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A review of bast fibres and their composites: Part 4 ~ organisms and enzyme processes

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Abstract: There is an urgent need to halt the degradation of the ecosystems on Earth. For the acquisition, modification and disposal of natural fibres and their composites, the use of organisms and the associated enzymes offers a promising route to sustainable composites. This would potentially reduce the required energy and use milder reaction conditions. This paper reviews the use of organisms and enzymes for (a) extraction of fibres from plant material (retting), (b) surface modification of fibres, and (c) end-of-life treatments, in the context of bast fibres and their composites. The use of enzyme processes at large scale is limited by the extended treatment times, costs of the enzymes and equipment, wastewater treatment and the current low level of adoption by industry.
Keywords: *Bacteria,* composites, enzymes, fibres, *Fungi*.

Introduction:

> **"**The decay of plant and animal debris is perhaps the most essential process in nature. Without decay, the nutrient cycle would grind to a halt, and life could no longer survive. ... The main constituent of leaf litter is cellulose, the commonest organic substance on earth. This is not easily degraded, if at all, by animals, plants, or bacteria, but many *Fungi* do have the requisite enzymes to break down cellulose into simpler compounds (principally glucose) and utilise it as a food resource" (Spooner and Roberts, 2005).

Bast fibres, extracted from the stems of plants, are finding increasing use in "sustainable" composites. To ensure the environmental credentials for such materials, a life cycle assessment should be conducted, rather than rely on the instinct that "green is good". Previous reviews on bast fibres and their composites include papers specific to reinforcements [1], use in composites [2], modelling [3], interfaces [4], manufacturing processes [5], life cycle assessment [6] and forensic techniques [7] with well over 100 review papers addressing this topic [8].

Life is divided into three domains, the *Archaea,* the *Bacteria,* and the *Eukarya* (the latter arising from a member of the *Archaea* taking up endosymbiont *Bacteria* to create mitochondria). The *Eukarya* then diverged to give rise to the six major divisions [9] listed in order of position of radiation: (i) *Archaeplastida* (containing green plants, C*hlorophyta*, and algae, (ii) *Hacrobia* (CCTH clade), (iii) *Harosa* (SAR clade), (iv) *Excavata*, (v) *Amoebozoa*, (vi) *Opisthokonta* (containing the *Animalia*, including humans, and the *Fungi*) A seventh division, the *Hemimastigophora*, has been proposed by Lax et al (2018) [10].

Fungi are closer to animals than to plants. The fungal Kingdom includes mushrooms, molds, mildews, plant pathogens (rusts and smuts) and yeasts. Cavalier-Smith [11] suggested the definition of 'true fungi' was dependent on characteristics such as chemistry of the cell wall, mode of nutrition, biosynthetic pathways and ultrastructure of the mitochondria. However, the taxonomy of *Fungi* is evolving rapidly due to recent research based on DNA comparisons [12, 13]. The classification of *Fungi* has changed dramatically since the 1990s. The kingdom now commonly divides into five "true" phyla (groups): *Ascomycota* (yeasts and sac *Fungi*), *Basidiomycota* (club *Fungi*), *Chytridiomycota* (chytrids), *Glomeromycota* (tree root symbionts) and *Zygomycota* (bread molds). *Blastocladiomycota* are under consideration for full phylum status by splitting *Blastocladiales* from the *Chytridiomycota* [14] (Lange and Olson, 1980). There is some uncertainty as to whether *Microsporidia* and *Neocallimastigomycota* are phyla of *Fungi* or *Protozoa* [15] (Læssøe and Petersen, 2019).

Fungi [15-17] (Spooner and Roberts, 2005. Money, 2016. Læssøe and Petersen, 2019) grow in soil and do not move around like animals. The diversity of *Fungi* is second only to that of insects, but they are a "poorly known group of organisms" due to their hidden life within various substrates except for the short period when they may produce fruit bodies [15] (Læssøe and Petersen, 2019). *Fungi* do not have chlorophyll and hence cannot photosynthesise. They are characterised by chitin, rather than cellulose, in their cell walls.

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The building blocks of all filamentous *Fungi* are hyphae (fine strands) which are hollow tubes containing nuclei, mitochondria and other organelles (specialised structures within a cell). All *Fungi* feed by absorbing small molecules (amino acids and simple sugars) inwards though the walls of the hyphae. Of particular interest in the context of natural fibres, polymers and composites are those *Fungi* that can produce digestive enzymes to attack complex molecules such as cellulose, lignin and starch and modify the material surface. Decomposition by *Fungi* appears to be primarily at the actively growing filament tips. *Fungi* in the lumen digest the fibre from the inside outwards.

Saphrophyes are plants, fungi, or microorganisms that process dead or decaying organic matter by extracellular digestion and are considered "decomposer organisms". Fungi are "the principal agents of natural decay and nutrient recycling" [16]. Brown rot is the residue of decomposers that break down the fairly easily degradable cellulose to leave brown lignin. White rot is the whitish fibrous residue of decomposers that break down both cellulose and lignin [15] (Læssøe and Petersen, 2019). The white rot fungi from *Basidomycetes* are the only fungi known to produce extracellular oxidases that expose and metabolise the cellulose and hemicellulose then degrade the lignin.

Other organisms can also degrade materials. Bacteria adhere to the fibre outer surface as particulate cells, release cellulases to promote biodegradation of cellulose to glucose, enlarge, then fission into daughter cells [18] (Siu, 1954). Dependent on the process objective, the progress of degradation by cellulases and the properties of the resulting products can be controlled by adjusting the treatment parameters, enzyme loading and the composition of the cellulase mixture [19, 20] (Kalia et al, 2013; Esteghlalian, 2002). Table 1 (animals), Table 2 (bacteria) and Table 3 (fungi) list the organisms in the papers reviewed below with their respective taxonomy.

