 8.8 - Percentage of CA carbon converted to CO_2 with time (no blank correction). This figure is a replicate of Figure 8.6 except that no blank correction has been applied.

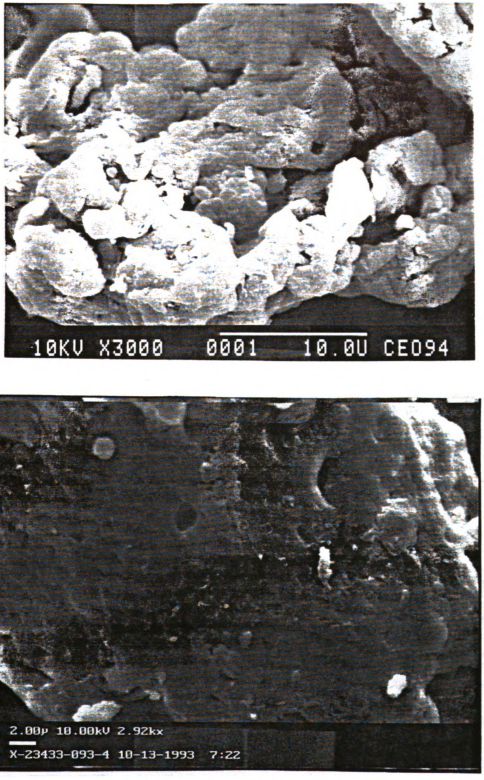
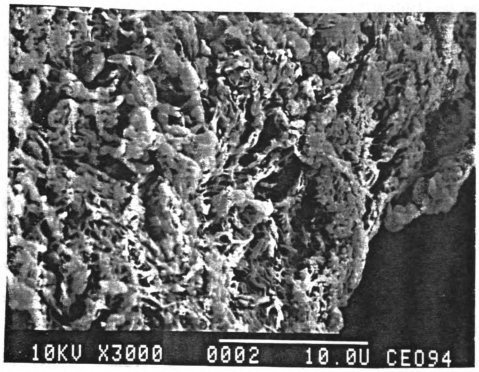


Figure 8.9 - SEM micrographs of CA-398-30 at 3000X



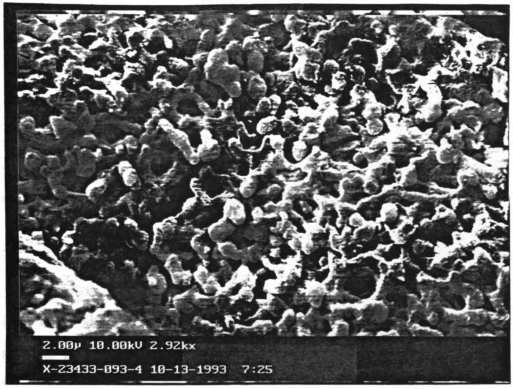


Figure 8.10 - SEM micrographs of JLF-68 at 3000X

then coated with gold on a sputter coater for 3 mins (21A thickness approx.). An accelerating voltage of 10kV, working distance 15 or 35 and condenser lens setting of 400 or 600 was used depending on the magnifications used.

8.6 EFFECT OF MORPHOLOGY - LITERATURE

It has been well established that for any substrate to be available for microbial attack, a direct contact needs to be established between the acting enzyme and the active site region. Cellulose has been studied to some extent as far as morphology effects are concerned, but cellulose derivatives have been studied very little. This area has been explored in terms fiber size, pore size etc., of the cellulosic substrate [Tanaka (1983), Wong (1988)].

Table 8.2 - Differences between the two cellulose acetates

	CA-398-30	JLF-68
BULK DENSITY	80 lb/cu.ft	26 lb/cu ft.
MORPHOLOGY	Closed Structure smooth surface	Open honeycomb structure

They have shown that the course of enzymatic solubilization of cellulosic materials is governed by the surface area of pores accessible to the enzymes. Moreover, only small cellulase components can diffuse slowly into small pores

