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Microbial Degradation of Flexible Polyurethane Foams by *Aspergillus niger* and *Aspergillus terreus*

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ABSTRACT

The ability of the fungi, Aspergillus niger and Aspergillus terreus*, to degrade palm-based flexible polyurethane foam was determined via the petri dish and shake flask tests. A dense fungal growth was detected by visual examination of foams inoculated on minimal nutrient agar/solution (MN) but not on the mineral salts agar/solution (MS). Both the palm-based and commercial flexible polyurethane foams incubated on MN suffered significant weight losses while slight increases were recorded by the samples incubated on MS. Under a scanning electron microscope (SEM), dense fungal growth was observed covering the samples incubated on MN but none on the samples incubated on MS. Palm-based foams were degraded much faster than the commercial foams in the shake flask test with* A. terreus *as shown by higher weight losses, deterioration and even complete decomposition of the samples. Complete decomposition of palm-based foam showed that it could be degraded in the environment in the presence of* A. niger *or* A. terreus*, with sufficient nutrients and maximum contact between the fungi and the foams.*

ABSTRAK

Kebolehan kulat Aspergillus niger *dan* Aspergillus terreus *menguraikan busa poliuretana fleksibel berasaskan sawit ditentukan melalui kaedah piring petri dan goncangan kelalang. Pertumbuhan kulat yang padat dapat dilihat dengan mata kasar pada busa yang dieram dalam larutan/agar nutrien minimum (MN) tetapi tiada pertumbuhan pada busa yang dieram dalam larutan/agar garam mineral (MS). Kedua-dua jenis busa yang dieram dalam MN mengalami kehilangan berat yang ketara manakala terdapat sedikit pertambahan berat pada busa yang dieram dalam MS. Pengamatan melalui mikroskop imbasan elektron (SEM) menunjukkan pertumbuhan kulat yang padat pada busa di dalam MN, tetapi tidak pada busa di dalam MS. Busa berasaskan sawit diuraikan lebih pantas dari busa komersial di dalam ujian goncangan kelalang bersama-sama* A. terreus *seperti yang ditunjukkan melalui kehilangan berat yang lebih tinggi dan penguraian sepenuhnya busa tersebut di dalam MN. Penguraian sepenuhnya busa berasaskan sawit menunjukkan busa ini dapat diuraikan di persekitaran dengan kehadiran* A. niger *atau* A. terreus*, nutrien yang mencukupi dan pendedahan maksimum kulat kepada busa tersebut.*

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

INTRODUCTION

Polyurethanes (PU) are a diverse group of synthetic polymers. They possess a wide range of chemical and physical properties which have been used by the industry, commerce, agriculture and medicine in an extensive range of materials such as foams, elastomers, paints, fibres, coatings, adhesives and sealants. Widely used in a range of industries, PU is often discarded after use. They would therefore, present an environmental problem because of their resistance to disintegration and biodegradation.

For years researchers have tried to solve the degradability problems by investigating modification or productions that could lead to biodegradable products. One solution to these

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problems would be to include a material in the formulation of PU that provides biodegradability. Starches, various polysaccharides and vegetable oil have all been used in the production of PU foam to promote foam disintegration (Borchardt, 2004).

Palm oil and its products have been found to be good alternative raw material for the production of PU. Palm oil, unlike petroleum, is a renewable resource. The starting materials used for the production of palm-based PU are polyols (derived from epoxidized palm oil and its products), methylene diisocyanates (MDI), water and additives (Parthiban *et al.,* 2001). The production process of palm-based PU is more environmentally friendly as water is used as the blowing agent and this introduces less environmental problems than the conventional petrochemical-based PU, which normally uses CFC as the blowing agent.

Studies have shown that PU were susceptible to microbial attack. Several workers have found that the polyester PU is more vulnerable to microbial degradation than the polyether PU (Ossefort and Testroet, 1966; Darby and Kaplan, 1968; Potts *et al.*, 1973; Pathirana and Seal, 1985; Nakajima-Kambe *et al.*, 1999). A number of microorganisms, particularly fungi, have been isolated and characterized for their ability to degrade polyester PU. Some of these fungi produce esterases capable of degrading PU.

