Pure & Appl. Chem., Vol.52, pp.399-407.
Pergamon Press Ltd. 1980. Printed in Great Britain.

0033-4545/80/0201-0399 $02.00/0

SYNTHETIC POLYMERS AND THE LIVING ENVIRONMENT

G.J.L. Griffin

Department of Non-metallic Materials, Brunel University, Uxbridge, Middlesex, UK.

Abstract - The interaction between synthetic polymers and the physical environment, especially in terms of the effects of oxygen and radiant energy, has been extensively studied and reported and directly useful results have been obtained by the use of experimental techniques of a kind very familiar to physical chemists. The introduction of living organisms to the system, however, adds to the acknowledged difficulty of polymer characterisation other problems more familiar to the biologist. Attempts to reduce the complexity of the system and create study models such as a single homodisperse polymer in contact with a single organism are very misleading because of the mutual dependancy of living things and the delicate balance between organisms, polymer, and auxiliary nutrition.

This field is reviewed here, and extended with observations on the interdependance of macro and micro biological phenomena in the destruction of plastics together with the significance of oxidation as an intermediate stage in the sequence of events.

INTRODUCTION

The primary object of this paper is to summarise the evidence for the breakdown of synthetic polymers in the natural environment under the influence of living organisms. Certain new experimental information is also offered on the question of an oxidative first step in the process of biodegradation of polyolefines. The precise meaning of the term biodegradation has been the subject of some questioning over the last decade. It would appear to imply a very clear organism/material relationship where a conjunction of the specific biological vector and the precise material will always produce a given set of changes implying that a particular process in the life action of the organism, such as the excretion of an identifiable enzyme, is linked to a chemically recognisable cleavage of the molecular structure of the substrate material. Current activity in this field is here reviewed as a survey of recent literature.

It can be equally argued, however, that a particular case of functional decay of a material, e.g. loss of strength, substance, transparency, or good dielectric properties should be termed biodegradation where it is known to be identifiable with exposure of the material to a living environment, which may itself be very complex, and the property loss may be attributable to physical or chemical actions as first steps in an elaborate chain of processes. The exposure of a cotton fabric based resin laminate to a humid tropical environment would be typical of this latter situation in which the initial activity could be the colonisation of the surface by a damp film of fungi encouraged by surface dirt with a subsequent swelling of the laminate surface layers caused by seepage of moisture along the cellulose fibres. The swelling of the fibres causing cracking and disintegration of the resin matrix would be the first stage of properties loss followed rapidly by 'true' biodegradation of the fibres. Biological attack on the resin matrix might, indeed, never occur or might itself be preceded by atmospheric oxidation of the fractured surfaces.

Finally, we must also acknowledge the contribution of a macrobiological vector, this being the mechanical destruction of plastics materials by creatures larger than the bacteria and fungi in the course of nest building , burrowing in search of food, or direct chewing or attrition due to a presumption of nutritive value. This latter situation may be deliberately encouraged by a modification of the plastic to make it attractive to particular insects or crustaceans whose habitat and life cycle is appropriate to achieving destruction of a plastic artefact after it has served its useful purpose and especially if it has been improperly discarded.

We can summarise the probable degradation routes thus :

i) Direct biodegradation, involving a direct enzymic scission of the macromolecule which may be followed by metabolisation of the cleavage products, or a progressive enzymic assimilation of the macromolecule starting from the chain ends.

(ii) Indirect biodegradation, oxidative cleavage of the macromolecule followed by metabolisation of the fragments.

(iii) Macrobiological degradation, mechanical attrition of the plastic by rodents, insects, crustacea etc. probably followed by type (i) or (ii) action accelerated by the fine state of subdivision of the material.

DIRECT INTERACTION BETWEEN SYNTHETIC POLYMERS AND ENZYMES

General

The one type of degradation where direct enzyme dissolution of synthetic polymers seems to be beyond dispute is in those cases where actual penetration of a solid polymer matrix by fungal hyphae can be established visually. The classic example is the interaction between polyester polyurethane elastomer and Ulocladium Chartarum as described by Levisohn (Ref. 2). A recent photomicrograph of a discrete event of this kind is seen in Fig.1.