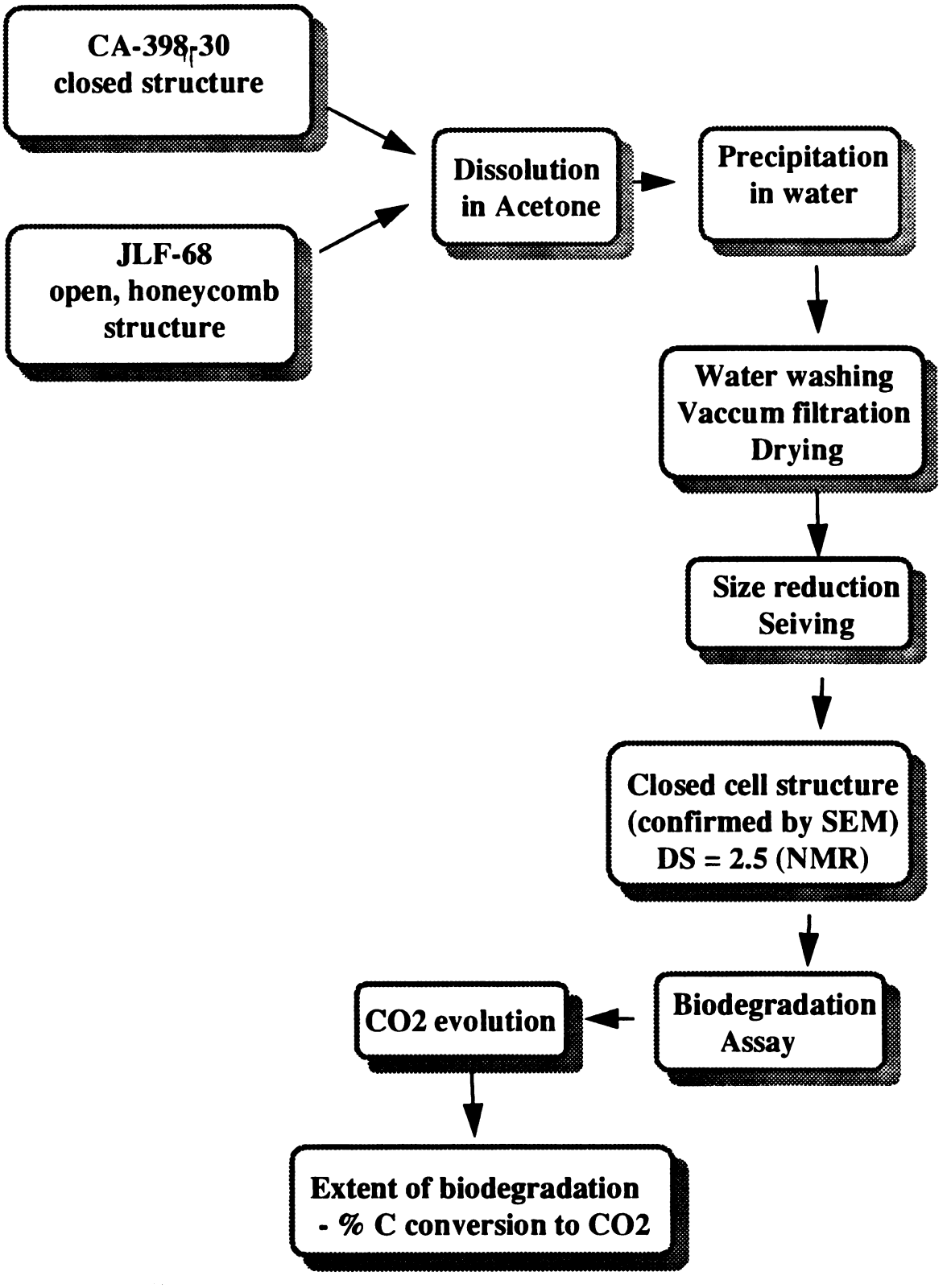


Figure 8.11 - Preparation of samples with defined morphology

inaccessible to the entire complex of enzymes, and once they enter in they are trapped there and thus prevent further molecular movement and synergistic action. The biodegradability of cellulose derivatives has also been explored in terms of the charge on the substrate. It has also been shown that positively charged cellulosic substrates attract enzymes more than the negatively charged ones [Boyer (1983)], which in turn affects the rate of hydrolysis.

8.7 CA WITH DEFINED MORPHOLOGY -EXPERIMENTALS

8.7.1 Preparation of CA samples with defined morphology

CA 398-30 and JLF68 were separately dissolved in acetone, and precipitated in water. The precipitates were thoroughly washed with water, vacuum filtered and dried. They were then ground to obtain a fine powder and sieved to obtain a size fraction between 120 and 140 mesh size. Figure 8.11 summarizes the procedure followed for the sample preparations. The DS of these two samples were obtained through proton NMR. They both had a DS of around 2.5 (Figure 8.12 and 8.13). SEM pictures were also taken and revealed a similar closed morphology for both the samples (Figure 8.14 and 8.15).

8.7.2 Inoculum and Test Solution

CA398-30 (ppt) = 1.6 g, carbon content = 0.8 g

JLF68 (ppt) = 1.6 g, carbon content = 0.8 g

Cellulose = 1.5 g, carbon content = 0.8 g

The stock solution used in each flask were as mentioned in chapter 3 except that 3 ml of ammonium sulfate were added. Water was added to the flask upto a level of 1500 ml. The C/N ratio maintained was approximately 33/1.

