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# MICROBIAL DEGRADATION OF THE MICROBIAL POLYESTER CO-POLYMER POLY HYDROXY BUTYRATE / VALERATE

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ABSTRACT

Screening of environmental specimens was performed in order to isolate polyhydroxy - butyrate / valerate degrading bacteria. Basal mineral salts medium supplemented with the polymer was used as enrichment medium. Isolates capable of degrading the polymer and hence clearing the opacity of the polymer containing solid medium were selected. The isolates capable of degrading the polyme were obtained and characterized a *Ekinella corrodenes* , *Alcaligenes faecalis* and *Klebsiella pneumoniae.* The three isolates were found to show extracellular polyhydroxy alkanoate ( PHA) depolymerase activity. Crude enzyme preparations were used to study the characteristics of these enzymes. The optimum temperatures and pHs for activity, substrate specificity , effect of dialysis , divalent cations , various inhibitors and detergents on depolymeterase and esterase activities were studied for the three isolates . The effect of cultural and nutritional conditions on enzymes production were studied for *E. corrodenes* .

## INTRODUCTION

At present , disposing of synthetic polymers after use is a growing problem and microbial degradation of these polymers has been a concern of polymer consumers, environmentalists industry and investigators [1-3].

Poly hydroxyalkanoates (PHA) are synthesized and accumulated intracellularly during unbalanced growth by a large variety of bacteria reaching up to 90% of the cell dry weight [4] . Poly 3- hydroxybutyrate (PHB) is produced by a wide variety of microorganisms. Some bacteria have been found to accumulate polyhydroxyalkanoates, P(3HA), other than P3 HB [5,6].

A copolymer containing 3-and 4-hydroxybutyrate units has been found in *Alcaligenes eutrophus* [7] . A copolymer of 3-hydroxy butyrate and 3 hydroxyvalerate (P 3HBCO 3HV) has been produced commercially by *A. eutrophus* from propionic acid and glucose by ICI ( Imperial Chemical Industries) under the name "Biopol" [8-10]. Poly 3-hydroxybutyrate and related PHA are regarded as very promising candidates for biodegradable themoplastic which do not pollute the environment [11] .

Aerobic and anaerobic microorganisms which produce depolymerase enzymes capable of degrading PHA polymers are widely distributed and have been isolated from variety of biological environments [12-15] .

To assess the microbial biodegradation of the co-polymer polydroxybutyrate/ valerate and various factors related to its clearance from environment , this study was undertaken .

## MATERIAL AND METHODS

### Chemicals :

### Polymers :

The polymers used in this study consisted of polyhydrox- alkanoates (PHAs) and as a co-polymers of 3-hydroxybutyrate with 3-hydroxyvalerate (PHB/V) with different percentages of the latter as denoted by the numbers as follows : PHB/V 7.3, PHB/V 11.9, PHB/V 15.6, PHB/V 24. These polymers were obtained from ICI Chemicals Polycaprolactone diol (PCL) polymers with varying approximate molecular weight as denoted by the numers were obtained from Aldrich Chemical Company and Comprised: PCL 530, PCL 930, PCL 1250, PCL 2000 and PCL 10, 000.

### Culture Media :

The culture medium used in all studies consists of basal mineral salts ( BMS) solution containing per liter: $K_2HPO_4$ $7H_2O$ 0.7g; $NaNO_3$, 1.0g; and trace mineral solution, 1 ml. The trace mineral solution contained per litre: NaCl 5g; $FeSO_4$ $7H_2O$ 2g ; $ZnSO_4$ $4H_2O$ 2g; $MnSO_4$ $H_2O$ 7g. The mineral salt solution was filter sterilized and added aseptically to the basal mineral. The phosphates were sterilized separately from the rest of the medium.

For preparing solid media 10g of Noble agars (Difco, Detroit, USA) was added per litre.

### Polymer preparation :

For PHB and PHB/V polymers, a fine suspension containing 1.0g per 100 ml distilled water was prepared by sonication of polymer pellets using probe sonicator (Bransonic 1510). This suspension was used in plate clearing assay as well as in tube depolymerase assay.

PCL suspension was prepared by dissolving 1.0 g of PCL pellets in 30 ml acetone to get a clear solution, and slowly adding distilled water with stirring to obtain milky white precipitate. The suspension was filtered through glass wool to remove any aggregates of particles. This suspension was used in plate clearing and in tube assay for depolymerase enzyme at 2% (v/v).

### Isolation of polymer degrading organism by enrichment culture:

Sediment samples (5g) were obtained from

natural water sediment (from Willimantic River, Connecticut, USA) initially cultured at room temp in 50 ml BMS (pH 7.3) containing 2% w/v PHB/V 15.6 as carbon source . After one week, 0.5 ml sediment enrichment was transferred to 10 ml MBS containing 5% of the polymer . Enrichment cultures were maintained through 4 biweekly transfers followed by 5 monthly transfers. Samples from the enrichment cultures were platted on MBS agar containing PHB/V polymer dispersion. After incubation, colonies surrounded by clear zones were picked, streaked on trypticase soy (TS) agar, and indentified according to previous standard methods (Bergey's Manual of Systematic Bacteriology, 1984 and API system) .

Fresh trypticase soy broth cultures of the isolated were stored at 4°C in 50% glycerol. Working cultures were transferreed weekly on TS agar and incubated at 25°C.

**Enzyme production :**

A loopful of the organism from a plate containing the polymer as sole carbon source was used to inoculate 50 ml of the TS broth as starter culture. The cultures were grown overnight on a reciprocating shaker 20°C before being used to inoculated 400 ml MBS containing PHB/V polymer in a 1500 ml beaker. Sterilized two pices of glass tubing and a magnetic stirring bar were placed and the beaker was covered with an inverted petri dish.

The PHB/V polymer covered microscopic slide were prepared by dropping clean microscopic slide to 3/4 of its length in chloroform solution of the polymer and allowing chloroform to evaporate. This process was repeated several times to provide a significant polymer film. Microscopic slides 3/4 of its length covered with PHB/V films and placed in a staining rack were immersed into the BMS above the level of the magentic stirring bar. The whole assembly was incubated at 20°C and continuously stirred over a magnetic stirrer for an appropriate time. The optical density and the depolymerase activity were measured periodically.

