ΕΠΙΛΕΓΜΕΝΕΣ ΒΙΟΜΕΤΑΤΡΟΠΕΣ ΜΕ ΧΡΗΣΗ ΜΥΚΗΤΩΝ

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ΤΟ ΠΡΟΒΛΗΜΑ – Η ΠΡΟΚΛΗΣΗ !

Λιγνινοκυτταρινούχα υπολείμματα και παραπροϊόντα


Wood Chips
Sugarcane Bagasse
Corn Stover
Switch Grass
Wheat Straw
Rice Straw

Table 14. Annual production of lignocellulose residues generated by different agricultural sources

Lignocellulosic residues	Millions of tons
Sugar cane bagasse	380
Maize straw	191
Rice shell	188
Wheat straw	185
Soya straw	65
Yuca straw	48
Barley straw	42
Cotton fibre	20
Sunflower straw	9.0
Bean straw	5.9
Rye straw	5.2
Pine waste	4.6
Coffee straw	1.9
Almond straw	0.49
Hazelnut husk	0.24
Sisal and henequen (*Agave*) straw	0.093

Table modified from Sanchez (2009) and based on Food and Agriculture Organisation (FAO) and similar official sources.

Η ΦΥΣΗ ΤΟΥ ΠΡΟΒΛΗΜΑΤΟΣ !

Σύσταση λιγνινοκυτταρινούχων υλικών

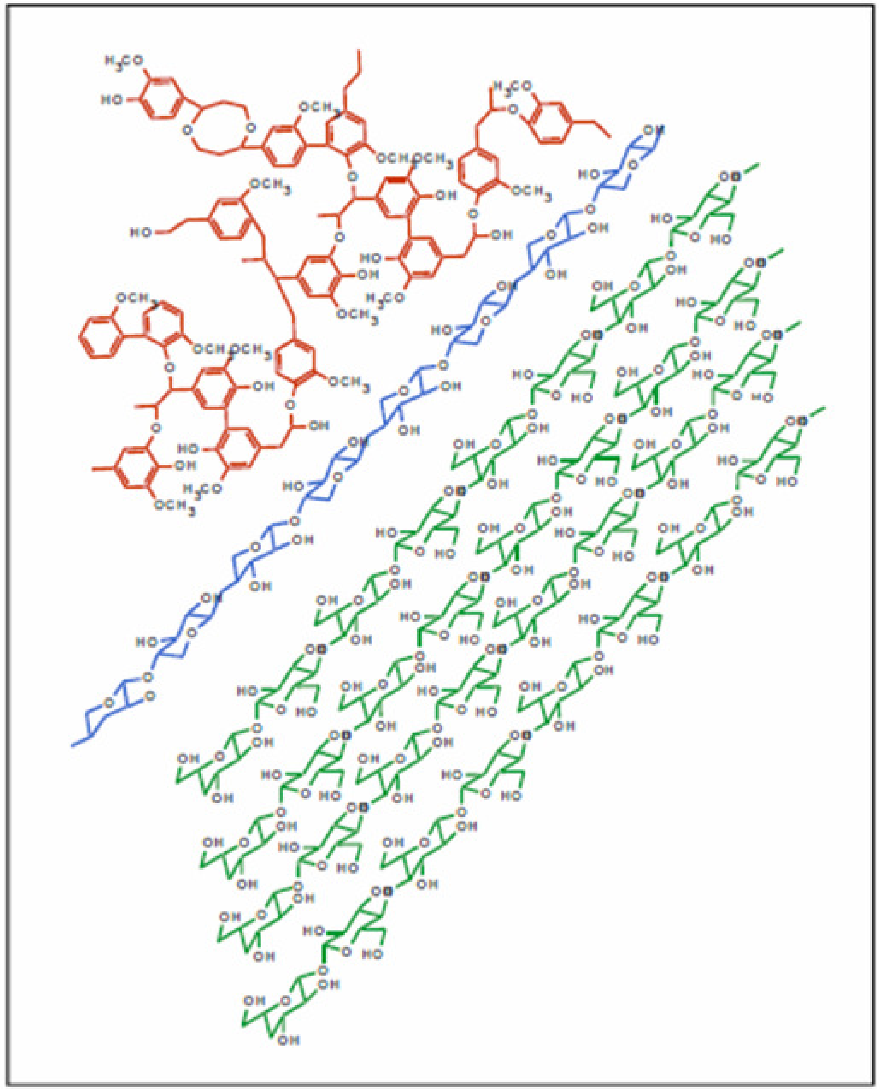
Wood, that is, plant secondary cell wall, is the most widespread substrate on the planet.