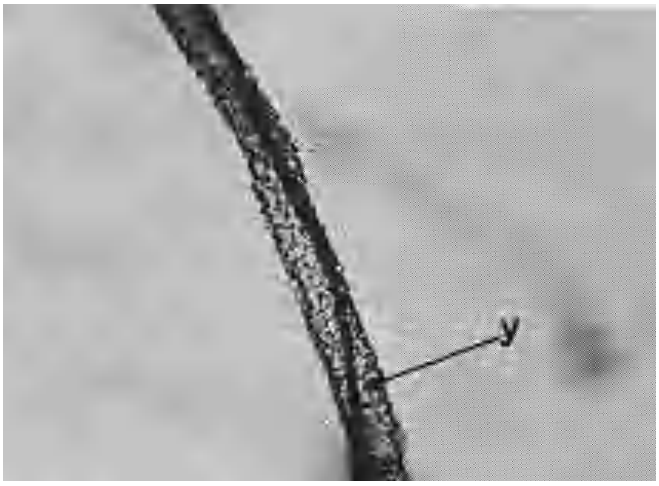

Fig.1. An optical photomicrograph of a fungal hypha in its tunnel etched into ICI 'Daltorol' cast polyester polyurethane elastomer. The hypha y is about 2 micrometres in diameter and the tunnel is about 10 micrometres in diameter.

Similar action has been noted in Starch/EVA blends by Griffin & Mivetchi (Ref. 43). The Author has found it possible to monitor the growth of single hyphal tips within small blocks of clear polyester polyurethane cast on glass cover-slips. Time-lapse cinematography through a microscope over periods of 6-10 hours gave excellent recordings of tip growth, although the extraction of quantitative data was made difficult by apparently random variations in direction, growth velocity, and branching.

It is clear to the observer that no mechanical forces are at work in this hyphal penetration, the fungal filament lies slack in its generous excavation whilst the hole simply grows longer ahead of the growing tip. It is therefore tempting to place the investigation on a more precise footing by attempting a formal analysis of the action. We can list the variables affecting such a venture as follows :-

Subject to direct visual observation -

i. Geometrical factors
ii. Growth kinetics
iii. Branching frequency
iv. Relative growth rates of branches

Measurable local environmental influences -

i. Ionic strength
ii. pH
iii. Surface charge
iv. Temperature
v. Concentration of auxiliary nutrients

The geometrical and kinetic data can be derived by the frame by frame analysis of the cinematographic records subject only to errors of growth velocity components along the optical

axis. Even this error can be minimised by rejecting records in which the growth path leaves the focal plane of the microscope to any significant extent.

The microenvironmental conditions at the hyphal tip present more serious experimental problems and would probably have to be derived from bulk values.

Biological Principles

Experience with bacterial cultures suggests the possibility of an induction of enzyme production by the substrate (Ref. 48). However, macromolecular substrates with their highly restricted molecular mobility pose the problem of access to the protein-synthesis activity, within the microorganisms, although general biological evidence might admit the possibility of surface receptors responding to such a substrate despite their gel or solid form. We have examples in bacterial membranes and in the hormone receptors of higher animals. There seems also, no fundamental reason why the surfaces of fungal hyphae should not be capable of responding to macromolecular substrates. In the case most studied, however, we do not know whether the PU degrading enzymes are constitutive (i.e. substrate independent), or induced.

In order to explain hyphal growth kinetics in terms of enzyme kinetics the enzymes responsible must first be isolated and studied in vitro. This done, then theoretical models could be formulated.

The simplest would involve two growth phases -

A) Initiation of tunnel & B) growth by extension of the tunnel.

A naive model would thus be a steady state kinetic scheme in which a constant concentration of active enzyme(s) is/are maintained in the growing zone and where growth is assumed to be restricted to the tip region. Given the surface area of the polymer tunnel at the growth zone (noting the problem of its indefinite boundary) and the enzyme concentration in the growth zone extracellular fluid we can calculate the local rate of dissolution of the tunnel end simply from the enzyme kinetics. The tunnelling geometry might be depicted simply as in Fig.2.

