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The Growth Of Microorganisms On Degradable Film Under Selected Disposal Conditions With Specific Reference To A d_2w^{tm} Product From Symphony Environmental.

Microstructure Investigation Report

Prepared for Michael Stephens
Technical Director
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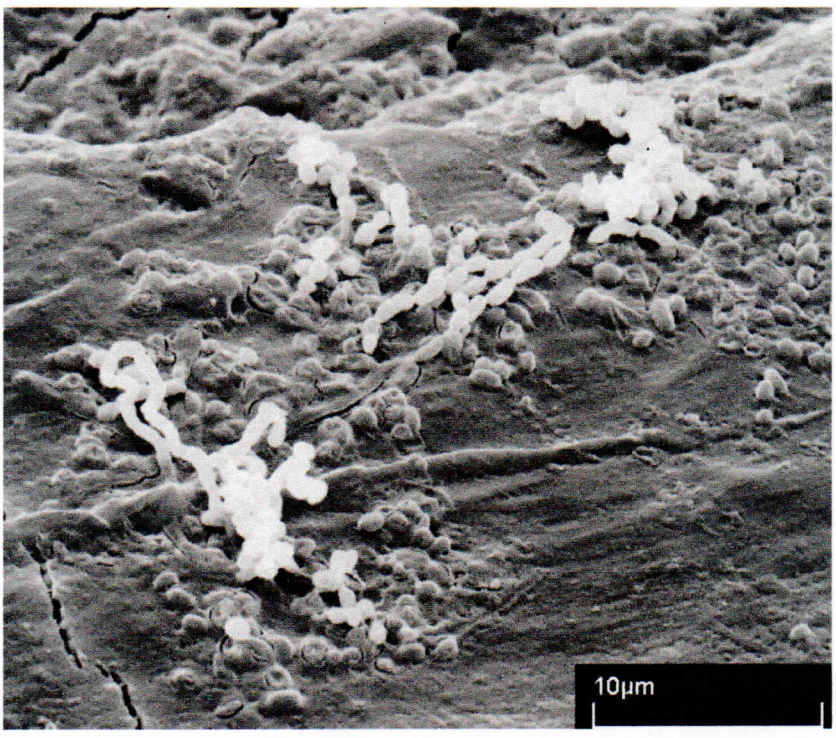
30th July 2005



The Growth Of Microorganisms On Degradable Film Under Selected Disposal Conditions With Specific Reference To A d_2w^{tm} Product From Symphony Environmental.

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1. Summary

This investigation undertaken to determine whether it is possible to demonstrate microbial colonisation of degradable polyethylene film based on Symphony Environmental's d_2w^{TM} technology. The experiment was designed to show whether the material can be colonised by microorganisms and, if so, whether these microorganisms demonstrated the capability of using the material as a carbon source, thus indicating that the material is biodegradable. To do this, samples of the material were placed in compost and in flowing water for 30 days and then examined by electron microscopy. It was found that the surface of the plastic was colonised by microorganisms and that there was evidence that these microorganisms had removed mass from the plastic within the time frame of the experiment. It can therefore be concluded that given a longer period of immersion this plastic can be expected to fully biodegrade in these environments. The growth of the microorganisms is evidently not inhibited by the transition metal based catalyst used in this product. A control sample added to the set showed limited microbial growth and no evidence of breakdown.

2. Objective

To determine whether polyethylene film based on Symphony Environmental's d_2w^{TM} technology could be shown to be colonised and consumed by microorganisms whilst in compost or in flowing fresh water.

3. Introduction

A sample of plastic film which had already begun to show signs of oxodegradation, creating small friable flakes, has been used to investigate biodegradation in the film.

A great deal of work has been done on the conditions leading to the physical breakdown of this material but reassurance that the material is physically colonised by organisms which promote breakdown was sought. Symphony Environmental's own website *(http://www.degradable.net)* states: "It has proven too difficult to measure what reduced molecular weight the PE must degrade to before it's "far enough" for the organisms to begin biodegradation, but currently figures in the order of 40,000 are now accepted as allowing initial microbial digestion." Physical evidence of colonisation and digestion would corroborate this.

Mixing friable material with compost means that it is very difficult to make meaningful measurements of mass loss. It was therefore decided that electron microscopy of the material and anything which might colonise it would be the most effective way of demonstrating whether it was becoming a substrate for microbial growth and whether the plastic was being consumed.

