river Yamuna. The data presented here may serve as a baseline to which all future data may be compared. All groundwater samples, whether collected from shallow or deep bore pumps, showed the presence of coliforms. The presence of coliforms of faecal origin in a majority of these samples showed that microbial contamination in groundwater was widespread and even deeper layers of groundwater may not be regarded as free from disease-causing micro-organisms. However, except for some samples, most drinking water samples were found to be devoid of any coliform count.

Vibrio cholerae O1 was isolated from all surface and waste water samples while only 5% of the groundwater samples showed the presence of *Vibrio cholerae* O1. *Vibrio cholerae* O139 however, could not be isolated from any of the water samples tested. Although outbreaks of cholera due to *Vibrio cholerae* O139 have been reported from Delhi in the past[8], there has been no information on the presence of this organism in aquatic environments. *Esherichia coli* O157 : H7 while being primarily associated with food-borne outbreaks, has also become an important public health concern as a water-borne pathogen. Though generally considered to be of concern to developing countries, a large-scale outbreak of haemorrhagic colitis due to water-borne *E. coli* O157 : H7 was reported from Africa[9]. In India, *E. coli* O157 : H7 has recently been isolated from dairy cattle and beef in Kolkata[10,11]. In our study, although a number of isolates which were non-fermentative on sorbitol McConkey agar ($SMAC^-$) and also lacked the enzyme methylumbelliferyl-β-D-glucuronidase (MUG^-) were obtained, none of these proved to be enterohaemorrhagic *E. coli* when tested with specific O157 and H7 antisera. Our inability to isolate *E. coli* O157 : H7 from waste water, surface and groundwater may be attributed to the absence of this organism in this geographical region, or its presence in extremely low numbers necessitating the use of more efficient isolation methods such as immunogenetic separation or direct PCR amplification of *stx* genes using specific primers[11]. Clearly, more rigorous surveillance is required that would help to clarify the public health significance of water-borne *E. coli* O157 : H7 in India. Earlier, *Yersinia enterocolitica*, another emerging food- and water-borne pathogen has been isolated from waste water collected from sewage treatment plants (STPs) located in various parts of the national capital of Delhi[12]. As regards other emerging water-borne pathogens, viz. *Cryptosporidium*, *Campylobacter jejuni* and *Microsporidia*, there is no information on the prevalence of these in the aquatic environment of India. Studies in this respect are warranted to safeguard ourselves against emerging water-borne pathogens.

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A potential *Aspergillus* species for biodegradation of polymeric materials

In recent years, considerable attention has been focused on biodegradability of polymeric materials mainly because pollution of the environment by waste polymers has become a worldwide problem. Such materials need to be resistant to degradation both prior to and during use and should be capable of being degraded, if discarded after use, without causing any environmental problems. Two possible approaches to reduce the 'vices of polymeric materials' are (a) to develop biodegradable commodity plastic[1] and (b) to identify potential micro-organisms and develop protocol to effectively biodegrade the polymeric materials[2].

The present study is an attempt to assess the potential of *Aspergillus foetidus* for polymer degradation. This fungal culture was isolated from a polymeric sheet under degradation due to fungal colonization. The sheet was used as a greenhouse covering at Pithoragarh, India. The fungus obtained from degraded polymeric sheet was cultured, brought in pure form and was identified as *Aspergillus foetidus* with the help of the Indian Institute of Microbial Technology, Chandigarh. The culture has not yet been reported to degrade polymer in the available literature.

To assess the potential of *Aspergillus foetidus* for polymer degradation under laboratory conditions, polyurethane was taken as a test material. Polyurethanes are a class of plastics which are widely used as raw material in various industries. The degradation of polyurethane is known for quite some time[3–5]. Despite its xenobiotic origin, polyurethane has been found to be susceptible to naturally occurring microorganisms[6–8]. Enzymatic attack on polyurethane could be contributed through hydrolysis by enzymes such as ureases, proteoses and esterases[9–11].

Polyurethane sheet (1 mm thick) was synthesized by the Composite Division at the Defence Materials and Stores Research and Development Establishment (DMSRDE), Kanpur (based on polyether polyol, cyclo aliphatic diisocynate and dibutyle tin dilaurate compound as catalyst). Test coupons were cut from this sheet in different shapes and sizes such as 4×3 cm for visual examination, 3×1 cm for estimation of change in mass, 1×1 cm for SEM observations and 15×1.25 cm (dumb-bell shaped) for tensile test. The test coupons were sterilized with the help of ethanol and kept in desiccators.