Several methods have been used to assess fungal degradation of PU and the degradation has been quantified in terms of weight loss and by changes in the physical properties of the PU (Darby and Kaplan, 1968; Potts *et al.*, 1973; Pathirana and Seal, 1984). The measurement of weight loss has been used to quantitatively assess the extent of degradation of the material (Pathirana and Seal, 1985). However, later investigations have shown that although fungal growth was apparent on test material, the weight losses were often insignificant (Seal and Pantke, 1986). Measurements of tensile strength and other physical properties have also been used by a number of workers and proved to be useful criteria to assess the extent of microbial degradation (Pathirana and Seal, 1985; Bentham *et al.*, 1987).

Previous study had shown that *Aspergillus niger* was able to penetrate into the foams and cause significant weight losses of the samples when incubated on MN in petri dish test for four weeks. However, the short test period was unable to demonstrate the ability of the foams as the sole carbon source for *A. niger* without additional nutrients.

This study investigated the ability of *A. niger* and *A. terreus* to degrade the flexible PU foams at a longer test duration, examine the effects of the fungal attack on the properties of the foams and the biodegradability of foams prepared from palm-based polyol. *A. niger* has been used in such studies before and is known to be able to degrade PU (Filip, 1979). *A. terreus* was isolated from oil palm plantation and its ability to degrade the foams was evaluated. The ability of both fungi to degrade the palm-based and commercial foams was assessed by the weight loss and changes in physical properties of the foams.

MATERIALS AND METHOD

Test Samples

Palm-based and commercial flexible PU foams (petroleum-based flexible foam) were used in this study. Foam was used 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 according to an in-house method using palm-based polyols [patent Singapore 55223, patent Malaysia MY 114189-A, patent application (Indonesia): P 962884]. The commercial foam was obtained from Rokisar Sdn Bhd, Malaysia.

The foams were cut into blocks (30.0 mm x 30.0 mm x 15.0 mm) ± 0.2 mm and also into dumb-bells with a dumb-bell cutter (Die A of Test Method ASTM D412). The initial weights and dimensions of the blocks and dumb-bells were measured using an analytical balance accurate to 0.01 g and an electronic digital caliper to 0.1 mm, respectively.

Preparation of Agar Media and Solution

Mineral salts agar/solution (MS) and minimal nutrient agar/solution (MN) were prepared as described in BS 6085. MN contained the same salts as MS but with added mycological peptone (1% w/v) and malt extract (1% w/v). MS was used to determine whether the foams might serve as a nutritive substance for the fungus. The agar and solution were autoclaved at 121°C for 15 min.

Fungi

A. niger (ATCC 10146) and *A. terreus* (isolated from oil palm plantation) were 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 was adjusted to 10^6 -10^7 spores ml^{-1}.

Degradation of Foams on Agar Plates (petri dish test)

The foam blocks were placed on MS and MN agar plates already inoculated with *A. niger* or *A. terreus*. The plates were sealed with parafilm to prevent evaporation. They were then incubated for three months at 37°C ± 1°C. The controls (palm-based and commercial foams) without inoculation, were incubated together with the other plates.

Degradation of Foams in Liquid Culture (shake flask test)

Besides agar plates, liquid culture was also used to maximize contact between the fungi and foams. Three replicates were placed in each 250 ml conical flask, containing either 100 ml MN or MS solution. All flasks were then incubated in an incubator shaker with continuous shaking at 200 rpm for two months at 37°C ± 1°C. Control samples were shaken together with the other flasks.

Test Procedures

Triplicate samples of each type of foam were taken from both agar plates and solutions monthly while in the shake flask test, triplicate samples were taken weekly. They were cleaned with tissue paper and conditioned to room temperature for 24 hr. The control samples were taken at the end of the test.

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

a. *Visual examination*. At specified intervals, three replicates of the samples were assessed visually. The samples were also examined for evidence of cracking.

b. *Microscopic examination*. Small portions of the samples were carefully cut out before start of test and after completion of the test, and were sent for scanning electron microscopy (Model Phillip XL 30) evaluation at Universiti Kebangsaan Malaysia for any indication of structural disruption.

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

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

$$\text{Weight loss (\%)} = \frac{\text{[Initial wt. (g) – Final wt. (g)] x 100\%}}{\text{Initial wt. of the sample (g)}}$$

Initial weights of samples were measured just after cutting the samples into blocks or dumb-bells.

d. *Changes in compression strength and tensile strength*. For the determination of tensile strength and compression strength, the block and dumb-bell samples were conditioned at room temperature for 24 hr. The compression and tensile tests were conducted using a Hounsfield S-Series Material Testing Machine (Model H10K-S).

