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Research Article

Biodegradable Potential of Bacillus amyloliquefaciens and Bacillus safensis Using Low Density Polyethylene Thermoplastic (LDPE) Substrate

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ABSTRACT

Increase of plastics accumulation in the environment cause ecological threats and has been one of the serious issue worldwide. In the current study, two bacterial isolated strains *Bacillus safensis* and *Bacillus amyloliquefaciens* were used for their plastic degradation capabilities. The biodegradation of low density polyethylene thermoplastic was assessed by weight reduction, Scanning electron microscopy (SEM) analysis and by culture media pH alteration. The results shows that *Bacillus safensis* was more efficient and degrade 18.6% LDPE than *Bacillus amyloliquefaciens* that degrade 18% of LDPE after incubation period of 30 days. Moreover, it was also noted that longer incubation periods results in higher biodegradation of low density polyethylene thermoplastic. It is concluded that the biodegrading ability of *Bacillus safensis* is more than *Bacillus amyloliquefaciens* as confirm from weight reduction of low density polyethylene thermoplastic.

Keywords: biodegradation, low density polyethylene, microbial degradation, Bacillus safensis, Bacillus amyloliquefaciens

INTRODUCTION

Land and water pollution caused by the extreme use of plastic is one of the major environmental problem worldwide. Plastic use has been incorporated into everyday life of human because of its low cost, various application, less weight and durability (Ghosh et al., 2013). Various type of plastics has been used continuously in our daily life such as polyethylene bags, polyvinyl chloride, and nylon etc (Smith., 1964). The demand for plastic production worldwide has been increased from 1.5 million to 245 million tons during 1950 to 2008 with a higher increase rate of 9% each year (S. Chanprateep et al., 2010).

However accumulation of huge amount of plastic waste is extremely dangerous to environment as it is non-biodegradable in nature, and have known negative consequences for both human and animal health. The usage of polyethylene is increasing continuously at a risk rate of 12% each year worldwide (Roy et al., 2008). Packaging plastic (polythene bags) comprises about 10% of the total municipal waste created worldwide (Barnes et al., 2009). Small part of this polyethylene waste is reused while a large portion of the wastes are buried in the ground for the sake of degradation but is takes several years or may be not degrade (Lederberg, 2000; Moore, 2008). Wasted polyethylene bags is a serious threat to aquatic and terrestrial life, it could cause blockage in the gut of fowls, fishes, and other aquatic animals (Denuncio et al., 2011). 267 species in the marine ecosystem, including mammals, sea turtles, and seabirds are affected by the plastic pollution (Coe and Rogers, 1997). The death of terrestrial animals, such as bovine was reported because of eating of polythene bags in the field (Singh., 2005). The biodegradable polymers are manufacture to degrade it rapidly by microorganisms because of their capability to degrade most of the chemical compounds (Sadocco et al., 1997). *Pseudomonas, Streptococcus, Staphylococcus, Micrococcus, and Moraxella, Aspergillus niger, Aspergillus glaucus, Actinomycetes sp. and Saccharomonospora* genus has been identified for their potent biodegradable properties (Swift et al., 1997).

The current study focuses on the biodegradation capabilities of two bacterial isolates *Bacillus safensis* and

Bacillus amyloliquefaciens against low-density polyethylene (LDPE).

MATERIAL AND METHODS

Microorganisms

The present study was intended to monitor biodegrading capability of two bacillus strains isolated and characterized previously at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar (IBGE) through conventional and molecular techniques.

Pure Culture

One hundred mL of nutrient Agar medium (5g yeast extract, 10g of NaCl, 3g of dextrose, 0.1g of NaH_2PO_4, 0.5g of KH_2PO_4, and15g of agar with adjusted pH of 7.4±0.2) was prepared and autoclaved by following standard procedures. Next day streaking was done under sterile condition at room temperature.

Inoculum Preparation

One hundred mL of nutrient broth medium composed of g/L: (yeast extract 5 g, NaCl 10 g, dextrose 3 g, NaH_2PO_4 0.1 g, KH_2PO_4 0.5 g with pH of 7.4±0.2) was prepared and incubated overnight at 30°C.

Pre-treatment for LDPE

Low-density polyethylene (LDPE) was cut into small fragments, washed with distilled water and sterilized in ethanol for 15 min. Residual ethanol was removed by further washing, following by the addition of 0.1% mercuric chloride and washing continuously.

Biodegradation Studies

Bacteria were isolated and recognized as plastic-degrading bacillus strains which were developed and grown in supplement media (5g peptone, 5g NaCl, 1.5g yeast extract, and 1.5g HM peptone with the adjusted pH of 7.4). The media was autoclaved at 121°C for 20 minutes. About 200 ml media was added into 250 ml sterilized Erlenmeyer flask. 1ml of overnight inocula from both strains with 1g of plastic was supplemented into broth.