Basic mineral salt medium was prepared in one litre capacity conical flasks, which were filled with 700 ml of mineral salt medium (MSM). MSM per litre contains $NaNO_3$ – 2 g, KH_2PO_4 – 0.7 g, K_2H-PO_4 – 0.3 g, KCl – 0.5 g, $MgSO_4$. $7H_2O$ – 0.5 g, $FeSO_4.7H_2O$ – 0.1 g and distilled water – 1 l. The pH of the medium was adjusted at 6.0–6.5. Flasks were plugged with cotton and sterilized at 120℃ for 20 min at 15-pound pressure.

Aspergillus foetidus was used as test organism. The culture was grown in sterilized potato dextrose agar (PDA) and incubated at 30 ± 2℃ for seven days for preparation of spore suspension in sterilized distilled water. 0.5% of Twin-80 was added as a non-fungicidal wetting agent. Ten millilitre of water was added in each of the culture tubes and sterilized nichrome wire was used for scraping on culture surface to liberate the spores. The liquid was then gently agitated to disperse the spores. The spores were filtered with the help of a funnel with sterilized muslin cloth in a sterilized conical flask. The flask was then shaken to break up any lumps of spores with the help of glass beads[12]. Inoculum concentration of 4.8×10^6 spores/ml was adjusted with the help of a haemocytometer. Test coupons were exposed in replicates of three (each replicate containing a set of three coupons) and were suspended in basic mineral salt medium in flasks with the help of sterilized thin nichrome wire. Flasks were inoculated with 3 ml/100 ml of test inoculum concentration. Abiotic controls were also kept. All the exposures were kept at 30 ± 2℃ in an incubator for 90 days. Regular shaking was carried out for all exposures.

After the 90 days exposure period, all types of test coupons were taken out. Visual examination for biogrowth on 4×3 cm coupons was carried out as per the recommendation of ISO-846-1978E[13]. The coupons of 3×1 cm were washed and desiccated and final weight loss (percentage) was recorded. The biofilm deposited on 4×3 cm coupons was used for biofilm characterization. The biofilm deposited on each set of nine replicates was extracted in sterilized distilled water. The pooled extract was shaken and diluted thousand times. One millilitre of this diluted extract was inoculated in sterilized PDA petri plates at 30 ± 2℃. Fungal colonies were counted on the fourth day of inoculation with the help of a microbial colony counter. ATP[14], protein[15] and carbohydrate[16] contents in biofilm were also estimated as per standard methods.

The 1×1 cm coupons were rinsed, fixed overnight in 2% glutraldehyde and dried. Scanning Electron Microscopy (SEM) observations were taken employing JEOL-JSM-35CF after gold coating. Mechanical properties of polyurethane samples were studied in terms of tensile strength, tensile modulus and elongation at break percentage employing ASTM test methods[17].

Little *et al.*[18] reported that microorganisms grow on material surface and produce viscoelastic layer or biofilm. The environment at the biofilm–material interface, in terms of pH, dissolved oxygen, organic and inorganic species is readily different from the bulk medium to which the material is exposed. In the present study, a significant biogrowth on the material surface was observed during visual examination, covering 25–50% area of test samples. On biogrowth characterization, 32.8×10^4 number of colonies/cm^2 were recorded on the material surface. ATP (17.5×10^{-9} g/ml), carbohydrate (1802.00 µg/ml) and protein contents (942.50 µg/ml) were estimated in the biofilm formed on material surface. 0.22 per cent loss in weight of test coupons exposed to *A. foetidus* was also recorded as compared to abiotic control.

Biodegradation of polymers has earlier been studied using SEM where it has been reported that SEM micrographs provide evidence of active biofilm development on the surface of such materials[19–21]. Mercedes *et al.*[22] observed through SEM that colonizing bacteria were interconnected by extracellular polymer and were attached to hydrocarbon droplets as well as to sheets of polymeric materials. In the present study, SEM micrograph of abiotic control sample showed smooth surface morphology, though some darkened area and white dots were seen in SEM (Figure 1). The polyurethane samples exposed to *A. foetidus* showed profuse biogrowth. The fungal mycelium and spores were also present in the micrograph (Figure 2). After wash-