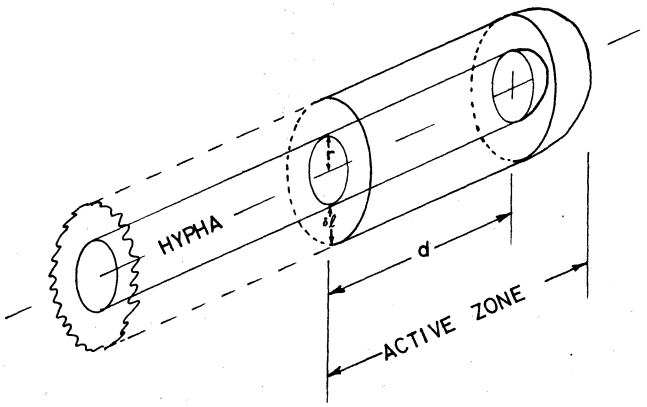

Fig.2. Diagrammatic representation of fungal penetration into an homogeneous polymer by direct enzyme etching.

However, the form of kinetic equation must depend on the mode of heterogeneous catalysis involved and there will be effective concentrations (that is local thermodynamic activities) of chain ends and loops. Different enzymes or different subunits of the same multimeric enzyme may be required to degrade these different sections of polymer. There may also be different rates applicable to areas of different chain enlargement. The differential etching of polymer surfaces by oxidising agents is a reminder of this possibility.

These observations will only relate to short time periods because there are statistical variations amongst the hyphae in a mycelium and the growth may even be oscillatory. The real situation with possible induced enzyme generation and multi-step enzyme processes could prove to be exceedingly complex (Ref. 49) but it is hoped that calculated averages might lead to bulk invasion rates being deduced and it is this latter quantity that related to the progressive destruction of the bulk polymer.

It is clear that useful contributions to this study will be derived from the large body of work existing on the degradation of natural macromolecules such as cellulose but the refractory nature of the common plastics and their often complex formulations combine with the complexity and variability of live biological systems to present some formidable problems.

INDIRECT BIODEGRADATION

General

It is generally accepted that high molecular weight is a major factor in inhibiting the microbiological assimilation of polymers. There is, therefore, no difficulty in accepting published descriptions (Refs. 50-51) of the biological assimilation of certain polymers after massive exposure to ultraviolet radiation in the presence of appropriate degradation catalysts. There has, however, been accumulating evidence that the 'difficult' polymers are also very slowly degraded by processes whose final stages are biological under certain environmental contact situations even in the absence of light. The earliest observation is the work of Oppenheimer and his colleagues in the medical field (Ref. 4) where implants of radioisotope labelled polymers, including polyethylene, were seen to be steadily and progressively losing carbon judging by the appearance of radioactivity in the urine of the experimental animals. Subsequently the observations of Wallhauser (Refs. 52-53-54) on polymer samples retrieved from landfill excavations and composting plants confirmed that very slow attack was occurring. Then we find the radioisotope labelling experiments of Nykvist (Ref. 55) designed to show the biological sensitivity of light degraded polyethylene but incidentally showing a small level of carbon metabolization occurring in the unexposed controls. This could have been attributed to scavenging of low molecular weight polymer normally present in the commercial material but when this work was extended by Albertsson (Refs. 44-45-46) in the form of experiments lasting several years, it became apparent that the tailing off of activity that would have been expected if the low molecular weight fraction explanation was correct did not occur, in fact an actual increase in activity was observed. Such a continuing metabolization must mean a progressive breakdown of the polymer.

The apparent contradiction is resolved if we accept that, under certain conditions a two stage process can operate in which the polymer molecular weight is first reduced by oxidation followed by biological scavenging of the oxidation products. Evidence in support of this action in the composting domestic garbage environment was put forward by Griffin (Ref. 56) on the basis of the detection of peroxides and carbonyl groups in low density polyethylene which had been exposed to warm fermenting garbage containing autoxidising fats.

Experimental

On the assumption that it must be possible to reproduce the composting results using clean and characterised materials the Author set up a series of compositions based on a low additives content polyethylene (I.C.I. Q.3188 with MFI of 2 and density 910kg m^{-3}) blended with 1% of various unsaturated oils and 0.01% of cobalt added in the form of a commercial cobalt naphthenate solution in xylene .