Once the optical density exceeded 1 and the depolymerase activity exceeded 2, the cells were removed by centrifugation at 10,000 g for 10 minutes and the pooled supernatant was filtered through 0.2 $\mu$ cellulose acetate membrane filter (Millipore). Supernatant with low activity levels was concentrated by ultrafiltration using Amicon YM10 membrane ultrafilter in a thin channel filtration cell. The process usually takes 8hr to concentrate 1 litre to 100 ml.

**Enzymes Assays :**

**Depolymerase Assay :**

A densitometric assay using a suspension of PHB/V polymers was used. The reaction mixture, with a final volume of 2ml, consisted of 0.5 ml phosphate buffer (pH 7.3) , 0.5 ml PHB/V suspension and 1 ml of enzyme preparation. The optical density at $\lambda$600 nm was followed for 10 minutes with readings taken every 2 min. A control of the buffer and polymer was monitored simulatneously. The depolymerization activity was expressed as units of enzyme .

The enzyme unit was defined as a change of 100 unit/ ml / min of a suspension of PHB/V.

**Esterase Assay :**

The substrate in assay was p-nitrophenyl caproate ( Sigma Chemicals ) . The substrate (5mM) was sonicated in phosphate buffer (pH, 7.3) , distributed into 3 ml aliquots and frozen at -20°C unitl just before use. The assay was initiated at room temperature by mixing 0.5 ml phosphate buffer, 0.5 ml substrate suspension and 1 ml enzyme preparation. The absorbance at $\lambda$-420 nm was recorded every 30 sec. for 5 min. A unit of esterase activity was defined as the change in absorbance by 1 unit/ ml/ min.

**Cell growth and release of depolymerase :**

The growth and depolymerase production were monitored by taking sample every 8h over the entire incubation period at 20°C . Depolymerase activity was followed by taking 5 ml samples , removing cells by centrifugation at 7,000 g and filtration through 0.2 $\mu$ and using in the test as described before .

**Effect of temperature on enzyme :**

For determination of the optimum temperatures, the component of the assay reaction were prewarmed to the desired temperature and maintained at that temperature during reaction. For determination of the heat stability of the enzyme , the enzyme preparation in 5 ml aliquots were held at varying temperature in water bath for 30 min before assaying its activity .

**Effect of pH on enzyme activity :**

A series of assay mixtures with varying pH between 5 and 12 as well as appropriate controls were prepared before adding the enzyme preparation and conducting the assay as before . This pH range was covered by Sorenson's phosphate buffer (pH 5-7), TRIS (tris-hydroxymethyl amino methane HCl , pH, 7.5-8.5) and Sorenson's glycine buffer (pH 9-12).

To study the effect of EDTA (0.2 , 0.4, 0.6, 0.8 and 1mM) were ordered in the reaction mixture. adjusting the pH to 7.3 before carrying out the assay

The effect of soybean trypsin inhibitor STI (Sigma Chemicals ) was studied by including STI solution in phosphate buffer (pH 7) in the reaction mixture with final concentration range from 0 to 2.5 mg/ml. Controls of STI and substrate were included

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To study the effect of detergents on depolymerase , the deterogents under test were included in the reaction mixture at final concentration of 0.1 M. The detergents used included tween 80 (American Research Company), hexadecyltrimethyl ammonium bromide, 3-(3 cholamidopropyl) dimethylammonio-1-propane sulfonate (CHAPS-1), 3 (3-cholamidopropyl) dimethylammonio -2hydroxyl -1 propane sulfonate (CHAPSO-1) and n-octyl β-D glucopyranoside ( Sigma Chemicals ) .

To study the effect of phenyl methyl sulfonyl fluoride (PMSF) on enzymes activities , it was included in the reaction mixture at a final concentration of 1mM.

**Substrate specificity of depolymerase :**

Various PHB/V and PCL polymers were included, one in time , in the reaction mixture and assay was followed as before .

**Effect of Chaoptrophic agents on depolymerase activity :**

The effect of KSCN and SDS on depolymerase was determined by adding the agent to the enzyme preparation at final concentration of 1% w/v and determination of the enzyme activity was cattied out after filtration. The chaoptropic agent - enzyme complex was dialyzed against 5 mM phosphate buffer and the activity of the dialyzed enzyme was tested.

**Determination of cofactor requirement :**

Aliquots of 100 ml of each enzyme preparation were dialyzed at 4°C for 24 h using cellulose dialyzing tube with MW cut off 12.000 Da against 3 litres of deionized water. The dialyzed enzyme preparation was assayed for both depolymerase and esterase activities. The reaction mixture was supplemented by addition of each of the following components BMS, filtrate from the concentrated enzyme preparation using Amicon filter with MW cutoff 10, 000 Da in the concentration process, 0.7 g/l $MgSO_4$ and BMS without $MgSO_4$.

## RESULTS

A screening programme was launched for isolation of bacterial strains capable of degrading PHB/V co-polymer from natural water sediment (from Willimantic River, Connecticut, USA) . After enrichment in basal salts medium containing the polymer as a sole carbon source and isolation on agar medium of the same composition, three isolates were obtained that demonstrated zones of clearance around their colonies. These isolates were identified as *Eikenella corrodens* , *Klebsiella pneumoniae* and *Alcaligenes faecalis* .

The increase in cell mass of *E. corrodens* and the exhibited depolymerase and esterase activities in the medium during growth on basal mineral solution containing the polymer as a sole source of carbon is presented in Fig. 1 The other isolates demonstrated more or less similar profiles .

Supernatants concentrated by ultrafiltration (using Amicon YM10 membrane ultrafilter) were used as crude enzyme preparation for further study . The effect of temperature on the activity and the stability of the crude depolymerase enzyme preparations of the three isolated are presented in Figure 2 and 3, respectively. The effect of pH of the depolymerase activity is presented in Figure 4.

The effect of EDTA on depolymerase and esterase activities of the three isolates is presented in table 1.

The effect of soybean trypsin inhibition (STI) on the depolymerase and esterase activities of the three isolates is presented in table 2. The effect of various detergents on depolymerase activity of the three isolates is presented in table 3.

The inhibitory effects of 0.1 mM phenyl methyl sulfonyl flouride (PMSF) on depolymerase and esterase activities of the three isolates are shown in table 4.

