Algal biofilms on polythene and its possible degradation

Today the use of polythene (high density and low density polyethylenes) has become an unavoidable entity of human life. As the consumption of polythene/plastic has increased manifold, its waste management is emerging as a parallel industry. Disposal of polythene material is a serious problem. Normally polythenes are thrown in water bodies as garbage material or discarded as landfill to decompose/degrade. During our freshwater algal floristic investigations from oligotrophic water bodies of Uttar Pradesh, some submerged polythene (carry bags) were found with heavy algal growth, slimy and soft textured, tearing easily with brittle nature. The tough and tear-resistant polythene bags totally disintegrated into pieces, after becoming soft in the waters. This could be partial decomposition/degradation of polythene due to a consortium of aquatic microbial activity.

Biofilms are a collection of microorganisms (algae, fungi bacteria) surrounded by the slime, an extracellular polymeric substance (EPS) they secrete, attached to either an inert or living surface and exist wherever the surface gets in contact with water. They are ubiquitous, and can be seen with the naked eye, coating the surface of the substrate with dangling slippery texture and green colour. Submerged polythenes of our collection showed the similar type of biofilms on their surface (Figure 1 *a* and *b*). Microscopic observations of these polythenes revealed profuse cracks on the polythene and heavy algal colonization on the surface as biofilm. This observation led us to observe submerged polythenes of different water bodies in and around Lucknow city. For polythene collection the following factors were taken into consideration: (i) slimy/mucilaginous algal/microbial growth on the polythene, (ii) softness of the polythene, which indicates the condition of decomposition, and (iii) tearing or breaking of the polythene (irrespective of the colour and texture of the sheet) into small pieces. Photodegraded polythene (directly exposed to sun for several days) was also collected. An experiment was set up at our laboratory using plastic and glass tubs to study the possible degradation of polythene by the microbial consortia, which was collected from the biofilms of polythene.

Among all the polythenes samples collected, only those from the oligotrophic water bodies were soft and decomposed, and tore easily. Scanning electron microscopic images revealed profuse cracks on polythene on the adherence of algal species (Figure 2 *a–f*). Majority of the dominant organisms of the biofilm were algae, with rare occurrence of bacteria. Fifteen algal taxa, including *Chaetophora*, *Coleochaete scutata*, *Coleochaete soluta*, *Aphanochaete*, *Gloeotaenium*, *Oedogonium*, *Oocystis*, *Oscillatoria*, *Phormidium*, *Chroococcus*, *Aphanothece*, *Fragillaria*, *Cocconis*, *Navicula* and *Cymbella* were identified. *C. scutata* and *C. soluta* colony proliferation on the surface of polythene was clearly seen under scanning electron microscope (Figure 2 *a* and *f*). A clear crack around the mucilaginous tips of the filaments on the polythene is evident at higher magnification (Figure 2 *b*, *c*, *e* and *f*). Algal biofilms are commonly found on submerged objects, cooling towers, spray ponds, etc. Polythenes collected from eutrophic waters and slum areas were tough, leathery and tear-resistant.

Polythenes are carbon and hydrogen polymers, remarkably resistant to biological decay. It is estimated that polythene would degrade less than 0.5% over 100 years and 1% if exposed to sunlight for two years[1]. Degradation mainly depends on temperature, light exposure, oxygen and moisture availability. Landfills do not ensure the degradation because they entomb the waste by not allowing light, oxygen, moisture and temperature. Environmental degradation by sunlight and oxygen may result in loss of tensile strength and enbrittlement as evident in scanning electron micrographs (Figure 3 *b* and *c*) without commensurate loss of mass (non-degraded polythene surface is plain even at 5000x magnification, Figure 3 *a*), while degradation by mechanical forces may simply reduce large pieces of plastic to smaller ones[2]. Biodegradation involves a biological agent utilizing the organic polymer as a substrate for growth and energy, so that the end product of complete biodegrada-