The compositions were mixed in a miniature internal mixture of 10g capacity in an atmosphere of nitrogen, the discharged material being immediately pressed into sheets, about 100 micrometres thick, between polyester foils in a hydraulic press with steam heated platens. These sheets, stripped of their protective foils, were examined by ESR spectroscopy whilst maintained at 80°C in an oxygen atmosphere. Specimens were also mounted for IR spectroscopy, maintained in a circulated air oven at 80°C, being removed for spectroscopy at regular intervals. A further set of 12mm wide strips were cut from the pressed sheets and mounted between roller grips in an Instron tensile tester set to record a 10% elastic elongation slope every hour. The sample and grips were maintained at 80°C in a precision oven fitted in the working area of the tester.

Similar results could be obtained by feeding cold preblends of polymer granules and oil additives into the hopper of a single screw extruder (45mm diameter screw of 20 : 1 LD ratio) attached to a film extrusion die and cooling tower. This technique doubtless exposed the polymer melt more to the atmosphere but the period of exposure was brief. Good film samples could be obtained speedily but larger quantities of material (circa 1 kilo) were required and the exact composition of the film was not as certain as in the small scale milling and pressing operation.

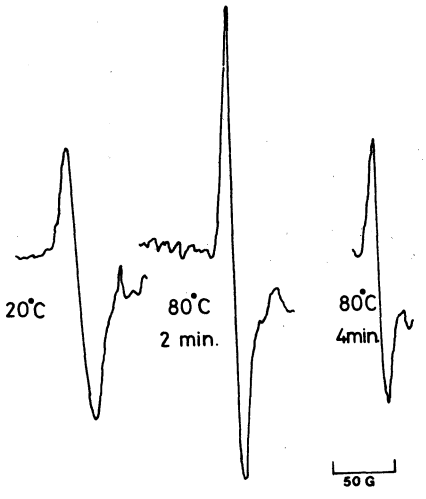

Fig. 3. Free radical ESR responses from a low density polyethylene film containing 1% alkali refined linseed oil and 0.01% cobalt as naphthenate.

Fig. 3 shows the way in which a typical ESR response appeared and diminished in an LDPE sample containing 1% of alkali refined linseed oil and 0.01% of cobalt as naphthenate. All the indications are that the spectrum is associated with hydroperoxy radials, although the shape is not quite as expected. Because of the competing reactions in this complex system it is difficult to ensure that the specimen is put into the spectrometer at such a stage in its oxidation as to produce the spectrum reliably and it may be desirable to simplify the procedure in some way.

By deriving a carbonyl concentration index from the infra red spectra it was possible to plot the growth of carbonyl content against time and this is shown in Fig. 4.

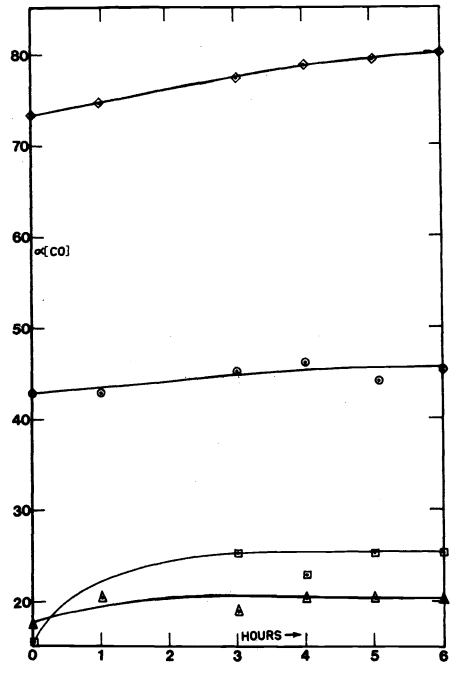

Fig. 4. The carbonyl content index of low density polyethylene film (△), the same polymer containing 0.01% of cobalt as naphthenate (□), 1% soya oil (○) and 1% soya oil with 0.01% cobalt as naphthenate (◇). The index is shown as a function of time at 80°C in air.

In this it is evident that cobalt alone has very little effect on LDPE under these mild conditions, whereas the combination in the case recorded of refined soya oil and cobalt

soap shows a dramatic increase far greater than with soya oil alone. The carbonyl level is greater than would be expected from the minute amount of oil actually present in the IR beam path but it is clearly necessary to demonstrate in some positive way that oxidative transfer is taking place between the oil and the polymer. The simplest demonstration of this effect is to show the corresponding changes in the elastic properties of the polymer which might be expected to stiffen initially with crosslinking and then degenerate as the molecular weight diminished. These changes can be seen clearly in Fig.5 which records the tangent modulus of LDPE containing these co-oxidants, and controls, as a function of time at 80°C. It is frustrating not to be able to get closer to zero time because of the technological problems of sample preparation, but the effect is clear enough. This work has been extended and confirmed by molecular weight change and this will be reported separately.

