PRELIMINARY STUDY ON MICROBIAL DEGRADATION OF FLEXIBLE POLYURETHANE FOAMS – PHYSICO-MECHANICAL AND WEIGHT CHANGES DURING FUNGAL DETERIORATION

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ABSTRACT

The ability of the fungus, Aspergillus niger*, to utilize palm-based flexible polyurethane foam as food was determined via the petri-dish test. A commercial polyurethane foam was used as the control. A dense fungal growth was detected by visual examination of foams inoculated on minimal nutrient agar (MNA) but not on the mineral salts agar (MSA). The weight changes for all samples were analysed after four weeks. Both the palm-based and commercial flexible polyurethane foams incubated on MNA suffered significant weight losses while slight increases were recorded by the samples incubated on MSA. Under a SEM, dense fungal growth was observed covering the samples incubated on MNA but none on the samples incubated on MSA. Instead, the presence of spores on the latter indicated that the fungus had not germinated sufficiently to degrade the polyurethanes. The compression strength for all the samples increased with time. The increase in hardness was more in the palm-based polyurethanes than in the commercial ones. This study showed that palm-based flexible foam can be degraded by* Aspergillus niger *in the presence of sufficient nutrients.*

Keywords: microbial degradation, flexible polyurethane foam, *Aspergillus niger*, petri-dish test.

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INTRODUCTION

Polyurethanes (PU) have found widespread applications in many industries and agriculture, and it is imperative to know more about their biological stability or degradability. Since their introduction commercially more than 50 years ago, PU have found a niche in the plastics industry in which they are assured a healthy future. Their superior properties of abrasion, oxidation, oil resistance, and elasticity have made them a most suitable material for electrical cable sheathings, shoe soles, motor car components, foams, adhesives and as alternatives to natural rubber (Pathirana and Seal, 1984).

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Although most synthetic polymers are resistant to biodegradation, PU, particularly the polyester type, are susceptible to biodegradation by a wide range of microorganisms, which break down the polyester by extra-cellular hydrolytic enzymes. Polyether PU are also susceptible to microbial attack by enzymatic hydrolysis of the urethane link (Wales and Sagar, 1985). Many studies, however, have shown that polyether PU are more resistant to microbial degradation than polyester PU (Wales and Sagar, 1985; Kaplan *et al.*, 1968). Kaplan *et al.* (1968) found that the ability of microorganisms to utilize PU as their sole carbon source depends on the PU molecular structures and the types of chemical links they contain.

The testing of PU for their resistance towards microbial degradation involves an assessment of the deleterious effects of microorganisms on the functional properties of the PU. The properties range from the purely visual to the distinctly mechanical, physical and electrical properties. The way in which microorganisms alter these properties is governed

by a range of factors related to the formulation of the PU, the processing and handling prior to the testing, the physical and organic environment in which the tests are carried out and the constitution of the inoculum (Seal and Pantke, 1986). There is thus much scope for variability and therefore poor reproducibility of the results, even from similar samples tested in the same laboratory.

Previous studies have shown that many fungi inhabit PU under different conditions (Evans and Levison, 1968; Hedrick and Crum, 1968; Kaplan *et al*., 1968), some only on the surface while others penetrate into the insides. Some of these fungi produce esterases capable of degrading PU. Pathirana and Seal (1983) reported that the fungus *Gliocladium roseum*, could only degrade PU if provided with additional nutrients. Klausmeier (1966) concluded that even polyether PU may be degraded if sufficient, readily metabolisable nutrients are available.

This study investigated the ability of *Aspergillus niger* to degrade the palm-based and petroleum-based PU, and examine the effects of the fungal attack on the properties of the PU. *A. niger* has been used in such studies before and is known able to degrade PU (Filip, 1979). The resistance of both PU to the fungal attack was assessed by their weight loss and changes in physical properties.

MATERIALS AND METHOD

Test Samples

Palm-based and a commercial flexible PU foams (petroleum-based flexible foam) were used in this study. Foam was considered a suitable test material as its porous nature provides a large surface area-to-volume ratio for the microorganisms to attack the material. The palm-based foam was prepared using an in-house method while the commercial foam was obtained from Rokisar Sdn Bhd, Malaysia. The foams were cut into blocks (60.0 mm x 60.0 mm x 20.0 mm) ± 0.2 mm. The initial weights and dimensions of the blocks were measured using an analytical balance to 0.01 g and an electronic digital calliper to 0.1 mm, respectively.

