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RESEARCH ARTICLE

SCREENING OF THE POLYTHENE DEGRADING FUNGI COLLECTED FROM WEST COAST OF GUJARAT

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ABSTRACT

At three different pH (pH 3.5, 7, 9.5) screening of the polythene degrading fungi was carried out based on percent weight loss and changes in tensile strength after 2 months of regular shaking at room temperature. Maximum percent weight loss (13.63 ±4.93%) of the polythene after 2 month of regular shaking was found with isolates JAMNF 3 at pH3.5 whereas minimum percent weight loss (0.00±0.00 %) was reported with JAMNF6 at pH 3.5. In the present study we also reported percent weight gain (24.41±6.99 % with JAMNF4 at pH 9.5) of the polythene instead weight loss that might be due to accumulation of fungal hyphae in the scions of polythene caused due to degradation of the polythene. Based on changes in tensile strength, fungal isolate SURF3 recorded maximum changes in tensile strength (19±0.00 Kg/cm²) at pH 9.5 as compare to control.

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INTRODUCTION

Polythene is widely used in our day to day life in various purposes due to their advantageous property i.e., mostly hydrophobic and highly durable nature in various aspect. Polythene is thermoplastic material that is produced primarily by the catalytic polymerization of ethylene gas (C₂H₄) at elevated temperature and pressure. Annually about 57 million tons of plastic waste is being generated around the globe (Bollag *et al.*, 2000). Due to hydrophobic nature its degradation in the environment is a great challenge, hence for marine life, non-degradable polythene waste is regarded as major threat. As per reports, it has been estimated that one million marine animals are killed every year due to blockage and intestinal choking (Spear *et al.*, 1995, Secchi and Zarzur, 1999; Rutkowska *et al.*, 2002). Non degradable plastic waste causes serious problem to mega-cities especially coastal ones. Iiyoshi *et al.* (1998) raised environmental concern that led us to seek ways to resolve the problem that unmanageable plastic which are accumulating on earth at alarming rate. Several hundred tons of plastic have been discarded in the marine environment every year (Sangale *et al.*, 2012). From prehistoric times the mangroves of the coastal areas are being used as dumping site for the plastic waste (Kathiresan and Bingham, 2001). In the present study an attempt has been made to screen the polythene degrading fungi

based on percent weight loss and change in tensile strength from the rhizosphere of *Avicennia marina* growing along the West Coast of Gujarat.

MATERIAL AND METHODS

Identification of the localities of *Avicennia marina* (Forsk.) Vierh.

Two localities of the *Avicennia marina* (Forsk.) Vierh. along the West Coast of Gujarat were identified using the regional floras and individual visits.

Collection of the rhizosphere soil of the *A. marina*

For collecting rhizosphere soil we uprooted 5-6 young plants of *A. marina* at each locality followed by the uprooting of 3-5 pneumatophorous aerial roots (NAR) of each plant. The young plants/NAR with adhered soil was carried to the working station in the sterile zip-lock bags for isolation of the fungi.

Isolation of the fungi

The isolation of the rhizosphere soil fungi was carried as per Chaturved *et al.* (2008). The fungal cultures were grown on Sabouraud's Agar (SA) media. The pure cultures were maintained in slants at 4°C (Aneja, 2003). After 6 days of incubation (Incubator, Classic Scientific, and India) at 28°C. The colony forming units of each isolate was determined using the Colony counter (HiMedia, India).

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Screening of the polythene degrading fungi

20 micron thick polythene carry bags were purchased from the local market at Pune. Polythene strips (2cm × 2cm) were made using the sharp blade. All the polythene strips were given pre-treatment as per Sharma and Sharma (2004) to remove the additives. The pre-treated polythene strips were weighed (Precision balance, Sartorius, Germany) and autoclaved (Steelmet, India). The sterile polythene strips (3strips/vial) were aseptically transferred to the sterile vials containing the Sabouraud's broth. After inoculation (3 vial/isolate), all the cultures were shaken (Rotary Shaker, Steelmet, India) at 92±7 rpm at room temperature for two months at selected three pH 3.5, 7 and 9.5. The experiment was carried out in triplicate set with control. The screening of the fungal isolates with polythene degradation potential was carried out by analyzing the 2 key characteristics (percent weight loss and tensile strength) of the polythene.