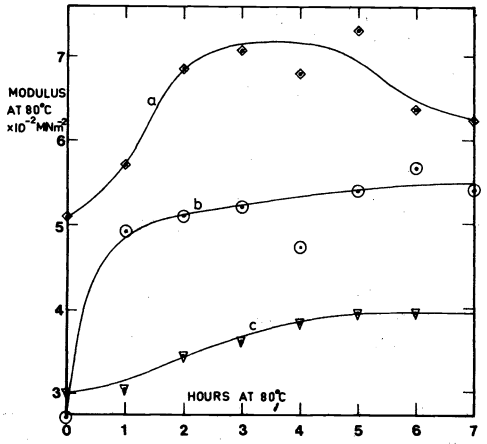

Fig.5. The change in tangent modulus with time of exposure to air at 80°C of low density polyethylene film alone (▽), the same polymer plus 0.01% cobalt as naphthenate (⊙), and with 1% soya oil and 0.01% cobalt as naphthenate (◇).

MACROBIODEGRADATION

The subject of plastics under attack by living creatures larger than bacteria and fungi was extensively reviewed by this Author with Turner in 1978 (Ref. 57) and need not, therefore, be considered in such detail here. Its scattered early literature, entirely concerned with the harmful influence of the attack on man-made artefacts, was given a more formal structure with the appearance of publications from Becker and his Berlin colleagues. For example on insect attack in 1962 (Ref. 58), and again in 1972 (Ref. 59). In a similar manner Schreyer wrote on rodent attack in 1972 (Ref. 60). An opposite view of the situation, i.e. macrobiological attack as a beneficial component of the natural cycle, became possible with the observation, reported initially in (Ref. 57) that controlled attractiveness of material to the animal vector could be achieved with the special case of the crustacean isopoda, the woodlouse, and the novel composite polyethylene/starch (Ref. 61). It now seems possible to engineer a composite by choosing filler ratio, filler particle size, and product geometry so as to create a particular degree of attraction to the woodlouse thus elevating this ubiquitous and attractive creature to the status of potential plastics litter scavenger. Current experimentation confirms the willingness of the woodlouse to discharge this function optimally when it is enjoying its normal humid environment and diet on decaying leaves or wood, that is to say it does not have to be starving to resort to eating starch/polyethylene composite. It is possible to make a film 50 micrometres thick such that 50mm squares will be totally consumed by woodlice in a few weeks. The polymer is discharged in the faeces of the crustacea as submillimetre particles harmless to the environment and in an ideal condition for slow indirect biodegradation to continue. It is clearly likely that other small scavengers could become involved in this action and, most important, the parallel creatures active in the marine environment could be dragooned into beneficial environmental housekeeping to deal with the alarming burden of novel artefacts finding their way into deep water (Ref. 62).

LITERATURE REVIEW

It is interesting to note how the early interest in polymer/environment interactions changed from a preoccupation with damage by micro-organisms to military equipment having plastics components and stored goods wrapped in plastics films, as is typified by the papers of Klausmeier (Ref. 1) to a concern with pollution by discarded plastics items made from embarrassingly long lived polymers. This latter interest built up a large body of publications starting from around 1970 many of which were more alarmist than informative. A mild conflict appeared between those who contended that many polymers were everlasting in the absence of UV radiation and those who thought otherwise. In 1966 a very factual paper appeared (Ref. 2) first as a PATRA report in which Miss Levisohn reported a clear case of a micro-organism/synthetic polymer interaction involving polyester PU. Later observations of polylactones (Ref. 3) originally synthesised for fibre improvements is a second such clear case. Meanwhile medical research work on implants and prostheses had stimulated studies of biocompatability in which as early as 1955 we find Oppenheimer (Ref. 4) using C14 labelling to demonstrate a progressive biological metabolisation of polystyrene in the bodies of experimental animals.