Preparation of Agar Plates

Two agar media were prepared as described in BS 6085 - mineral salts agar (MSA) and minimal nutrient agar (MNA). MNA contained the same salts as MSA but with mycological peptone (1% w/v) and malt extract (1% w/v) added. MSA was used to determine whether the foams might serve as a nutritive substance for the fungus.

Fungus

A. niger (ATCC 9642) was used in this test. The stock culture was maintained on potato-dextrose agar. A well-sporulating culture was used to prepare the spore suspension. The spore density was counted and the concentration adjusted to 10^6 – 10^7 spores ml^{-1}.

Degradation of PU on Agar Plates

The foam blocks were placed on both MSA and MNA plates already inoculated with *A. niger*. The plates were incubated for four weeks at 37ºC ± 1ºC. The controls were without inoculation, incubated together with the other plates.

Triplicate samples of each foam were taken from both agar plates weekly, cleaned with tissue paper and conditioned to room temperature for 24 hr. The control samples were taken at the end of the test.

Test Procedures

After the conditioning period, the following tests were conducted on the samples.

Visual examination. Several methods have been used to assess fungal degradation of PU, including a rating scheme to assess the extent of fungal contamination. Hitz *et al.* (1967) assessed the resistance of PU to fungal attack by the degree of fungal growth. After incubation for one, two, three and four weeks, the samples were assessed visually and rated according to the schedule in *Table 1*. Three replicates were used for each assessment. The results were interpreted according to *Table 2*. The samples were also examined visually for evidence of cracking.

Weight changes. Assessment of the weight loss gave a clear indication of whether the foams were resistant or susceptible to the microorganisms. The method has been used to quantify the assimilation of materials by microorganisms (Kay *et al*., 1991).

TABLE 1. RATING SCHEME ACCORDING TO HITZ *et al.* (1967) FOR ASSESSMENT OF FUNGAL GROWTH ON PLASTIC SAMPLES

Rating	Evaluation
0	No growth seen under a light microscope
1	Fungal growth barely visible to the naked eye but quite apparent under a light microscope
2	Growth on the surface visible to the naked eye but covering less 25% of the test surface
3	Growth on the surface covering more than 25% of the test surface

TABLE 2. INTERPRETATION FOR RATINGS ACCORDING TO HITZ *et al.* (1967)

Rating	Evaluation of test material
0	Material of no nutritive value to microorganisms; it is inert or fungitoxic.
1	Material contains nutritive substances or is contaminated to such a small degree that it permits only slight growth.
2,3	Material not resistant to fungal attack and contains nutritive substances suitable for development of microorganisms.

Following the visual examination, the samples were weighed and the percentage weight loss calculated by:

$$\text{Weight loss (\%)} = \frac{[\text{Initial wt. (g)} - \text{final wt. (g)}]}{\text{Initial wt. of the sample (g)}} \times 100\%$$

Microscopic examination. Small portions of the 0-week (initial) samples and the final week samples were carefully cut out and sent for Scanning Electron Microscope (Model Phillip XL 30) evaluation in Universiti Kebangsaan Malaysia for any sign of structural disruption.

Changes in compression stress. The compression hardness test was conducted according to DIN 53577. Samples were compressed between two flat plates at the rate of 10% of the samples' initial thickness (mm min^{-1}) using a Hounsfield S-Series Material Testing Machine (Model H10K-S). Based on the stress *vs*. strain curve, the compressive stress values for the flexible foams were recorded weekly.

RESULTS AND DISCUSSION

Visual Examination

The results based on the rating scheme by Hitz *et al.* (1967) are summarized in *Table 3*. Dense growth of the fungus was observed on the surface of the palm-based and commercial flexible foams incubated on MNA (*Figure 1*). No growth was observed on the samples inoculated on MSA (*Figure 2*), indicating that *A. niger* was unable to use the foams as its sole carbon source. The inclusion of mycological peptone (1% w/v) and malt extract (1% w/v) in the MNA promoted the growth of *A. niger*. No signs of cracking or any other deterioration were observed on all samples.

Determination of Mass Changes

The average mass losses as percentages of the original masses of the samples are shown in *Table 4*. Palm-based and the commercial flexible foams

TABLE 4. MASS CHANGES OF POLYURETHANES (PU) SAMPLES AFTER FOUR WEEKS

Sample	Weight Loss (%) after 4 weeks*
Palm-based flexible foam (MSA agar)	-0.455
Palm-based flexible foam (MNA agar)	40.20
Commercial flexible foam (MSA agar)	-0.87
Commercial flexible foam (MNA agar)	25.31

Note: * Means of three readings.

