

A light microscopy study on the cell walls decay of biodegraded coir waste

(Kajian reput dinding sel gabus habuk kelapa yang telah dikomposkan, menggunakan mikroskop biasa)

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Key words: coir waste, biodegradation, light microscopy, cell walls decay, cavity

Abstrak

Mikroskop biasa telah digunakan untuk mengkaji reput dinding sel gabus habuk kelapa yang telah dikomposkan oleh sekumpulan kulat mikro (*Chaetomium globosum*, *Hemicola grisea*, *Trichoderma reesii*, *Penicillium citrinum* dan *Aspergillus niger*). Kajian ini memberi bukti yang nyata tentang penembusan hifa, kolonisasi dan reput dinding sel oleh kulat reput berair (*C. globosum* dan *H. grisea*). Hifa-hifa kulat reput berair menembusi sel-sel yang berdekatan melalui liang sel dan mereputkan dinding sel melalui pembentukan rongga. Kulat mol (*T. reesii*, *P. citrinum* dan *A. niger*) pula hanya menembusi dinding sel dan tidak menyebabkan reput yang serius.

Abstract

A light microscopy study on the cell walls decay of biodegraded coir waste by a group of micro-fungi (*Chaetomium globosum*, *Hemicola grisea*, *Trichoderma reesii*, *Penicillium citrinum* and *Aspergillus niger*) was conducted. This study gave conclusive evidence of hyphal penetration, colonisation, cell wall decay and destruction by the soft rot fungi (*C. globosum* and *H. grisea*). The hyphae of these soft rot fungi penetrated through pits into adjacent cells and severely degraded the cell walls by formation of cavities. However, the moulds (*T. reesii*, *P. citrinum* and *A. niger*) only penetrated the cell walls and did not cause any severe cell walls destruction.

Introduction

Coir waste or coir dust, is a by-product of coconut (*Cocos nucifera* L.) fibre or coir. It is essentially the waste material produced when the coconut fruit mesocarps (husks) are shredded for coir production. When the coconut husks are shredded, only 30% are long fibres which are considered suitable for industrial use and the remaining 70% are the 'pith' tissues or 'dusts' which are regarded as wastes (Del Mazo Suarez et al. 1986).

Enormous quantities of coir waste are accumulated over time as a by-product of coir manufacturing and these are often left unutilized or burnt in the open.

Attempts had been made for industrial use of coir waste like manufacturing of hard board, building slabs and rubberized flooring but with limited success (Child 1974). Of late, due to environmental concerns and also diminishing supply of peat soils for horticultural substrates, coir waste is being

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considered as a renewable substitute for peat soils in horticultural use.

As a horticultural substrate, coir waste is often mixed with other organic or inert materials. Handreck (1992) recommended that the coir dust should make up not more than 30% by volume of mixtures, with other coarser components. Although coir waste could be used by itself, a mixture with charcoal dust (2:1 v/v) was better for growing tomatoes (Teo and Tan 1993). In choosing coir waste as a soilless container medium, the irrigation and nutritional regimes had to be adjusted accordingly on a crop by crop basis as its physical and chemical properties varied markedly with supply (Meerow 1994; Evans et al. 1996; Noguera et al. 1997). When fresh coir waste was used as a plant substrate, plant growth was inhibited because it contained phytotoxic elements (Radjagukguk et al. 1983; Verdonck et al. 1983). Verdonck et al. (1983) suggested that fresh coir waste should be decomposed first before it could be used as a horticultural substrate.

This paper presents the results of a light microscopy examination of cell walls decay of coir waste which had undergone 3 months of biodegradation by a group of micro-fungi.

Materials and methods

Coir waste biodegradation

The coir waste was air dried for 72 h in the laboratory at approximately 22 °C and 60% RH. Each 100 g sample was packed and sealed in a clear polythene bag and was subsequently sterilized using gamma irradiation at a dosage of 25 kGy overnight. Spore suspensions of individual fungus namely the moulds (*Aspergillus niger*, *Penicillium citrinum*, *Trichoderma reesii*), the wood soft rot fungi (*Humicola grisea* and *Chaetomium globosum*) and the mixture of all the five fungi were used to enhance the biodegradation of coir waste.