Percent weight loss

After two months of regular shaking the polythene strips were removed from each vial aseptically in separate petriplates and were washed with absolute alcohol (once) and water tap water (twice). The washed polythene strips were then dried in oven overnight at 40°C. The dried polythene strips were again weighed. The percent weight loss of the polythene was calculated using the formula

$$\text{Percent weight loss} = \frac{X - Y}{X} \times 100$$

Where X: average fresh weight (mg) and Y: average weight after 2 months of regular shaking

Standard deviation to the mean of each triplicate was calculated using Micro Soft excel 2007 software.

Tensile strength

After two months of regular shaking the tensile strength (Kg/cm²) of the degraded polythene strips was measured using the tensile testing machine (Veekay Testlab) as per the method of ASTM D 638 (1998) at Dutech India laboratories, Pune, India. All the experiments were performed in triplicates and standard deviation to the mean was calculated using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Collection and isolation of fungal isolates

From West Coast of Gujarat rhizosphere soil sample was collected from Jamnagar and Surat (Fig.1). The GPS details of each locality are given in the Table 1. Total 10 fungal isolates were isolated from both the localities (Table 2).

Screening of the polythene degrading fungi

Based on the percent weight loss

After two months of regular shaking at room temperature percent weight loss of the polythene strips was recorded at three different pH (Fig. 2).

pH 3.5

Among the ten fungal isolates maximum percent weight loss (13.63±4.93%) of the polythene after 2 month of regular shaking was reported with JAMNF 3. The minimum percent weight loss (0.00±0.00) reported with JAMNF6. Instead of percent weight loss percent weight gain was also reported. Maximum weight gain (14.06±4.07%) was documented with JAMNF2 and minimum percent weight gain (1.64±0.74 %) was reported with the JAMNF1.

pH 7

SURF3 recorded maximum percent weight loss (1.38±0.54%) among the ten isolates whereas minimum percent loss (0.75±0.22%) was recorded with the isolate SURF6. Similar to pH 3.5 percent weight gain was also reported at pH7. JAMNF3 leads to maximum percent weight gain (4.33±0.48%) and the least percent weight gain (0.64±0.21%) was reported with SURF2.

pH 9.5

At pH 9.5 JAMNF5 documented maximum percent weight loss (1.56±0.56%) whereas minimum percent weight loss (0.64±0.22 %) was recorded with the isolate SURF1 (Fig. 3). Maximum percent weight gain (24.41±6.99 %) was reported with JAMNF4 followed by least percent weight gain (0.66±0.18 %) by the isolate SURF4.



Fig. 1. Map showing collection sites along the West Coast of Gujarat, India

Table 1. GPS details of the *A. marina* localities along the West Coast of Gujarat

Sr. No.	Locality	District	Latitude	Longitude	Altitude (m)
1	Dumas Beach	Surat	21° 08' 01.85"	72° 71' 51.85" E	1
2	New Bedi Port	Jamnagar	22° 54' 24.86" N	70° 03' 44.94" E	4.5

Table 2. Locality wise details of the fungal isolates

Sr. No.	Locality	State	District	No. of Fungal isolates
1	Jamnagar	Gujarat	Jamnagar	6
2	Surat	Gujarat	Surat	4

