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Optimization of Culture Conditions to Enhance Polyethylene Enzyme Production by using Pseudomonas

Botany

KEYWORDS : Polyethylene degrading enzyme, Pseudomonas, optimization

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ABSTRACT

In the present research work, Pseudomonas was isolated from mangroves of pichavaram.Screening was performed with the help of clear zone method. LDPE act as a sole carbon source for the production of polyethylene degrading enzyme. Mineral salt medium was used for the production of enzyme. Enzyme assay was performed to nullify the producing ability of microorganisms. Protein content of the enzyme also calculated. The activity of enzyme production was optimized with the study of culture condition like, incubation time, temperature and pH. The maximum activity was showed in pH 7.5and at temperature 35˚ C after two days of incubation period. The goal of this work was to enhance the polyethylene degrading enzyme production by pseudomonas to be applied in the enzymatic degradation of polyethylene in future.

INTRODUCTION

Polyethylene is a synthetic polymer of carbon, hydrogen and oxygen which are derived from petrochemicals. Due to the development of the petrochemical industry, about one hundred million tons of chemically synthesized plastics are produced per year, and environmental contaminations rising because the chemically synthesized plastics are not biodegradable. Enzymes are, first and foremost catalysts. Structurally, enzymes are complex and highly specialized proteins, which produced by the cell, in order to catalyze specific types of chemical functions. Ability of microorganism to degrade extracellular polymers depends on the secretion of specific depolymerases that hydrolyse the polymer to water soluble products [1]. Many microorganisms grow on polymeric materials as their sole source of carbon. Enzyme characterization regarding optimum pH and optimum temperature as well as pH and thermal stabilities during the reaction time is very important for the application of byproducts with catalytic activity. In this present study deals about optimized condition for polyethylene degrading enzyme by using Pseudomonas.

MATERIALS AND METHODS

ISOLATION OF BACTERIA

Soil samples were collected from Polyethylene sheets of Pichavaram mangroves in Tamilnadu, India. (11°29'N; 79°46' E; southeast coast of India)Then samples were transferred into the conical flask having 99 ml of sterile water. This content, which was shaken vigorously for its equal distribution, was serially diluted. The pour plate method was adopted using the Nutrient agar medium for bacteria. For each dilution, three replicates were made. The plates were then incubated at 30°C for 24-48 hrs. Individual Bacterial colonies were isolated & pure cultures were maintained for further study.

IDENTIFICATION OF MICROORGANISMS:

The bacterial strains were identified based on the keys detailed by Holt et al [2]. Colony morphology, Gram staining, Motility, Biochemical characters were used for identification and compared with standard manuals of soil Bacteria.

MATERIALS

Low density polyethylene powder (LDPE) was obtained from Sigma Aldrich Chemical Co. (Germany).

SCREENING OF POLYETHYLENE DEGRADING MICROORGANISMS BY CLEAR ZONE METHOD

Low density Polyethylene powder was added in mineral salt medium at a final concentration of 0.1% (w/v) respectively and the mixture was sonicated for1hour at 120 rpm in shaker. After sonication the medium was sterilized at 121°C and pressure for 15 lbs/inch2 for 20minutes. About 15 ml sterilized medium was poured before cooling in each plate. The isolated organisms were inoculated on polymer containing agar plates and then incubated at 25-30°C for 2-4 weeks. The organisms, producing zone of clearance around their colonies were selected for further analysis. [3]

POLYETHYLENE DEGRADDING ENZYME ASSAY

Polyethylene degrading enzyme assay was performed according to the method described by Kobayash [4]. About 0.3% Polyethylene powder was suspended in 50mM Tris-HCl buffer, pH 7.5 and the suspension was sonicated for 20min in a 300ml flask immersed in as ultrasonic water bath (35KHz, 285W) prior to the dilution to 0.03% in the same buffer. Culture supernatant 0.1ml was added to 0.9ml of the substrate suspension and incubated for 24 hours at 30°C. Activity was measured as the decrease in OD, as measured at 650nm through spectrophotometer, against substrate buffer blanks.

UNIT OF ACTIVITY

One unit is defined as the activity resulting in a decrease in OD 650 per 24 hours [4].

PROTEIN ESTIMATION

Protein concentration estimation was performed for each enzyme sample using method suggested by Lowry [5]. Color developed during the assay procedure was read at 650nm. Finally concentration of protein in the culture supernatant was determined using standard curve of bovine serum albumin.