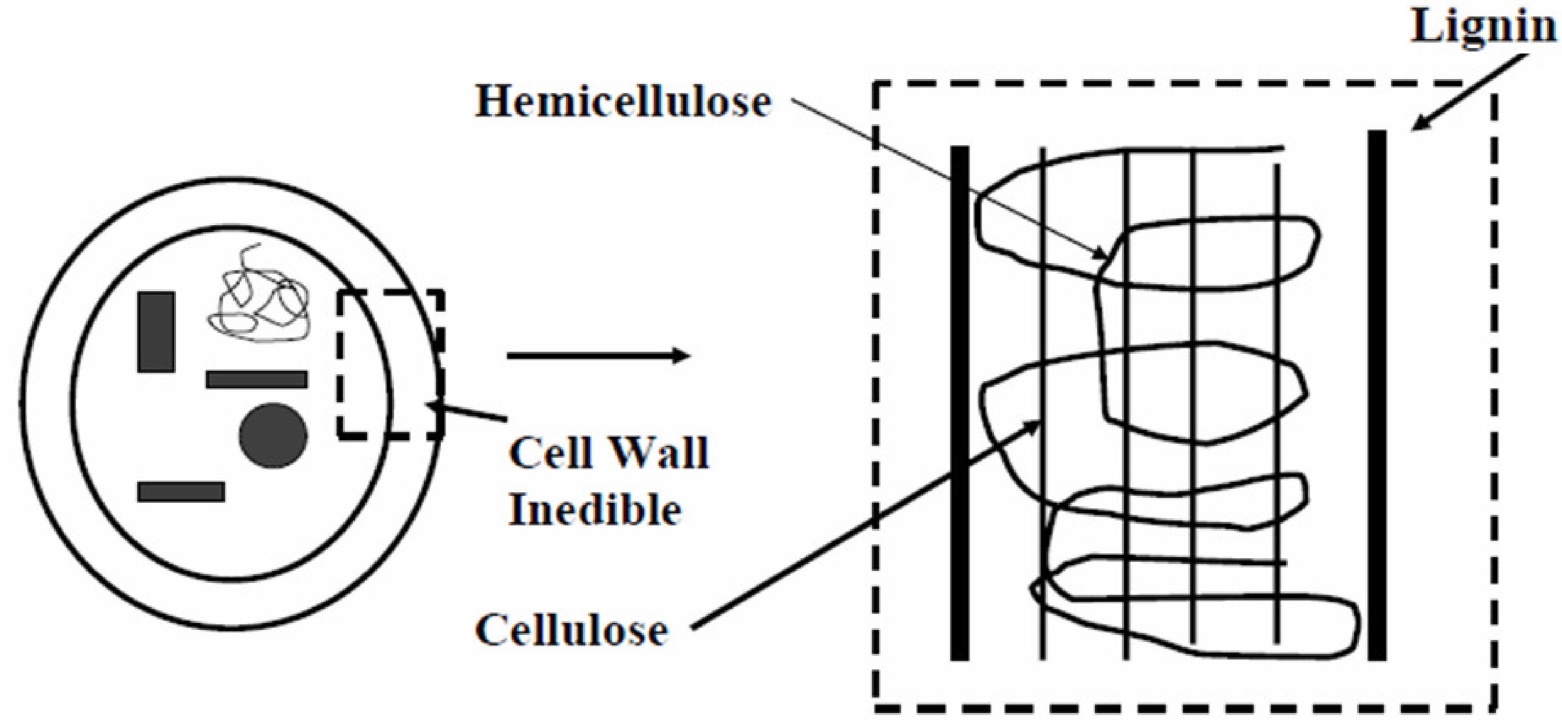
Except for lignin, the bulk of plant cell biomass consists of the polysaccharides cellulose, hemicelluloses, and pectins in varying proportions depending on the type of cell and its age.

Even though wall components predominate, the cytoplasm of dead cells contributes lipids, proteins and organic phosphates to the remains; however, wood itself is relatively poor in nitrogen and phosphorus.

Plant biomass does not consist of neatly isolated packets of polysaccharide, protein and lignin; these three (and other materials) are intimately mixed together, so that it is better to think of the degradation of lignocellulosic and/or lignoprotein complexes.