To study the effect of chaoptropic agents on depolymerase activities, potassium isothiocyanide (KSCN) and sodium dodecyl sulfate (SDS) were used at final concentration of 1% w/v and dialyzed before assaying for the activities . KSCN caused complete loss of the depolymerase activities of the three isolates. SDS caused partial inhibition of depolymerase activities of the three isolates by 50% , 50% and 37% for *E. corrodens, K. pneumoniae,* and *A. faecalis,* respectively.

In order to study the substrate specificity of the depolymerase and the effect of increasing valerate ratio in the PHB/V co-polymer as well as increasing the MW of polycaprolactone (PCL) polymer on polymer degradation were tested and the results are presented in table 5 and 6 . Binding of the depolymerases to the polymers was determined by adding the polymer to the enzyme preparation, at a 1% rate of equilibration for few minutes. This was followed by filtration and determination of the residual activity in the filtrate. The results are presented in table 7.

For investigation of a possible co- factor involvement , the effect of dialysis and addition of various ions on the enzyme activities were tested . The results of such treatments are presented in table 8.

To study the effect of cultural and nutritional factors on the growth and depolymerase production of *E. corradens,* the effect of various carbon and nitrogen sources as well as minerals were tested . The effects of carbon source on growth of *E. corrodens* and on depolymerase production are shown in Fig. 5 and 6. The addition of 0.1% of sodium acetate, Tween 80 or

**Table (1) :** The effect of EDTA on depolymerase and esterase activites of *E. corrodens. A. faecalis* and *K. pneumoniae.*

| Enzyme activity units / EDTA conc. mM | *E. corrodens* | | *A. faecalis* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|
| | Depolymerase | Esterase | Depolymerase | Esterase | Depolymerase | Esterase |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 0.2 | 80 | 95 | 99 | 100 | 90 | 100 |
| 0.4 | 80 | 95 | 95 | 100 | 90 | 97.5 |
| 0.6 | 75 | 92.5 | 95 | 95 | 85 | 97.5 |
| 0.8 | 68.75 | 90 | 92.5 | 95 | 80 | 90 |
| 1% | 62.5 | 90 | 90 | 93 | 79 | 90 |

**Table (2) :** The effect of STI on depolymerase and esterase activites of *E. corrodens. A. faecalis* and *K. pneumoniae.*

| Enzyme activity units / EDTA conc. mM | *E. corrodens* | | *A. faecalis* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|
| | Depolymerase | Esterase | Depolymerase | Esterase | Depolymerase | Esterase |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 0.5 | 95 | - | 100 | - | 100 | - |
| 1 | 95 | 85 | 98.5 | 100 | 97.5 | 78 |
| 1.5 | 90 | 80 | 97.5 | 95 | 95 | 75 |
| 2 | 90 | 74 | 97.5 | 100 | 95 | 70 |
| 2.5 | 89 | 70 | 95 | 100 | 91 | 65 |

**Table (3) :** Effect of detergents on depolymerase activity of *E. corrodens. A. faecalis* and *K. pneumoniae.*

| Enzyme activity / Detergent | *E. corrodens* | *A. faecalis* | *K. pneumoniae* |
|---|---|---|---|
| Control | 100 | 100 | 100 |
| Tween 80 | 70 | 90 | 87 |
| CHAPS | 62 | 80 | 43 |
| SDS | 40 | 34 | 15 |
| CHAPSO | 30 | 75 | 87 |
| CTAB | 0 | 0 | 0 |
| Octyl β-glucopromide | 50 | 60 | 40 |

**Table (4) :** The effect of phenyl methyl sulfony flouride (0.1 mM) on depolymerase and esterase activities of *E. corrodens*, *A. faecalis* and *K. pneumoniae*.

| Enzyme activity units / EDTA conc. mM | *E. corrodens* | | *A. faecalis* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|
| | Depolymerase | Esterase | Depolymerase | Esterase | Depolymerase | Esterase |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 10 | 52.5 | 40 | 40 | 30 | 45 | 35 |
| 20 | 35 | 30 | 30 | 25 | 41.2 | 20 |
| 30 | 30 | 16 | 20 | 15 | 25 | 0 |
| 40 | 20 | 0 | 10 | 0 | 10 | |
| 50 | 10 | | 5 | | 0 | |
| 60 | 0 | | 0 | | | |

**Table (5) :** Effect of valeric acid percent in the copolymer on the activities of PHA depolymerase of *E. corrodens*, *A. faecalis* and *K. pneumoniae*.

| Depolymerase activity (units) / % of valeric ml | *E. corrodens* | | *A. faecalis* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|
| | | % | | % | | % |
| 0 | 141.6 | 100 | 254 | 100 | 33 | 100 |
| 7.3 | 141.6 | 100 | 225 | 88.6 | 41.66 | 126 |
| 11.9 | 137.5 | 97.1 | 195.8 | 77.08 | 50 | 151.5 |
| 15.6 | 133 | 93.92 | 138 | 54.33 | 58 | 175.75 |
| 24 | 50 | 353 | 133 | 52.36 | 66 | 200 |

**Table (6) :** Effect of molecular weight of polycarprolcatone (PCL) polymer on its degradation by depolymerase from of *E. corrodens*, *A. faecalis* and *K. pneumoniae*.

| Enzyme activity (units) / PCLMW | *E. corrodens* | | *A. faecalis* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|
| | | % | | % | | % |
| 530 | 41.66 | 100 | 85 | 100 | 48.3 | 100 |
| 930 | 38.33 | 92.8 | 78.3 | 92 | 48.3 | 100 |
| 1250 | 31.66 | 76 | 70 | 82.35 | 43.3 | 89.64 |
| 2000 | 31.66 | 76 | 70 | 82.35 | 38.3 | 79.29 |
| 10.000 | 11.66 | 28 | 53.3 | 62.7 | 18.3 | 37.88 |

**Table (7) :** Binding of Depolymerase to PHB/V and PCL polymers ( relative activity represents residual activity).

| Depolymerase activity (%) | Max. activity (no binding) | Binding to PHB/V 7.3 | Binding to PHB/V 24 | Binding to PHB/V 10.000 |
|---|---|---|---|---|
| *E. corrodens* | 100 | 70 | 60 | 85 |
| *K. pneumoniae* | 100 | 57 | 43 | 71 |
| *A. faecalis* | 100 | 71 | 57 | 79 |

**Table (8) :** The effect of analysis and addition of magnesium ion, basal salt and dialysate to the dialysed preparation on depolymerase and esterase identities.

| Treatment | *E. corrodens* | | *A. faecalis* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|
| | Depolymerase | Esterase | Depolymerase | Esterase | Depolymerase | Esterase |
| Concentration enzyme preparation | 100 | 100 | 1000 | 100 | 100 | 100 |
| Dialyzed enzyme | 40 | 25 | 125 | 110 | 50 | 20 |
| Dialyzed enzyme + Mg ion | 57 | 40 | 100 | 100 | 65 | 35 |
| Dialyzed enzyme + BMS with Mg | 60 | 50 | 110 | 100 | 70 | 40 |
| Dialyzed enzyme + BMS without Mg | 60 | 40 | 100 | 100 | 60 | 40 |
| Dialyzed enzyme + concentrated filtrate | 100 | 100 | 100 | 100 | 100 | 100 |

* Percentage as activity

tributyrin to the culture with depolymerase in declining phase ( after 4h) did not affect the activity. .