The test fungi were first sub-cultured in test tube slopes of 2% Malt agar medium containing 20 g/L Malt extract powder and

12 g/L agar. The test fungi were incubated at a constant temperature of 27 °C. After 21 days, the fungal cultures were used for the preparation of spore suspension. Taking each culture in turn, 10 mL of the sterilized wetting agent solution (0.5 g dioctyl sodium sulfosuccinate/L) was added. A sterilized platinum wire loop was used to scrape gently the surface of the culture to liberate the spores. The spore suspensions were then slowly decanted into a separate container. The number of spores in a sample of the spore suspensions prepared from each fungus was determined using a haemocytometer counting chamber. Only spore suspensions containing at least $\times 10^5$ spores/mL were used for inoculation of coir waste. For the treatment with a mixture of five fungi, 2 mL of spore suspension from each fungus was used. The procedure of preparing the spore suspension followed that described in the European Prestandard For Wood Preservatives ENV 807 (Anon. 1993).

A 10-mL spore suspension of each fungus and 350 mL of distilled water plus 2.5 g urea as N source were then added to each 100 g sterilized coir waste sample. The sample was then thoroughly mixed to ensure that it was uniformly moistened and carefully placed into a clear polythene bag and then sealed. Each determination was carried out in three replications. The treated samples were then placed in an incubator with a constant temperature of 30 °C for 3 months under semi-sterilized conditions.

Light microscopy

A light-microscope was used to examine the cell walls decay of biodegraded coir waste. The samples were first dehydrated followed by pre-infiltration, infiltration and embedding processes before preparation of thin sections, staining and mounting. A weighted index from 0 to 4 was used to score the severity of decay.

Preparation for embedding Fresh and biodegraded coir waste samples were embedded in a methacrylate resin before

they could be sectioned for microscopy study. The resin used was a commercial preparation of 2-hydroxyethyl methacrylate (HEMA) resin (Kulzer Technovit 7100) which consisted of a Base Liquid, Hardener 1 and Hardener 2. The method of embedding followed that described in the user instruction manual, and Igersheim and Cichocki (1996). Before embedding, the coir waste samples were first dehydrated in 70% and 90% ethanol for 2 h each followed by an hour in 100% ethanol. After the dehydration series in ethanol, the main procedures were pre-infiltration, infiltration and embedding.

Pre-infiltration and infiltration The pre-infiltration liquid consisted of 100% ethanol and the base solution in a ratio of 1:1. The samples were left in the pre-infiltration liquid for 2 h at room temperature. The samples were then transferred into the infiltration liquid, which was prepared using 1 g of Hardener 1 (ready weighed 1 g/bag) mixed thoroughly with 100 mL of chilled (4 °C) Base Liquid. The samples in the infiltration liquid were placed under vacuum (700 mm Hg) for half an hour to ensure proper infiltration. The samples remained in the infiltration liquid for a further 24 h at 4 °C refrigeration.

Embedding Embedding was carried out using the embedding liquid that contained a mixture of 1 mL of Hardener 2 and 11 mL of infiltration liquid. The coir waste sample was picked and placed in the centre of a mould (TAAB mould 19 x 13 x 5 mm) which was then filled up with the embedding liquid. A stub (TAAB embedding stub 25 mm diameter) was carefully placed on top of the mould. The mould was then kept at room temperature for an hour or until the liquid had become viscous before transferring it into an oven at 40 °C for 1 h to complete the polymerisation process.

Sectioning Sectioning was carried out using a rotary microtome (Model Spencer

820). The cutting thickness was set at 12 nm. The block was constantly kept moist with distilled water using a pointed paint brush during sectioning to avoid cracking due to brittleness of the resin. The sections were carefully picked from the surface of the cutting knife using fine forceps and then placed onto the surface of the distilled water to help uncurl them. The sections were then placed and stretched on a glass slide with the help of a wet paintbrush. The glass slides were then placed on a warm hot plate (40 °C) to dry.

Staining and mounting The thin sections were stained with 1% aqueous safranin followed by lactophenol cotton blue. This staining method produced red stain for the cell wall while decayed cell wall and fungal tissues were stained blue. This method produced quite satisfactory results on staining bamboo sections decayed by fungi (Murphy et al. 1991; Sulaiman 1993).