Evaluation of Degradation

The plastic pieces were removed from the bacterial culture at day 10, 20, and 30. The collected pieces were rinsed with distilled water to remove any attach microbe to its surface. The pieces were then air dried in oven at 60^0C and weighed to determine weight loss. The same procedure was done for all other samples.

pH Evaluation

Estimation of pH change was studied to confirm any metabolic activity of the microbial isolates in supplemented medium. If the pH increased that show metabolism microbial cells incredibly support that the strains are dynamic and provide a proof of degradation. The pH of each bacterial culture was calculated at an interval of 10, 20 and 30 days respectively during the study.

Optical Density

Optical density was calculated after 5, 10, 20, and 30 days using UV/VIS Spectrophotometer at 610 nm in order to ensure growth of the bacteria.

Final Weight Determination

Final weight of polythene bag was determined to check extent of degradation. The plastic pieces were removed after 10, 20 and 30 days of incubation and were sterilized, dried until removal of moisture and weighed for final weight. The extent of degradation of LDPE pieces by *Bacillus safensis* and *Bacillus amyloliquefaciens* was determined by calculating the percentage of weight loss of plastic pieces which was calculated by the given formula:

Percentage of weight loss = Initial weight - Final_weight / Initial weight x 100

RESULTS

Biodegradation Studies

After incubation process of 10, 20 and 30 days, the degrading ability of the two bacterial strains *Bacillus safensis* and *Bacillus amyloliquefaciens* were evaluated and were interpret using different parameters.

Weight Decrease

Weight loss is the primary mean for the determination of polymer degradation. Microorganisms that grow on polymer cause weight loss of LDPE. The changes occurred due to microbial activity were qualitatively measured by the weight loss of the LDPE after inoculation with *Bacillus safensis* (pwg^{-7}) and *Bacillus amyloliquefaciens* (c_2ssk^{-8}). The weight loss was monitored on regular interval of 10, 20 and 30 days. The weight reduction of LDPE was 9.9%, 16.9% and 18.6% after 30 days of incubation with *Bacillus safensis* and 6%, 13.4 and 18% with *Bacillus amyloliquefaciens* respectively. The weight loss results are illustrated in **Figure 1**. Among the two isolates in the experiment, *Bacillus safensis* was found to be more efficient than *Bacillus amyloliquefaciens* in degradation of polythene bag after 30 days of incubation that was recorded about 18.6%.

pH

The deviation in pH of both bacterial culture before and after the biodegradation process are illustrated in **Figure 2**. Bacterial isolates, *Bacillus safensis* and *Bacillus amyloliquefaciens* demonstrated the generation of compounds and metabolites with the indication of pH change supporting the metabolic activity of strains on the LDPE and its degradation.

Change in Optical Density

Changes in optical density of medium within 30 days incubation treatment are illustrated in **Figure 3.** Optical density was increased from day first to day 30. The highest optical density was observed for *Bacillus safensis* after 30 days of treatment.

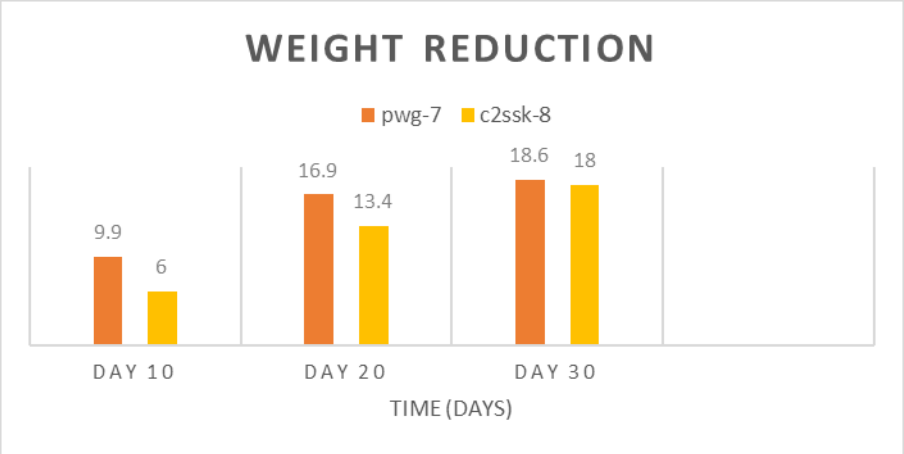

Figure 1. Weight Reduction of LDPE by using *Bacillus safensis* and *Bacillus amyloliquefaciens*