On the other hand, addition of 0.02% w/v of bovine serum albumin (BSA) or gelatin at this stage resulted in transient increase in depolymerase activity before declining , while 0.02% sodium caseinate or 0.5mM ammonium sulfate had slight or no effect (Fig. 7). After the initial potentiation, further additionn of BSA had no effect on depolymerase activity but affected further potentiation in protease activity .

## DISCUSSION

The environmental problems associated with disposal of plastics have spurred efforts to develop biodegradable plastics. Designing a plastic material that exhibits structural and functional stability during storage and yet susceptible to microbial and environmental degradation upon disposal without any adverse environmental impact is a real challenge. The design of appropriate biodegradable material requires clear understanding of factors influencing material performance properties and biodegradability .

The properties of PHB as biodegradable thermoplastic material have attracted attention for more than 20 years [4,16,17] . Although a lot of effort has been made to explore the physiological and molecular basis for the synthesis and accumulation of PHA [4, 19-21] little work has been done on the degradation of these polyesters [18, 22, 23, 24, 25] . One of the commercially attractive features of PHAs is their biodegradability in the natural environment, effected principally by enzymatic activities of microorganisms [ 15, 25, 26 ]. In the present study, the search for bacterial isolates capable of utilizing PHA as a sole carbon source in

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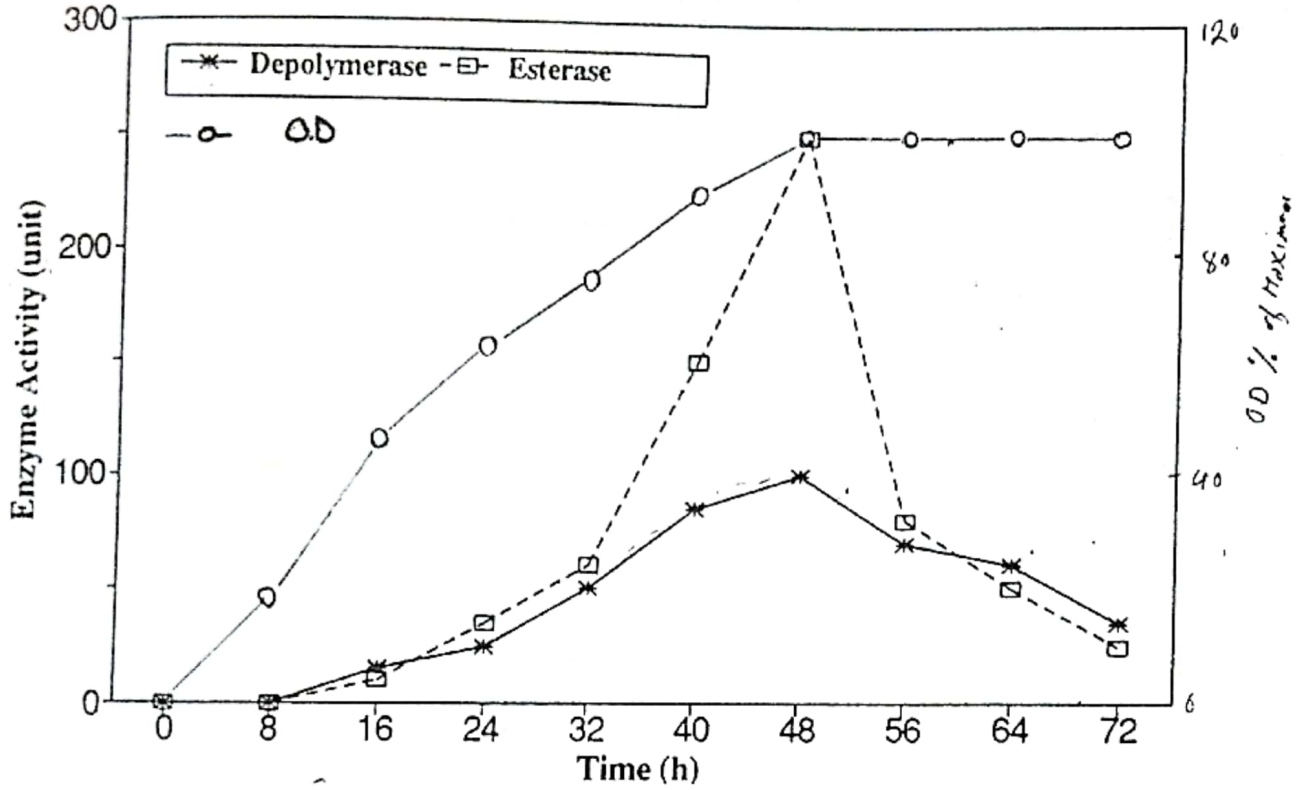