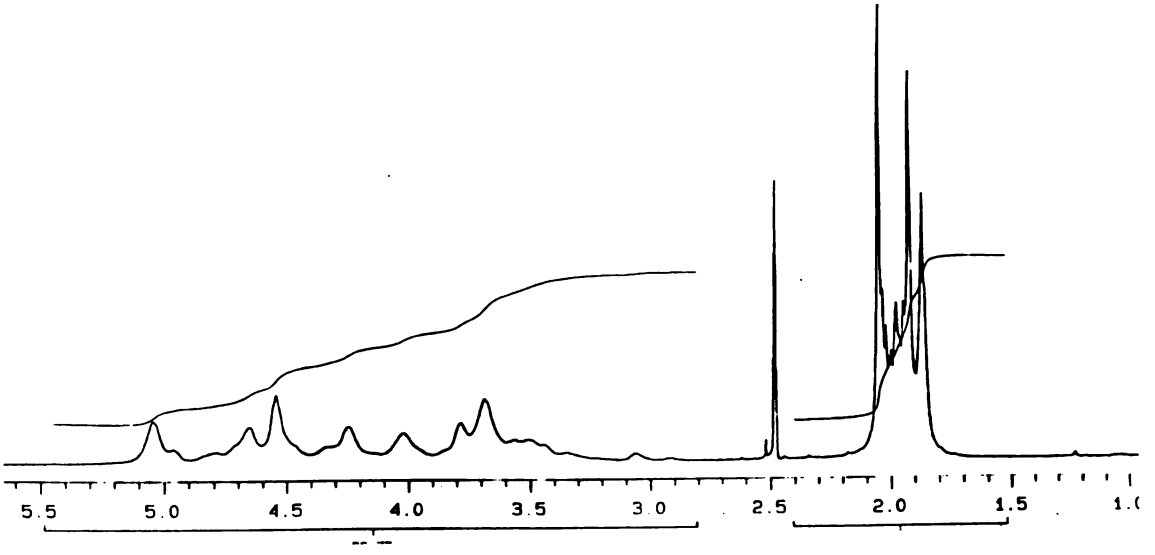


Figure 8.12 - Proton NMR of precipitated CA-398-30

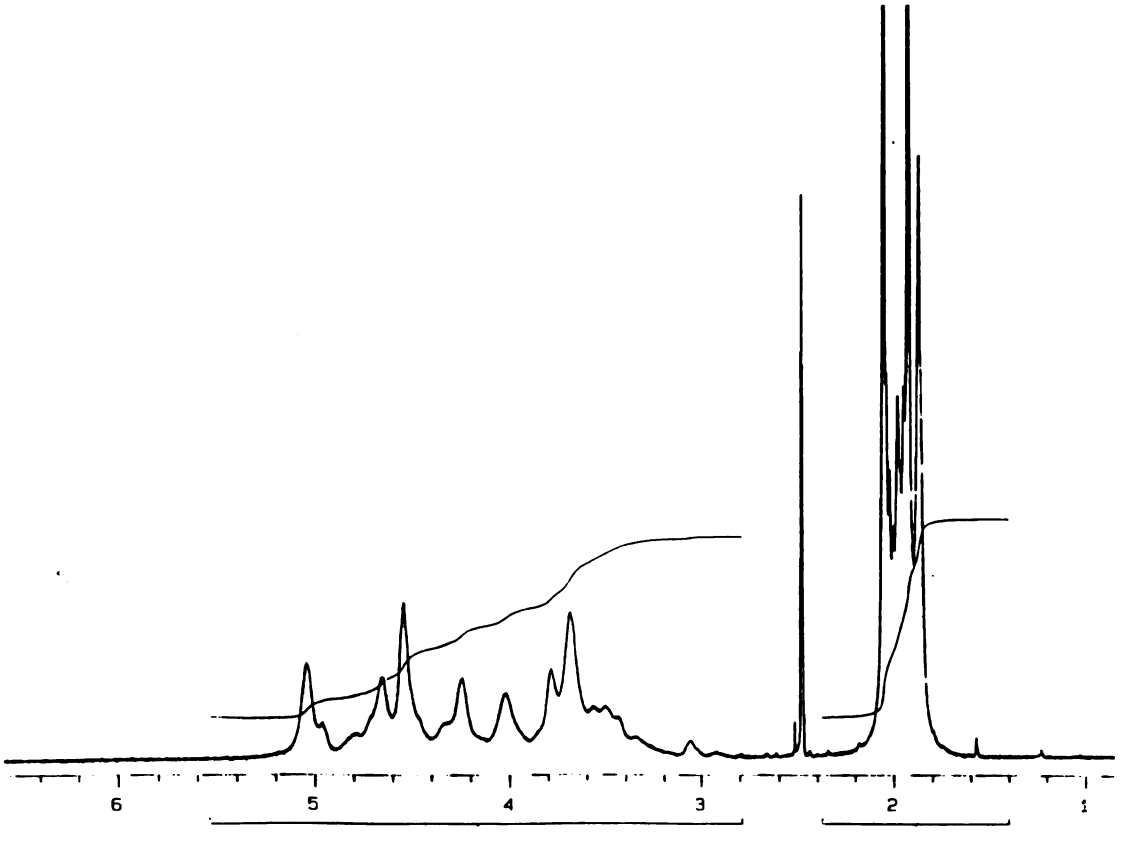


Figure 8.13 - Proton NMR of precipitated JLF-68

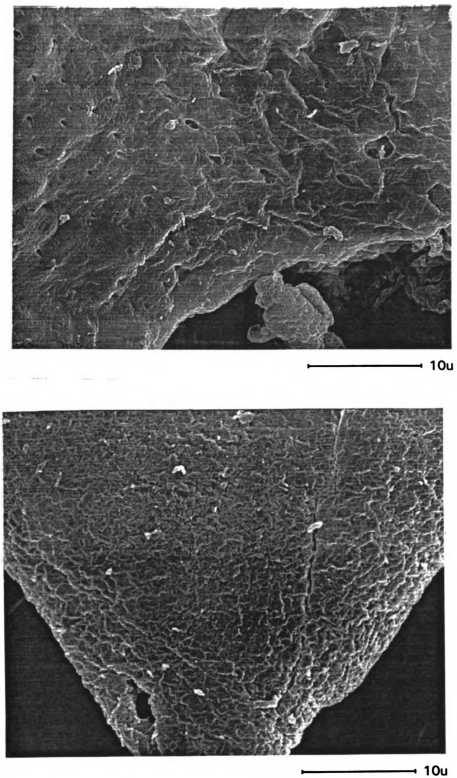


Figure 8.14 - SEM micrograph of precipitated CA398-30 at 3000X

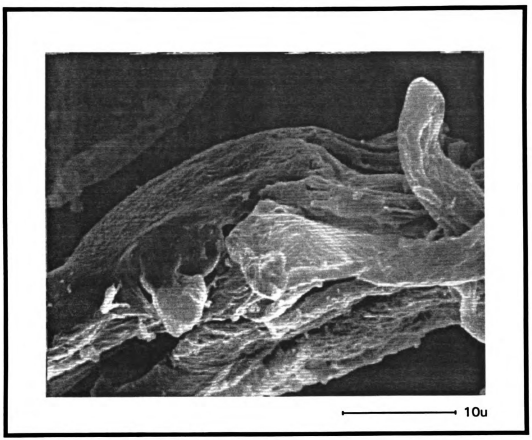
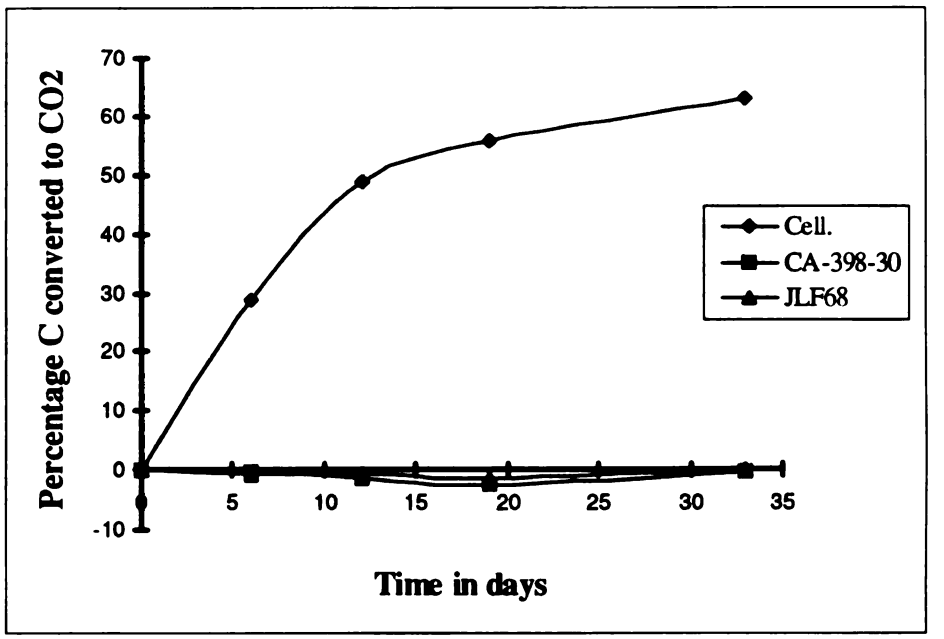