TABLE 3. GROWTH OF *A. niger* ON POLYURETHANES (PU) SAMPLES RATED ACCORDING TO HITZ *et al.* (1967) AFTER FOUR WEEKS

Sample	Intensity of fungal growth*	Evaluation
Palm-based flexible foam (MSA agar)	0	Material of no nutritive value to the microorganism
Palm-based flexible foam (MNA agar)	3	Material not resistant to degradation and contains substances for development of the microorganism
Commercial flexible foam (MSA agar)	0	Material of no nutritive value to the microorganism
Commercial flexible foam (MNA agar)	3	Material not resistant to degradation and contains substances for development of the microorganism
Controls for all samples in both types of agar	0	Material of no nutritive value to the microorganisms

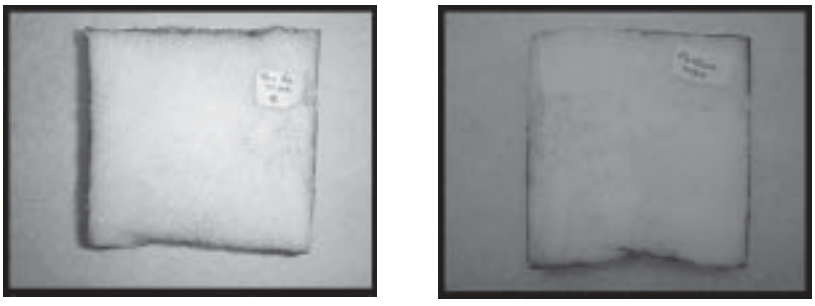
Note: *Means of three assessments.

0 week 1 week 2 weeks

3 weeks 4 weeks

Figure 1. Growth of A. niger *on palm-based flexible foam at 0, one, two, three and four weeks' incubation on minimal nutrient agar (MNA).*

0 week 1 week 2 weeks



3 weeks 4 weeks

Figure 2. No growth of A. niger *on palm-based flexible foam at 0, one, two, three and four weeks' incubation on mineral salts agar (MSA).*

inoculated on MNA showed weight losses of 40.2% and 25.3%, respectively after four weeks. The higher loss in the palm-based foam suggested that it was degraded faster than the commercial foam by *A. niger.*

The foams inoculated on MSA, however, slightly increased in weight after four weeks. Since there was no fungal growth on them, no microbial degradation would have occurred to cause any weight loss, and the slight increase in weight may be due to absorption of microscopic particles, most probably water, into the porous foams.

Microscopic Examination

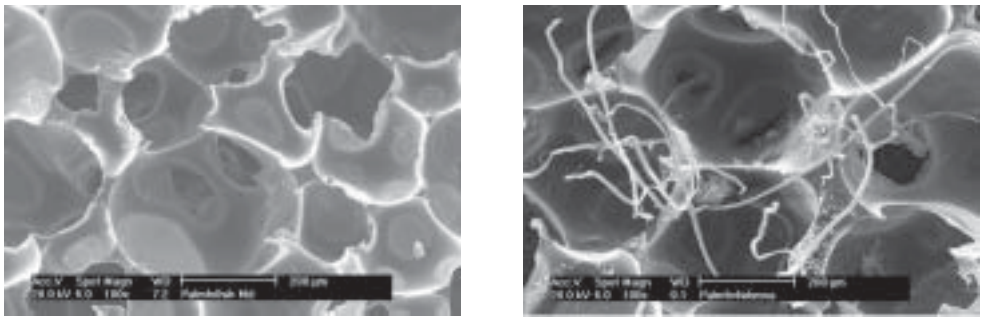
Microscopic examination of the 0-week (initial) and four-week samples revealed dense of fungal hyphae on the surface of the samples incubated on MNA (*Figure 3*). However, only a few of the hyphae penetrated into the sample. Since an attack by an organism proceeds from the surface inwards (Hitz *et al.*, 1967), it was primarily the outer surface of the foams that was being attacked. No hyphae growth however, was observed in the samples incubated on MSA (*Figure 4*) and in the control samples. A lot of spores were seen on the MSA samples, indicating that the fungus had not fully germinated to degrade the foams (*Figure 5*). This may be the reason for the insignificant weight changes in the foams incubated on MSA.

According to Kay *et al.* (1991), degradation took the form of cracking on the lamellae between the foam cells. In this test however, no cracking of lamellae was detected on any of the samples.