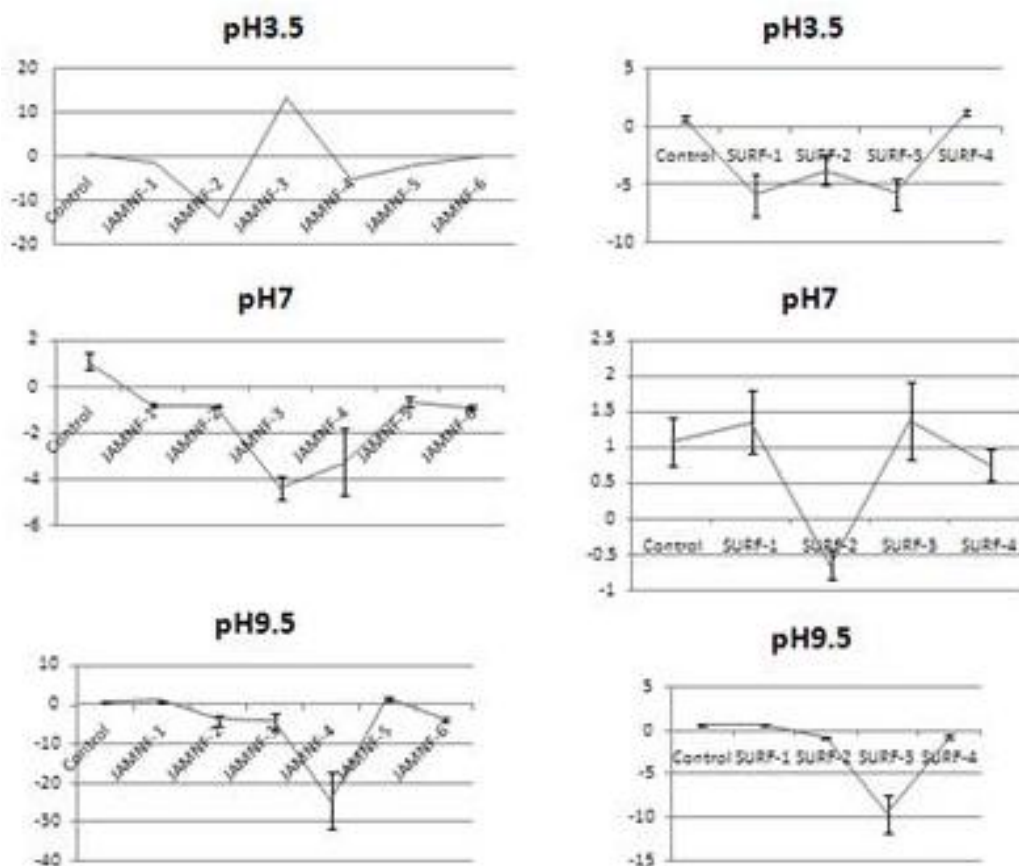


Fig. 2. Percent weight loss based degradation of polythene using fungal isolates at pH3.5, 7 and 9.5 (JAMNF: Jamnagar fungal isolates; SURF: Surat fungal isolates)

Change in tensile strength

Similar to weight loss, tensile strength was also measured after two months of regular shaking at room temperature (Fig.3).

pH3.5

At pH 3.5 maximum changes in tensile strength ($21.83 \pm 0.5 \text{ Kg/cm}^2$) was reported with SURF1 as compared to control followed by the least change ($23.83 \pm 1.15 \text{ Kg/cm}^2$) was recorded with SURF2.

pH7

JAMNF1 leads to maximum change ($20.67 \pm 1.52 \text{ Kg/cm}^2$) at pH7 whereas the least change in tensile strength ($24 \pm 1.52/1 \text{ Kg/cm}^2$) was documented with JAMNF5 and SURF4 respectively.

pH9.5

Highest change in tensile strength ($19 \pm 0.00 \text{ Kg/cm}^2$) was reported with SURF3 followed by least change in tensile strength ($23 \pm 1.64 \text{ Kg/cm}^2$) by SURF3.

Polythene is very important polymer used in daily life. Due its slow degradable nature it takes almost 1000 years to degrade under natural environmental conditions (Sangale *et al.*, 2012). Raman *et al.* (2012) reported 8 to 12% weight loss of the polythene after 30 days with *Aspergillus* spp. isolated from polythene polluted sites around Chennai. After 55 days of incubation maximum percent weight loss of polythene ($11.01 \pm 0.51\%$) was reported with *Aspergillus niger* (Kannahi and Sudha, 2013).