4 Materials & Methods

A self-sealing polythene bag containing fragments of degrading blue film made from the d_2w^{TM} product was sent to the Fritwell Laboratory. The film was very friable.

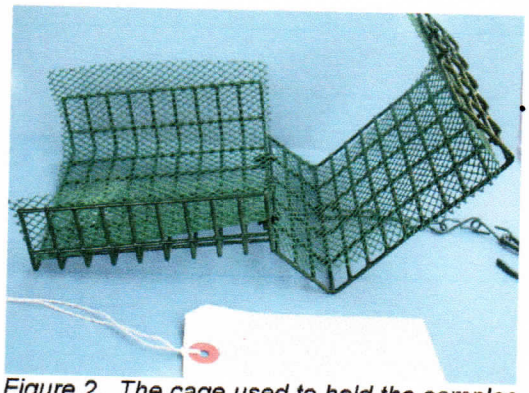


Figure 2. The cage used to hold the samples

Samples of the degrading film were placed in wire cages to enable them to be put into the flowing water and retrieved again. Within the cage, fine mesh netting was used to prevent loss of fragments of film. (Figure 1) Labels were tied to the cages and the cages immersed in water under a cascade in a pool containing fish. (Figure 2) At the time of immersion, the water temperature was 8 degrees Celsius. The samples to be placed in compost were too small to be simply placed in a compost heap so they were packed into a small box with typical garden compost consisting of grass clippings and other garden residues which had reached 45 degrees Celsius within the heap. The extracted compost, mixed with the film and a control non-degradable film was then placed in an incubator at 45 degrees Celsius.

Figure 3: cages hanging in water beneath the cascade.

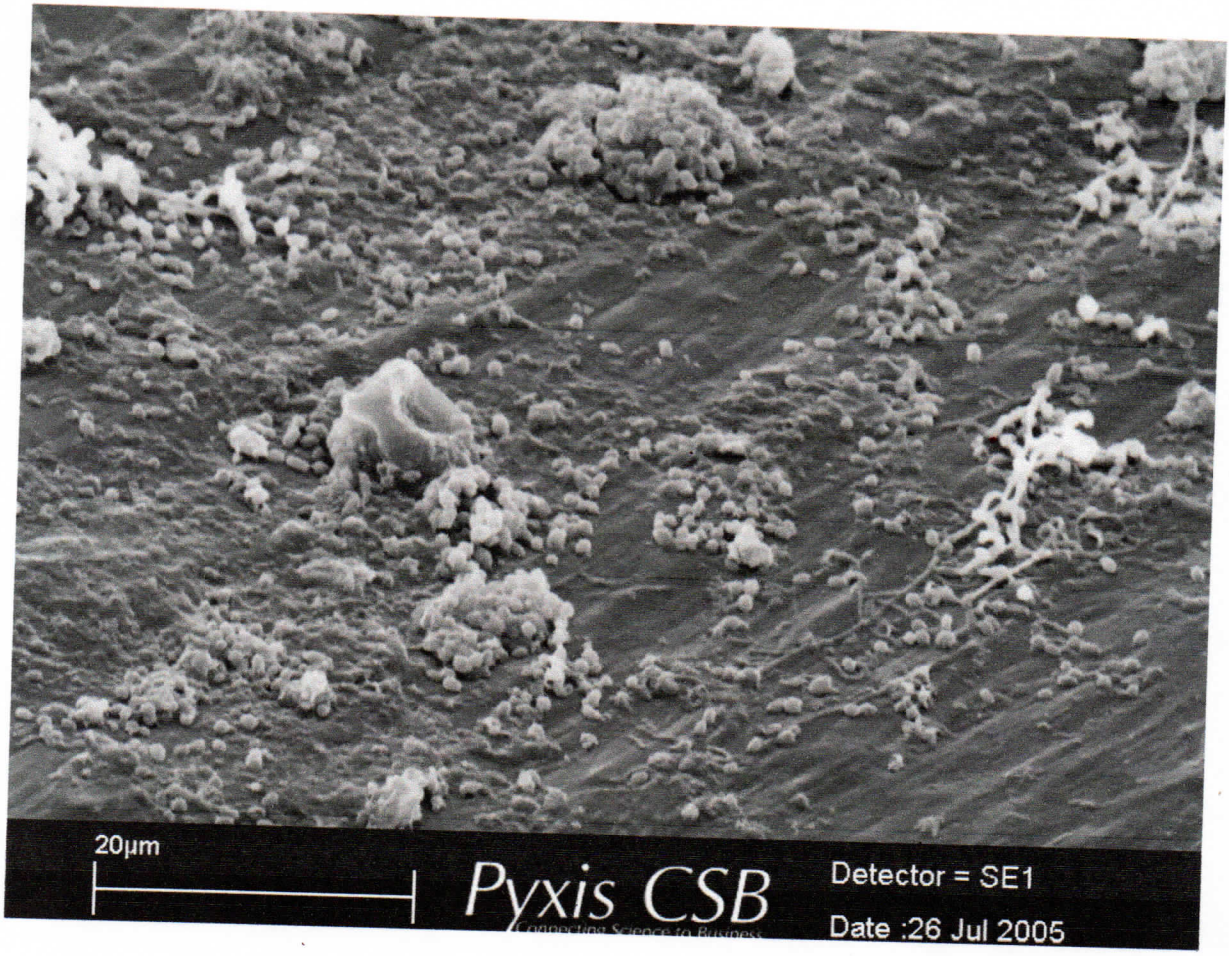
The samples remained in place for thirty days. They were then retrieved from the incubator and from the flowing water.