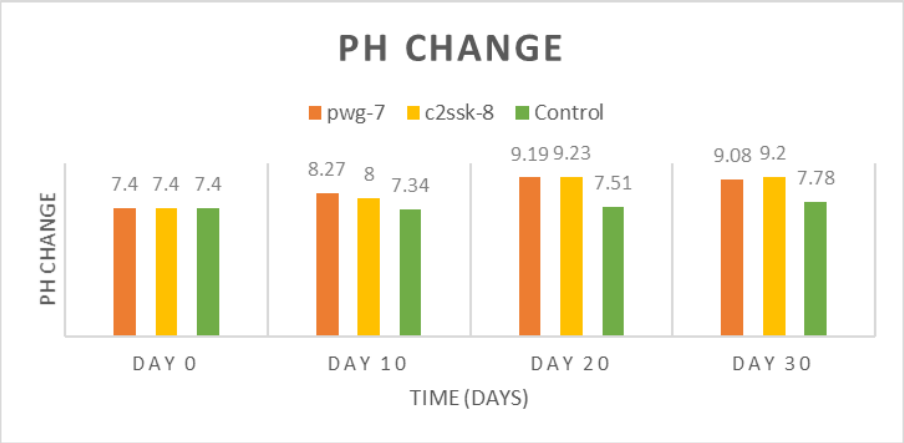

Figure 2. Changes of pH value due to microbial activity during biodegradation

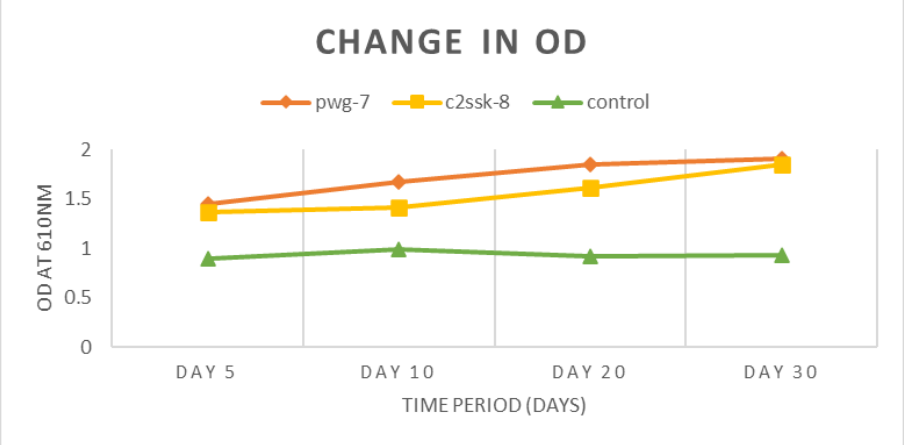

Figure 3. Changes in optical density of the culture medium during the process

DISCUSSION

The biodegradation capability of *Bacillus amyloliquefaciens* has been reported many time (Das Kumar et al., 2015). *Bacillus safensis* isolated in this study had not been testified before and is a novel bacteria that can degrade LDPE. Biodegradation of organic compounds through microbes require oxygen, nutrients and physio-chemical conditions like temperature and pH (Fred, 2001). We provide these control condition to our bacterial culture that have the capability to degrade carbon compounds by utilizing them as sole source of energy and carbon by using its own enzymatic system (AMSA, 2004; Dagly, 1984). In this study two of the bacterial strains were used *Bacillus safensis* and *Bacillus amyloliquefaciens*. *Bacillus safensis* showed a high degradation activity against LDPE as compare to *Bacillus amyloliquefaciens*. During biodegradation bacteria used plastic as sole source of carbon and energy which result in weight reduction of the LDPE. Results showed that the weight reduction was 18.6% for *Bacillus safensis* and 18% for

Bacillus amyloliquefaciens after incubation while there was no changed occurred in untreated LDPE.

pH is an essential aspect for the survival and action of microbes that guarantee the production of enzyme which results in beginning of degradation (Xu et al., 2011). These bacterial isolates secret some extracellular and intracellular enzyme in the suspension media that responsible for degrading ability. Most favorable pH for the biodegradation process of hydrocarbons is 6-9 (Mentzer and Ebere, 1996). The results revealed variation in the pH of culture media through the 30 days of incubation period with *Bacillus safensis* and *Bacillus amyloliquefaciens*. The pH of the aqueous media continuously increased toward alkalinity that might be considered for degradation.

CONCLUSION

Both strains *Bacillus safensis* and *Bacillus amyloliquefaciens* has the capability to degrade low density polyethylene (LDPE). It was found that the degrading ability of *Bacillus safensis* is more than *Bacillus amyloliquefaciens* as confirm from weight reduction. As plastic is the major threat to human health and environment, we can easily eliminate plastics by using these strains.

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