Figure 8.15 - SEM micrograph of precipitated JLF-68 at 3000X



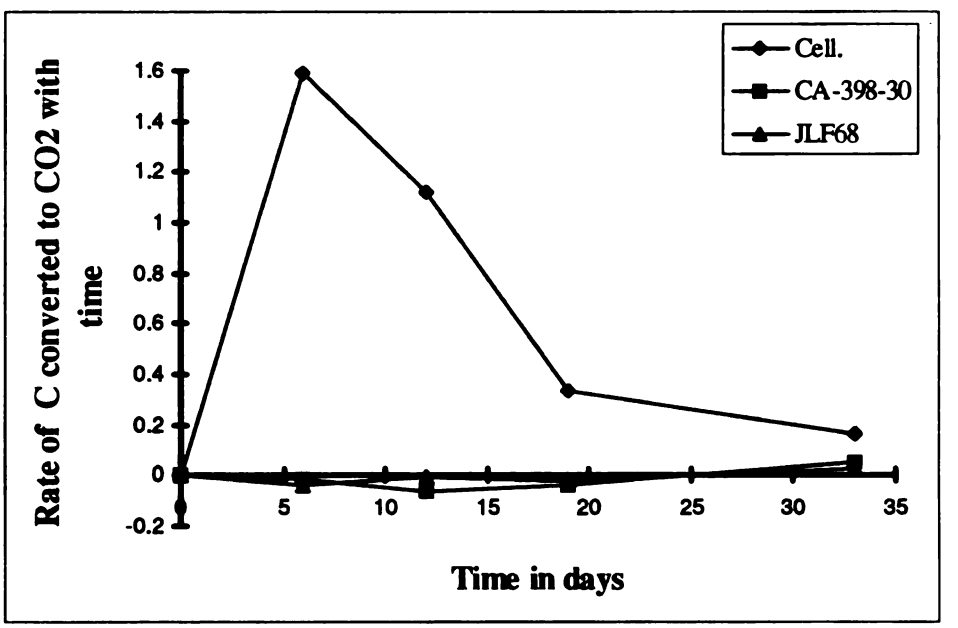
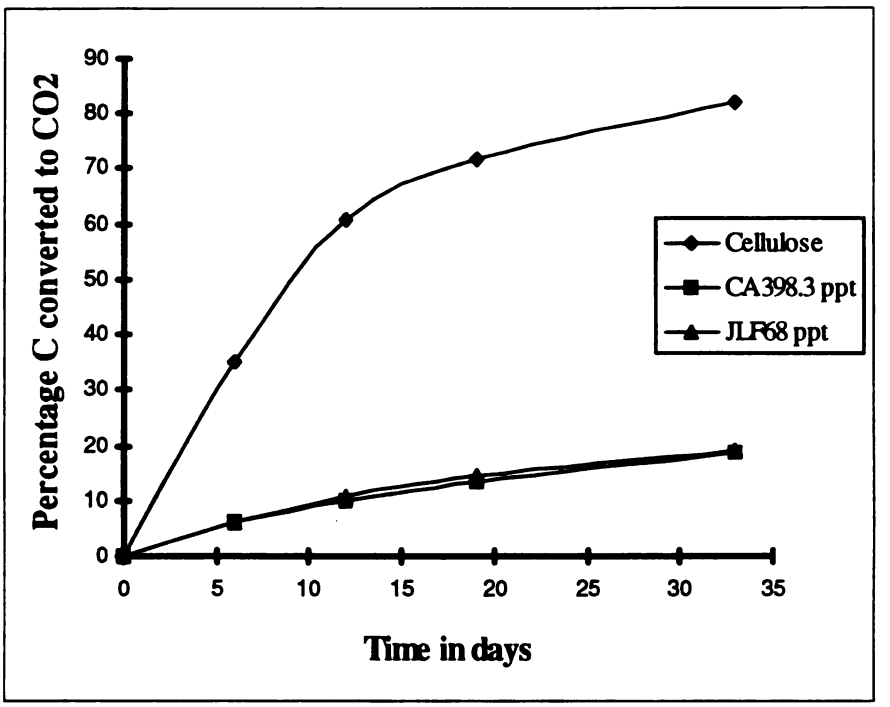


Figure 8.16 - CA- carbon conversion to CO_2 with time for the precipitated CAs. Carbon conversion to CO_2 was measured for the precipitated CA products with similar morphology. Each curve represents an average of duplicate runs.