Samples were removed from the cages and from the mixed compost in the box in the incubator, rinsed in distilled water to remove loosely adhering material and placed on analysis stubs ready for examination in the electron microscope the following morning. Whilst this was being done, it was noted that the samples, particularly those removed from the compost, appeared to have become significantly more brittle.

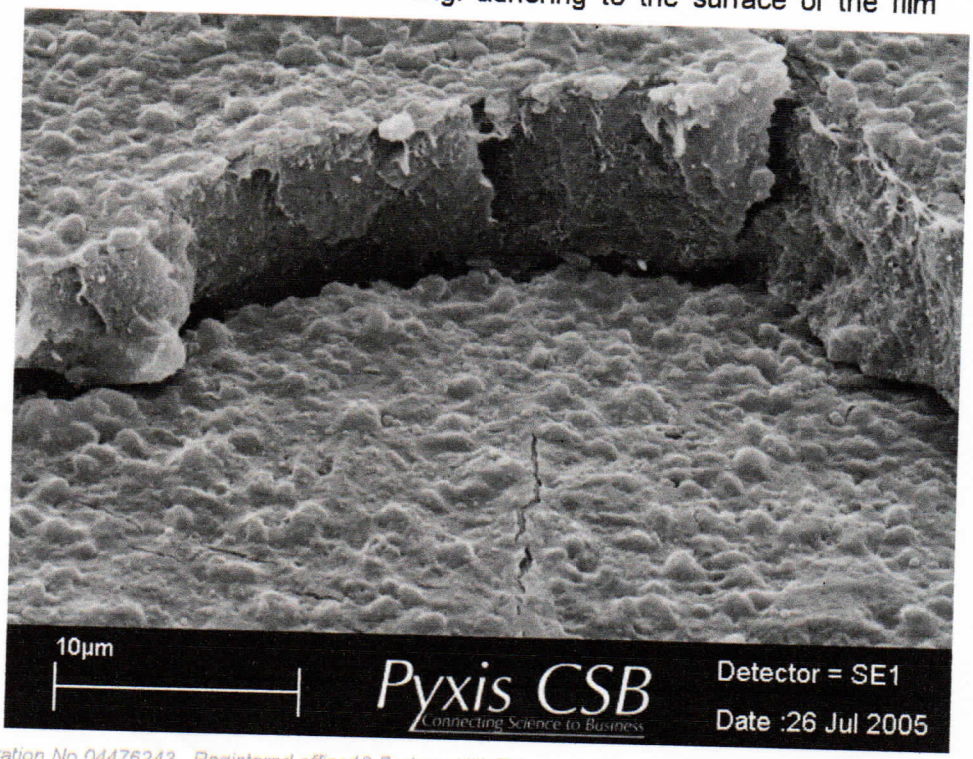
The samples were examined in a Leo 435VP variable pressure scanning electron microscope and examined at high vacuum using an accelerating voltage of 20KV and a beam current of 200 picoamperes. Charging of the material was prevented by coating the samples with 5 nanometres of gold in a sputter coater. Images were taken with the film samples tilted at 45 degrees to maximise the topographic detail visible on the film surfaces.