Enzymes [19,21] (Kalia et al, 2013; Prior, 2013) are natural chemicals produced by *Bacteria*, *Fungi*, protozoans, termites, plants and animals. They catalyse selective chemical synthesis and/or decomposition at low concentrations under mild (temperature, humidity, pH) conditions and consume less energy and water than conventional chemical processes. Tailored enzymes have high reaction specificity, work under milder reaction conditions and can result in non-destructive transformations on the surface of polymers. Factors that limit the use of enzymes at large scale include the high cost for enzymes and equipment, wastewater treatment systems and the lack of industry support.

Enzymes [21] (Prior, 2013), like true catalysts, are not consumed by the reactions they facilitate. An enzyme enables another molecule, termed the substrate, to undergo a reaction by forming a temporary complex with that substrate. The active site on the enzyme must have the correct chemical nature and perfect conformation for the specific substrate. For example, cellulose is the substrate for the cellulase enzymes.

Enzyme function depends on the precision of the Activated Enzyme-Substrate Complex (AESC). A substrate and its enzyme bind together like a key in a lock (known as the Lock and Key Analogy). The AESC forces the substrate into a stressed form making the bonds more susceptible to reaction (the induced fit hypothesis) when the required energy exists. Enzymes: A Very Short Introduction will soon be published [22] (Engel, 2020).

Each enzyme has been classified using an Enzyme Commission number (EC number: "EC" followed by four integers separated by full stops (periods in US English)) based on the hierarchy of the catalysis to progressively describe the chemical reaction of the enzyme (Tables 4 & 5) [23, 24]. Preliminary EC numbers include an 'n' as part of the fourth (serial) digit (e.g. EC 3.5.1.n3). BRENDA (BRaunschweig ENzyme DAtabase) < https://www.brenda-enzymes.org/ > is a comprehensive enzyme database offering free-of-charge functional data to the scientific community. ENZYME < https://enzyme.expasy.org/ > is one alternative repository of information for the nomenclature of enzymes, primarily based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB) [25] (Bairoch, 2000).

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Cellulases are a class of enzymes that hydrolyse cellulose (catalyse cellulolysis), most often acting on the β-(1,4)-linkage. Cellulases are the enzymes that cleave sugar from the cellulose molecule. They consist of three different synergistic enzymes:

- endoglucanases (EG) randomly hydrolyse the β-(1,4)-linkages in the water-insoluble cellulose chain,
- cellobiohydrolases (CBH) hydrolyse the linkages at the reducing ends of the cellulose chain to form cellobiose and cellobiases, and
- β-glucosidases convert water-soluble cellobiose into two glucose residues [19, 26] (Kalia et al, 2013; Almeida and Cavaco-Paulo, 1993).

Organisms that possess cellulases include *Bacteria*, some flagellate and ciliate protozoa, and *Fungi* [27] (Scott, 2002).

Pectinases (pectinolytic enzymes) are naturally occurring chemicals generated by *Fungi* [28] (Singh et al, 1999), actinomycetes [29] (Beg et al, 2000), yeasts [30] (Blanco et al, 1999) or *Bacteria* [31, 32] (Dosanjh and Hoondal, 1996. Kapoor et al, 2001). Pectinases produced from fungal sources dominate the commercial products [33] (Henriksson et al. 1997). The most common fungal species used in industrial production of pectinolytic enzymes is *Aspergillus niger* [34] (Jayani et al, 2005). *Bacillus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc* are effective bacterial sources for fermentation processes that produce pectinolytic enzymes [35] (Kouhoundè et al, 2014).

This paper reviews the use of organisms and enzymes for (a) extraction of fibres from plant material (retting), (b) surface modification of fibres, and (c) end-of-life treatments. Table 6 compiles the process parameters reported for the respective processes. Those parameters (where quoted) fall in the range ambient-60°C, 65-85% RH, pH 3.5-10 with durations up to 40 days. Whatever the chosen route, there should be risk assessment for economic, environmental (including escape of organisms into the ecosystem) and health and safety when handling these systems. Quantitative Life Cycle Assessment (QLCA) should reveal the extent of environmental burdens imposed.

Retting

Sisti et al. [36] (2018) have comprehensively reviewed the retting process as a pre-treatment for the acquisition of natural fibre reinforcements for polymer composites. Retting, also known as degumming, is the process of softening harvested plant stems for fibre extraction. Retting permits separation of the fibre bundles, and individual fibres, from the vegetal skin and the woody core cells. The process involves soaking (water retting) or exposure to moisture (dew-, or field-, retting) using pectin enzymes naturally secreted by indigenous microflora. Monitoring the progress of the retting process is essential to obtain high quality fibres.

Retting uses pectinases or pectinolytic enzymes to loosen the fibres from the other stem tissues by hydrolytic depolymerisation of the pectic substances. Pectinolytic enzymes are classified according to their main catalytic reaction mechanisms. Polygalacturonases are fundamental to the retting process as they catalyse hydrolytic cleavage of polygalacturonic acids [34, 37, 38] (Zhang et al. 2000. Evans et al, 2002. Jayani et al. 2005). Pectin lyases drive a non-hydrolytic breakdown of pectates and pectinases by a trans-elimination splitting the pectic polymer and so are potentially important for retting bast plants [39-41] (Sakai et al, 1993. Akin et al, 2007; Bruhlmann et al, 2000). Pectin esterases may have a lesser role. This heterogeneous group of enzymes are widely distributed in higher plants and microorganisms where they aid cell wall extension and soften plant tissues during maturation and storage. They also contribute to maintenance of ecological balance by decomposing and recycling waste plant materials [42, 43] (Mohnen, 2008. Ridley et al. 2001).