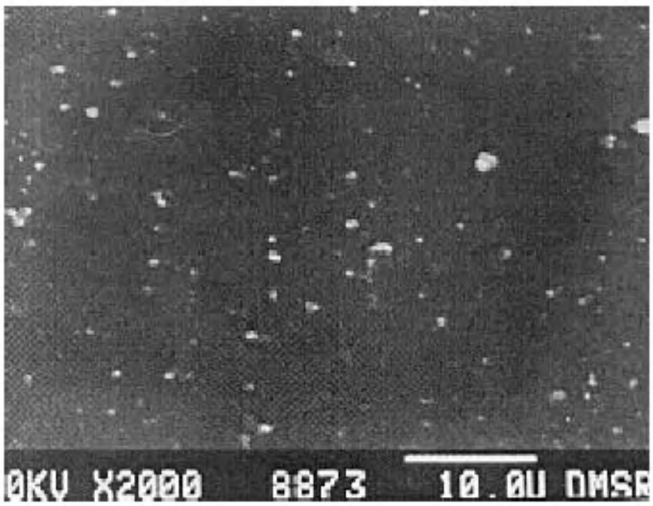

Figure 1. Scanning electron micrograph of polyurethane coupon: Control.

ing the biogrowth, whitened areas with ridges and furrows were seen. The rough surface of the coupons showed its degradation due to *A. foetidus* (Figure 3).

A decrease in tensile strength of adhesive PVC tapes exposed to two bacterial and one fungal cultures has also been reported[23]. In contrast to this, Seal and Pantke[24] observed significant increase in tensile strength of PVC films exposed to fungal cultures. In the present study, the growth of *A. foetidus* induced significant reduction in mechanical properties such as tensile strength, tensile modulus and elongation at break percentage of test polyurethane coupon (Table 1). A loss of 11.15% in tensile strength, 1.87% in tensile modulus and 9.15% in elongation at break was noted as compared to abiotic control. Statistically a significant reduction ($P < 0.05$) was observed in tensile strength, however, there was no significant difference in tensile modulus. A significant reduction was also observed in the case of elongation at break percentage when compared to abiotic control.

This study suggests the hitherto not reported degradation potential of *A. foetidus* for polymers. Further studies are in progress to enhance this ability of the culture to degrade waste polymeric materials.

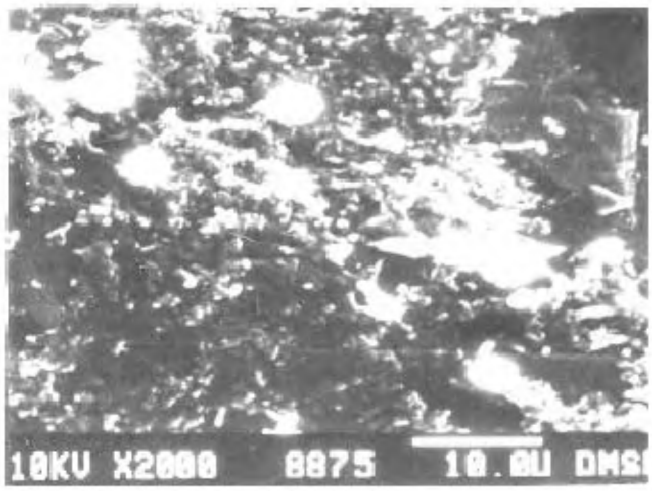

Figure 2. Scanning electron micrograph of polyurethane coupon exposed to *A. foetidus* showing biogrowth.

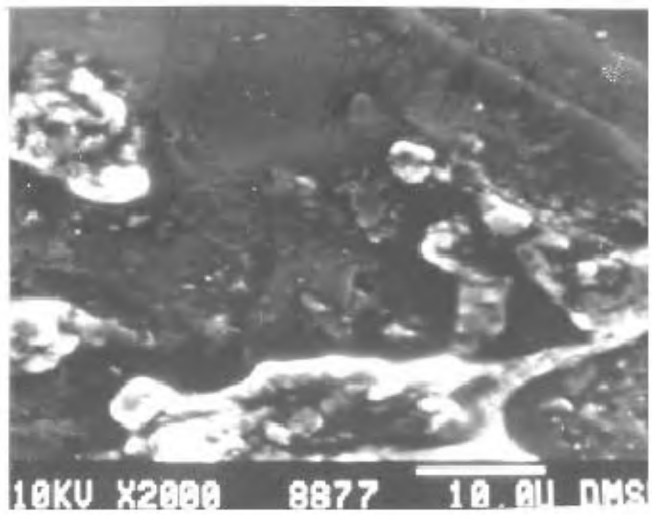

Figure 3. Scanning electron micrograph of polyurethane coupon exposed to *A. foetidus* after washing the surface biogrowth.