5 Results

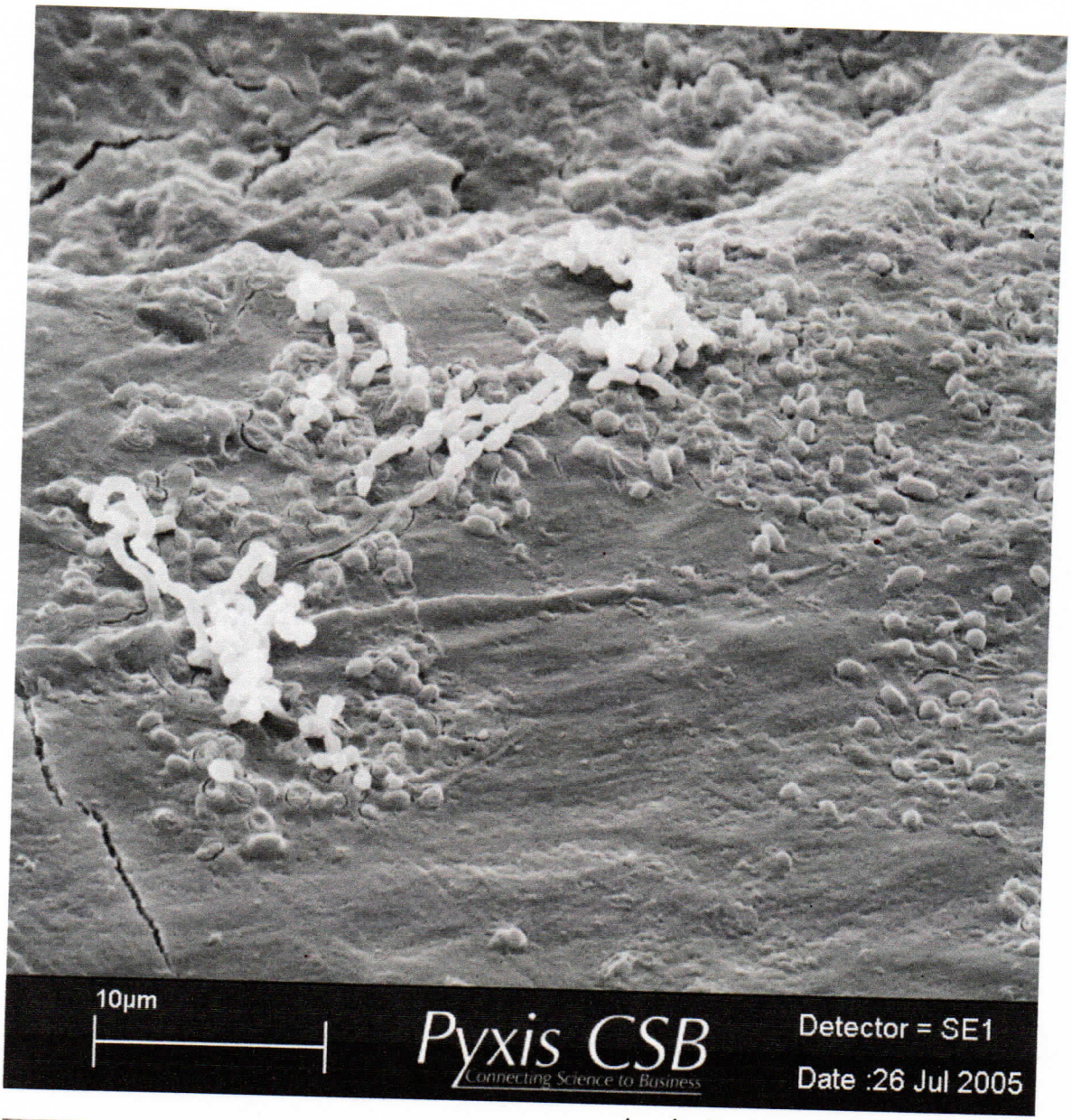
The following micrographs show what was observed.