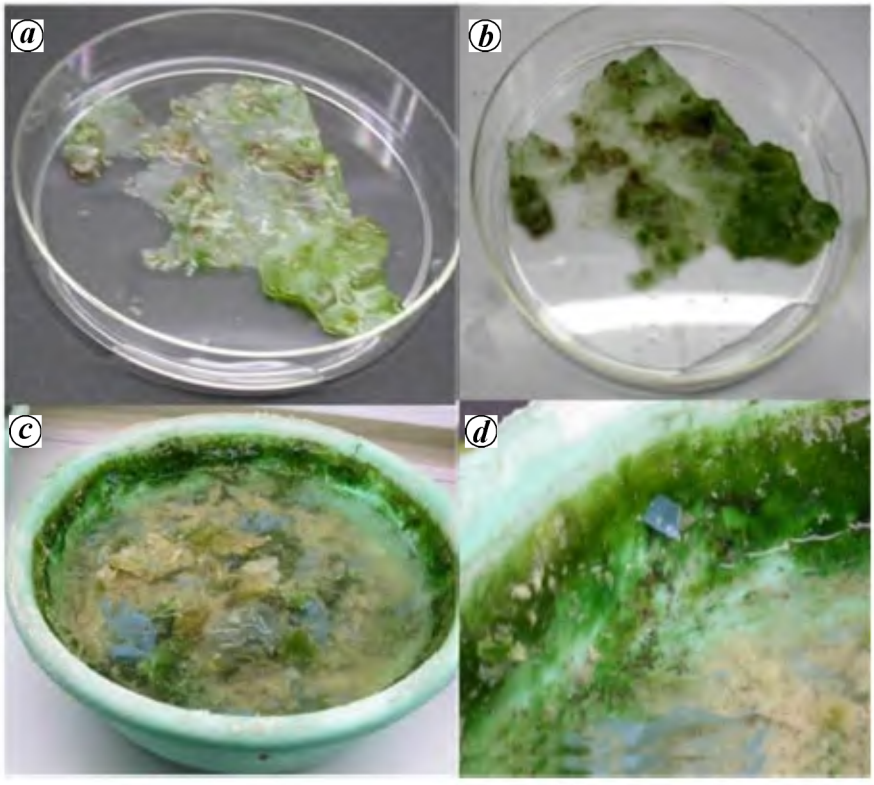

Figure 1. ***a***, Green, slimy biofilm on polythene. ***b***, Green, dangling slippery, bio-film on polythene. ***c***, Algal colonization on the inner surface of the plastic tub. ***d***, Close-up view of (***c***).

tion will be microbial biomass in an aerobic environment. Some substrates may not serve directly as either carbon or energy sources; instead they may be co-metabolized in the presence of another source to form alternate compounds. These products of co-metabolism may be either assimilated and therefore decomposed to CO_2 and water or not able to assimilate[3], in which case no reduction in the amount of plastic occurs. The nature of polythene samples from the oligotrophic water bodies revealed that there was certain amount of degradation/decomposition due to the microbial colonization.

Some of the blue-green algae are capable of solubilizing bound (tricalcium) phosphate, making it available for uptake by themselves and other organisms[4]. Extracellular substances of blue-green algae are able to chelate the surrounding medium leading to greater solubility and availability of nutrients[5]. Microorganisms, especially algae with mucilaginous secretion of extracellular polymeric substances (EPS) are the primary colonizers of building materials, walls, rocks, etc. and play a significant role in their biodeterioration[6]. Slimy mucilage of Chaetophorales alga, *Trentipohlia* softens the rock material and degrades it into powder[7]. As these microorganisms are capable of solubilizing, degrading/deteriorating hard materials like rock, cement, etc. the possibility of algal biofilm capable of degrading polythene cannot be overruled. Chaetophorales algae, diatoms and blue-green algae (phytoplankton) produce extracellular polymeric mucilaginous substance and adhere to any type of the substratum[8]. Phytoplankton EPS has high content of sugars like rhamnose, xylose and mannose[9] which helps in foaming and flocculation[10]. These algal species along with other aquatic microbes are the primary colonizers that form biofilm and serve as cue for other larger organisms to colonize on the surface. Colonization of larger microorganisms is called fouling, which affects the strength and performance of submerged objects[11]. Microbial biofilms on metal surfaces are involved in regulation of corrosion. Algal attachment was found on the inner walls of plastic tubs in our laboratory where the experiment was set up (Figure 1 *c* and *d*), whereas no algal attachment was found on the walls of the glass tub. Factors like electrostatic forces, surface charges, hydrophobicity of the surface and availability of cation play an important role in forming biofilms[12]. Although EPS may not be directly involved in inducing or inhibiting corrosion of metals, the presence of EPS in biofilms helps in creating an ideal environment that influences the corrosion process[11]. Here the combined effect of algal mucilage and other microbes might have reacted with the polymer of the polythene and softened it.