**Fig. (1) : Depolymerase and esterase of *e. corrodens* .**

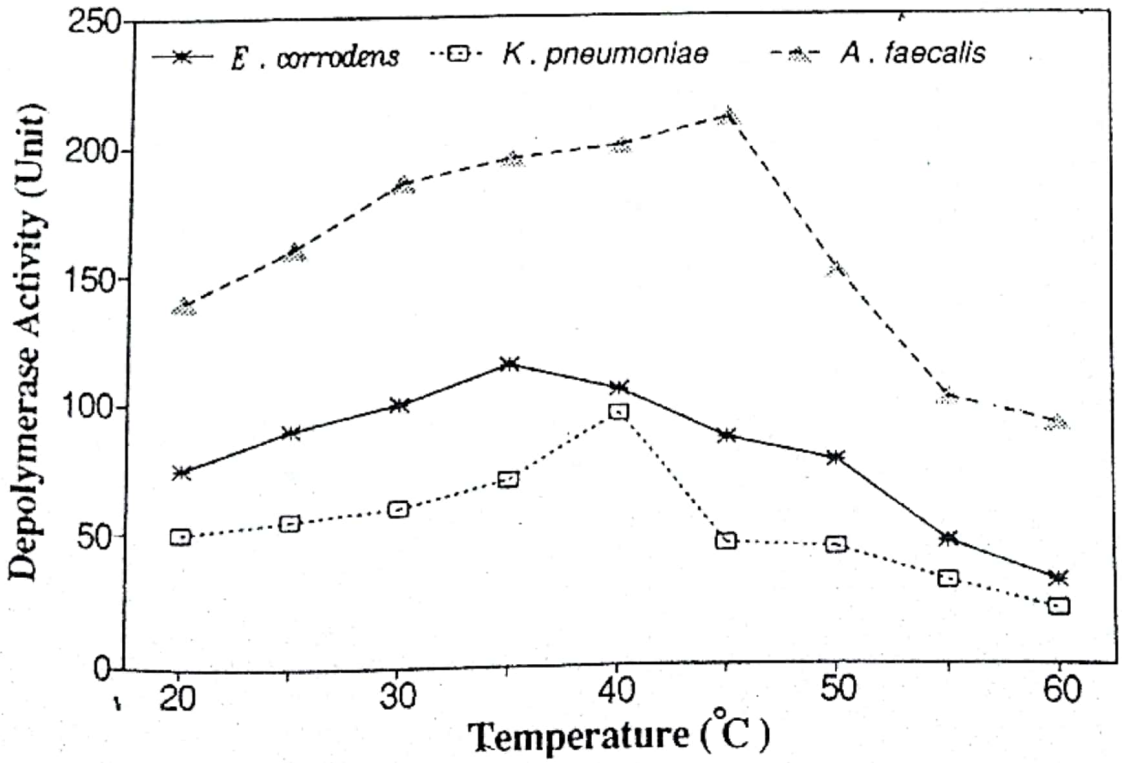


**Fig. (2) : Depolymerase optimum temperature.**

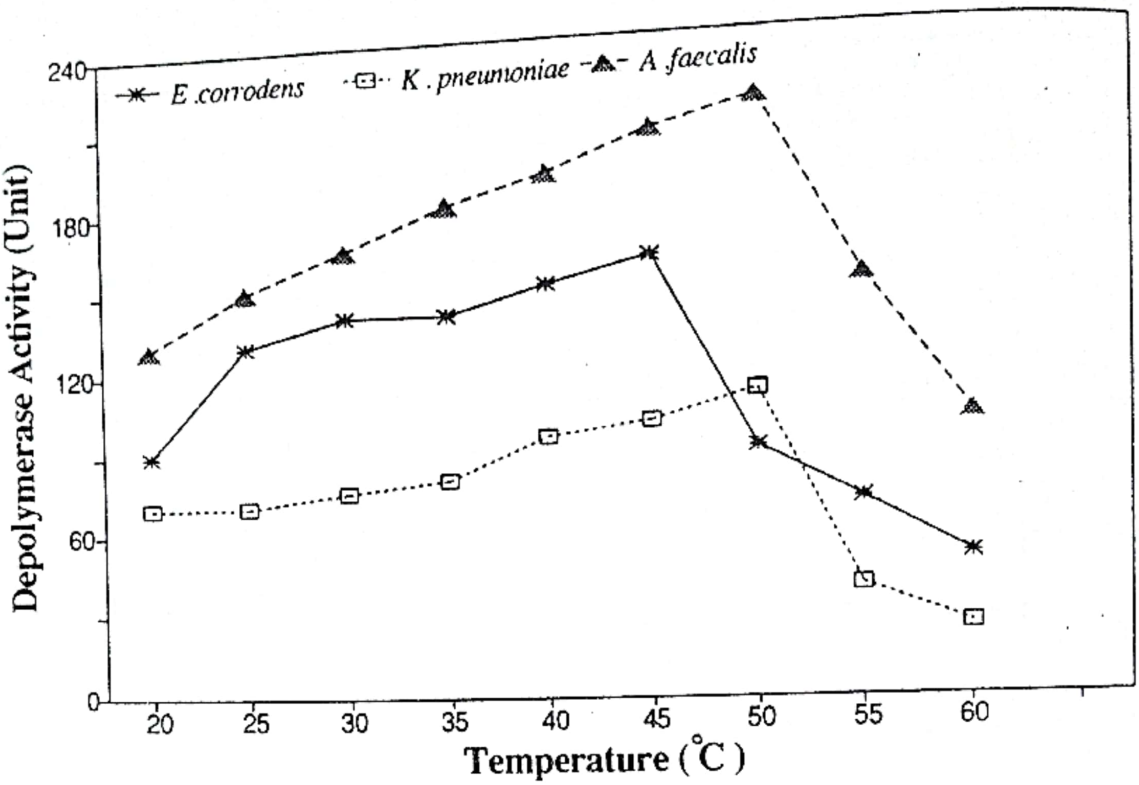


**Fig. (3) : Depolymerase temperature stability.**

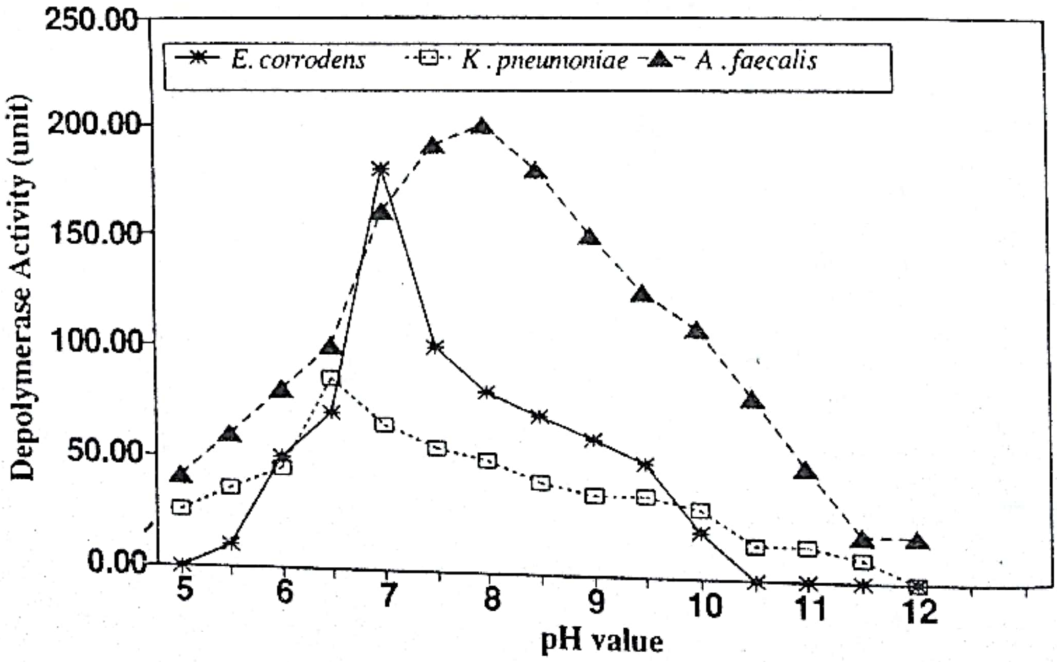


**Fig. (4) :Effect of pH on depolymerase activity.**

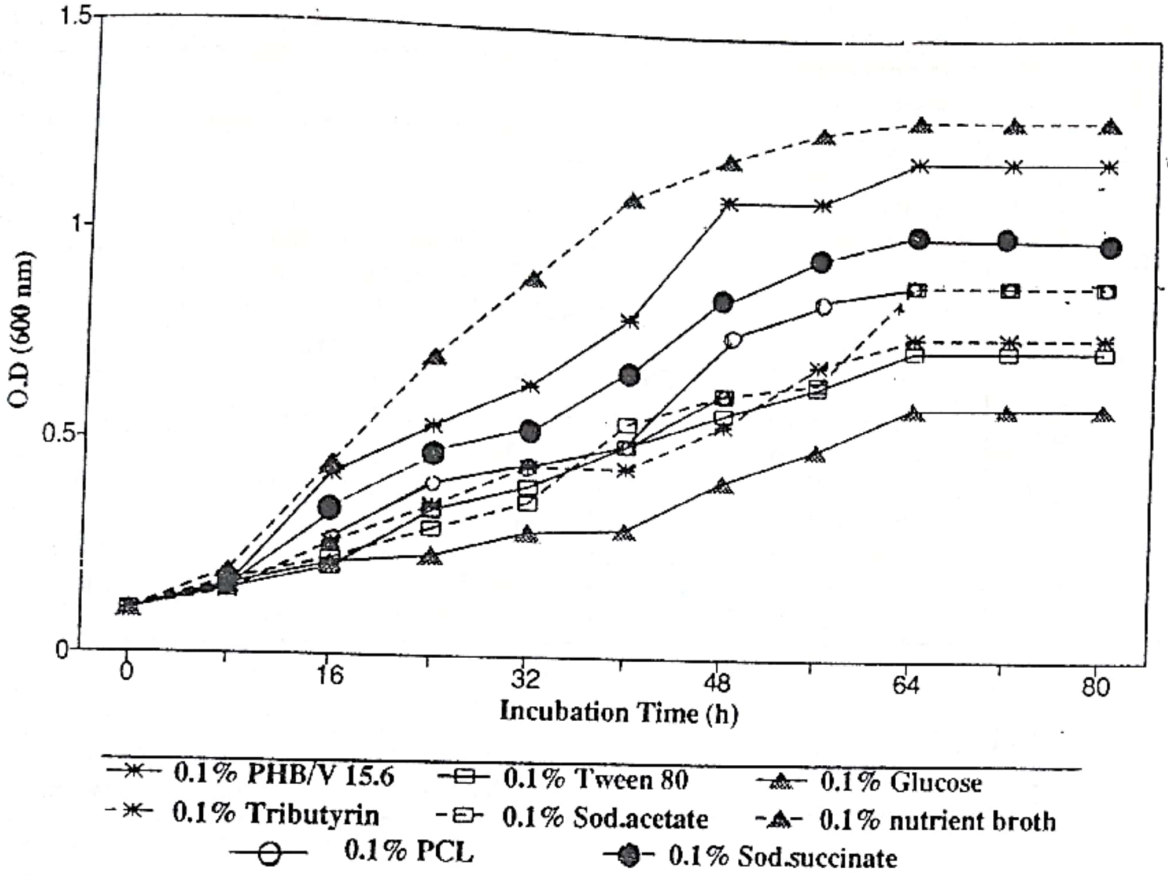


**Fig. (5) : Effect of different carbon sourcees on growth of *E. corrodens*.**

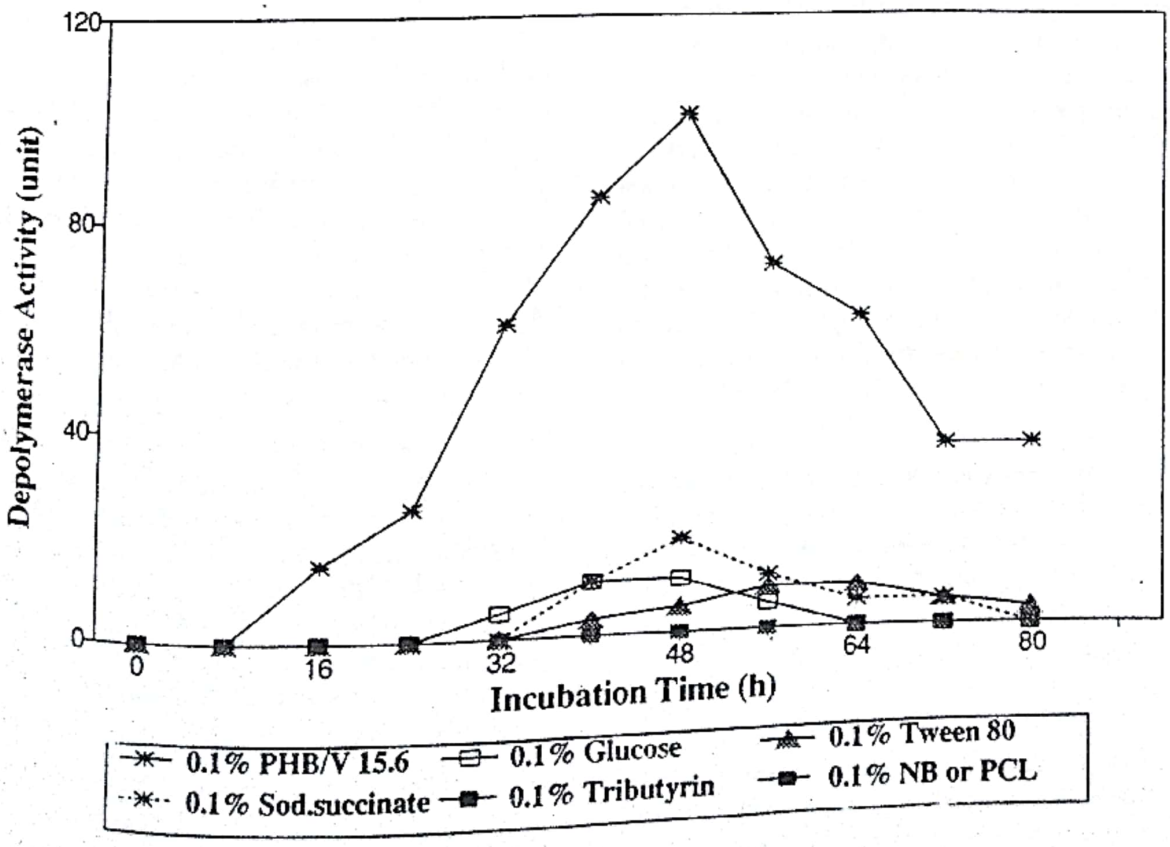


**Fig. (6) : Effect of different carbon sourcees on *E. corrodens* depolymerase.**

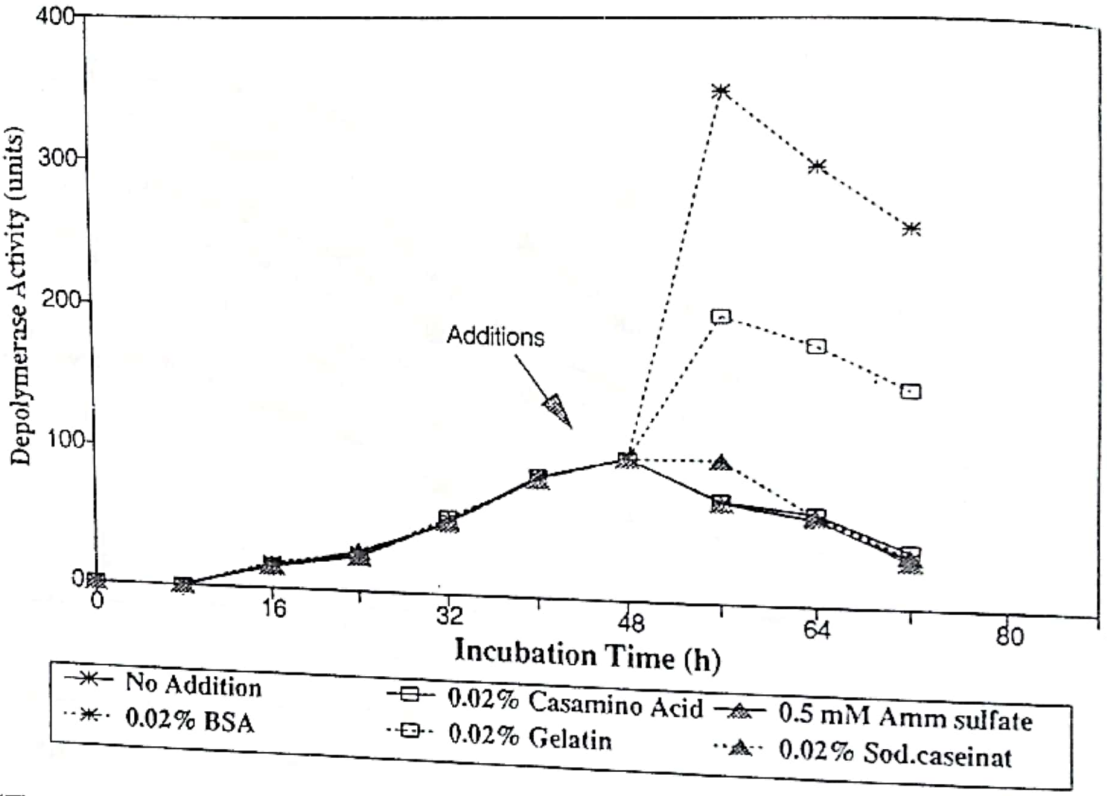


**Fig. (7) : Effect of different nitrogen addition on depolymerase of *E. corrodens***

natural water samples was undertaken . Using PHA as a carbon source in enrichment and solid media