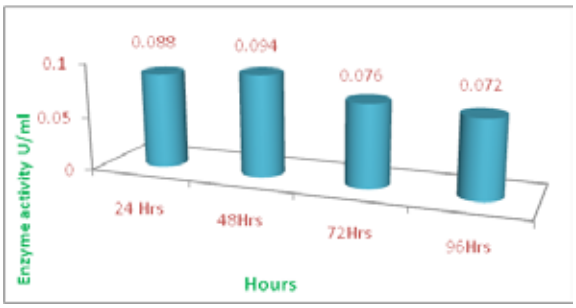
RESULTS

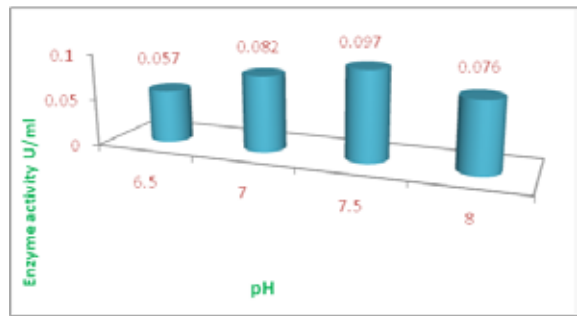
EFFECT OF INCUBATION TIME FOR POLYETHYLENE DEGRADING ENZYME PRODUCTION

The organism inoculated in Mineral Salt Medium containing 0.1% of LDPE powder at different incubation period from 1 to 4days and incubated at 37˚C. Enzyme activity was observed in spectrophotometer at 650 nm.

Table- 1; Fig- 1 Effect of incubation time

S.No	Incubation time	O.D Value
1	24 Hrs	0.088
2	48Hrs	0.094
3	72Hrs	0.076
4	96Hrs	0.072



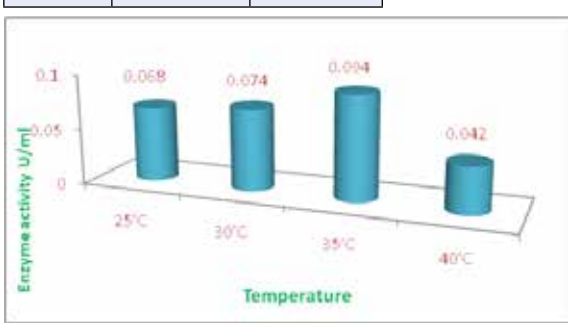


EFFECT OF TEMPERATURE FOR POLYETHYLENE DEGRADING ENZYME PRODUCTION

Mineral salt medium containing 0.1% of LDPE powder at pH 7 was inoculated with1ml Culture. Various temperature were maintained between25 to 40 (25,30,35 and 40) Enzyme activity was observed in spectrophotometer at 650 nm.

Table- 2; Fig- 2 Effect of Temperature

S.No	Temperature	O.D Value
1	25°C	0.068
2	30°C	0.074
3	35°C	0.094
4	40°C	0.042



EFFECT OF PH FOR POLYETHYLENE DEGRADING ENZYME PRODUCTION

Flasks containing mineral salt medium, 0.1% of LDPE powder, with different Ph ranges,(6.5, 7,7.5 and 8) were prepared, inoculated with 1ml Culture suspension and incubated at 37°C for 48hours. pH was adjusted using 1N NaOH and 1N HCl. Enzyme activity was observed in spectrophotometer at 650 nm.

Table- 3; Fig- 3 Effect of pH

S.No	pH	O.D Value
1	6.5	0.057
2	7.0	0.082
3	7.5	0.097
4	8.0	0.076

DISCUSSIONS

Microorganisms secret continuously low amounts of various extracellular hydrolyzing enzymes into their surroundings. The depolymerization products thereby produced are taken up into the cell, where they can induce the synthesis of appropriate amounts of the required or favorable hydrolyzing enzyme [6]. In the present study ,the bacterial species identified from the sample were Bacillus sp., Staphylococcus sp. and Micrococcus sp(Gram- positive bacteria); Escherichia coli,Moraxella and Pseudomonas sp. (Gram-negative bacteria); There were five bacterial and two fungal species, commonly and predominantly found detected in both polythene and plastics[7]. According to the clear zone formation, isolates were selected, it belongs to Pseudomonas sp. The extracellular hydrolyzing enzymes secreted by the target organism hydrolyze the suspended polyesters in the turbid agar medium into water soluble products thereby producing zones of clearance around the colony [8]. In our findings, the activity of enzyme was found maximum 0.092U/ml. Protein Content of the enzyme was found to be 0.32µg/ml .An enzyme was secreted by Pseudomonas in a mineral salt medium, when supplemented with LDPE [0.1%]. The culture medium is key factor for the growth as well as metabolites production by microorganisms. In this present study efficient enzyme production was observed at optimum incubation time was 2 days, pH 7.5 and temperature35°C.

CONCLUSION

From this present investigation it was confirmed that the Pseudomonas species has produced the enzyme and it can be used as a catalyst for the degradation of polyethylene which is an abundant Recalcitrant. Activity of enzyme found maximum at 0.092U/ml and their protein contentwas0.32µg/ml. From our studies also revealed the optimized cultural conditions such as incubation time, temperature and pH. This work will be helpful in enhancing the enzyme production and their activity

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