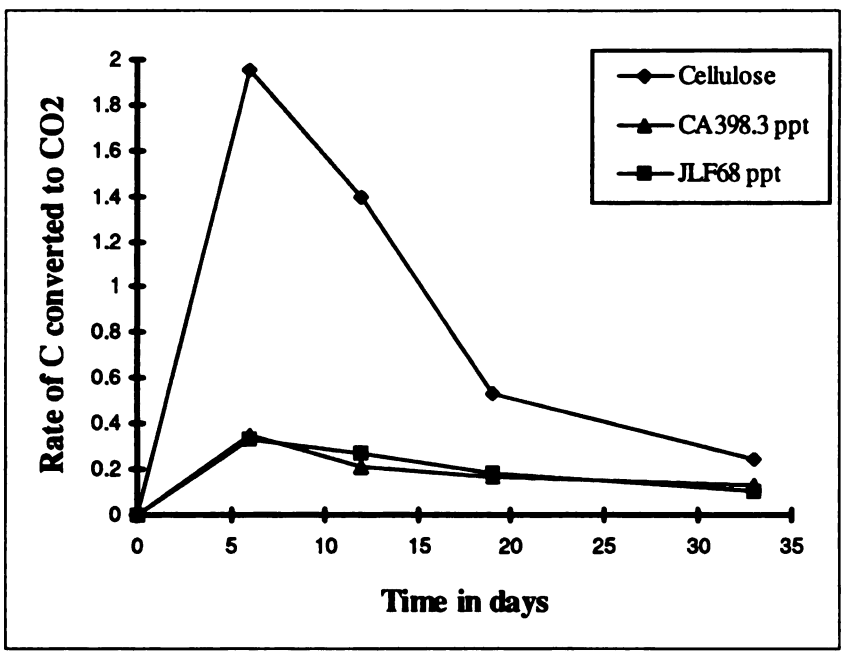


Figure 8.17 - CA carbon conversion to CO_2 for the precipitated CAs (no blank correction). This figure is a replicate of Figure 8.16 except that no blank correction has been applied here.

8.7.3 Biodegradation Results

Figures 8.16 and 8.17 show the biodegradation results of the above experiment. Table 8.3 summarizes the biodegradation results of both the experiments. Both the materials reached approximately the same CO_2 evolution level within the time frame of the experiment which was 35 days. This confirmed the fact, that the morphology of the material played an important role in the biodegradabilities of the two materials tested here.

Table 8.3 - Biodegradation results - Percentage C converted to CO_2 with time

	Unprecipitated (39 days)	Precipitated (33days)
Cellulose	60.14 (69.1) %	62.94 (82.07) %
JLF68	61.17 (70.17) %	0.28 (19.07) %
CA398-30	0.48 (9.48) %	0.07 (18.86) %

The bracketed values are the ones with no blank correction

8.8 DISCUSSION

The difference in the biodegradation rates of the two different morphology samples of cellulose acetate may be related to the accessibility of the polymer backbone active sites to the degrading enzymes. It is seen that the cellulose and the JLF68 biodegradation curves are very similar. This may be an indication that since JLF68 is very porous, the active sites on the cellulose acetate backbone are easily accessible to the enzymes, and hence undergoes biodegradation with the same kinetics as the cellulose itself. This theory is supported in part by a work done earlier by Fujii et al. on cellulose derivatives in which he refers to the molecular

weight of the polymer as a limiting factor instead of the porosity. He claims that below a certain molecular weight the reaction kinetics of the cellulose derivatives are the same as that of pure cellulose. On the other hand, CA398-30 has a closed structure which does not allow the enzymes to access the active sites on the polymer backbone. This limits the rate of hydrolysis of the cellulose backbone and also the extent of hydrolysis.

8.9 PROPOSED MECHANISM FOR CA BIODEGRADATION

Two mechanisms have been proposed for the biodegradation of Cellulose acetate of high DS. They are:

a) Chain scission of the polymer backbone followed by assimilation of the monomer glucose acetate (Narayan et al).

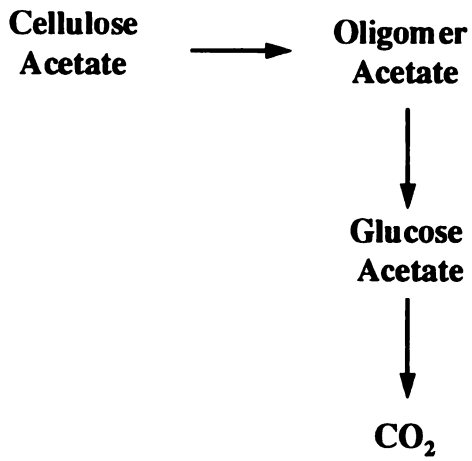


Figure 8.18 - Proposed mechanism for aerobic enzymatic degradation of CA

b) Deacetylation followed by chain scission of the resultant polysaccharide backbone and assimilation of the generated glucose units (Buchanan et al).

The results obtained from the morphology studies suggest that the accessibilty of the polymer backbone may be a key factor in the biodegradability of the high DS cellulose acetate. Some initial studies were conducted on the samples during the biodegradation experiments. It was found that after a 35% C conversion to CO_2 was reached for the CA, the DS of the sample (extracted, freeze-dried and studied through NMR), was slightly more than the original value, i.e., DS = 2.7. This suggested that after the unsubstituted units along the CA backbone were attacked, further CO2 generation was obtained by chain scission of the CA backbone. Based on these findings, the first mechanism was proposed by Narayan et al, which is schematically represented in Figure 8.18. This is only a hypothesis and needs to be investigated further for confirmation.

The key points in this hypothesis are as follows -

- Cellulose acetate is "intrinsically" biodegradable - i.e., it is capable of being utilized by microorganisms with no gratuitous or co-metabolism.
- Deacetylation may not be essential for biodegradation.
- Accessibility and transport of enzyme to the active site is the rate limting step for biodegradation of cellulose derivatives.
- Degree of substitution is not the rate limiting factor except as it modifies the enzyme transport mechanism.