Plant diseases and spoiling of fruit and vegetables are major manifestations of pectinolytic enzymes. In consequence, they have a role in extraction and treatment of plant fibres, extraction and clarification of fruit juices and vegetable oils, tea and coffee fermentation, bleaching of paper, poultry feed additives, and are used in the alcoholic beverages and food industries as well as for wastewater treatment [34] (Jayani et al, 2005).

Water retting

Water retting in rivers or ponds was widespread around five-six decades past. The harvested bast stems are immersed in water for one to two weeks so that the water penetrates into the centre of the stalk leading to breaking of the outer layer and hence more rapid moisture absorption. The treatment time is dependent on the water used, the temperature and the bacterial community [44, 45] (Bismark et al. 2005; Donaghy et al.

1990). Initially, growth of aerobic microorganisms consumes dissolved oxygen, creating an environment where anaerobic organisms can thrive. The active aerobic phase Bacillus microorganisms include *B. macerans (*now classified as *Paenibacillus macerans)*, *B. mesentericus*, *B. polymyxa* (now classified as *Paenibacillus polymyxa)* and *B. subtilis* [32, 46-48] (Ali 1958; Kapoor et al. 2001; Tamburini et al. 2003; Munshii and Cathoo 2008). The anaerobic phase *Clostridium* genus microbiota found in retting water include *C. acetobutylicum*, *C. aurantibutyricum*, *C. felsineum* and *C. tertium* [45, 47-50]] (Donaghy et al. 1990; Di Candilo et al. 2000; Zheng et al. 2001; Tamburini et al. 2003; Munshii and Cathoo 2008).

The quality of fibres from water retting is generally higher than from dew retting [51, 52] (Amaducci and Gusovious 2010; van Sumere 1992). However, the water retting process imposes high environmental burdens as it consumes and contaminates large volumes of both water [53] (van Dam and Bos 2004) and energy [54] (Van der Werf and Turunen 2008). It would be timely to consider alternative water sources for water retting to address freshwater scarcity and reduce watercourse pollution. The process is now conducted in large tanks. Zhang et al. [55, 56] (2008a/b) have suggested that seawater may be considered as an abundant, inexhaustible resource. They studied seawater retting with pectinolytic *Stenotrophomas maltophilia* and *Ochrobactrum antrophi* species with good retting results. Bismark et al [44] (2005) and Sisti et al. [57] (2016) conducted artificial warm water retting with a bacterial inoculum to produce clean, homogeneous, high-quality fibres in just 3-5 days.

Dew/field retting

In dew/field retting, the harvested plants are spread thinly on the field for 2-10 weeks. Microorganisms present in the soil and/or on the plants degrade the non-cellulosic material, especially the binding pectins and hemicelluloses of the parenchyma cells and the middle lamellae without damaging the cellulose fibres. The organisms are primarily filamentous *Fungi* and/or aerobic *Bacteria*. The field may not be available for the next crop for a number of weeks while dew retting occurs. Contaminated soil, and the *Fungi*, may also be undesirable for agricultural and economic reasons.

Fungal and bacterial species isolated from dew-retted plants include *Cladosporium sp.*, *Penicillium sp.*, *Aspergillus* and *Rhodotorula sp.* [58-60] (Fogarty et al. 1972; Ahmed and Akhter 2001; Ribeiro et al. 2015). *Fungi* isolated during flax dew retting, include *Cladosporium herbarum*, *Epicoccum nigrum*, *Alternaria alternate*, *Fusarium sp.*, *Aureobasidium pullulans*, *Phoma sp.*, *Mucor sp.*, *Rhizomucor pusillus*, and *Rhizopus oryzae* [32, 61-65] (Sharma 1986; Henriksson et al. 1997; Akin et al. 1998; Molina et al. 2001; Booth et al. 2004; Xiao et al. 2008). Lignin accumulates in plant stems as they age, so different enzymes may be needed for retting lignified fibres, and complementary mechanical methods are often necessary [60] (Ribeiro et al. 2015).

Currently, dew retting is the most used process for the industrial production of bast fibres, mainly flax and jute, because of its low cost [66] (Bacci et al. 2010). Unfortunately, the method is limited to geographic regions, where the weather is suitable for *Fungi* proliferation. Moreover, often low and inconsistent fibre quality is produced compared to other methods, such as water retting. Under-, or over-, retting leads to difficulties in separation or weakens the fibre respectively [67] (Jankauskiene et al. 2015). For example, cellulotic enzymes secreted by the microbiota can damage the fibres after extended exposure.

Over recent years, dew retting has been investigated in a controlled artificial environment with the variable parameters including fungal type, temperature and duration of treatment, in order to lower costs, increase efficacy and decrease environmental burdens [68] (Pickering et al. 2007).

Enzyme retting

In enzyme retting, also known as bioscouring, the enzymes are added to the substrate in a bioreactor vessel. The technique shows promise as an alternative to traditional retting methods with potential benefits including time-saving (typical duration of just 8-24 h), convenience and low environmental burdens. However, a balance must be sought between increased energy input and single-use enzymes compromising the cost-effectiveness of the process [69] (Tahir et al, 2011). Enzyme retting promises to improve fibre quality, but has not yet replaced pond/immersion or field/dew retting at large scale [19, 70-72] (Akin et al 2002; Akin 2013; Kalia et al, 2013; Lee et al, 2011).

