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A COMPARATIVE STUDY ON MICROBIAL GROWTH AND SUBSTRATE UTILIZATION KINETICS

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Abstract

Microbial growth and substrate utilization kinetics have been studied by many researches time to time in order to estimate the specificity of the particular microbe as potential bioremedial tool. In this present study, bacterial strains were screened and identified for low density polyethylene (LDPE) degradation. Two of the bacterial isolates genetically identified as *Bacillus amyloliquefaciens* BSM-1 and *Bacillus amyloliquefaciens* BSM-2 exhibited significant potential for LDPE degradation of 11% and 16% respectively after 60 d of incubation. This study was carried out to understand and reveal that the variation in specific growth rate and doubling time on LDPE supplemented media compared to that of nutrient broth. The specific growth rate of BSM-1 was $8.3X10^{-3}$ h^{-1} and generation time 4.66 h while for BSM-2 was 0.011 h^{-1} with generation time 5.25 h for supplemented media. In case of nutrient broth, doubling time for BSM-1 and BSM-2 was 1.8 h and 2.4 h respectively whereas specific growth rate for BSM-1 was 0.44 h^{-1} and for BSM-2 was 0.625 h^{-1}.

Keywords: *Bacillus amyloliquefaciens*, LDPE degradation, Specific growth rate, doubling time.

Introduction

Conventionally, plastics are non-degradable and stable organic compounds whose huge applications and simultaneous increasing accumulation in the ambient environment causes a major threat to the living systems. They are polymeric compounds with high molecular weight. These compounds may be degraded by some physico-chemical and biological reaction. Diverse metabolic capabilities of microorganisms have been exploited by man in diverse ways in the biodegradation of waste materials [1]. Microorganisms are using biological catalysts which can degrade either completely

or incompletely these higher molecular compounds to lower one. The potentiality of microbes as agents of degradation of several compounds thus indicates biological treatment as the major promising alternative to attenuate environmental impact caused by pollutants [2]. The microbes are using these degraded compounds as nutrient source that means carbon and energy source. But the organisms utilizing normal carbon sources are exhibiting different microbial growth curve pattern compare to those utilizing these degraded monomers. Microbial degradation of a solid polymer like polyethylene requires the formation of a biofilm on the polymer surface, to enable the microbes to efficiently utilize the non-soluble substrate by enzymatic activities [3].

Growth curves reflect the lifetime interrelationships between an individual's inherent impulse to grow and mature in all body parts and the environment in which these impulses are expressed [4]. In the growth of a bacterial culture, a succession of phases, characterized by variations of the growth rate, may be conveniently distinguished. This is a classical conception, but the different phases have not always been defined in the same way. The following phases are: lag phase: growth rate null; acceleration phase: growth rate increases; exponential phase: growth rate constant; retardation phase: growth rate decreases; stationary phase: growth rate null; phase of decline: growth rate negative. But the modern concept was clearly pointed out that the size of the population after duration of time depends on three factors i.e. Lag growth, Growth rate, ultimately on nutrient. It was also stated that during the growth, cells may vary in size even in same generation [5, 6].

Microbial growth on environmental pollutants using as substrate for growth and proliferation is not new to scientific world and is naturally established which ultimately leads to removal of contaminant form environment. In the recent years there has been a prime interest to bio remediate the contaminants in vitro with an option of microbes [7]. Determination of specific growth rate and saturation constant helps to find out affinity of a particular microbial strain towards a specific compound supplemented as substrate and to be used as nutrient source for microbial growth and proliferation.

This paper is an attempt to report the efficacy of the test organism ability to use low density polyethylene (LDPE) as carbon source for its growth and side by side comparison to that of nutrient broth from kinetics point of view.

Materials and Methods

Preparation of LDPE powder

LDPE films were cut into small pieces, immersed into xylene and boiled for 15 min. It was then crushed with blender at 3000 rpm while it was warm. The obtained powder of LDPE was later washed with ethanol to remove residual xylene, air dried to evaporate ethanol and dried in hot air oven at 60 $^{\circ}$C for overnight to get dry powder, stored at room temperature for further use [8].

Isolation and screening of LDPE degrading bacteria

The LDPE plastic soil samples were collected from the municipal solid waste landfill area, Pallikaranai, Chennai, India. To isolate the LDPE degrading bacteria, the plating was done through spread plate technique using 10^{-6} dilution on synthetic medium agar plates containing 0.3% LDPE powder. The composition of medium was as follows: (g/l: K_2HPO_4 1, KH_2PO_4 0.2, $(NH_4)_2SO_4$ 1, $MgSO_4.7H_2O$ 0.5, NaCl 1, $FeSO_4.7H_2O$ 0.01, $CaCl_2.2H_2O$ 0.002, $MnSO_4.H_2O$ 0.001, $CuSO_4.5H_2O$ 0.001, $ZnSO_4.7H_2O$ 0.001 Agar 15) with pH 7.0. The plates were incubated for 24 h at 37°C. The colonies grown on these plates were purified and maintained on nutrient agar slant at 4°C.