- Lignin: 15%–25%
    - Complex aromatic structure
    - Very high energy content
    - Resists biochemical conversion
- Hemicellulose: 23%–32%
    - Xylose is the second most abundant sugar in the biosphere
    - Polymer of 5- and 6-carbon sugars, marginal biochemical feed
- Cellulose: 38%–50%
    - Most abundant form of carbon
      in biosphere
    - Polymer of glucose, good biochemical feedstock




Lignin
Hemicellulose
Cell Wall
Inedible
Cellulose

Cellulose is the most abundant organic compound on Earth and accounts for over 50% of organic carbon; about 10^{11} tons are synthesised each year.

It is an unbranched polymer of glucose in which adjacent sugar molecules are joined by β 1→4 linkages (Fig. 1); there may be from a few hundred to a few thousand sugar residues in the polymer molecule, corresponding to molecular masses from about 50,000 to approaching 1 million.

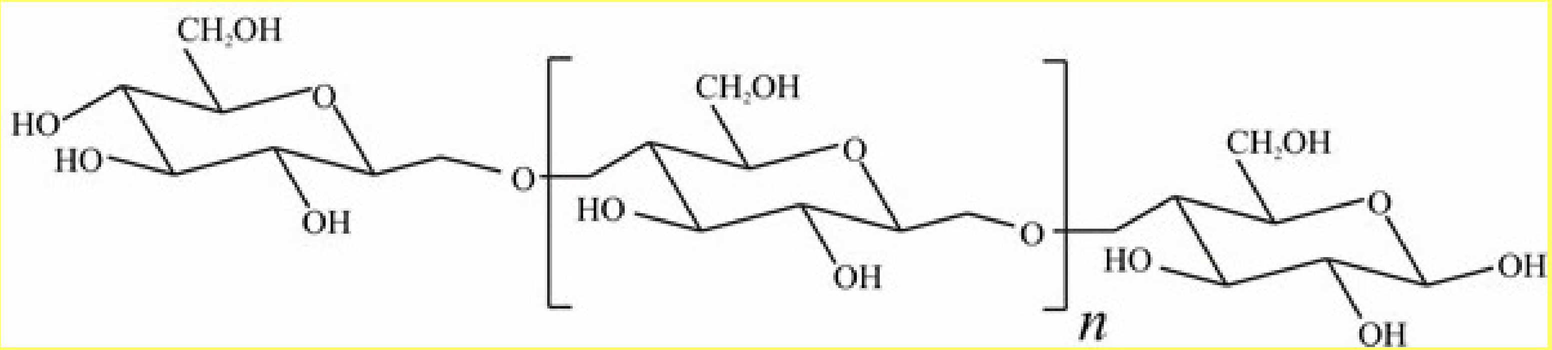
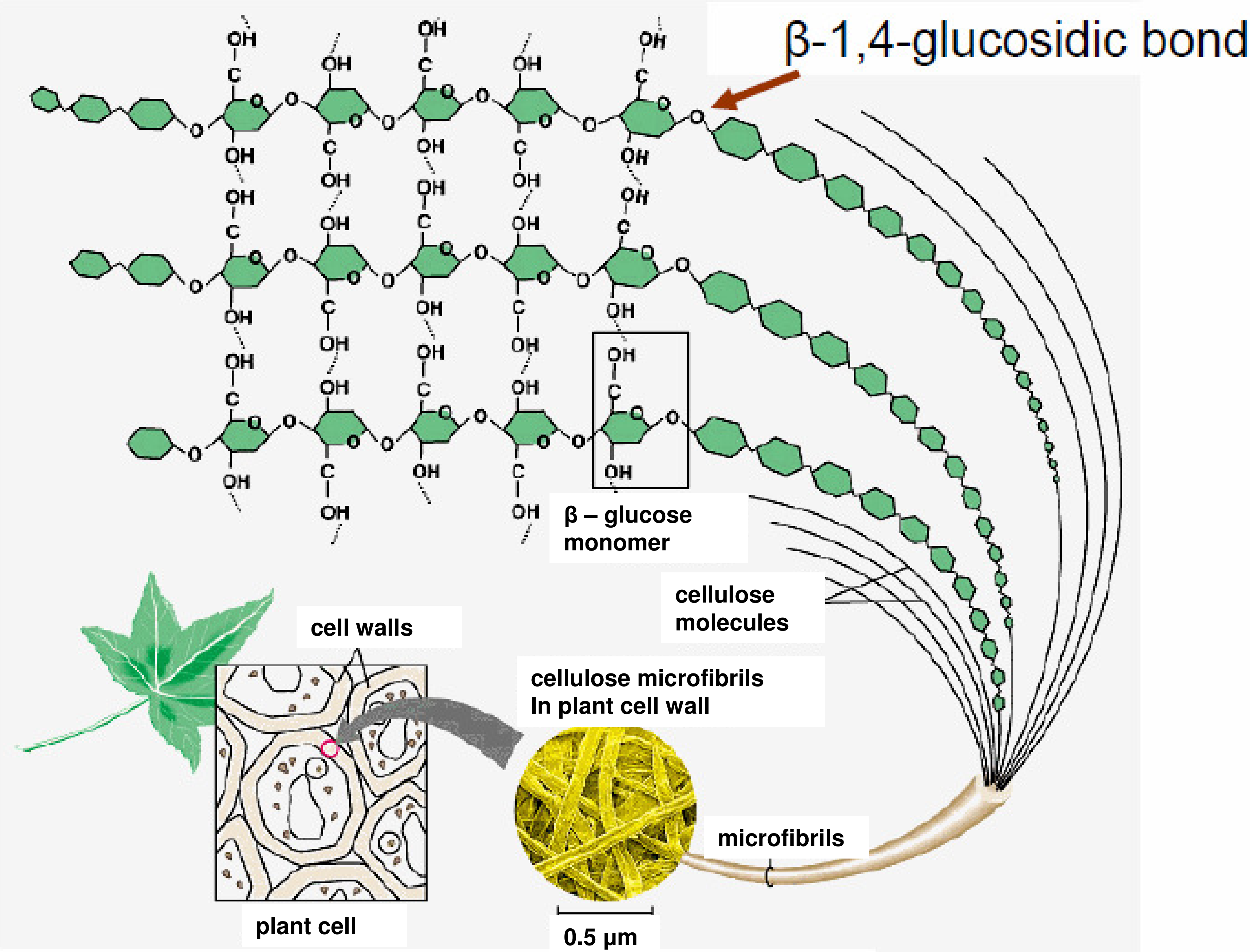