Table 1. Alterations in mechanical properties of polyurethane coupons exposed to *A. foetidus* (Mean ± SE)

Condition	Tensile strength (kg/cm^2)	Tensile modulus (kg/cm^2)	Elongation at break (%)
Positive control	284.10 ± 4.32^a	32.35 ± 0.45^a	922.60 ± 13.33^a
Abiotic control	281.30 ± 4.32^a	31.55 ± 0.67^a	889.40 ± 9.05^a
Aspergillus foetidus	249.94 ± 6.07^b	30.96 ± 0.75^a	808.00 ± 2.28^b

Mean values with different superscripts in a column differ significantly at $P < 0.05$.

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Possible amelioration of hyperthyroidism by the leaf extract of *Annona squamosa*

Excess secretion of thyroid hormones (hyperthyroidism) is often considered as a causative factor for some of the common health problems including diabetes mellitus and heart disease[1]. Although several scientific studies have been made considering plant extracts in relation to different health problems, investigations on the herbal regulation of hyperthyroidism are meagre[2,3]. In our endeavour to find a suitable plant extract, for the regulation of hyperthyroidism, an attempt was made with the leaf extract of *Annona squamosa* to study its relation to the regulation of thyroid function.

Annona squamosa L. (family Annonaceae), commonly known as custard apple, is cultivated throughout India, mainly for its edible fruit. This plant is attributed with some medicinal properties including anti-tumour, diuretic, antifertility activities in mice and rats[4–6]. Its thyroregulatory activity, if any, was hitherto uninvestigated, despite the fact that some of the reported medicinal properties are regulated by thyroid hormones[7]. This paper reports an attempt to investigate the effect of its leaf extract in the regulation of hyperthyroidism considering laboratory mouse as working model.

Sodium dodecyl sulphate, Diethylenetriamine penta-acetic acid (DTPA), *Tris*, L-T_4 and thiobarbituric acid (TBA) were supplied by E. Merck, Mumbai, India. Other chemicals were of reagent grade and were obtained from Loba Chemie, Mumbai, India. Radioimmunoassay (RIA) kits for the estimation of total serum triiodothyronine (T_3) and thyroxine (T_4) were purchased from Bhabha Atomic Research Centre (BARC), Mumbai, India.

Healthy leaves of *Annona squamosa* were collected locally, dried under shade and powdered with the help of an electric grinder. An aqueous extract was prepared by the cold percolation method using 35 g of dried leaf powder in 500 ml doubled distilled water (DDW). After filtration, the extract was concentrated and dried under vacuum. The yield of the extract was 17.4%. The powdered extract was dissolved in double distilled water to prepare the required doses for the final administration.

Colony bred, two and half-month-old adult Swiss albino male mice weighing 28 ± 2 g were acclimatized for 7 days in a light-and-temperature controlled (14 h light : 10 h dark, 27℃ ± 1) animal room with the provision of food (Gold Mohur mice feed, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. Thirty-five animals were divided into five groups of seven each. Group I animals receiving only 0.1 ml distilled water everyday served as control, Gr. II, Gr. III, IV and V were treated with pre-standardized dose (0.5 mg/kg) of L-thyroxine (L-T_4) initially for 12 days to render them hyperthyroidic[8]. While Gr. II continued to receive only T_4, Gr. III, IV and V animals were orally administered *Annona squamosa* leaf extract at three different doses (37.5, 75.0 and 150 mg/kg respectively) along with an equivalent dose of T_4 for the last 8 days before termination. On the 21st day, overnight fasted animals were sacrificed by cervical dislocation, blood from each one was collected, allowed to clot, centrifuged and serum samples were stored at – 20℃ until assayed for T_3 and T_4 concentrations.

Serum concentrations of T_3 and thyroxine T_4 were estimated by radioimmunoassay following the protocols of the RIA kits as done earlier[2,3]. In brief, RIA was performed using *Tris* hydroxymethyl amino methane (THAM buffer 0.14 M, containing 0.1% gelatin; pH 8.6) as assay buffer. The antisera, hormone standards, radio-labelled hormones (^{125}I-T_4 and ^{125}I-T_3) and the control sera were reconstituted with assay buffer/double-distilled water as mentioned in the kit. Incubation was done at 37℃ (30 min for T_4 and 45 min for T_3), which was terminated by the addition of PEG. The tubes were then centrifuged at 2000 g for 20 min. After decanting the supernatant the tubes were subjected to radioactive counting. Hormones of the samples were calculated from the standard curves of T_3 and T_4.