Four weeks may not be sufficient time for the fungus to fully develop and attack the foams, especially when incubated on MSA agar. A longer test may be required for detectable degradation.

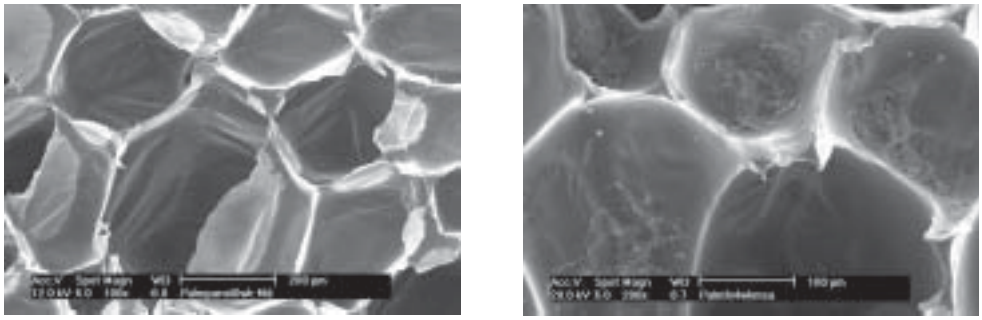
Changes in Compression Strength

Compressive strength is the resistance to collapse when pressure is applied perpendicular to the surface of the foam. The 40% strain is a characteristic value for flexible foam at which the cell structure starts to deform. *Figure 6* shows the stress *vs.* strain curves of flexible foams. Each point on the graphs represents the mean value of three replicates. The hardness of all the samples increased with time. Palm-based flexible foam seems to harden faster than the commercial foam. This increase in hardness may be due to water being absorbed from the surrounding and filling the foam cells or reacting with excess isocyanate in the formulation and making the foam harder.



0 week 4 weeks

Figure 3. Ramification of fungal hyphae incubated on minimal nutrient agar (MNA) as viewed under SEM.



0 week 4 weeks

Figure 4. No fungal hyphae on the surface of a sample incubated on mineral salts agar (MSA) as viewed under SEM.

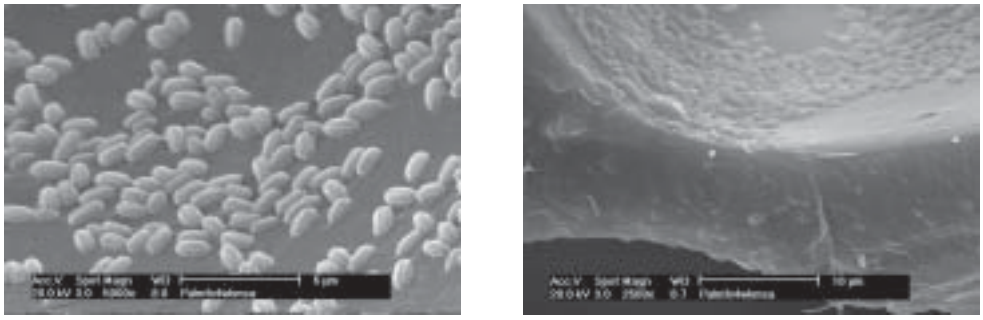


Figure 5. Spores in samples after four weeks' incubation on mineral salts agar (MSA).

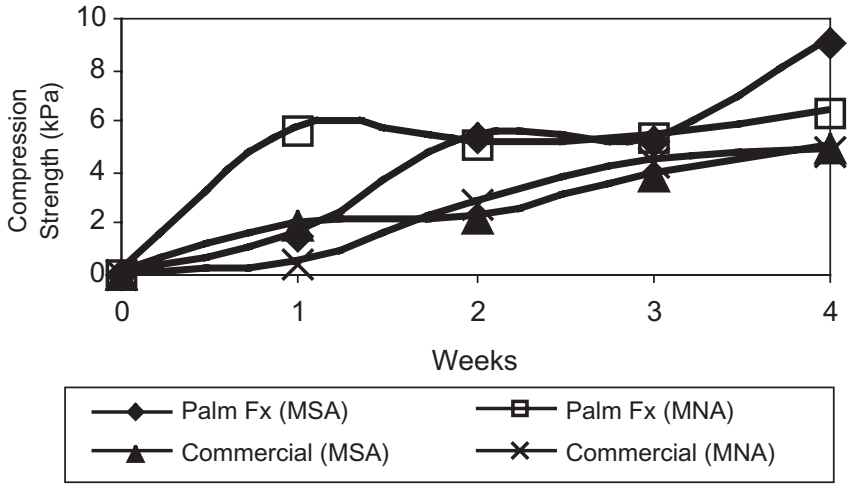


Figure 6. Compression strength values at 40% strain for palm-based (Palm Fx) and commercial flexible foam (commercial) in both mineral salts agar (MSA) and minimal nutrient agar (MNA) over time.