If we look now at the more recent literature spanning 1976 to 1978 it is interesting to note a sharp growth in research work on biosensitive polymers intended for medical work. Vezin & Florence at Glasgow are investigating Poly-N-alkyl cyanoacrylates (Ref. 5) whilst at the University of Naples Ferruti & others (Ref. 6) are examining Polysaccharide succinic esters as biodegradable drug release matrices.

At the University of Connecticut Bell, Huang, Knox & Others have been studying polyurethanes, -benzylated nylons, polyurea, poly (ester-urea) blends with phenylalanine and hydroxyacid copolymers (Refs. 7-11). At North Carolina State University Penn (Ref. 12) & Lynn (Ref. 13) have been working on block copolymers such as those containing amylose units.

At the National Cancer Institute, Bethesda, Bergman (Ref. 14) and others are examining the biodegradation of polylactic acid in the form of urethral grafts. The US Army Medical Bioengineering Laboratory at Fort Detrick are reviewing biocompatability by biodegradation testing in order to achieve short term evaluation of materials (Refs. 15-16).

At the University of Maryland, Bailey (Refs. 17-18) has started work on rather complex polyamides which, by ingenious chemistry, achieve hydrophilicity and biodegradability. The US Army Dental Research Institute in Washington D.C. has been looking at the biological hazards of using polylactate and polyglycollate materials as implant materials (Ref. 19). These same polylactic acid materials have interested Chang at McGill (Ref. 20) and are a commercial development of the Dynatech Research and Development Co (Ref. 21). A variation is seen with the work of Schindler and others (Ref. 22) at the Research Triangle Institute N.C. using caprolactone dilactide for drug release systems. At the University of Groningen, Netherlands, Marck and others (Ref. 23) have looked at the biodegradability of random copolymers of l-leucine, l-aspartic acid and l-aspartic acid esters. An extensive study of the biodegradation of Polylactones by hydroxybutyrate specific bacteria and fungi has been described in three papers by Tanaka of the Industrial Fermentation Research Institute, Chiba, Japan (Refs. 24-25).

Another approach to surgical implant elastomers is to be found in work at the University of Liverpool by Reed and others (Ref. 26) using poly (ethyleneoxide)-poly (ethyleneterephthalate) copolymers. Rather more exotic chemistry comes from Japan with Tabuse and colleagues at the Kohjin Chemical Co. claiming biodegradability for polyol modified phosphonitrilic polymers (Ref. 27), and from Russia where Sukharukova and others have been producing biodegradable polyurethane semicarbazides bearing alkyl thio side groups (Ref. 28).

A common feature of all this medically oriented work is a willingness to accept complex chemistry with attendant high cost. The actual goal of direct, i.e. enzyme chain breaking, biodegradation seems to be achievable provided only that at least the continuous simple carbon chain backbone is avoided by introducing N substituted amide links, ester links or, under some circumstances, ether links. A warning note by the US Army Bioengineering and Dental Units reminds us that the breakdown products of these exotic polymers cannot automatically be assumed as acceptable in toxicological terms but we should remember that they are concerned with the very specialised environment of body tissues.

When we consider non-medical application areas we find a much poorer yield. The USDA laboratory at Peoria describes various cellulose and starch graft copolymers (Refs. 29-30) and also water sensitive films cast from ethylene/acrylic acid copolymers extended with soluble starch (Refs. 31-32). The water sensitive route to biodegradation is also reflected in studies by Casey and Manley in an elaborate examination of Polyvinyl alcohol metabolisation in activated sludge (Ref. 33), a field also reviewed by Hahn and his colleagues at Hydroscience Inc of New Jersey (Ref. 34). Evidence of commercial activity using PVA in applications other than as textile sizes, comes from the Patent of Comerford and Kapur of Personal Products Inc. USA (Ref. 35).

Early observations of the biodegradability of ester plasticisers has led to a few studies of polyesters as biodegradable polymers, for example Tsuji of the Kuriraay Co. Japan, has

recorded Alternaria Sp. degrading polyhexamethyleneadipate and polyethylenepropylene adipate (Ref. 36), a field also reviewed by Weisfeld of Seton Hall University, South Orange, N.J. (Ref. 37). We also have Hatakeyama and his colleagues at the Industrial Products Research Institute, Tokyo, working steadily on methoxy-hydroxystyrene polymers, seen as models of the natural material lignin (Refs. 38-40). Finally the Author's work on starch-autoxidant polyolefine formulations as economically feasible degradable films and mouldings continues to develop (Refs. 41-43).