Before staining, the thin sections were rehydrated in 100, 90, 70, 50, 30% ethanol and distilled water for 1 min respectively. They were then stained with 1% aqueous safranin solution for 30 s. The sections were then rinsed twice in distilled water before they were stained with lactophenol cotton blue for 1 min. The sections were then rinsed thoroughly in distilled water and dehydrated in 70% and 100% ethanol for 3 min each. The sections were dried with a piece of clean filter paper before they were covered with a few drops of clove oil for 5 min. After that, they were washed with xylene for 2 min. For permanent mounting, a drop of Artmount mount was placed on top of the section and a cover slip was placed on top. Two spring clips were used to clamp the cover slip to ensure the sections remained flat during drying on a hot plate (60 °C) for 12 h.

Viewing The thin sections were examined using a light microscope (Model Leitz Diaplan) and photomicrographs taken on Fuji 35 mm coloured slide films.

Assessment of decay severity The assessment of severity of cell walls decay was carried out on the 3-month biodegraded samples. The severity of decay was assessed based on a weighted index from 0 to 4 which was adopted with slight modifications from the assessments of soft rot decay used by Wyles (1987), Murphy et al. (1991) and Sulaiman (1993) (*Appendix 1*).

Results and discussion

The light microscopy was used as one of the tools to assess the degree of decay caused by different groups of fungi. The decay of plant material was evident in some samples (*Table 1*).

The samples treated with the soft rot fungi particularly the *C. globosum* and *H. grisea* or the mixture of fungi showed severe decay of cell walls with a decay index of 3 while those treated with the moulds scored an index of 1.5 which was between slight and moderate decay (*Table 1*). The control sample was relatively clean with very little or no visible fungal attack as shown in the photomicrograph (*Plate 1*). The ground tissue parenchyma cells were relatively clear of fungal attacks. This indicated that the cell wall fraction of the coir waste was still predominantly undegraded.

For the moulds like *A. niger*, the hyphal penetration into the cell walls was visible. The hyphae grew in the fibre cells

Table 1. Decay severity index of 3-month biodegraded coir waste by various micro-fungi

Treatment	Decay severity index
Control	0.5
<i>Aspergillus niger</i>	1.5
<i>Penicillium citrinum</i>	1.5
<i>Trichoderma reesii</i>	1.5
<i>Humicola grisea</i>	3
<i>Chaetomium globosum</i>	3
Mixture of 5 fungi	3

0 = no visible decay, 1 = slight decay,
2 = moderate decay, 3 = severe decay,
4 = very severe decay

and the ground tissue parenchyma cells with only some slight cell wall erosions (*Plate 2*). For the soft rot fungi and the mixture of fungi, the hyphae penetrated through the cell walls. The hyphae colonised the tissues extensively and sometimes branched and formed new hyphae in the cell wall region. The hyphae penetrated through pits between the cells of coir waste (*Plate 3*). The cell wall degradation by the soft rot fungi was severe as observed from the photomicrographs (*Plate 4* and *Plate 5*). The addition of N was reported to increase the extent of biodegradation of coir waste when soft rot inoculum was added (Yau and Murphy 1998). All these decay patterns were typical of soft rot, as formation of cavities was evident (*Plate 4* to *Plate 6*).

A 'cavity' is referred to as a zone of lysis of cell wall substance produced by hyphae which is growing within the cell wall parallel to the presumed direction of cellulose microfibrils (Nilsson 1974). Before the formation of cavities, hyphae of soft rot fungi penetrated through cell walls. At times, the proboscis hypha was seen at the tip of the cavity (*Plate 4*). Under the polarised light microscopy, soft rot cavities were clearly shown. Most of the cavities were cylindrical or oval with conical ends (*Plate 4* to *Plate 6*).

Conclusion

The light microscopy is a useful tool in assessing the degree of decay in the cell walls of biodegraded coir waste caused by different groups of fungi. The soft rot fungi were more effective in degrading the cell walls of coir waste during biodegradation compared to the moulds. From the decay assessment, the coir waste that was treated with soft rot fungi showed severe cell wall degradation. The hyphae penetrated through pits into the adjacent cells and severely degraded the cell walls by formation of cavities. The proboscis hyphae at the tip of the cavity were also visible under the polarised light. While for the moulds, the hyphae only penetrated the cell walls and did very little destruction to the cell walls.