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Specific retting conditions, especially pH, temperature, and enzyme concentration, are required dependent on the chosen enzyme(s) as the activity can differ dramatically. Akin et al [73] (2003) reported that pre-soaking with distilled water before enzyme-retting "increased fine fibre yield in some cases, but fibre strength at times was reduced". Enzyme retting efficiency can be enhanced by chelators that withdraw calcium from the solution and by surfactants employed in formulations to improve activity. Calcium (II) chelators, such as Ethylene Diamine Tetracetic Acid (EDTA), improve retting by destabilising chemical bridges to separate the epidermal/cuticle material from the fibre and fibre bundles [74] (Akin et al, 2004).

Foulk et al [75] (2008) stated that when using a specific composition of an enzyme mixture, enzyme retting can tailor fibres, or their bundles, to produce particular properties, notably fineness and strength. Pectinase enzyme retting can produce consistent high strength renewable reinforcement fibres [76] (Foulk et al, 2011). Strength is maintained by retting with relatively pure pectinases, whereas these enzymes in mixtures with cellulases tend to shorten fibres, especially by attacking the nodes limiting their use to paper pulp or injection moulding compounds [75] (Foulk et al, 2008).

Customised enzyme blends enhance the dew retting process, e.g. Inotex Texazym® SER sprayed on the field before pulling, or within the first 3 days of dew retting. The flax long fibre yield increased by >40%. These enzymes, in combination with mild mechanical treatment, can eliminate the aggressive and energy-intensive processing otherwise required [77] (Antonov et al, 2007).

Fibre surface treatment

Fungal treatment has potential as an environmentally friendly and efficient process for surface modification of natural fibres. This biological treatment removes the non-cellulosic components (e.g. wax) from the fibre surface by the action of specific enzymes. Extracellular oxidase enzymes, from *e.g.* white rot *Fungi* reacts to remove lignin constituents from the fibre, and to increase the solubility of hemicelluloses. The net effect is reduced hydrophobicity. The fungal hyphae create fine holes at the fibre surface that roughen the fibre surface and provide a better mechanical interlock with the matrix of the composite [78] (Kabir et al 2012).

Enzymes with chelators have been used to modify flax fibre surfaces, by removing pectin and calcium, accompanied by wall stripping and generation of fine fibrils, making the surface more hydrophobic and enhancing the fibre/matrix interfacial adhesion [19, 79] (Kalia et al, 2013; Adamsen et al. 2002).

Enzymes (including cellulases, hemicellulases, pectinases, xylanases and laccase (EC 1.10.3.2)) have been used for surface treatment of jute fibres leading to removal of lignin and hemicellulose constituents and reduction of breaking strength of the treated fibres by 15-25% [80] (Kamiko et al 2002).

Ouajai and Shanks [81] (2005) performed bioscouring of hemp using pectate lyases (EC 4.2.2.2, Scourzyme L) by varying concentration, treatment time and substrate concentration to obtain the reaction kinetic constants. SEM indicated smooth surfaces and separated fibres. Thermogravimetry indicated complete removal of pectins while FTIR and WAXD suggested there was no destruction of the cellulose crystalline structure of the fibres.

Pickering et al [68] (2007) studied fungal treatment of hemp fibres to enhance their bonding in natural fibre reinforced polypropylene composites. Fibres were pre-treated in 10% NaOH for 45 minutes at up to 160°C. The hemp was incubated at 10 mg *Fungi*/12 g of sterilised fibre at 27°C for two weeks. The five Fungi used were:

- three *Basidiomycetes* white rot *Fungi* (*Phanerochaete sordida* (D2B), *Pycnoporus* species (Pyc) and *Schizophyllum commune* (S.com)),
- *Ophiostoma floccosum* (F13) ascomycetes, and
- *Absidia* (B101) zygomycete.

The treated fibres were characterised using X-ray diffraction (XRD), ξ-potential, TAPPI lignin testing, DTA and TGA thermal analysis, and scanning electron microscopy (SEM). A combined alkali and *Fungi* treated fibre composite produced the highest tensile strength of 48.3 MPa, an increase of 32% compared to composites with untreated fibre. Among *Fungi*, the white rot fungus (*Basidiomycetes*) is the only one able to degrade non-cellulosic compounds from natural fibers, thus improving the mechanical properties of the resulting natural fibre reinforced composites.

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White rot *Fungi* cellulase enzymes singly and in combination (cellulases, xylanases and pectinases) were used to treat jute fibres and degraded the lignin polymers leading to a reduction in flexural rigidity and tenacity [82] (Jayapriya and Vigneswaran, 2010).

Acero et al [83] (2014) used laccases (EC 1.10.3.2), p-diphenol dioxygen oxidorecductases, from *Trametes hirsuta* as a biocatalyst for direct grafting of different types of functional phenolic and amine molecules onto flax fibres. The different chemical moieties generated could increase compatibility with polymeric matrices.

Bio-composite core materials for sandwich panels

The fibrous networks of mycelia (the vegetative part of *Fungi*) may be used as sustainable alternatives to synthetic foams for the cores of sandwich structures. The topic has been reviewed by several authors [84-87] (Jones et al, 2017; Attias et al, 2019; Girometta et al, 2019; Hyde et al, 2019).