of basal minerals yielded three isolates that were capable of degrading the polymer. These isolates were tentatively identified as *E. corrodens, A. faecalis* and *K. pneumoniae*. The polymer was used as sole carbon source to avoid catabolic repression in the presence of soluble substrates [15]. For *E. corrodens,* the depolymerase activity increased with growth to its maximum and started to decline rapidly after growth planting. The decline in depolymerase activity could be attributed to its denaturation or its degradation by proteases.

The close association between depolymerase and esterase activities during growth was also obtained by **Bunch ,** [27] . This may indicate that the enzyme which depolymerizes the polyester is capable of breaking ester bonds.

The depolymerases of the three isolates are relatively heat stable up to 50°C . The optimum activities of these depolymerase were 35°C for *E. corrodens,* 40°C for *K. pneumoniae* and 45°C for *A. faecalis* . These depolymerase work at pHs between 6 to 10 with optimum pHs 7, 6.5 and 8 for *E. corrodens, K. pneumoniae* and *A. faecalis* , respectively.

Divalent cations (e.g. $Mg^{2+}$) seen to have a role in depolymerase activities of *E. corrodens* and *K. pneumoniae* that were partially inhibited by the chelating agent EDTA. Such inhibition never exceeded 40% of the maximum activity at concentration of 1 mM EDTA . This concentration affected the esterase activity to a lesser extent with less than 20% inhibition. On the other hand, the depolymerase and esterase activities of *A. faecalis* were only slightly affected, with less than 10% inhibition by 1 mM EDTA.