CONCLUSION

Although palm-based and commercial flexible foams may not be the sole carbon source for *A. niger*, the foams were not completely resistant to microbial attack. They were degraded when incubated on MNA, *i.e.* when sufficient, readily metabolisable nutrients are available.

Significant weight losses were recorded for both the foams incubated on MNA, in addition to dense fungal growth after four weeks. The higher weight loss in the palm-based foam showed that it would degrade faster in the environment with *A. niger*. Insignificant weight changes in foams incubated on MSA suggested that the fungus requires nutrients to germinate and degrade the foams.

Under the SEM, dense fungal hyphae was observed on the surface of the foams incubated on MNA, but only spores were detected on the foams incubated on MSA. Again, this indicated that the fungus requires nutrients to grow.

The compression strength of all the samples increased with time and was higher on the palm-based flexible foams. These samples hardened faster than the petroleum-based samples due to the presence of water.

The presence of nutrients promoted the growth of *A. niger*. This fungus could degrade both the flexible foams. However, four weeks were not sufficient time to demonstrate the ability of these foams to be used as the sole carbon source for *A. niger* without additional nutrients. A longer test will be required for the fungus to fully exert its degradative power on the foams. The results, nevertheless, suggested that palm-based flexible foams can be degraded by *A. niger* and should therefore not pose any environmental problem after their disposal.

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REFERENCES

BS 6085 (1981). *Determination of the Resistance of Textiles to Microbiological Deterioration*. British Standards Institution, London.

DIN 53577 (1988). *Determination of Compression Stress Value and Compression Stress-Strain Characteristic for Flexible Cellular Materials Characteristics*. Deutsche Industrie Normen, Germany.

EVANS, D M and LEVISON, I (1968). Biodeterioration of polyester-based polyurethane. *International Biodeterioration Bulletin, 4:* 89-92.

FILIP, Z (1979). Polyurethane as the sole nutrient source for *Aspergillus niger* and *Cladosporium herbarum. European J. Appl. Microbiol. Biotechnol., 7:* 277-280.

HEDRICK, H G and CRUM, M G (1968). Effects of jet-fuel microbial isolates on a polyurethane foam. *Appl. Microbiol.*, *16*: 1826-1830.

HITZ, H; MERZ, A and ZINKERNAGEL, R (1967). Determination of the resistance of plasticized PVC to attack by fungi and bacteria by the weight loss *Method and Evaluation of Mechanical Properties.* p. 271-296.

KAPLAN, A M; DARBY, R T; GREENBERGER, M and ROGERS, M R (1968). Microbial deterioration of polyurethane systems. *Developments in Industrial Microbiology*, *9:* 201-217.

KAY, M J; MORTON, L H G and PRINCE, E L (1991). Bacterial degradation of polyester polyurethane. *International Biodeterioration, 27:* 205-222.

KLAUSMEIER, R E (1966). The effect of extraneous nutrients on the biodeterioration of plastics. *SCI Monograph 23, Microbiological Deterioration in the Tropics.* p. 232-243.

PATHIRANA, R A and SEAL, K J (1983). *Gliocladium roseum* (Bainier), a potential biodeteriogen of polyester polyurethane elastomers. *Biodeterioration 5* (Oxley, T A and Barry, S eds.). John Wiley, New York. p. 679-689.

PATHIRANA, R A and SEAL, K J (1984). Studies on polyurethane deteriorating fungi. Part 1. Isolation and characterization of the test fungi employed. *International Biodeterioration, 20(3)*: 163-168.

SEAL, K J and PANTKE, M (1986). An interlaboratory investigation into the biodeterioration testing of plastics, with special reference to polyurethanes. Part 1: petri dish test. *Material and Organismen, 21:* 151-164.

WALES, D S and SAGAR, B F (1985). Effect of structure on susceptibility of polyurethanes to biodegradation. *Biodeterioration and Biodegradation of Plastics and Polymers* (Seal, K G ed.). Biodeterioration Society, Surrey, UK. p. 56-69.