However, to claim that CA is biodegradable in composting or waste disposal systems,

- CA must be engineered to biodegrade in the operating time frame of the disposal system (composting, sewage/waste water treatment facilities, soil, etc.) to an appreciable extent, greater than 50%.

- Trace the disposition of the residual carbon - cell mass, humic substances, oligomers and determine the rate of biodegradation and time taken for complete biodegradation/assimilation.

In order to create a kinetic model for the the proposed biodegradation mechanism of cellulose acetate, cellulose degradation kinetics were studied theoretically as a first attempt. A kinetic model was developed for the aerobic biodegradation of cellulose in the experimental test flask.

8.10 PROPOSED KINETIC MODEL FOR THE AEROBIC BIODEGRADATION OF CELLULOSE

8.10.1 ASSUMPTIONS

A set of assumptions were imposed in deriving a comprehensive mechanistic model for the aerobic enzymatic hydrolysis of cellulose. These assumptions were based on Fan and Lee (1983) , as well as P.L. Beltrame's (1984) assumptions.

1) The hydrolysis of cellulose occurs in two steps: the insoluble cellulose is hydrolysed first to soluble cellulose by the synergistic action of endo-glucanase and cellobiohydrolase, and the cellobiose is further hydrolysed to glucose by the action of b-glucosidase. The first two enzymes have been lumped together as a single enzyme complex, E, and the third enzyme referred to as K_β.

2) The two fractions of cellulose, the crystalline and the amorphous are not distinguishable.

3) The overall biodegradation rate is not affected by diffusion or mass transfer, i.e., the product concentrations are governed by the reaction kinetics.

4) The rate of glucose production is equal to the rate of glucose cosumption to give CO_2. This assumption was based on negligible glucose concentrations of samples taken out from the experimental flasks periodically during the period of biodegradation. This has been explained further.

5) The hydrolysis of cellulose to cellobiose occurs at the cellulose surface by the adsorbed enzyme. This reaction is non-competitively inhibited by cellobiose. No inhibition by glucose takes place owing to negligible concentration of glucose in solution. The decomposition of cellobiose to glucose takes place in the aqueous phase, and following the previous assumption, is not inhibited by glucose.

8.10.2 COMPETITIVE AND NON-COMPETITIVE INHIBITION

The difference between competitive and non-competitive inhibition is shown in

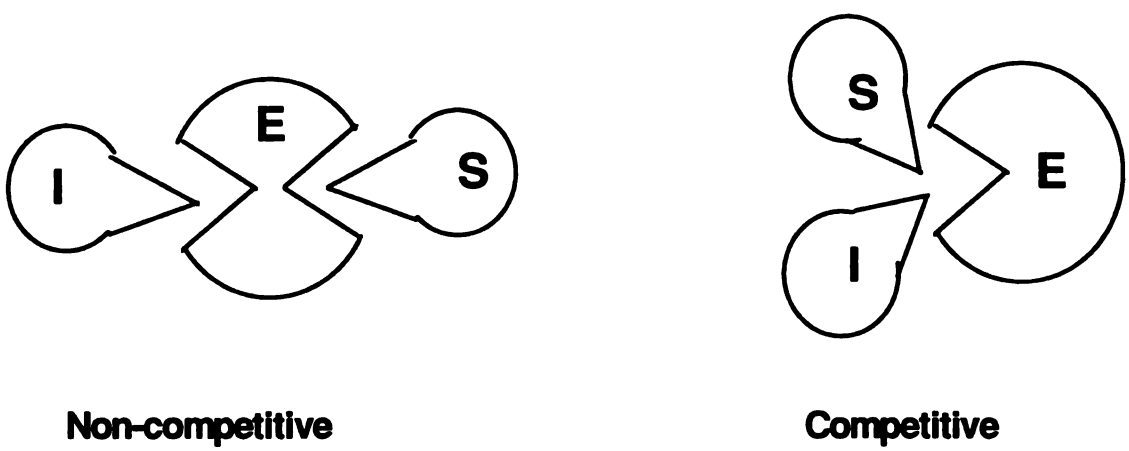


Figure 8.19 - Competitive and Non-competitive inhibition

Figure.8.19. Competitive inhibition is a situation where there is an inhibitor molecule binding to the enzyme active site, thus blocking it, so that the substrate molecule cannot bind on to the same active site. Non-competitive inhibition on the other hand, binds away from the active site, thus giving free access to the active site for the substrate. In both the cases, there is a reduction in the affinity of the enzyme for the substrate, but no reduction in reaction rates.

8.10.3 MECHANISM OF DEGRADATION

The assumptions in this model gives rise to two major steps in this overall reaction.

8.10.3.1 Decomposition of cellulose to cellobiose

The decomposition of cellulose to cellobiose is a heterogeneous reaction, which includes the adsorption of the enzyme, and the formation of the enzyme-substrate complex. The following reactions are taking place in this step.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad (1)$$

$$ES + C \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ESC \qquad (2)$$

$$E + C \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EC \qquad (3)$$

$$ES \xrightarrow{k_3} E + C \qquad (4)$$

where, E = lumped enzyme complex

S = cellulose substrate

ES = enzyme-substrate complex

C = cellobiose

EC = enzyme-cellobiose complex

ESC = enzyme-substrate-cellobiose complex in non-competitive inhibition by cellobiose

k_1 and k_{-1} = forward and reverse rate constants for equation (1)

k_2 and k_{-2} = forward and reverse rate constants for equation (2) and (3)

k_3 = rate constant for equation (4).