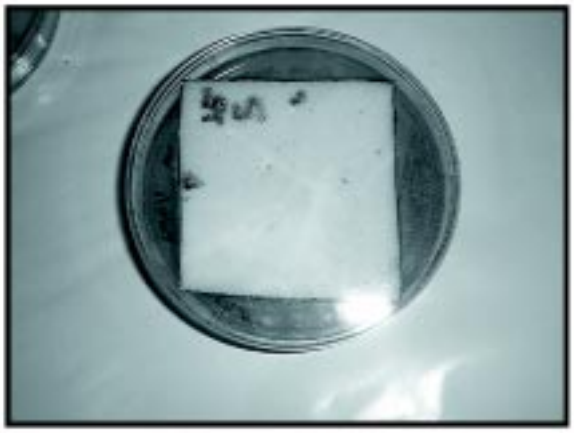
The compression test was performed on the blocks according to standard method DIN 53 577. Samples were compressed between two flat plates at the rate of 100 mm min^{-1}. Based on the stress *vs*. strain curve, the compressive stress values for the flexible foams were recorded.

The tensile properties are important characteristics of the strength of any material. The specimen is stretched at a constant rate until it breaks. The tensile strength is the maximum stress the material withstands before rupture. The tensile test on the dumb-bells was conducted with a crosshead speed of 500 mm min^{-1} according to standard method ASTM 3574.

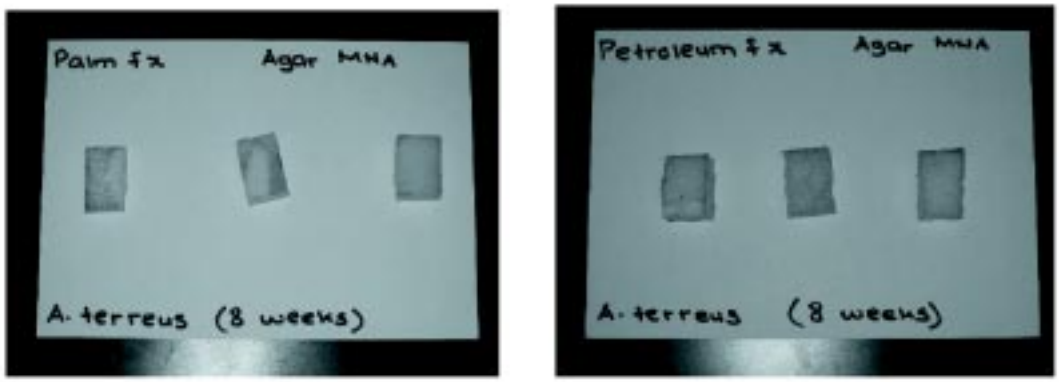
RESULTS AND DISCUSSION

Visual Examination

Dense growth of the fungi was observed on the surface of the palm-based and commercial flexible foams incubated on MN agar in the petri dish test (*Figure 1*). No growth was observed on the samples inoculated on MS agar, indicating that the fungi were unable to use the foams as its sole carbon source. Nakajima-Kambe *et al.* (1999) found that PU degradation by fungi



0 week



8 weeks

Figure 1. Growth of A. niger *and* A. terreus *on palm-based flexible foam (left) and commercial foam (right) at 0 and 8 weeks' incubation on MN agar in petri dish test.*

requires the addition of several nutrients and no PU-degrading fungi can utilize PU as the sole carbon source. The inclusion of mycological peptone (1% w/v) and malt extract (1% w/v) in the MN promoted the growth of the fungi. No signs of cracking or any other deterioration were observed on all blocks.

Due to the maximum contact between fungi and foams, more sample deterioration was observed in the shake flask test than in the petri dish test. After one month, some of the dumb-bells in MN deteriorate, lost their shape or broke. After two months, palm-based foams fully degraded and precipitated at the bottom of the flask (*Figure 2*). Commercial foams however, only lost their shape or broke into smaller fragments (*Figure 3*). Palm-based foams incubated in MS solution also showed certain degree of deterioration (*Figure 4*), unlike commercial foams that retained their original form (*Figure 5*). No deterioration could be detected in any of the control samples.