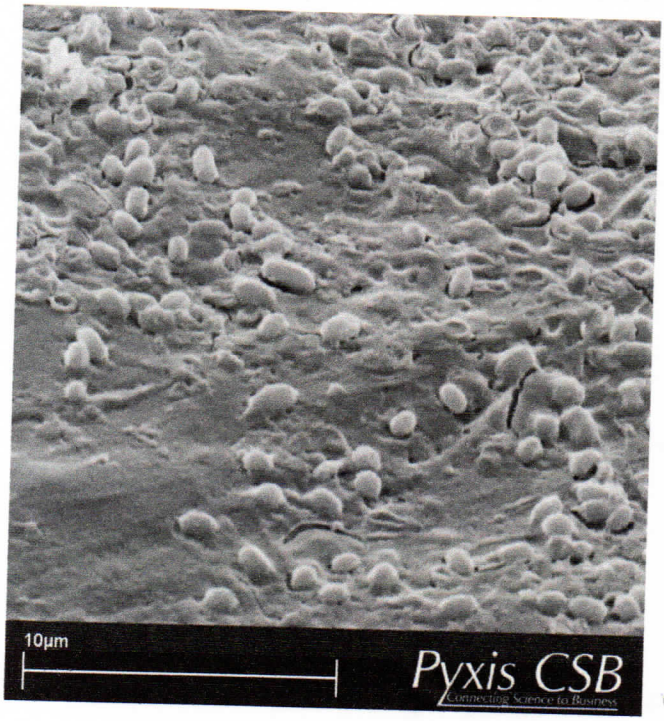


Micrograph 1, above, shows both bacteria and fungi adhering to the surface of the film sample after extraction from the compost. The bacteria are in chains and clusters showing that they have multiplied where they are. The fungal hyphae are appressed to the surface of the film.



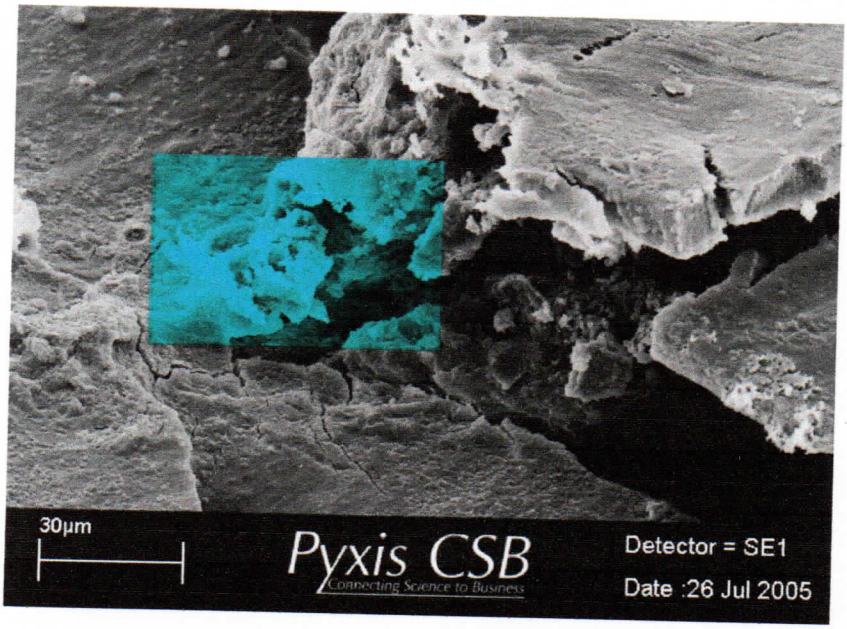
The plastic can be seen to be breaking up. In micrograph 2 (right), the film shows a deep crack and in the foreground is a