Few papers are devoted specifically to studying the 'mechanics' of biodegradation and the work of Albertsson at Stockholm is unique in many respects (Refs. 44-46) reporting an impressively lengthy investigation using the C14 labelling technique.

Also interesting is a Patent by Yogo and Minoda of the Mitsubishi Oil Co (Ref. 47) claiming biodegradation of Polyethylene by Acinetobacter calco aceticus and cunninghamella elegans. In this work it is claimed that HDPE loses 8.9% of its weight in 60 days incubation in a medium of nutrients at 30°C. The specified presence of Fe in the nutrient may be significant in view of the importance of oxidation in these situations.

ACKNOWLEDGEMENTS

The Author would like to record his gratitude to his son, Dr. W.G. Griffin, for valuable suggestions and discussions on the subject of enzyme/polymer interactions. He is also grateful to Dr. R.A. Lott of the Chemistry Department, Brunel University, for assistance with ESR spectroscopy. and to Mr. C. Bewick for skilful experimental assistance with time-lapse photography.

REFERENCES

1. Klausmeier, R.E. Internat. Biodet. Bull. 8, 1, 3 (1972).
2. Levisohn, I. Patra (UK) Printing Laboratory Report 71 (1966).
3. Potts, J.R. Glendinning, R.A. and Ackart, W.B. P.I. Conference Preprints 12-1. Deg. of Polymers and Plastics, London (1973).
4. Oppenheimer, B.S. et al Cancer Research 15, 335-340 (1955).
5. Veein, W.R. and Florence, A.J. J.Pharm. Pharmacol 30, Brit. Pharm. Conf. Supp 5, (1978).
6. Ferruti, P. Tanzi, M.C. Vaccaroni, F. Makromol. Chem. 180, 2, 375-82 (1979).
7. Huang, S.J. Knox, J.R. et al J.App.Pol. Sci. 23, No.2, 429-437 (1979).
8. Huang, S.J. Knox, J.R. A.C.S. Symp. Series 49, Text. Pap. Chem. Technol. Symp. 73-80 (1977).
9. Leong, K.W. Diss. Abs. Intl. B. 38(1), 216 (1977).
10. Bell, J.P. Huang, S.J. and Knox, J.R. U.S. NTIS Rep. Issue AD-A 029935 (1975).
11. Huang, S.J. Bell, J.P. and Knox, J.R. et al. Proc .3rd Int. Biodegradation Symp. 731-741 (1976).
12. Penn, B.J. Diss. Abs. Int. B. 39(5) 2330-1 (1978)
13. Lynn, M.M. Diss. Abs. Int. B. 39(5) 2330 (1978)
14. Bergman, S. et al. Invest. Urol. 16, 48-49 (1978)
15. Megyeli, A.F. et al. Coll. Proc. of 1st and 2nd Conference on short term in vitro test for Carcinog. Mutagen. Tox. 50-75. Pub. Franklin Inst. Press, Phil. Pa.
16. Wade, C.W.R., Hegyeli, A.F. and Kulkarni, R.K. J.Test.Eval. 5,(5) 397-400 (1977).
17. Bailey, W.J. et al. Proc. 3rd. Int. Biodegradation Symp. 765-773 (1976).
18. Wu, Y.S.H. Diss. Abstr. Int. B. 38 (10) 4837-8 (1978).
19. Nelson, J.F. et al. Oral Surg. Oral Med, Oral Path 43 (6) 836-843 (1977).
20. Chang, T.M.S. J.Bioeng 1 (1), 25-32 (1976)
21. Wise, D.L. Acta. Pharm. Suec. 13, (Supplement) 34 (1976).
22. Schindler, A. et al. Contemp. Top. Polym. Sci. 2, 251-289, (1977).
23. Mark, K.W. J.Biomed. Mater. Res. 11, (3) 405-422 (1977).
24. Tanaka, H. et al. Biseibutsu Kogyo Gijutsu Kenkyusho Hokoku 48, 67-74 and 75-79 (1976).
25. Tanaka, H. et al. Nippon Nogei Kagaku Kaishi 50, (9), 431-436 (1976).
26. Reed, A.M., Gilding, D.K. and Wilson, J. Trans. Am. Soc. Art. Int. Organs. 23, 109-115, (1977)
27. Tabuse, I. et al. Japan Patent No.76134800 of 20 May(1975). Japan Kokai Pub.22 (1976).
28. Sukhurukova, S.A. et al. Sint. Fiz. Khim. Polim. 18, 24-27 (1976).
29. Dennenberg, R.J. et al. J.App. Pol. Sci. 22 (2), 459-465 (1978).
30. Bagley, E.B. et al. US Patent 4026849 (1975).
31. Otey, F.H. and Westhoff, R.P. US Patent 4133784 (1977)
32. Otey, F.H. and Westhoff, R.P. US Patent App. 837931 (1977)
33. Casey, J.P. and Manly, D.G. Proc. 3rd Internat. Biodegradation Conf. 819-833 (1976)
34. Hahn, W.H. Barnhart, E.L. and Meighan, R.B. Proc. Ind. Waste Conf. 30, 530-539 (1977).
35. Comerford, J. and Kapur, C. Fr.Pat. 2308656 (1975)