Composites end-of-life

Fibres

Once the natural fibres are incorporated into a composite, the ultimate (<~25μm apparent diameter) or technical fibres or bundles (>~25μm apparent diameter) are enclosed by a different polymer as the matrix for the composite. Deacon [88] (2019) states that the fungal hyphae are typically 5-10 μm diameter, and thus of a similar size to the synthetic high-performance reinforcement fibres (aramid or carbon). For an ultimate fibre, access will be limited to ~ 20 hyphae travelling parallel to each other.

Leonowicz et al [89] (1999) reviewed the biodegradation of lignin, as a constituent of wood, by white rot *Fungi*. Wood rotting basidiomycetous *Fungi* directly attack the "lignin barrier", penetrate the wood and metabolise the carbohydrate components of the material. The hypothetical mechanisms of lignocellulose transformation by white rot enzymes are presented in Figure 4 of reference [89].

Stamboulis et al [90] (2000) have observed that moisture causes fungus development on the fibre surface after a couple, or three, of days of exposure, resulting in degradation of the fibres and the decrease of their mechanical properties.

While all-cellulose composites do exist (e.g. [91-94] Nishino et al, 2004. Nishino and Peijs, 2014. Reddy et al, 2014. Li et al, 2018.), they are comparatively rare, but both the fibre and matrix could potentially be decomposed by cellulases, lignases and pectases acting singly or in combination.

Biodegradation of cellulose to glucose is the principal route for treatment of plant fibres at end-of-life. Degradation by cellulases can be controlled by adjusting the treatment parameters, including time, temperature, enzyme loading and the cellulase composition.

Milner et al. [95] (2008) and Anon. [96] (2008) have reported a new strain of thermophylic *Bacteria* that can break down cellulose waste to produce useful renewable fuels for the transport industry. The *Geobacillus* family normally synthesise sugars and produce lactic acid as a by-product when they break down biomass in a compost heap. The re-engineered TM242 strain is claimed to produce ethanol more efficiently (yields of 10 to 15%) and cheaply than in traditional yeast-based fermentation.

Acetylation of lignocellulosics provides protection against fungal attack and hence may not be desirable for end-of-life composites. Suttie [97] (1999) suggests that the substitution of the hydroxyl (-OH) groups on cellulose with acetyl moieties makes the cell wall polymers incompatible with the enzymes associated with *Fungi*.

Matrix

For composites that will be highly stressed, where the glass transition temperature of the matrix is above ambient temperature, thermoplastic matrix composites will generally be processed at temperatures that degrade the bast reinforcement fibres and hence the composite system may not be useful after initial, or re-, processing. Poly(lactic acid) (PLA) is an exception with Tg ~65°C and Tm ~175°C. Thermosetting matrix composites are normally disposed of at end-of-life by chemical or thermal methods or sent to landfill.

For end-of-life bast fibre composites other than self-reinforced polymers, a cocktail of *Fungi/Bacteria/*enzymes may be required to degrade the composite. However, it is possible that some mixed systems likely to be antagonistic and hence below optimal efficiency.

The matrix systems used for many bast fibre composites are synthetic polymers. The polyolefins (polyethylene, polypropylene, etc.) are often used as the matrix for (injection moulded) short bast fibre composites as they can be melt processed below the degradation temperature of the fibres. Given their chemical similarity to petrochemical fuels, they might be processed using the fungal systems that "spoil" those fuels [16] (Spooner and Roberts, 2005), once they are no longer suitable for reprocessing at their end-of-life.

Bombelli et al [98] (2017) reported fast bio-degradation of poly(ethylene) (PE) by larvae of the wax moth *Galleria mellonella*, which feed on beeswax, to produce ethylene glycol. The authors were "not clear whether the hydrocarbon-digesting activity of *G. mellonella* derives from the organism itself, or from enzymatic activities of its intestinal flora previously reported by Yang et al [99] (2014) as biodegrading PE. Weber et al [100] (2017) disagreed with the methodology and conclusions from the Bombelli et al paper stating that "weight loss alone is clearly insufficient to prove the proposed biodegradation of polyethylene".

Poly(lactic acid) (PLA) is a bio-based thermoplastic that can be processed at similar temperatures to the polyolefins, and has the additional advantage that the glass transition temperature is above ambient so the materials can take stress at room temperature. Gross and Kalra [101] (2002) report that even at high humidity, it is "uncommon to encounter contamination of high molecular weight PLA by fungi, mold, or other microbes". In consequence, PLA may need to be composted in municipal facilities. Tokia and Calabia [102] (2006) have reviewed the biodegradability and biodegradation of poly(lactide). The microorganisms considered include the PLA-degrading bacteria *Pseudonocardiaceae* genera such as *Amycolatopsis*, *Lentzea*, *Kibdelosporangium*, *Saccharothrix* and *Streptoalloteichus*.

Brueckner et al [103] (2008) treated PET fabrics with cutinase(s) from *Thermobifida fusca* (GBF Braunschweig) and lipases from *Thermomyces lanuginosus* (Novozymes) and observed formation of novel carboxyl and hydroxyl groups after treatment. A new esterase class of enzymes (PETases) has recently been identified that can catalyse the hydrolysis of poly(ethylene terephthalate) [104-107] (Han et al, 2017. Austin et al, 2018. Chen et al, 2018. Son et al, 2019). Although specific to a high-performance (high melting point) thermoplastic system, it does indicate that it may be practical to develop similar systems to process unsaturated polyester thermoset resin matrices.

Magnin et al [108] (2019) have developed screening strategies to identify enzymes for depolymerisation of polyurethanes. Esterases depolymerise PCL-based thermoplastic polyurethane (TPU). The combination of esterases and amidases was synergistic in the hydrolysis of urethane bonds.