Fig. 1. Structural formula of cellulose. There may be from a few hundred to a few thousand sugar residues in the polymer molecule, corresponding to molecular masses from about 50,000 to approaching 1 million. Modified from Moore, 1998.

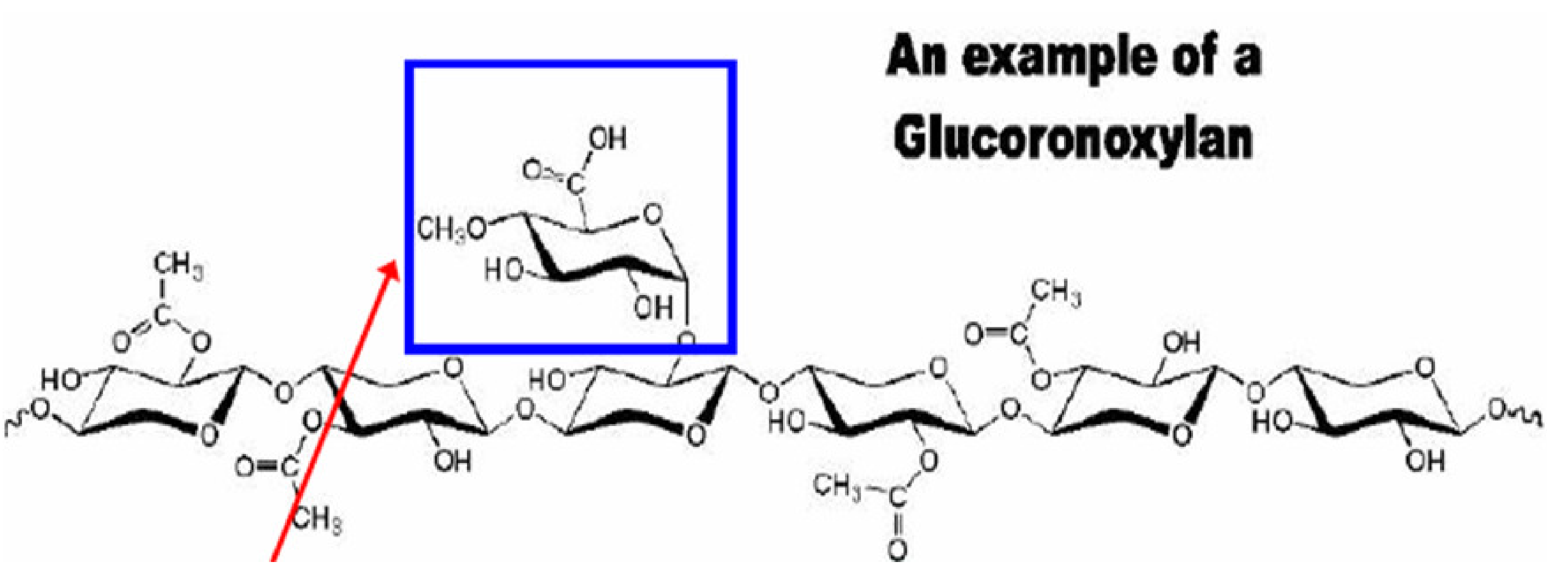


http://kentsimmons.uwinnipeg.ca/cm1504/Image74.gif

Hemicellulose is a name which covers a variety of branched-chain polymers containing a mixture of various hexose and pentose sugars, which might also be substituted with uronic and acetic acids.

The main hemicelluloses found in plants are xylans (1→4-linked polymers of the pentose sugar xylose), but arabans (polyarabinose), galactans (polygalactose), mannans and copolymers (e.g. glucomannans and galactoglucomannans) are also encountered.

The major angiosperm hemicellulose is a xylan with up to 35% of the xylose residues acetylated, and it is also substituted with 4-*O*-methylglucuronic acid in dicotyledonous plants.



<u>Lignin</u> is a highly branched phenylpropanoid polymer that comprises about 20-25% of wood