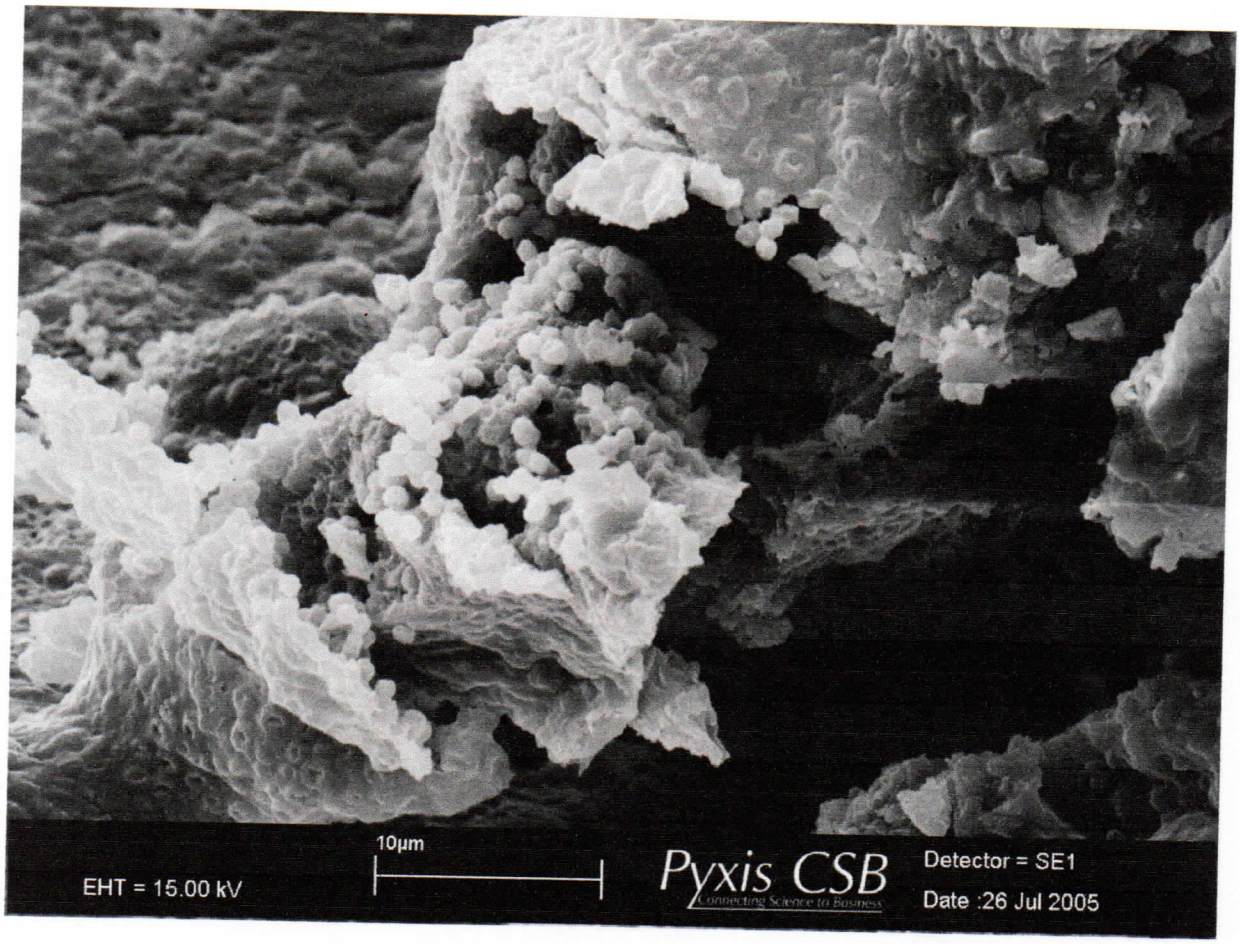


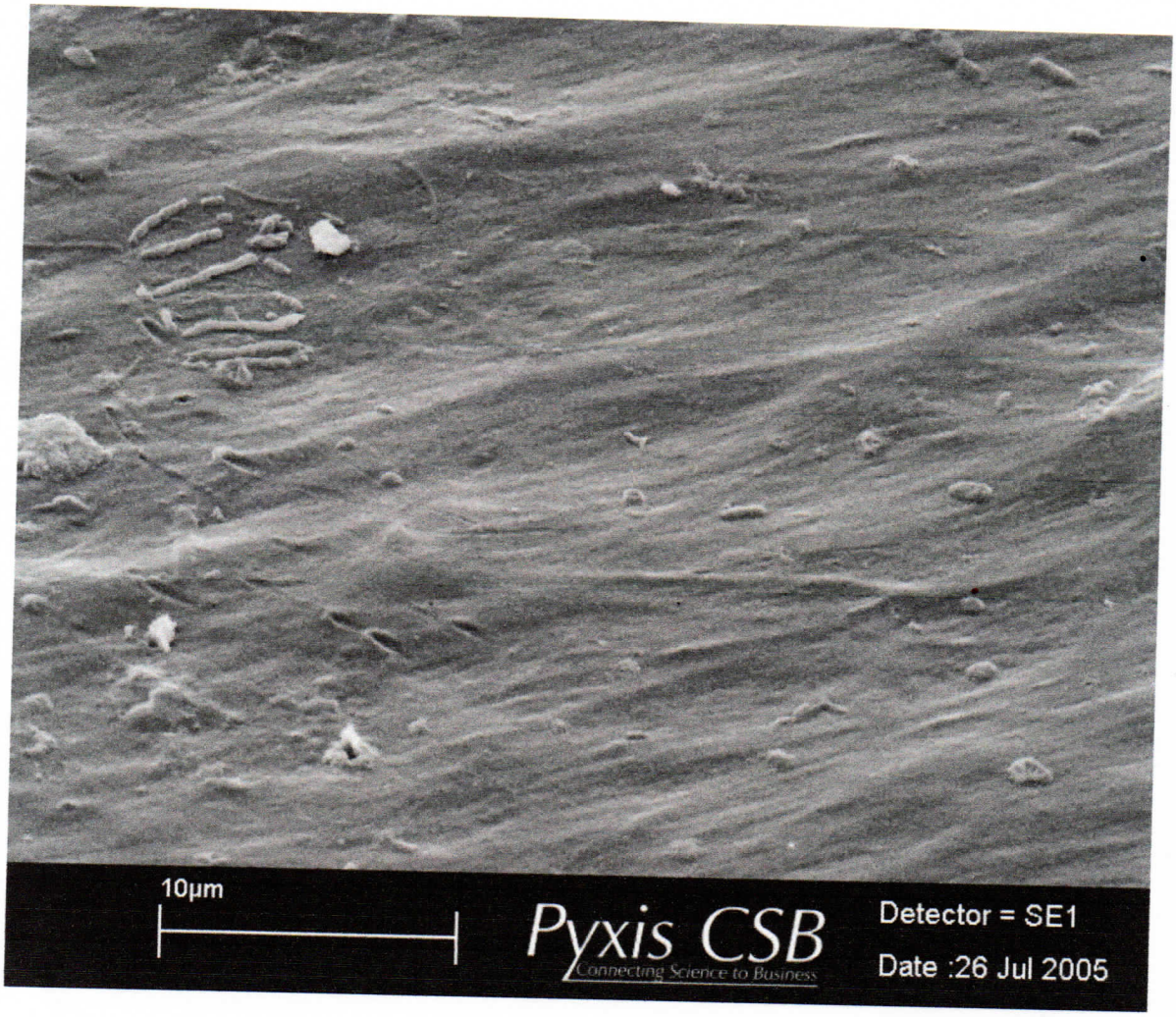
developing crack. The surface of the film is coated with bacteria, and these are immersed in a biofilm coating the surface of the plastic. Micrograph 3 (above) shows fungi sporing on the surface and again the film is cracking. Fungal hyphae and bacteria have digested the surface of the film and are partly immersed in it. Weak regions in the plastic film are giving rise to cracks along the length of the fungal hyphae.