Composites

Gu et al [109] (1996) inoculated pre-sterilised samples of glass/fluorinated polyimide or carbon fibre reinforced bismaleimide or epoxy or PEEK to a fungal consortium for five weeks at ~22°C and found fungal attack in all samples. Unfortunately, the poor quality of the images in the paper makes it difficult to understand their claim of fungal penetration into the glassfibre composite.

Gu et al [110] (1997) exposed samples of epoxy-matrix sandwich panels with glass fibre skins over a unidirectional IM6G carbon fibre core to a "mixed culture of *Fungi* ... enriched from degraded composite materials" for up to 179 days and inferred that the *Fungi* used the resins or fibre sizing chemicals as carbon and energy sources. Scanning electron microscopy indicated fungal colonisation of the composites with localised penetration of fungal hyphae into the interior of the composites. However, mechanical testing was limited to interlaminar shear strength (ILSS) tests where "No significant difference of [ILSS] was detected between the inoculated and the control composites" although the "resultant fracture indicated that bonding strength between fibres and resin was weakened after inoculation with *Fungi* compared to the [sterile] control.

Tufan et al [111] (2016) conducted decay tests to European standard EN 113 [112] using brown rot *Fungi*, *Coniophora puteana* (Schumach.: Fr.) P. Karst (Mad-15) and white rot *Fungi*, *Trametes versicolor* L.Pilat (Mad-697) to determine the degradation of the sisal (leaf) with carbon fibre polypropylene matrix hybrid composites. Mechanical/thermal properties and biological durability improved with the increasing proportions of carbon

fibre in the hybrid composite. After 16 weeks exposure, the mass loss rate was very limited (<7% for white rot and <5% for brown rot) in the hybrid composites.

Terzi et al [113] (2018) studied the decay resistance of composite sandwich panels with woven jute fabric skins over a wood particle and short glass fibre core in an unsaturated polyester matrix manufactured by VARTM. The fungal tests used brown rot (*Fomitopsis palustris*) and white rot (*Trametes versicolor*) *Fungi* with 12 weeks exposure. The rot *Fungi* weight losses were <5%, so the hybrid composites were classified as "highly resistant" according to ASTM D2017-05(2010) [114]. Mould tests used a mixture of *Aspergillus niger* 2.242, *Penicillium chyrsogenum* PH02 and *Trichoderma viride* ATCC 20476 *Fungi* with four weeks exposure. The lowest mold growth rates were found in panels without the jute skin.

Conclusion

For the acquisition, modification and disposal of natural fibres and their composites, the use of organisms and the associated enzymes offers a promising route to sustainable composites. This would potentially reduce the required energy and use milder reaction conditions. This paper reviews the use of organisms and enzymes for (a) extraction of fibres from plant material (retting), (b) surface modification of fibres, and (c) end-of-life treatments, in the context of bast fibres and their composites. The use of enzyme processes at large scale is limited by the extended treatment times, costs of the enzymes and equipment, wastewater treatment and the current low level of adoption by industry. It will be essential to undertake risk assessment before laboratory experiments or commercial use of organisms and enzymes to address economic, environmental (including escape of organisms into the ecosystem) and health and safety issues. Further, a quantitative life cycle assessment (QLCA) should demonstrate that the chosen route incurs minimal environmental burdens.

CRediT authorship contribution statement

John Summerscales: Conceptualization, Investigation, Writing - original draft

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Summary of animals mentioned in this review.

Kingdom	Phylum	Class	Order	Family	Type	Genus	Species
Animalia	*Arthropoda*	*Insecta*	*Lepidoptera*	*Pyralidae*	*Phycitini*	*Plodia*	*interpunctella*
Animalia	*Arthropoda*	*Insecta*	*Lepidoptera*	*Pyralidae*	*Galleriinae*	*Galleria*	*mellonella*

Table 2: Summary of bacteria mentioned in this review.

Kingdom	Phylum	Class	Order	Family	Genus	Species
Bacteria	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Nocardiopsaceae*	*Thermobifida*	*fusca*
Bacteria	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Amycolatopsis*	
Bacteria	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Pseudonocardia (Amycolata)*	
Bacteria	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Kibdelosporangium*	
Bacteria	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Lentzea*	
Bacteria	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Saccharothrix*	
Bacteria	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Pseudonocardiaceae*	*Streptoalloteichus*	
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	*mesentericus*
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	*subtilis*
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Paenibacillaceae*	*Paenibacillus*	*macerans*
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Paenibacillaceae*	*Paenibacillus*	*polymyxa*
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillu*	
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Leuconostoc*	
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Pediococcus*	
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*acetobutylicum*
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*aurantibutyricum*
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*felsineum*
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*	*tertium*
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Brucellaceae*	*Ochrobactrum*	*antrophi*
Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Ideonella*	*sakaiensis*
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Enterobactrales*	*Enterobacteriaceae*	*Enterobacter*	*asburiae*
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Stenotrophomas*	*maltophilia*

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Table 3: Summary of fungi mentioned in this review (taxonomy may be contentious).