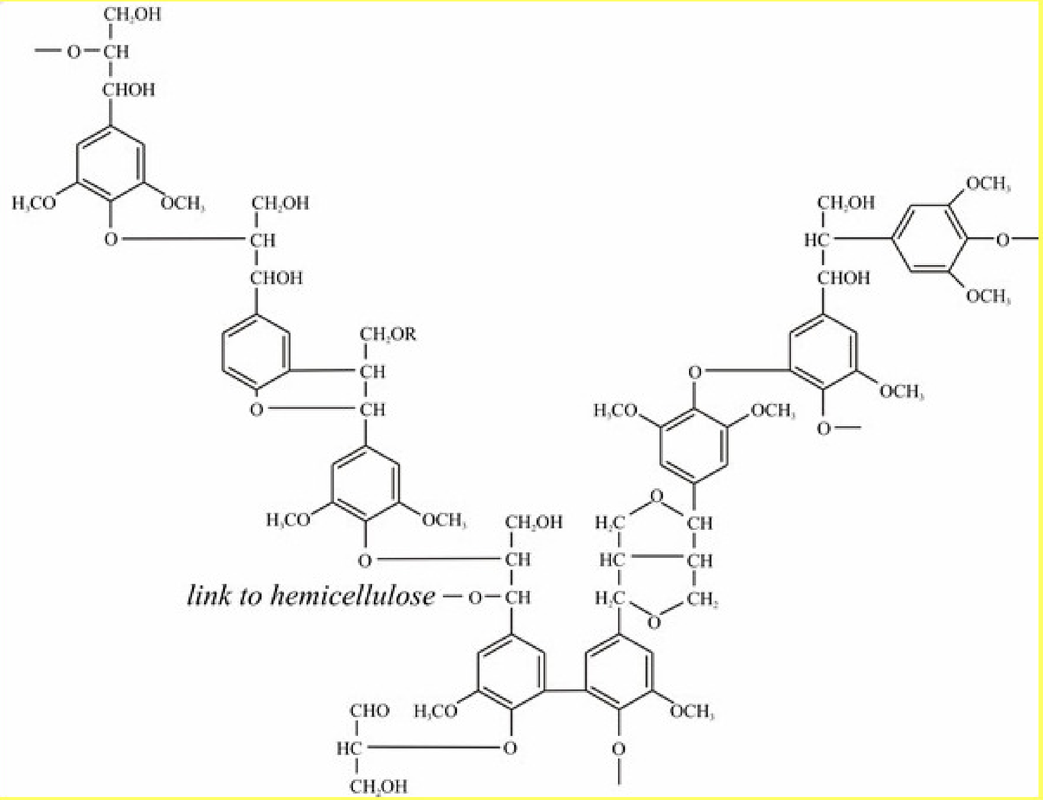


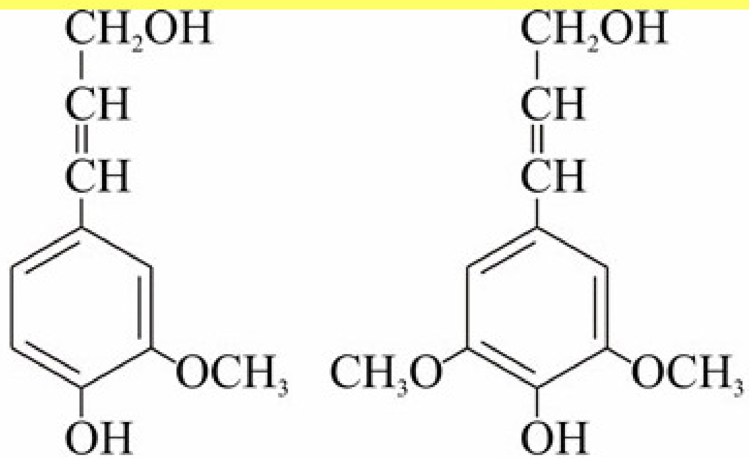
Fig. 6. Schematic formula of the polymer structure of angiosperm lignin. Note the way the three phenylpropanoid alcohols are used in the structure; note the predominance of ether linkages and carbon-carbon bonding, and the presence of a few hydroxyls that can take part in cross links to other polymers (polysaccharides and proteins). Above all, note the predominance of benzene rings. Modified from Moore, 1998.

Noteworthy are the three phenylpropanoid alcohols are used in the structure; the predominance of **ether** linkages (-C-O-C-) and **carbon-carbon bonding**, and the presence of a few hydroxyls that can take part in cross links to other polymers (polysaccharides and proteins, for example).

But above all, it is very essential to note the predominance of **benzene rings**.

Lignins are high-molecular weight, insoluble plant polymers, which have complex and variable structures.

They are composed essentially of many methoxylated derivatives of benzene (phenylpropanoid alcohols, also called monolignols), especially coniferyl, sinapyl, and coumaryl alcohols, the proportions of these three differ between angiosperms and gymnosperms, and between different plants.



coniferyl alcohol   sinapyl alcohol   *p*-coumaryl alcohol

Fig. 5. Chemical structures of the phenylpropanoid alcohols used to construct the lignin polymer. These are also called monolignols. The proportions of these three phenylpropanoid alcohols differ between the lignins of angiosperms and gymnosperms, and between lignins of different plant species.

ΔΙΑΧΕΙΡΙΣΗ ΤΟΥ ΠΡΟΒΛΗΜΑΤΟΣ ?