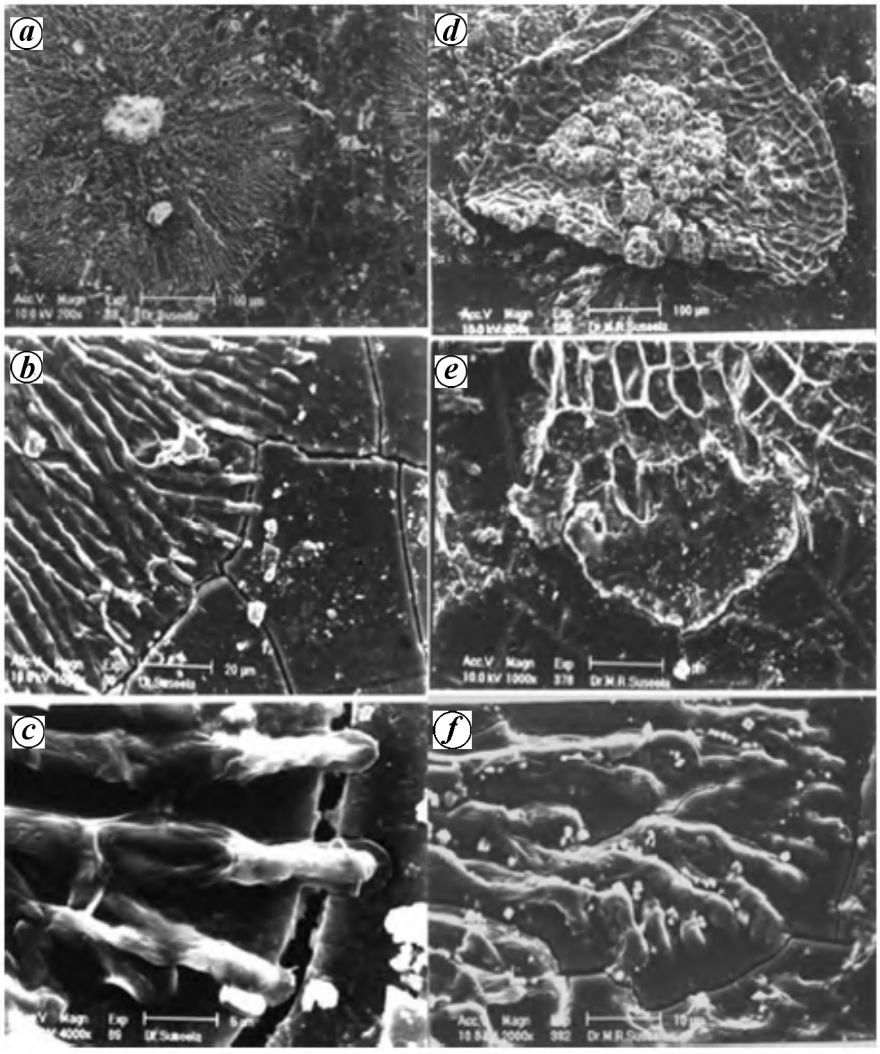

Figure 2. Scanning electron micrographs of biofilm; algal colonization on the surface of polythene. ***a***, *Coleochaete scutata* colony and cracks on the surface of polythene. ***b***, *C. scutata* filament proliferation. ***c***, *C. scutata* filaments at higher magnification. ***d***, *Coleochaete* sp. colony. ***e***, *C. solouta*. ***f***, *C. soluta* filament proliferation.

At this point it is difficult to say whether algae and other aquatic microbes have used the polythene as a carbon source but for primary colonizers, polythene certainly acts as a substratum. Later, the secondary colonizers (fouling) affect the strength and performance of polythene. In summer, when the water bodies are dry, the partially decomposed/degraded polythenes exposed to the sun, split into small pieces with bacterial and algal attachment and are released into the environment. In due course of time these polythenes further degrade in the soil or in any suitable environment. Biofilm microorganisms enhance the degradation of polythene[13].