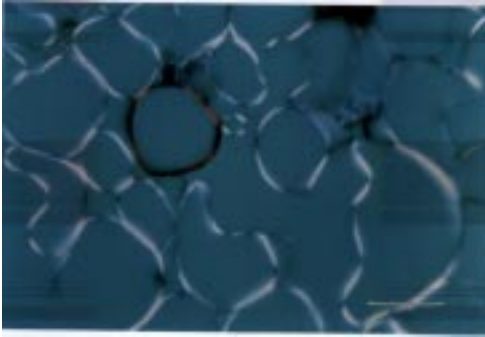


Plate 1. Surface of ground tissue parenchyma cells of coir waste in the control treatment (after 3 months of incubation) showing no visible fungal attack. Note the birefringence of cells. (polarised light, bar = 50 μm)

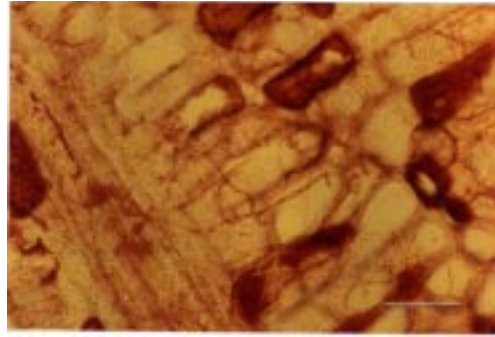


Plate 2. The treatment with *Aspergillus niger* (after 3 months of incubation). Note the hyphae invading the fibre and the parenchyma cells with slight cell wall erosion (bar = 50 μm)

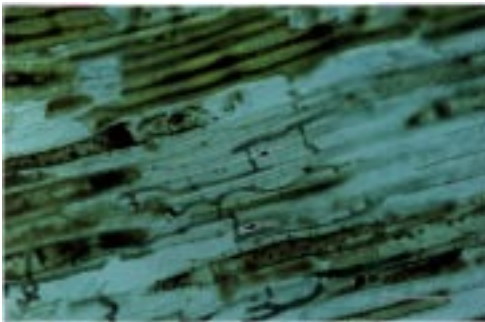


Plate 3. *Chaetomium globosum* (after 3 months of incubation) hyphae penetrated through possible pits into the adjacent fibre cells (arrow) (bar = 50 μm)



Plate 4. *Humicola grisea* (after 3 months of incubation) hyphae penetrated through parenchyma cells and formed cavities. Severe cell wall decay was observed under polarised light. Note the proboscis hypha at the tip of cavity (arrow) (bar = 10 μm)

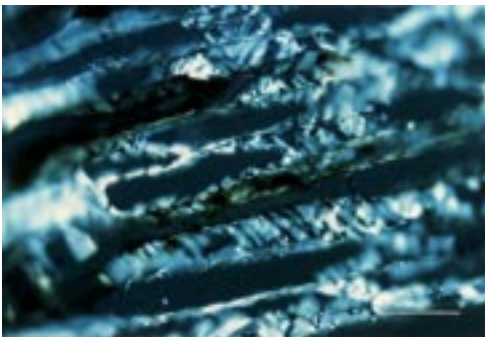


Plate 5. *Chaetomium globosum* (3 months of incubation) hyphae caused cavity formation in the cell walls of the fibre bundle. The decay pattern is typical of soft rot with cavity formation clearly shown under polarised light (bar = 50 μm)

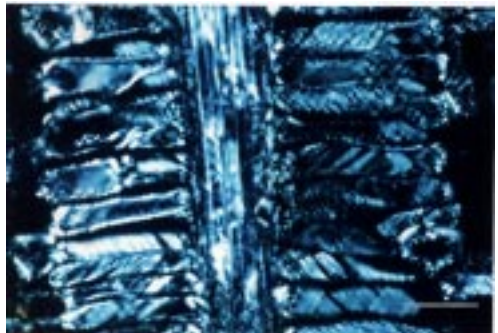


Plate 6. Cell wall decay of coir waste treated with the mixture of fungi (after 3 months of incubation). Both the fibre and parenchyma cells showed severe decay. The decay pattern is typical of soft rot with cavities clearly shown under polarised light (bar = 100 μm)

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Appendix 1. The decay severity index

Index	Descriptions	Area of disintegrated wall (%)
0	No visible decay	0
1	Slight decay	1–10
2	Moderate decay	11–40
3	Severe decay	41–90
4	Very severe decay	91–100