The difference in depolymerase and esterase activies due to EDTA could be attributed to the presence of two different enzymes, the presence of more than one class of active site, or the difference in substrate solubilities . [15] reported that divalent cations did not affect PHB depolymerase activity of *A. faecalis* while PHB depolymerase of *Comamonas sp* was found to be inhibited by 1mM EDTA [28] . PCL depolymerase and estease activities of *C. laurentii* were inhibited by EDTA with depolymerase activity more affected [29].

The proteinase inhibitor soybean trypsin inhibitor (STI) was used for a possible distinction between depolymerase and esterase activities [30] . The results showed moderate inhibition of esterase activities of *E. corradens* and *K.pneumoniae* by STI but no

significant inhibition was seen with depolymerases. Neither depolymerase nor esterase activities of *A. faecalis* were affected by STI. The results favours the assumption that these enzymes have different active sites .

Cationic detergents, e.g. cetyltrimethyl ammonium bromide and hexadecyltrimethyl ammonium bromide completely inhibited the depolymerases of the three isolates at 0.1 mM concentration. Equivalent concentrations of anionic detergents e.g. sodium dodecyl sulfate (SDS) , had a moderate inhibitory effect on depolymerase activities . Nonionic detergents e.g. Tween 80, CHAPSO, CHAPS and Octyl β–D-glucopyranoside showed little inhibitory effect on depolymerase activity . The results may indicate the presence of hydrophobic region in vicinity of the active site for depolymerase activity . Detergents such as deoxycholate (0.1%) and trition X-100 (non-ionic surfactant) showed strong inhibitory effect on depolymerases in other studies [15, 26, 31]. **Fukui et al.,[32] and Shiraki et al.,[26]** showed that depolymerase enzyme of *A. faecalis* contains a hydrophobic active site and was inhibited by the presence of detergents.

The complete inhibition of depolymerases by phenylmethyl sulfonyl fluoride (PMSF) indicates the presence of active serine residue in their active sites analogous to that of *Pseudomonas fluorescens* [25] , *A. faecalis* and *Ps. Lemoigni* [26].

Incorporation of valeric acid and increasing its ratio in the copolymer resulted in a progressive decrease in the depolymerase activities of *E. corrodens* and *A. faecalis* and an increase in the depolymerase activity of *K. pneumoniae*. These results may reflect differences in structure and active sites between these enzymes. Depolymerases of *A. faecalis* T1, A1 and *P. picketti* were shown to degrade PHB or co-polymers with a high 3-HB contents but poorly degrade PHV. Conversely. *A. entrophus* enzyme degraded copolymers with a high PHV content faster than PHB[26].

In comparison with PHA , PCL is less susceptible to depolymerase activity of the three isolates and the activity of the three isolates and the liability to degradation decreased with increase in MW of PCL. Decrease in MW decrease surface hydrophobicity, increase the concentration of OH and possibly COOH groups, thus increasing solubility in reaction medium and exposure to enzymes sites resulting in greater degradation [33,34]. SDS seen to have denatured the depolymerases causing approximately 50% loss in their maximum activities , while KSCN caused complete loss of enzyme activities . These chaoptropic agents would affect hydrogen bonding between water molecules making the enzymes untable and more liable to disruption.

Dialysis resulted in a decrease in depolymerase and esterase activities of *E. corrodens* and *K. pneumoniae* and an increase in that of *A. faecalis*. Addition of $Mg^{2+}$, MBS or broth were partially restored the lost activity of depolymerases and esterases of *E. corrodens* and *K. pneumoniae* while abolishing the increased activity of those of *A. faecalis* induced by dialysis . On the other hand, addition of the dialysate completely restored activities. These results suggest a possible role of small molecules or cofactors in the activies of these enzymes [35].

Cultural studies demonstrated a good growth on nutrient broth, tributyrin, Tween 80, sodium acetate, sodium succinate, PCL or glucose as carbon source. However, the highest depolymerase activity was obtained when the isolates were grown on BMS containing PHB/V as the sole carbon source. These results show that the enzyme is inducible . The basal insignificant depolymerase activity could be attributed to the formation of PHA granules inside bacteria . The presence of readily assimilable carbon sources seem to cause repression of the utilization of the polymer by catabolite repression [12] . The addition of nitrogenous compounds like ammonium sulfate, sodium caseinate and casamino acids had no effect on depolymerase activity. However, bovine serum albumin and gelatin temporarily increased the depolymerase activity. This can be explained by the assumption that these proteins are preferentially used as substrates for protease enzymes that would degrade the depolymerases.

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# التحلل الميكروبى للبولي إستر المكون من حمضي هيدروكسي بيوتريك وحمض الفاليريك الميكروبي

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للمساهمة فى التخلص من النفايات البلاستيكية من أثر ميكروبى بشكل أمن للبيئة وفى محاولة للبحث عن سلالات ميكروبية لديها القدرة على تكسير البولى استر المكون من حمض هيدروكسى بيوتريك والفاليريك أمكن عزل ثلاث عزلات بكترية من رواسب نهرية لديها القدرة على ذلك .

وبالتعرف على هذه العزلات تبين أنها ايكنيلا كورودينز و كليبسلا نيمونيا والكاليجينز فيكاليز وقد أظهرت هذه العزلات مقدرتها على تكسير البوليمر وذلك بزوال عتامة الوسط الغذائى الصلب والمتسبب بوجود البوليمر حول المستعمرات البكترية .

وتبين أن ذلك يرجع إلى افراز انزيم يكسر البولى هيدروكسى الكانوات وقد تم تحضير خلاصات خام هذه الانزيمات من الوسط البكتيرى لهذه العزلات واجراء دراسة مبدائية لبيان خصائصها وأنشطاتها المختلفة وتأثير درجة الحرارة ودرجة الحموضة على فعاليتها وكذلك ثباتها الحرارى كما تم دراسة تأثير أيونات المعادن ثنائية الشحنة ومثبطات الأنزيمات المختلفة وكذلك المنظفات الصناعية ونوع البوليمر ووزنه الجزئى على أنشطة الانزيمات المختلفة وبخاصة تكسير البوليمر وتحلل روابط الاستر.

وقد أظهرت خلاصات الأنزيمات الأولية تفاوتا فى خصائصها . وقد تم أيضا دراسة تأثير الوسط الغذائى وظروف نمو البكتيريا على أنتاجية الانزيم لعزلة ايكنيلا كورودينز