Polythene, a xenobiotic polymer which has been under large-scale production only since 1950s has become a global problem today. To protect our planet from the plastic menace, further studies on understanding biofilms, microbes (algae and other aquatic microbes) and their metabolism, physico-chemical analysis of water and polythene, polymer chemistry and its degra-

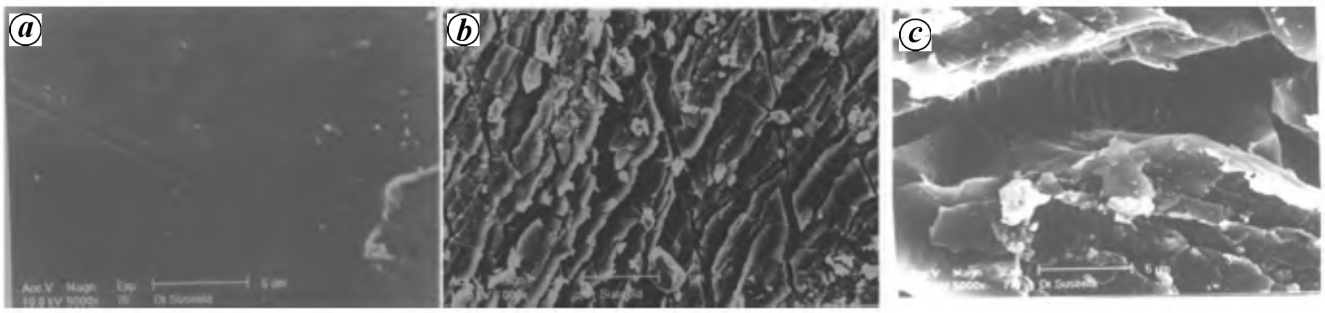

Figure 3. Scanning electron micrographs of polythene. ***a***, Plain surface of non-degraded polythene at 5000x magnification. ***b***, Photodegraded polythene showing cracks. ***c***, Stretching of polymer in the crack of photodegraded polythene.

dation process, etc. are required through inter-disciplinary collaboration.

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In vitro clonal propagation of *Casuarina equisetifolia* Forst. from mature tree-derived explants

Casuarina equisetifolia Forst. (Australian or Whistling pine) is an evergreen, xerophytic tree with multiple uses. It is widely used for sand stabilization, soil rehabilitation and as shelter belts[1–5]. The impact of the recent tsunami was reduced in the coastal areas of Tamil Nadu (India) where *C. equisetifolia* were planted as shelter belts[6]. Propagation is through seeds or by rooting of cuttings. The viability of seeds is low[4]. The seed-propagated progeny is not true-to-type. Multiplication by rooting of cuttings is slow. The rooting frequency declines sharply with aging of ortets[5]. Rooted cuttings from mature trees often show varying degree of plagiotropic growth[7]. Therefore, methods like *in vitro* clonal propagation are advantageous over conventional methods in obtaining rapid multiplication and true-to-type plantlets. Earlier reports on *in vitro* studies in *C. equisetifolia* deal with regeneration of plants from juvenile and mature stem segment explants through organogenesis[5], micropropagation using immature female inflorescence explants[7] and seeds[8,9] and callogenesis and organogenesis from apical meristem explants from two-year-old trees[10]. Here we report efficient *in vitro* clonal propagation of *C. equisetifolia* from mature tree-derived explants.

Fresh, green, healthy twigs were collected from 30-year-old flowering trees in the campus of National Chemical Laboratory, Pune (India). The needle-like branchlets or cladodes (15–20 cm long) were trimmed (0.5–1.0 cm) and the twigs were cut to prepare explants of 3–4 cm length. The explants were treated with 0.1% (v/v) Labolene (Qualigens Fine Chemicals) for 5 min and placed under running tap water for 2 h. Surface sterilization was done with 5% (v/v) Savlon for 5 min, ethanol for 30 s and 0.1% (w/v) mercuric chloride for 5 min followed by rinsing thrice with sterile distilled water. The explants were inoculated in Gupta and Durzan (DCR)[11] basal medium containing 784.5 μM carbendazim, a systemic fungicide (Bavistin). Sucrose (3%, w/v) was used as carbon source and agar (0.8%, w/v) was used as gelling agent. To determine the most suitable period for culture establishment the plant material was collected each month from March 2003 to February 2004.

After culture establishment period of 30 days, the sterile explants were transferred to DCR medium supplemented