Identification of test organisms

Primarily isolated organisms were subjected to biochemical tests and identified through morphological characters and further genetically identified through 16S rRNA sequencing using forward primer (5'-GAGTTTGATCCTGGCTCA-3') and reverse primer (5'-ACGGCTACCTTGTTACGACTT-3') [9]. The nucleotide sequence of the 16S rRNA gene was compared with published 16S rRNA sequences using BLAST search at data base of National Center for Biotechnology Information (NCBI).

Determination of growth kinetics of bacterial isolates

Growth pattern of isolated strains were seen in synthetic media and nutrient broth (NB) separately. In these experiments, 100 ml of synthetic media was autoclaved, 0.3% LDPE powder was added in synthetic media and 5 ml of 24 h old microbial culture in nutrient broth was added as inoculum. These flasks were kept in rotary shaker at 130 rpm; 37° C. Growth of each bacterial isolate at different time intervals was tested by taking growth O.D (UV-VISIBLE spectrophotometer-8500 II) at 600 nm and graph was plotted using ln (OD/OD_0) vs time. From the graph, the value of

Specific growth rate (μ) and doubling time (t_d) were calculated. Similarly procedure followed for the cultures inoculated in nutrient broth medium and values were calculated.

Monod equation for growth curve $\mu = \frac{\mu max \times S}{Ks+S}$ ….....1

μ was calculated from the relation $\mu = \frac{2.303\ (\log OD2 - \log OD1)}{t2-t1}$ ….....1(a)

where t_2-t_1 represents doubling time.

t_d may be calculated from the relation $td = \frac{\ln 2}{\mu}$ .....1(b)

Results and Discussion

In order to determine the potential of bacterial isolates for plastic degradation to use LDPE as sole carbon and energy source, the growth kinetics of those strains in LDPE were studied and compared with that of usual nutrient broth.

Genetic identification of LDPE degrading bacteria

Two bacterial strains were found effective for LDPE degradation. The isolates were identified as *Bacillus amyloliquefaciens* BSM-1 and *Bacillus amyloliquefaciens* BSM-2. The sequences were submitted in NCBI GenBank and assigned accession numbers were obtained, KC924446 for BSM-1 and KC924447 for BSM-2.

Growth kinetics for bacterial isolates in nutrient broth

In case of nutrient broth, it is normal media which is suitable for optimal growth of the most of bacterial isolates and contains simple sugars as carbon source due to which the bacteria are able to use it easily and grow fast, the isolated strains were inoculated in the broth and its growth was observed to get its generation time (Fig. 1 & 2). Based on the growth curve obtained from the plotted graph, doubling time was calculated and found to be 1.8 hours for BSM-1 with μ value of 0.44 h^{-1} while BSM-2 was found to have doubling time of 2.4 hours with μ value of 0.625 h^{-1}.