From these observations (*Figures 2* to *5*), palm-based foams were degraded much faster than the commercial foams. Lack of nutrients inhibited the degradation process as seen in samples incubated in MS and both fungi were unable to use the foams as the sole carbon source.

Microscopic Examination

Microscopic examination of the samples after completion of the test showed dense fungal hyphae on the surface of the samples incubated on MN (*Figure 6*). The fungi grew extensively in the lesions and destroyed the structural frame of the foams. Tunnels and channels appeared and were filled with mycelium and fructification bodies of the fungi. Broken network of the foams and several damaged areas could be observed especially at the surface of the foam. Since an attack by an organism proceeds from the surface inwards, it was primarily the outer surface of the foams that was attacked. No hyphae growth

Figure 2. Palm-based foams incubated in MN solution in shake flask test after one (left) and two months (right).

Figure 3. Commercial foams incubated in MN solution in shake flask test after one (left) and two months (right).

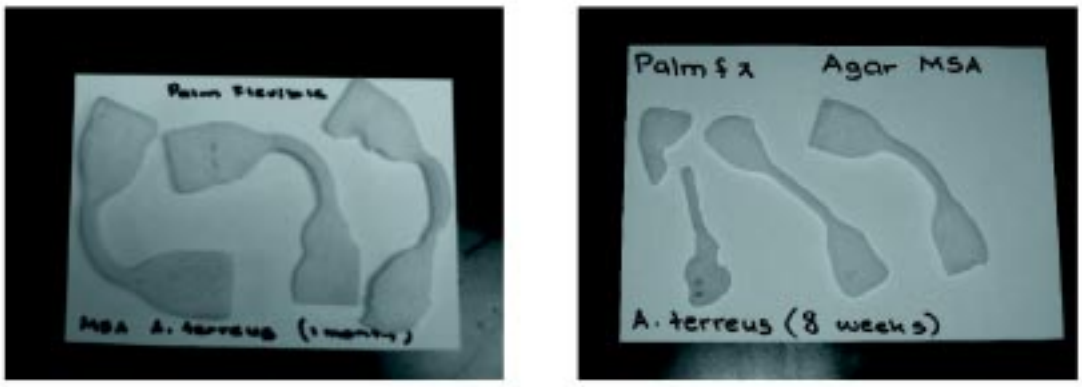


Figure 4. Palm-based foams incubated in MS solution in shake flask test after one (left) and two months (right).

Figure 5. Commercial foams incubated in MS solution in shake flask test after one (left) and two months (right).

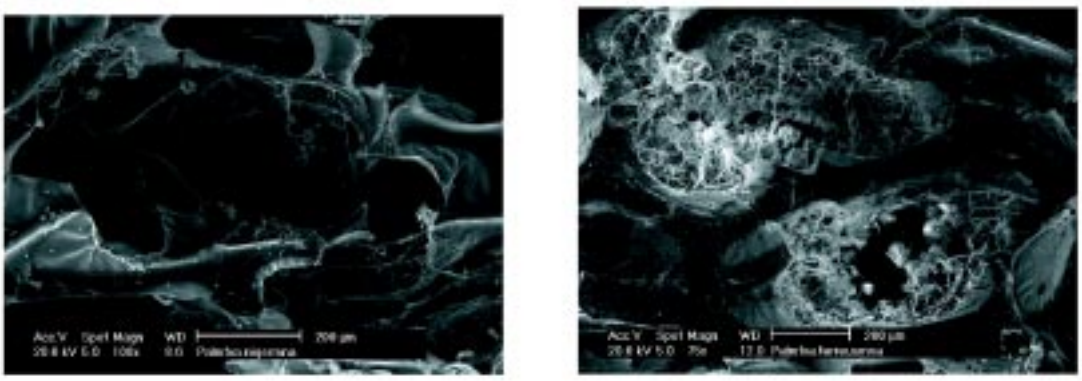


Figure 6. Ramification of fungal hyphae of A. niger *(left) and* A. terreus *(right) on foams incubated on MN agar as viewed under SEM.*