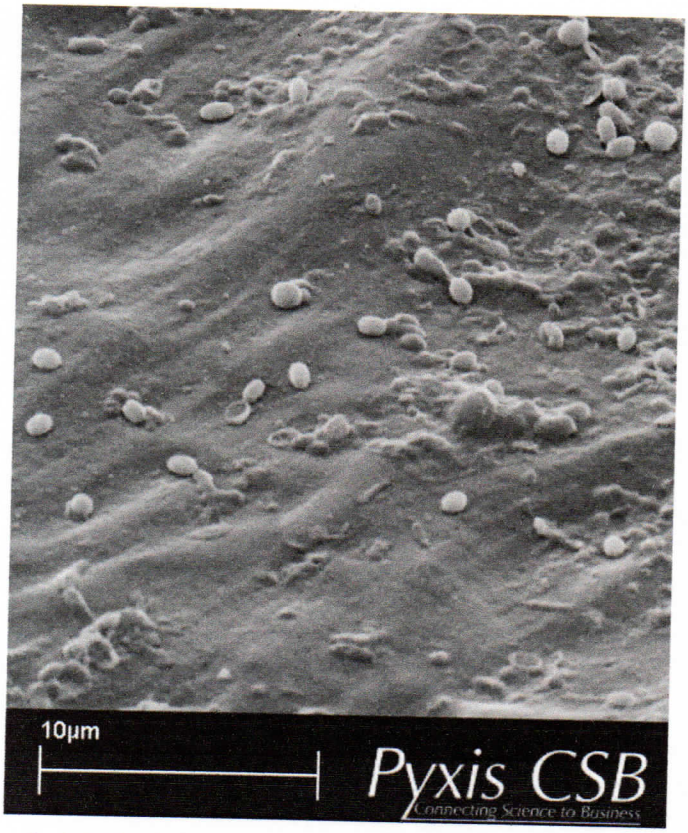
Micrograph 4 (left) also shows bacterial cells investing the surface of the plastic film. Numerous pits and hollows show where the film is being broken down by enzymes produced by the bacteria.



Where the film is breaking up it is readily colonised. Micrograph 5 (left) is of an area of extensive cracking. The area highlighted in blue is seen in greater detail in micrograph 6 below. Numerous bacterial cells and fungal spores are colonising the cracked area and the whole depth of the film through the crack is showing signs of microbial attack.







The samples placed in water have a biofilm of algae and bacteria forming on their surfaces. However, at the relatively low temperature of the pool (8-10 degrees Celsius) during the course of the experiment the rate of growth was very much slower than the growth in compost. Micrograph 7 (above) shows some chains of multiplying bacteria on the surface of the degradable film after immersion in water.

The control material which does not contain catalyst was also examined in the electron microscope. (micrograph 8, left). What microorganisms were present after immersion in compost were sparse and no adherent fungal hyphae were observed Few bacteria were seen and they were not forming colonies.

6 Discussion

It is clear that over the period of the experiment, bacteria and fungi in compost colonised the surface of the degradable plastic. In the case of the non-degradable control, there were few signs of microbial activity on the surface.

Clearly the d_2w^{TM} product provides a substrate for the growth of micro-organisms. Some selection may be involved but those species which do grow on the surfaces, both in water and in compost, appear to thrive. Growth in these species is not inhibited by the transition metal catalyst residues or the breakdown products of the polymer.

Where bioactivity has occurred, there is evidence that the plastic film is being digested. The surface is becoming pitted and where fungal hyphae are adhering, furrows are appearing in the surface. Cracks are associated with these furrows and it is this has made the film more brittle. Additional cracking can be expected in compost at higher temperatures.

Clearly, thirty days at 45 degrees Celsius is far too short a time to be able to unequivocally confirm that the material will break down completely to water and carbon dioxide in these environments. However, the speed and extent of microbial colonisation in this period leads us to expect that an equally rigorous study over a long timescale, covering the life of the material in each of these environments, will confirm that this is what does take place.

The experiment also indicates that the formulation and breakdown products are not ecotoxic. The colonisation and relatively rich microbial flora developed in the short time-frame of the experiment are reassuring in this respect but, again, this should be confirmed by the use of marker species over a lifetime decay analysis.

7 Conclusion

This experiment sought to demonstrate that the d_2w^{TM} product provides a substrate for microbial growth as a means of confirming that the material will biodegrade in compost or in watercourses. What the electron microscope has illustrated is that, in the conditions of this experiment and with the material used, biodegradation will take place. This formulation of plastic is colonised by fungi and bacteria in the case of compost, and algae and bacteria in the case of water immersion, although the process is much slower at the lower temperature of the water. The growth of these organisms is evidently not inhibited by the transition metal based catalyst used in the product and consequently there is no evidence that it is ecotoxic.

It is therefore concluded that, based on this experiment, products with this formulation of catalyst are biodegradable. The time that the process will take will depend on temperature, moisture levels and the amount of UV irradiation that the material has received.

A long-term experiment would therefore be expected to demonstrate the complete breakdown of the material to carbon dioxide and water and, if used in the formulation, traces of the metal used as the cation for the organic salt providing the catalyst for depolymerisation.

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