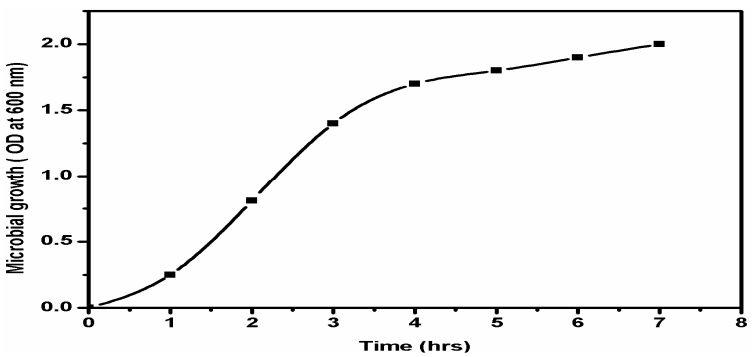

Figure 1: Growth curve for BSM-1 isolate on nutrient broth

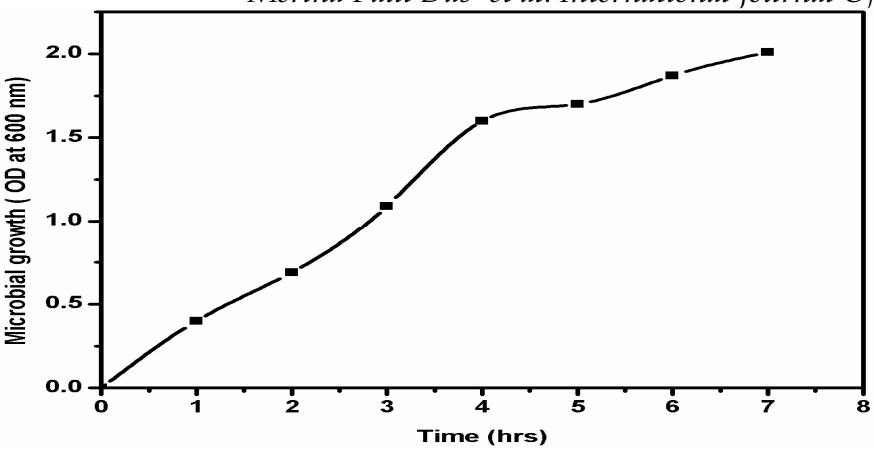

Figure 2: Growth curve for BSM-2 isolate on nutrient broth

Growth kinetics for bacterial isolates in synthetic media

When the medium contains more complex components then microbes find it difficult to utilize the supplemented medium and need time to get adapted with modified nutrient which changes its normal doubling time (Fig. 3 & 4). Thus was calculated on the basis of graph plotted from the data obtained after inoculation of bacterial isolates on LDPE powder as only carbon and energy source and generation time was found to be 4.66 hours for BSM-1 with μ value of $8.3X10^{-3}$ h^{-1} while BSM-2 was found to have doubling time of 5.25 hours with μ value of 0.011 h^{-1}.

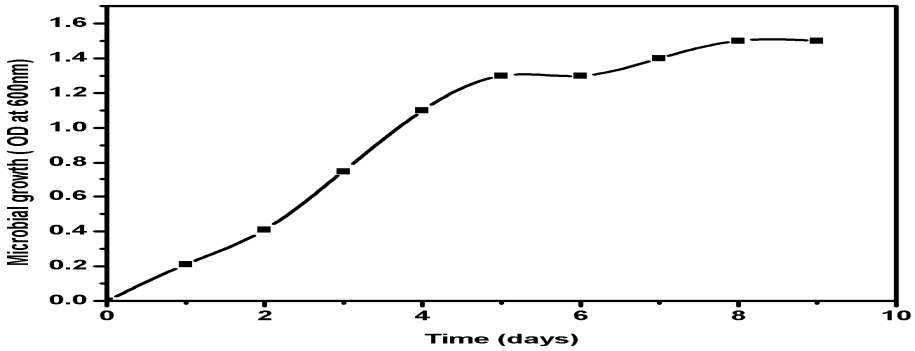

Figure 3: Growth curve for BSM-1 isolate on synthetic media supplemented with LDPE

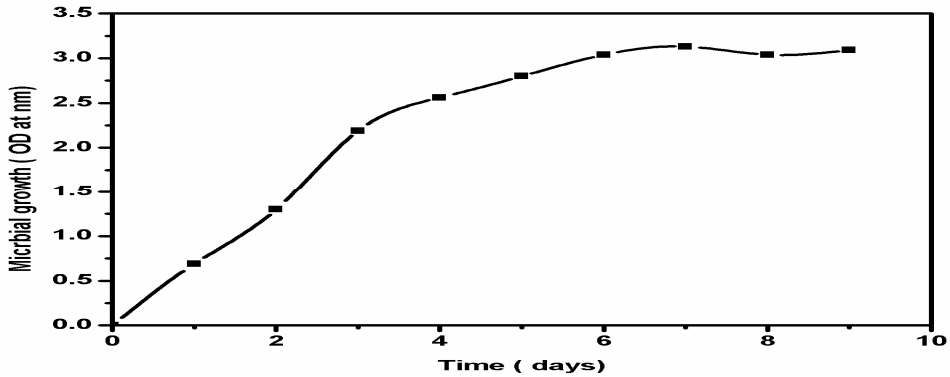

Figure 4: Growth curve for BSM-2 isolate on synthetic media supplemented with LDPE

Generally the best possible explanation for this phenomenon originates from the operon system. The microbes are known for their nature of plasticity i.e. ability to produce suitable enzyme and survive in harsh environment through adaption. The growth pattern of both bacteria implies that there was a probability for genetically modification of *Bacillus amyloliquefaciens* BSM-1 to *Bacillus amyloliquefaciens* BSM-2 and furthermore, both the bacteria were isolated from the same source. In case of such condition where the complex media is available then the population tends to utilize it through bringing some environmental adaptive changes in enzyme system and produce the enzyme which may utilize the available substrate and validate the theory of survival of fittest. This also explains why the two closely related strains from same genus and species having some mutational changes in base pairs shows huge variation in the substrate utilization capacity. Therefore, BSM-1 (unmodified) was grown in nutrient broth more faster rate than the LDPE supplemented media whereas in case of BSM-2 (may be modified due to survival in complex media i.e, plastic enrich soil) also showed the same growth curve but in different pattern. Microorganisms can utilize the normal growth media than the organic complex media and thus BSM-2 had higher specific growth rate than BSM-1 in LDPE supplemented media, because of that BSM-2 had higher polyethylene degradation capability (16%) as compared to BSM-1 (11%).

Conclusion: From this study, it is clear that the specific growth rate for BSM-1 and BSM-2 in nutrient broth was quite high than that of synthetic media supplemented with LDPE, but the doubling time was less in NB than synthetic media which proves that microbes can grow easily in simple medium than the complex one. The growth curve was steeper in case of BSM-2 than BSM-1 in synthetic media compared to the nutrient broth.

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