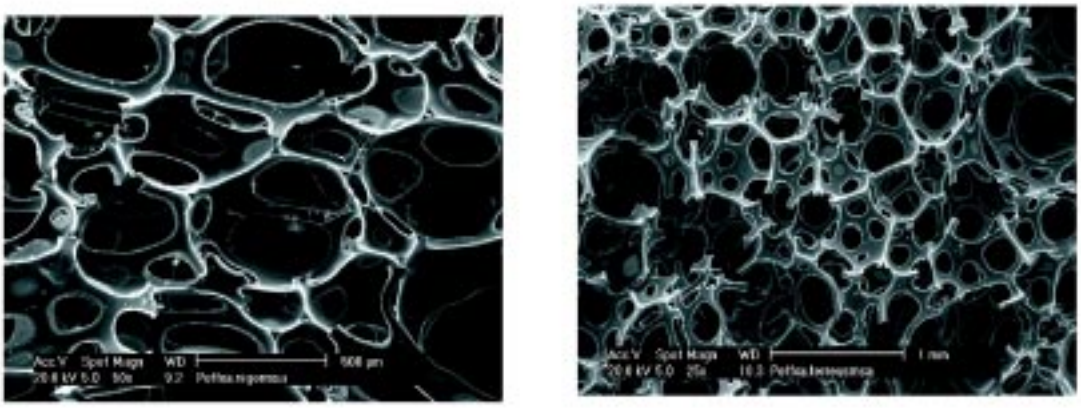


Figure 7. No fungal hyphae on the surface of a sample incubated on MS agar as viewed under SEM.

however, was observed in the samples incubated on MS (*Figure 7*) and in the control samples. Again, this shows that lack of nutrients inhibits the growth of the fungi in samples incubated in MS and both fungi were unable to use the foams as the sole carbon source.

Determination of Mass Changes

The average weight losses as percentages of the original weight of the samples are shown in *Figures 8* and *9*. Both foams were degraded much faster in the shake flask test with MN media as seen visually and also through higher weight losses of the samples. Higher weight losses were observed in the palm-based foam and this suggested that it was degraded faster than the commercial foam by the fungi. The higher weight losses were indicated by deterioration and even complete decomposition of the samples, especially in the shake flask test.

The foams inoculated on MS media however, showed a slight increased in weight after two and three months. 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.

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. *Figures 10* and *11* show the reduction in compression strength *vs.* incubation time curves of palm-based and commercial flexible foam blocks in the petri dish test, respectively. Each point on the graphs represents the mean value of three replicates.

Only small deteriorations of the cells could be detected in the petri dish test due to the minimum contact between the foams and MN agar (on only one side of the foams).

Changes in Tensile Strength

The effects of nutrients on the changes of tensile strengths with incubation time supported the observations made with changes in weight losses on the same media. Losses in tensile strength depend on the number of points at which the molecular chains are split in a unit cross-sectional area and not the amount of soluble units produced by the splitting of one molecular chain.

A. niger and *A. terreus* were able to degrade palm-based flexible foams incubated in MN solution in shake flask test as shown by complete decomposition of samples in two months, while the tensile strength of the commercial foams were reduced by only 7%-9% (*Figure 12*). Even in MS solution, palm-based foams experienced reduction in tensile strength and this correlated well with visual observation where the dumb-bells lost their shape and deteriorated to a certain degree.

Palm-based foams degraded much faster in the presence of nutrients in the shake flask test. The foams deteriorated without additional nutrients (*Figure 4*), but it may take longer for the fungi to fully degrade the foams.

CONCLUSION

Although palm-based and commercial flexible foams may not be the sole carbon source for *A. niger* and *A. terreus*, the foams were not completely resistant to fungal attack. They were degraded when incubated on MN media, *i.e.* when sufficient, readily metabolisable nutrients were available. Microscopic examination confirmed these findings where dense fungal hyphae could only be seen on samples incubated on MN while none could be detected on samples in MS. PU foams were not utilized as a carbon source but were degraded as a result of co-metabolism.

Higher deterioration of samples was observed in the shake flask test due to maximum contact between fungi and samples. At the end of the test, palm-based foams were fully degraded in this test while commercial foams were still intact or only lost their shape. *A. terreus* was able to degrade the foams much faster than *A. niger*.

Significant weight losses were recorded for both palm-based and commercial foams incubated on MN media. Both foams were degraded much faster in the shake flask test as shown through higher weight losses and, by deterioration and even complete decomposition of the samples. Complete decomposition of palm-based foam showed that it could be degraded in the environment in the presence of *A. niger* or *A. terreus*, with sufficient nutrients and maximum contact between fungi and the foams.