Equations (1) and (2) represent the decomposition to give cellobiose, whereas (3) and (4) represent the inhibition reactions by cellobiose

8.10.3.2 Decomposition of cellobiose to glucose

In this step the decomposition of cellobiose to glucose takes place in the aqueous phase, and includes the hydrolysis of the cellobiose to glucose with no inhibition from glucose. The following reactions take place in this step

$$E_\beta + C \underset{k_{-\beta}}{\overset{k_\beta}{\rightleftharpoons}} E_\beta C \qquad (5)$$

$$E_\beta C \xrightarrow{k_4} E_\beta + G \qquad (6)$$

where, G = glucose formed

E_β = enzyme β-glucosidase

$E_\beta C$ = enzyme-cellobiose complex.

k_4 = the rate constant for equation (6).

k_β and $k_{-\beta}$ $\stackrel{!!}{=}$ forward and reverse rate constants for equation (5)

8.10.4 DESCRIPTION OF THE MODEL

The following mathematical assumptions are made for the model.

1) Equations (1) and (2) and (3)are in equilibrium and the equilibrium constants are given as,

$$K_1 = \frac{k_1}{k_{-1}} \qquad K_2 = \frac{k_2}{k_{-2}} \tag{7}$$

Instead of equilibrium, we can also assume that equations, (1) and (4) follow a pseudo-steady state condition, i.e.,

$$\frac{dES}{dt} = 0$$

$$k_1 (E) (S) - k_{-1} (ES) - k_3 (ES) = 0$$

However, k_3 is relatively very small, i.e., the reaction (4) is a very slow one. Hence, the approximation that equation (1) is in equilibrium has been used.

2) The complexes, (EC), and (ESC) are dead end-complexes, i.e., they do not break down to give the product.

3) The reaction (5) is in equilibrium, and the equilibrium constant is given by,

$$K_\beta = \frac{k_\beta}{k_{-\beta}} \tag{8}$$

Instead of equilibrium, we can also assume that equations (5) and (6) follow a steady state condition, as in the case of (1) and (4). But since, k_β is very small, the equilibrium assumptions is applied.

3) The process setup is a batch reactor with the schematic shown in Figure 8.20.

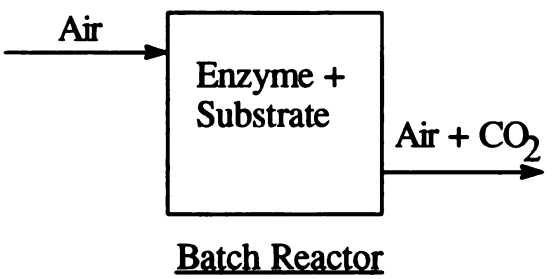


Figure 8.20 - Process Schematic for the batch reactor

The assumption made here is that the rate of production of glucose by the enzymatic hydrolysis equals the rate of consumption of glucose by aerobic conversion to CO_2, i.e.,

Rate of C conversion to CO_2 = rate of C production as glucose.

Since, the glucose produced is rapidly being removed from the solution as CO_2, the inhibition by glucose in both steps of the mechanism have been disregarded. Thus,

$$v = \frac{dC_{CO_2}}{dt} = \frac{dC_{glucose}}{dt}$$

8.10.5 DEVELOPMENT OF THE MODEL

8.10.5.1 Reactions with enzyme E

The reaction mechanism mentioned earlier has been schematically depicted in Figure 8.21.

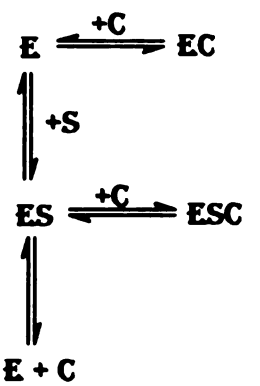


Figure 8.21- Reaction mechanisms for E and E_β

a) From equation (1),

$$k_1(E)(S) = k_{-1}(ES)$$

$$K_1 = \frac{k_1}{k_{-1}} = \frac{(ES)}{(E)\,(S)} \tag{9}$$

From equation (2),

$$k_2\,(ES)\,(C) = k_{-2}(ESC)$$

$$K_2 = \frac{k_2}{k_{-2}} = \frac{(ESC)}{(ES)\,(C)} \tag{10}$$

From equation (3),

$$k_2\,(E)\,(C) = k_{-2}(EC)$$

$$K_2 = \frac{k_2}{k_{-2}} = \frac{(EC)}{(E)\,(C)} \tag{11}$$

A total enzyme balance in the batch reactor for E yields

$$E_t = E + (EC) + (ES) + (ESC) \tag{12}$$

where, E_t = total enzyme E in the batch reactor.

From equation(7),

$$(E) = \frac{(ES)}{K_1(S)} \tag{13}$$

From equation (8)

$$(ESC) = K_2 (ES)\,(C) \tag{14}$$

From equation (7) and (9),

$$(EC) = \frac{K_2\,(C)(ES)}{K_1 (S)} \tag{15}$$

Substituting equations (11), (12), (13) in equation (10), we obtain

$$E_t = \frac{(ES)}{K_1 (S)} + \frac{K_2 (C)(ES)}{K_1 (S)} + (ES) + K_2 (ES)\,(C)$$

$$(ES) = \frac{E_t}{\frac{1}{k_1 (S)} + \frac{K_2 (C)}{K_1 (S)} + 1 + K_2 (C)} \tag{16}$$

Thus, we get, from equation (4), the concentration profile of the intermediate product C with time as,

$$\frac{dC}{dt} = k_3 (ES) = \frac{k_3 E_t}{\frac{1}{k_1 (S)} + \frac{K_2 (C)}{K_1 (S)} + 1 + K_2 (C)} \tag{17}$$

8.10.5.2 Reactions with enzyme E_β

The reaction mechanism mentioned earlier has been depicted in Figure 8.22.

$$E_\beta \underset{}{\overset{+C}{\rightleftharpoons}} E_\beta C \longrightarrow E_\beta + C \qquad (18)$$