36. Tsuji, M. and Icihara, Y. Hakko Kogaku Kaishi 56 (6) 799-801 (1978)
37. Weisfeld, J. Diss. Abstr. Internat. B 38 (4) 1939 (1977)
38. Nakamura, K. and Hatakeyama, H. Plast. Age 24 (8) 88-9 (1978).
39. Hatakeyama, H. Plast. Age 23 (4) 88-91 (1977)
40. Hatakeyama, H., Hayashi, E. and Haraguchi, T. Polymer 18 (8), 759-63 (1977).
41. Griffin, G.J.L. US Patent 4125495 (1973).
42. Griffin, G.J.L. Brit. Patent 1 489 050 (1973).
43. Griffin, G.J.L. and Mivetchi, H. Proc. 3rd Int. Biodeg. Symp. 807-813 (1976)
44. Albertsson, A.C. J.App. Pol. Sci. 23(12), 3419-33 (1978).
45. Albertsson, A.C. and Ranby, B. Proc. 3rd Int. Biodeg. Conf. 743-51 (1976).
46. Albertsson, A.C. J.App. Pol.Sci. 22 (12), 3435-47, (1978).
47. Yogo, M. and Minoda, Y. Japan Patent No.77 56175 (1975).
48. Watson, J.D. Molecular Biology of the Gene, 2nd Ed. 438. Pub. Benjamin, New York (1970).
49. Boyer, P.D. The Enzymes, Kinetics and Mechanism, Vol.II, pub. Academic Press, New York (1970).
50. Potts, J.E. Glendinning, R.A., Ackart, W.B. & Niegisch, W.D. Polym.Sci.Tech. 3, 61-79 (1973).
51. Guillet, J.E. Regulski, T.W. & McAneney, T.B. Envir.Sci. & Tech., 8, No.10 923-925 (1974).
52. Wallhauser, K.H. Verpackungs Rundschau, 3, 266 (1972).
53. Wallhauser, K.H. Mull. U. Abfall, 1, p10 (1972).
54. Wallhauser, K.H. Plastics & Rubber Inst. Conf. Degrad. of Polymers, Preprint Vol. London (1973).
55. Nykvist, N.B. Plastics and Polymers, Vol. 195-199 (1974).
56. Griffin, G.J.L. J.Polym.Sci. Symposium No.57, 281-286 (1976).
57. Griffin, G.J.L. and Turner, Ruth D. International Biodeterioration Conference, Berlin, August 1978 (In press).
58. Becker, G. Zeit fur Angew. Zoo. 49, 95-109 (1962).
59. Becker, G. and Seydlitz-Kirzbach, E. Material u Organismen, 7, 161-176 (1972).
60. Schreyer, G. Konstruiren mit Kunstoffen, Pub. Carl Hanser Verlag, Munchen, 743 (1972).
61. Griffin, G.J.L. Advances in Chemistry, Series 134, Fillers and Reinforcements for Plastics, A.C.S., 159-170 (1974).
62. Cundell, A.M. Environmental Conservation, 1, 63-68 (1974).