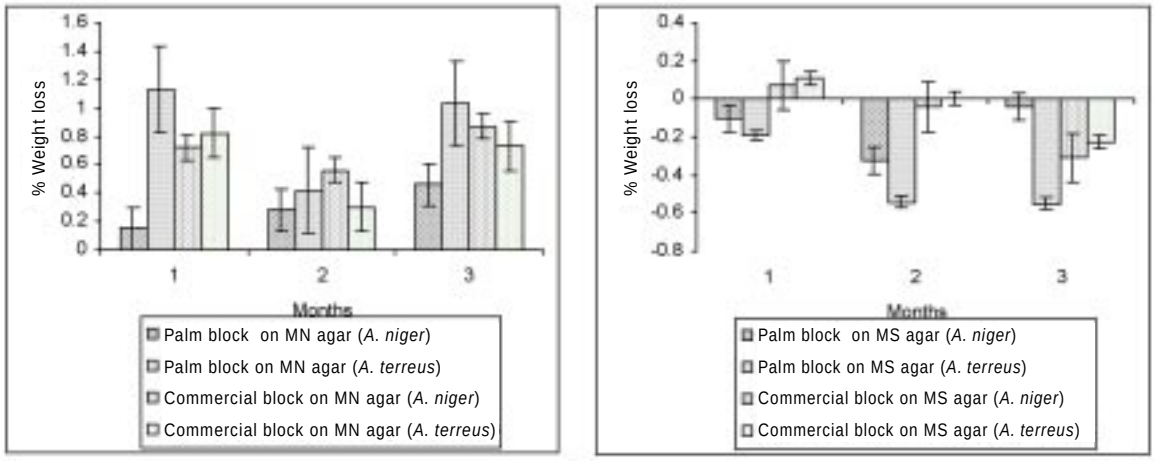


Figure 8. Weight losses (%) of palm-based and commercial foams incubated on MN (left) and MS (right) agar in petri dish test.

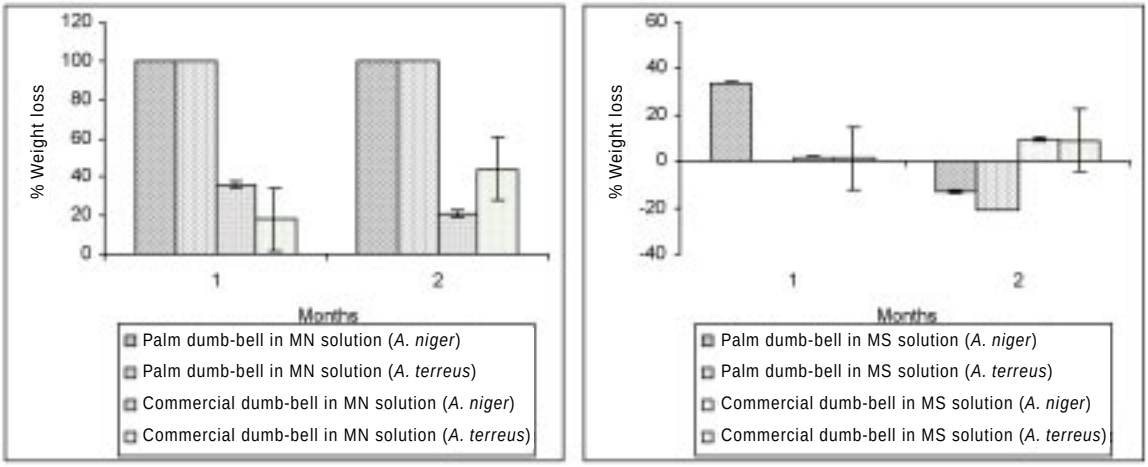


Figure 9. Weight losses (%) of palm-based and commercial foams in MN (left) and MS (right) solution in shake flask test.