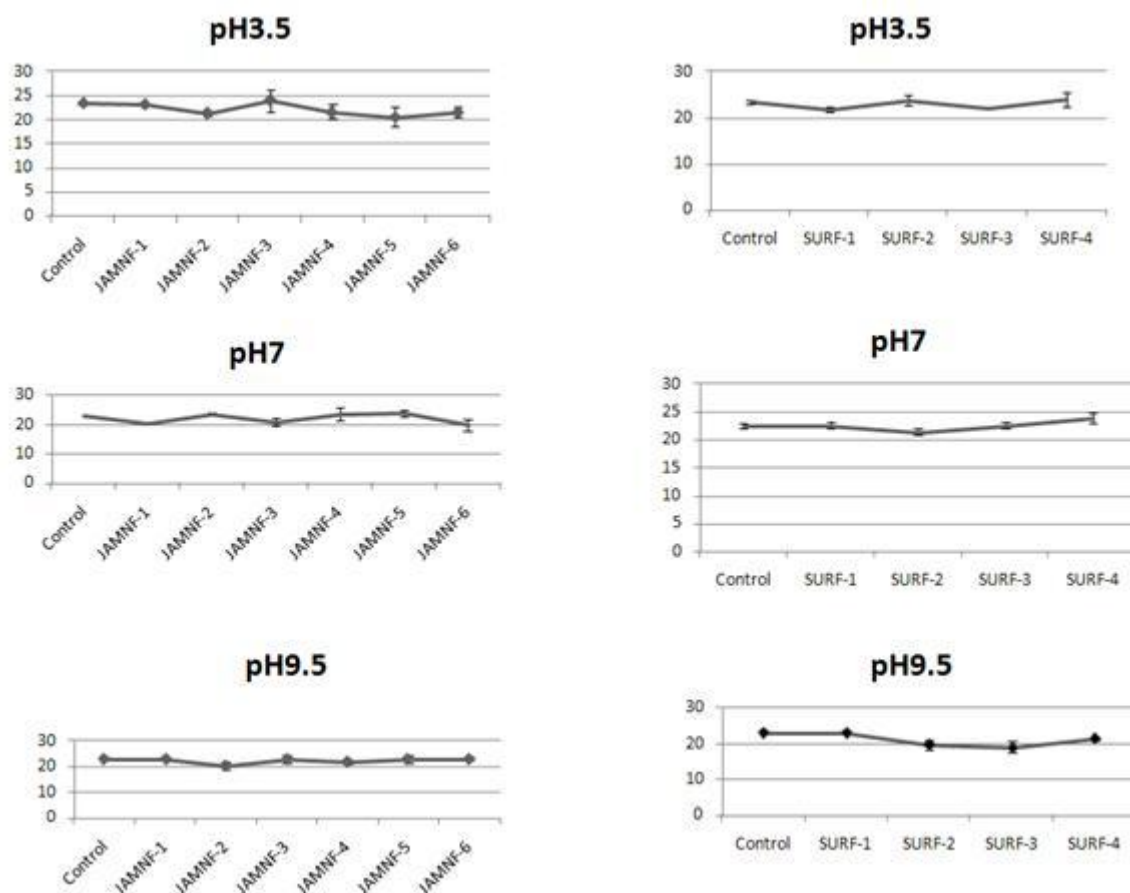


Fig. 3. Changes in tensile strength of the polythene degraded by fungal isolates at pH3.5, 7 and 9.5 (JAMNF: Jamnagar fungal isolates; SURF: Surat fungal isolates)

Our results are in agreements with these reports. To the best of our knowledge this is the preliminary study of polythene degradation by the fungi from West Coast of Gujarat. The Scientist working at CSIR-Central Salt and Marine Chemicals Research Institute, GB Marg, Bhavnagar 364002, Gujarat, India and Academy of Scientific and Innovative Research, CSIR, New Delhi, India reported polythene degrading bacteria (Harshvardhan and Jha, 2013) from pelagic waters, Arabian Sea Coast of Gujarat, India.

Conclusion

Among the ten fungal isolates overall maximum percent weight loss ($13.63 \pm 4.93\%$) of the polythene after 2 month of regular shaking was reported with JAMNF 3 at pH3.5. In terms of tensile strength maximum changes ($19 \pm 0.00 \text{ Kg/cm}^2$) were reported with SURF3 at pH 9.5. The identification of these two fungal isolates is under progress.

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