Kingdom	Phylum	Class	Order	Family	Genus	Species
Fungi	*Ascomycota*	*Dothideomycetes*	*Capnodiales*	*Davidiellaceae*	*Cladosporium*	*cladosporioides*
Fungi	*Ascomycota*	*Dothideomycetes*	*Capnodiales*	*Davidiellaceae*	*Cladosporium*	*herbarum*
Fungi	*Ascomycota*	*Dothideomycetes*	*Dothideales*	*Dothioraceae*	*Aureobasidium*	*pullulans*
Fungi	*Ascomycota*	*Dothideomycetes*	*Pleosporales*	*Didymellaceae*	*Phoma*	
Fungi	*Ascomycota*	*Dothideomycetes*	*Pleosporales*	*Pleosporaceae*	*Alternaria*	*alternate*
Fungi	*Ascomycota*	*Dothideomycetes*	*Pleosporales*	*Pleosporaceae*	*Epicoccum*	*nigrum*
Fungi	*Ascomycota*	*Euascomycetes*	*Hypocreales*	*Hypocreaceae*	*Trichoderma*	*viride*
Fungi	*Ascomycota*	*Eurotiomycetes*	*Eurotiales*	*Pleosporales*	*Aspergillus*	*niger*
Fungi	*Ascomycota*	*Eurotiomycetes*	*Eurotiales*	*Pleosporales*	*Aspergillus*	*versicolor*
Fungi	*Ascomycota*	*Eurotiomycetes*	*Eurotiales*	*Trichocomaceae*	*Penicillium*	*chrysogenum*
Fungi	*Ascomycota*	*Eurotiomycetes*	*Eurotiales*	*Trichocomaceae*	*Thermomyces*	*lanuginosus*
Fungi	*Ascomycota*	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	*Fusarium*	
Fungi	*Ascomycota*	*Sordariomycetes*	*Ophiostomatales*	*Ophiostomataceae*	*Ophiostoma*	*floccosum*
Fungi	*Ascomycota*	*Sordariomycetes*	*Sordariales*	*Chaetomiaceae*	*Chaetomium*	
Fungi	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Schizophyllaceae*	*Schizophyllum*	*commune*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Boletales*	*Coniophoraceae*	*Coniophora*	*puteana*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Fomitopsidaceae*	*Fomitopsis*	*palustris*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Phanerochaetaceae*	*Ceriporiopsis*	*subvermispora*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Phanerochaetaceae*	*Phanerochaete*	*chrysosporium*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Phanerochaetaceae*	*Phanerochaete*	*sordida*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Polyporaceae*	*Pycnoporus*	
Fungi	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Polyporaceae*	*Trametes*	*hirsute*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Polyporaceae*	*Trametes*	*versicolor*
Fungi	*Zygomycota*	*Zygomycetes*	*Mucorales*	*Mucoraceae*	*Absidia*	
Fungi	*Zygomycota*	*Zygomycetes*	*Mucorales*	*Mucoraceae*	*Rhizomucor*	*pusillus*
Fungi	*Zygomycota*	*Zygomycetes*	*Mucorales*	*Mucoraceae*	*Rhizopus*	*oryzae*

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Table 4: Enzyme classification

First integer	Major class	Characteristics
1	Oxidoreductases	Transfer a hydrogen atom or hydride ion ($H:^-$) or act on H_2O_2 or O_2
2	Transferases	Transfer acyl, amino, glycosyl, or phosphoryl groups
3	Hydrolases	Cleave a wide variety of substrates by adding water across a bond
4	Lyases	Cleave carbon-containing groups bound to carbon, nitrogen, or oxygen non-hydrolytically; the products contain one more double bond than the reactants
5	Isomerases	Include racemases, epimerases, intramolecular oxidoreductases; intramolecular transferases.
6	Ligases	Mediate ATP- or nucleoside triphosphate-dependent condensation reactions
7	Translocating enzymes (new at end of 2019)	Moves protons over a membrane: EC 1.9.3.1 at the moment, but will move to EC 7.x.x.x

Table 5: Enzyme or general type [23] (Boden, 2020)

System	Description
amidase **esterase** **lyase** **oxidase**	general types (not specific enzymes) describing a commonality of reaction, not a specific substrate. Variously hydrolases, oxidoreductases, or group-transferases, *e.g.* oxidase adds an "O" from O_2 and produces either H_2O or more usually H_2O_2 as a secondary product.
cellibiohydrolase	about four enzymes in EC. 3.2.1.*x*
cutinase	*sensu stricto* EC 3.1.7.4, but also used for two unrelated hydrolases. One acts on plastics (EC 3.1.1.101), and one on triacylglycerol lipids (EC 3.1.1.3).
endoglucanase	probably usually means EC 3.2.1.6 but 11 other enzymes have this as a synonym.
beta-glucosidases	(that is now the way it is styled, usually) are about a dozen enzymes, all now with got specific names.
hemicelluloase	refers probably to EC 3.1.1.73 but do check the reaction. No other enzymes have hemicellulose listed as a substrate
keratinase	numerous keratinase producing micro-organisms [24] (Srivastava et al, 2020)
laccase	a single enzyme, EC 1.10.3.2.
lignase	a colloquialism for "stuff that acts on lignin", not a real single enzyme, e.g. lignin peroxidases or EC 3.1.1.73 which has some activity with lignin.
lipase	27 thereof, now per beta-glucosidases and endoglucanases above - split up with specific names for easier specific identification.
pectinase	4 of these, again, each with a specific functional name now.
xylanase	a bunch with specific function names but EC 3.1.1.73 (above) has some xylanase activity too and could mean that.