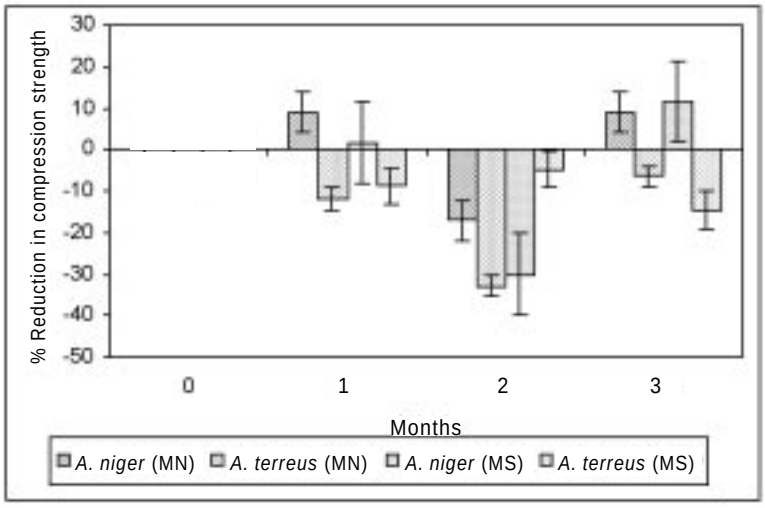


Figure 10. Reduction in compression strength (%) of palm-based foams in petri dish.

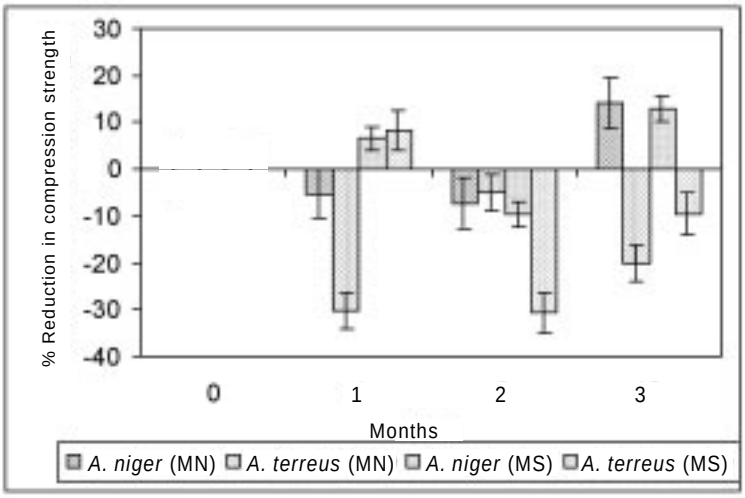


Figure 11. Reduction in compression strength (%) of commercial foams in petri dish test.

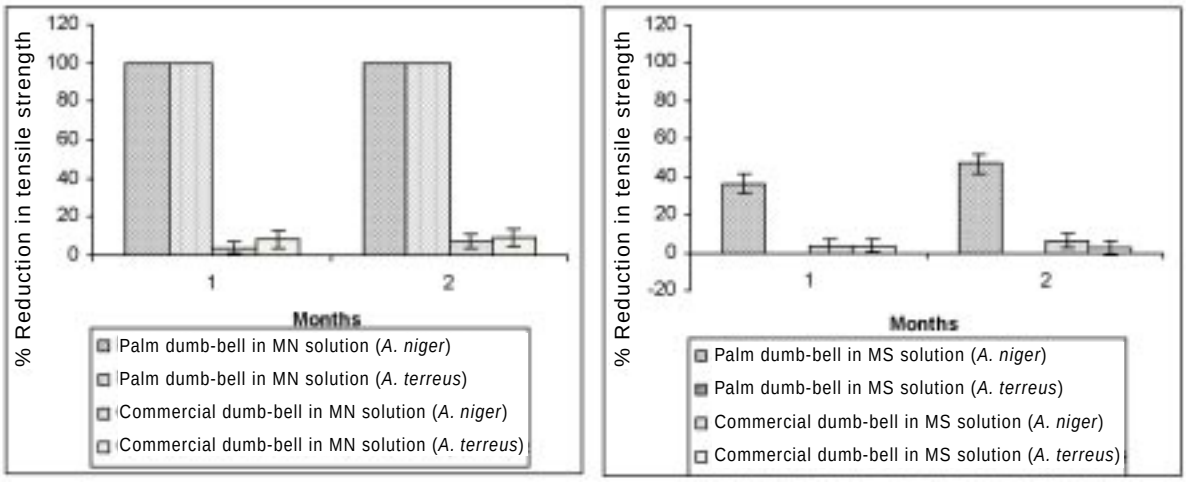


Figure 12. Reduction in tensile strength (%) of palm-based and commercial foams in MN (left) and MS (right) solution in shake flask test.

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