Figure 8.22 - Reaction scheme for E_β

From Eq. (5),

$$k_\beta (E_\beta)(C) = \underline{k}_\beta (E_\beta C)$$

$$K_\beta = \frac{(E_\beta C)}{(E_\beta)(C)} \qquad (19)$$

Also, from a total enzyme balance $E_{\beta t}$ in the batch reactor, we get,

$$E_\beta t = (E_\beta) + (E_\beta C) \qquad (20)$$

So, from (17) and (18), we get,

$$E_\beta t = (E_\beta C)\left(\frac{1}{K_\beta (C)} + 1\right)$$

Thus, we get from equation (6), a concentration profile for the final product glucose as,

$$v = \frac{dG}{dt} = k_4(E\beta C) = \frac{k_4 E\beta t}{\left(\frac{1}{K\beta\,(C)} + 1\right)} \qquad (21)$$

8.10.5.3 Mass balance (grams of carbon) in reactor

A carbon balance can also be done in the batch reactor to account for the concentrations of substrate(cellulose), cellobiose and glucose as,

$$S_0 = S + C + G \qquad (22)$$

where, S_0 = concentration of substrate at time, t=0

8.10.5.4 Summary

We thus have three variables S, C and G, and the three equations,

$$\frac{dC}{dt} = k_3(ES) = \frac{k_3 E_t}{\frac{1}{k_1(S)} + \frac{K_2(C)}{K_1(S)} + 1 + K_2(C)}$$

$$v = \frac{dG}{dt} = k_4(E\beta C) = \frac{k_4 E\beta t}{\left(\frac{1}{K\beta\,(C)} + 1\right)}$$

$$S_0 = S + C + G$$

These three equations can be solved simultaneously to give the concentration profiles with time.

CONCLUSIONS AND RECOMMENDATIONS

The current focus of the materials industry, specially packaging and disposables, on the redesigning of materials keeping in mind their ultimate environmental fate, provided the thrust for this work. Studies were conducted on the aerobic biodegradation of polymeric materials in the presence of municipal sewage sludge on a laboratory scale.

A viable method was evolved based on ASTM-D5209 to quantitatively measure the aerobic biodegradation of polymers in a liquid inoculum derived from municipal sewage sludge. It utilized the polymeric carbon as a single carbon source producing carbon dioxide which was periodically measured. This method was used successfully to quantify the extent of biodegradation of polymers based on percentage of original carbon conversion to CO_2. The results from all the experiments were reproducible within a maximum of ± 10% of the presented results. The cellulose standard showed consistent carbon dioxide evolution levels for all the experiments within the experimental time frames applied (≅ 60% within 30-35 days with blank correction) thus confirming the viability and reproducibility of the inoculum used. The rate of CO2 evolution was also found in all cases, to reach a maximum within the first 7 - 10 days from the start of the experiment, and

then slowly lag off to a steady rate after 25 - 30 days. This can be attributed to the growth cycle of the microorganisms present.

A theoretical analysis was done of the biodegradation process and the effect of certain process parameters on the biodegradation measurement around the experimental system. The effects of speed of agitation and inoculum acclimatization were studied experimentally. It was found that the extent of biodegradation increased with increasing speed of agitation. This was because of increased contact, hence increased surface area exposure of the materials to microorganisms with increasing agitation speed. Acclimatization of the inoculum was found to appreciably decrease the standard deviations between the replicates (< 3%), but did not increase the extents of biodegradation. Acclimatization for a longer period of time and serial transfers could result in a more uniform and concentrated inoculum with respect to the favorable microorganisms. This is an important area for further investigation. Another parameter that can be investigated further is temperature since this would raise up questions about the type of organisms actively involved in the biodegradation processes.

Several commercial as well as other polymers of interest were tested for their biodegradabilities and the results reported. Cellulose, Starch, 9-11 acid and glycerol triacetate showed appreciable biodegradation extents of >60%. PCL and the PCL/starch blend had relatively slower but steady CO_2 production rates and reached biodegradation extents of $\cong$ 40% within the experimental time frame. PVOH and EVOH showed very little biodegradation contrary to reported literature. This could be due to the insolubility and the high crystallinity of the two materials. Aqua Coat was found to show low levels of biodegradation, indicating

that when coated on paper, it will retard and maybe limit the biodegradation of otherwise naturally biodegradable paperboard. This material was studied because of the large use of coated paperboard as food cartons and other packaging material in the food industry.

Considerable attention was paid to the cellulose and starch esters, owing to their potential as biodegradable plastics. Both these chemically modified esters are economically attractive owing to their natural abundance and possess good mechanical and plastic properties comparable to most of the commercially available plastics. Starch propionates of different DS were characterized and tested for their biodegradabilities. It was found that the biodegradation decreased considerably with increasing DS owing to increased hydrophobicity and decreased accessibility of the active sites by the degrading enzymes. The correlation of biodegradability to positional substitution and method of synthesis is an interesting topic for further investigation.

Morphology was found to affect the biodegradation of cellulose acetate DS = 2.4 to a great extent. A CA sample with a porous, honeycomb structure was found to have a much higher biodegradation rate as compared to a CA sample with a closed smooth morphology. Re-precipitation of both the samples and reproduction of the closed smooth morphology resulted in almost negligible biodegradation. It was therefore concluded that the accessibility of the polymer backbone (i.e., the glycosidic bonds) is the governing factor in the biodegradation of the starch and cellulose esters. Based on these conclusions a mechanism was proposed for the biodegradation of CA which involved polymer chain cleavage, followed by monomer/oligomer assimilation by the microorganisms. As a first attempt, a

kinetic model was developed for the aerobic biodegradability of cellulose under the given experimental conditions.

In order to track down the mechanism of CA biodegradation experimentally, periodic measurement of chemical changes need to be done during the biodegradation assay. This can be done by periodic extraction and freeze drying of the CA samples from the experimental test flask followed by characterization using NMR for determining DS and GPC for determining molecular weight. An enzyme assay could also provide some interesting insights into the biodegradation mechanism.

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