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Table 6: Process conditions for biological action on bast fibres or polymers (pressures not specified and assumed ambient unless stated)

Organism/enzyme	Substrate	Temperature	Environment/pH	Reference	Notes
					Retting
Aspergillus niger	flax (Ariane)	27°C for 6.5 h 40°C for 22 h	pH 5.0	Zhang et al, 2000 [37].	retting
Aspergillus niger PGase *Rhizopus* PGase Viscozyme L	flax (Ariane)	40°C for 20 h	pH 5.0	Evans et al, 2002 [38].	retting
Pectate lyase Viscozyme L	Flax (Ariane, Hermes, Omega, York)	50°C for 1 h 40°C for 24 h	pH 8.74 pH 5	Akin et al, 2007 [40].	retting
Amycolata (Pseudonocardia) pectate lyase	ramie	"room" for 15h	pH 7	Bruhlmann et al, 2000 [41].	degumming
Bacillus sp. PGase	Ramie and sunn hemp	50°C for 12 h 60°C for 11 h	pH 10.0	Kapoor et al, 2001 [32].	degumming
Bacillus sp alkalophilic bacteria	ramie	37°C for 24–48 h	pH 10.0	Zheng et al, 2001 [50].	degumming
Trametes hirsuta	flax	37°C for 4 h	pH 4.5	Acero et al, 2014 [83].	surface treatment
Trametes versicolor	wheat	30°C for 40 days		Zhang et al, 2008a [55].	retting
Ochrobactrum anthropi *Stenotrophomnas maltophilia*	hemp	28°C for 36-48 h		Zhang et al 2008b [56].	retting
Clostridium felsineum *Bacillus subtilis*.	hemp (Tiborszallasi)	35°C for 3 days		Sisti et al, 2016 [57].	retting
18 filamentous fungi	(unspecified) plant fibres	30-37ºC for 3 days	pH 7.0	Molina et al, 2001 [63].	degumming
field environment	hemp	ambient outdoors	ambient outdoors	Booth et al, 2004 [64].	retting
0.05% Viscozyme L plus 1.8% Mayoquest 200	flax	40ºC for up to 24h	pH 5.0	Akin et al, 2003 [73].	retting
Various enzymes	flax	25-60ºC	pH 3.5-9	Foulk et al, 2008 [75].	retting
PL-BRI bacterial pectinolytic enzyme with lyase activity (E.C.4.2.2.2)	flax	42ºC up to 46 h	pH 8.5	Foulk et al, 2011 [76].	retting
Texazym SER-3 and SER-4	flax	Sprayed in field		Antonov et al, 2007 [77].	retting

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					Surface modification
White rot fungi / extracellular oxidases enzymes	plant-based natural fibre	27ºC for 2 weeks		Kabir et al, 2012 [78].	surface modification
Viscozyme, Ultrazyme or Denilite, then Cellusoft L/UL	jute	40-60ºC for 72 h then 1-4 h	neutral or pH 8.0	Kamiko et al, 2002 [80].	degumming
Scourzyme L pectate lyase (EC 4.2.2.2),	hemp	55°C up to 24 h	pH 8.5	Ouajai and Shanks, 2005 [81].	bioscouring
white rot fungi (*Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora*), cellulase enzyme, mixed enzymes (cellulase, xylanases, and pectinases)	jute	40°C for 90 min	pH 5.0-5.5	Jayapriya and Vigneswaran, 2010 [82].	biosoftening
Trametes hirsute laccase (EC 10.3.2)	flax (and coconut)	37°C for 3 h	pH 4.5	Acero et al, 2014 [83].	surface functionalisation
Trametes hirsuta	flax	37°C for 4 h	pH 4.5	Acero et al, 2014 [83].	surface treatment
					End-of-life
Galleria mellonella wax moth caterpillars	poly(ethylene) film	ambient		Bombelli et al, 2017 [98].	degradation
Enterobacter asburiae YT1 and *baciilus sp.* YP1 from *Plodia interpunctella* larvae guts	poly(ethylene) film	30°C and 85% RH for 28 days		Yang et al, 2014 [99].	degradation
Cutinase from *Thermobifida fusca* lipase from *Thermomyces lanuginosus*	InoTEX PAT fabrics	37 or 60°C for 120 h	pH 7 or alkaline	Brueckner et al, 2008 [103].	degradation
PET hydrolase (cutinase-like enzyme from *Ideonella sakaiensis* 201-F6	poly ethylene terephthalate film	30°C for 42 h	pH 9.0	Han et al, 2017 [104].	degradation
Ideonella sakaiensis PETase or engineered enzyme S238F/W159H	poly ethylene terephthalate film	30°C for 96 h	pH 7.2	Austin et al, 2018 [105].	degradation
Ideonella sakaiensis PETase or *Thermobifida fusca* DSM43793 cutinase 2 (TfCUT2)	poly ethylene terephthalate	30-70°C for 18-72 h		Son et al, 2019 [107].	degradation
esterase (E3576) and amidase (E4143) mix	PCL-based PU	37°C for 40 h	pH 7	Magnin et al, 2019 [108].	degradation
Aspergillus versicolor, *Cladosporium cladosporioides*,	carbon/bismaleimide, carbon/epoxy,	22±2°C for 5 weeks in a dark room		Gu et al, 1996 [109].	degradation

Chaetomium spp.	carbon/PEEK, glass/ fluorinated polyimide.				
"previously isolated fungal consortium in .. a malt broth medium" [Gu et al, 1996]	glass surface, carbon- or graphite-fibre core reinforced epoxy sandwich	30 days		Gu et al, 1997 [110].	degradation
white rot: *Trametes versicolor* L. Pilat (Mad.=-697) brown rot: *Coniophora puteana* (Schumach.: Fr) P.karst (mad-15)	Sisal carbon hybrid poly(propylene) composites	24±2°C and 75% RH for 16 weeks		Tufan et al, 2016 [111].	degradation
brown-rot fungus: *Fomitopsis palustris* white-rot fungus: *Trametes* (Coriolus) versicolor		26 °C and 65% RH		Terzi et al, 2018 [113].	degradation
					Table ends