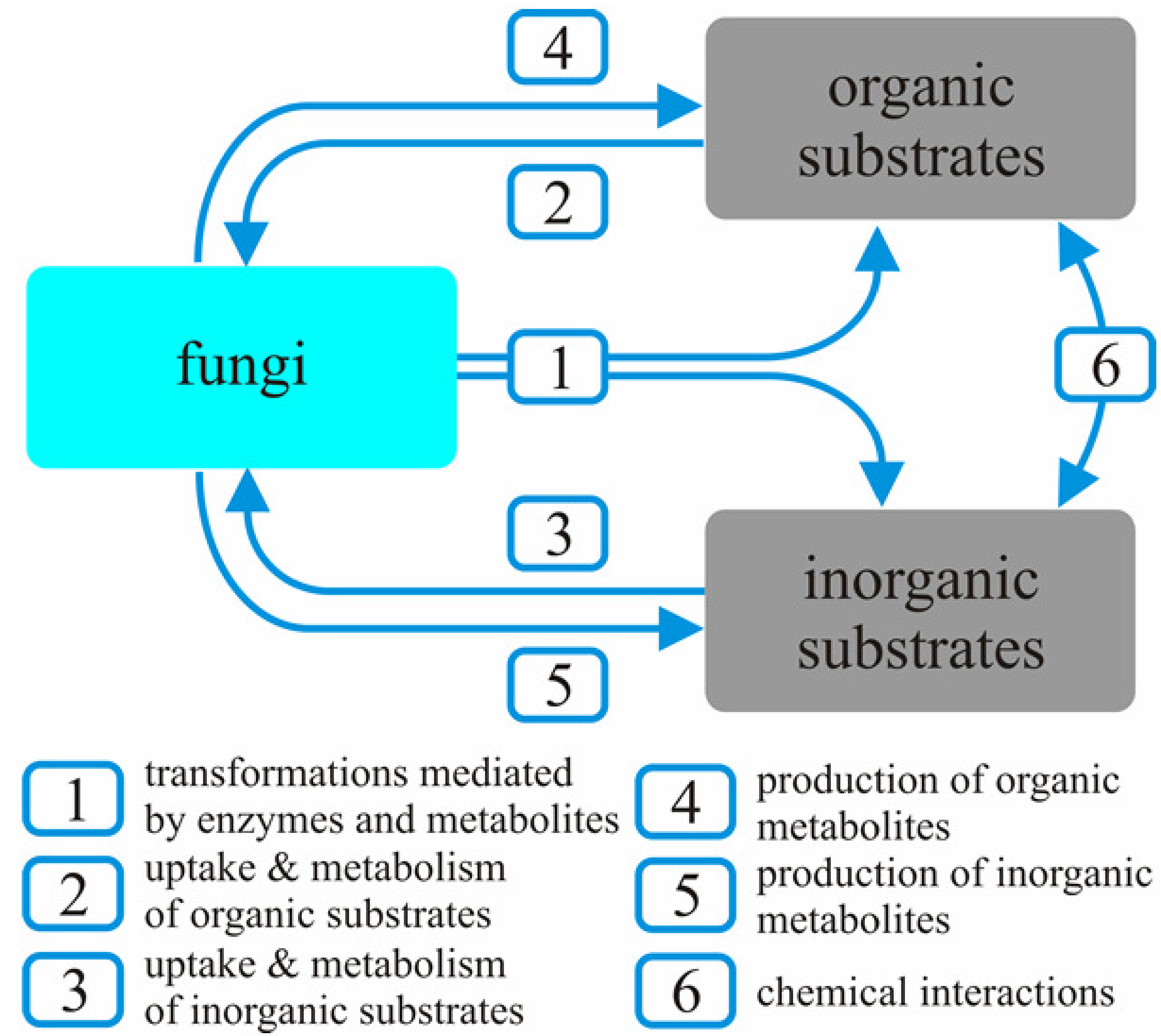
Οι Μύκητες

(και το ενζυματικό τους «οπλοστάσιο»…)

Saprotrophs are the decomposers, the category probably covers the majority of fungi.

Biotrophs are found on or in living plants and they do not kill their host plant rapidly. They may have very complex nutrient requirements, so they either cannot be grown in culture or grow only to a limited extent on specialised media.

Saprotrophic fungi decompose many things, and because they can digest and extract nutrients from so many of the materials that exist on, within and under the soil, fungal mycelia act as sinks of organic carbon and nitrogen in the soil.
In many ecosystems, a fair proportion of the carbon fixed by photosynthesis ends up in fungal mycelium because of the prevalence of the mycorrhizal symbiotic association between fungus and plant roots.



Diagrammatic representation of fungal action on organic and inorganic substrates which may be naturally-occurring and/or man-made (Gadd 2004).

About 70% of the mass of wood is made up of cellulose, hemicelluloses and pectins, the rest being lignin.

A particularly important ability of fungi is that they are the only organisms that can digest wood; that is, plant secondary cell wall, which is the most widespread substrate on the planet, constituting about 95% of the terrestrial biomass.

The lignin, which is complexed with hemicelluloses and cellulose in wood, is extremely difficult to degrade and has evolved in part to be a deterrent to microbial attack on long-lived plant parts.

Lignin digestion is restricted to fungi, mostly Basidiomycota but including a few Ascomycota, which between them consume an estimated $4{\times}10^{11}$ metric tonnes of plant biomass each year.

A typical agricultural residue, like cereal straw or sugar cane bagasse, contains 30-40% cellulose, 20-30% hemicellulose and 15-35% lignin.

Organisms that contribute to recycling may differ widely in their ability to degrade components of this mixture.
On this sort of basis wood-decay fungi have been separated into white-rot, brown-rot and soft-rot species:

- <u>white-rot fungi </u>(about 2 000 species, mostly Basidiomycota) can metabolise lignin,

- <u>brown-rot fungi </u>(about 200 species of Basidiomycota) degrade the cellulose and hemicellulose components without much effect on the lignin, and

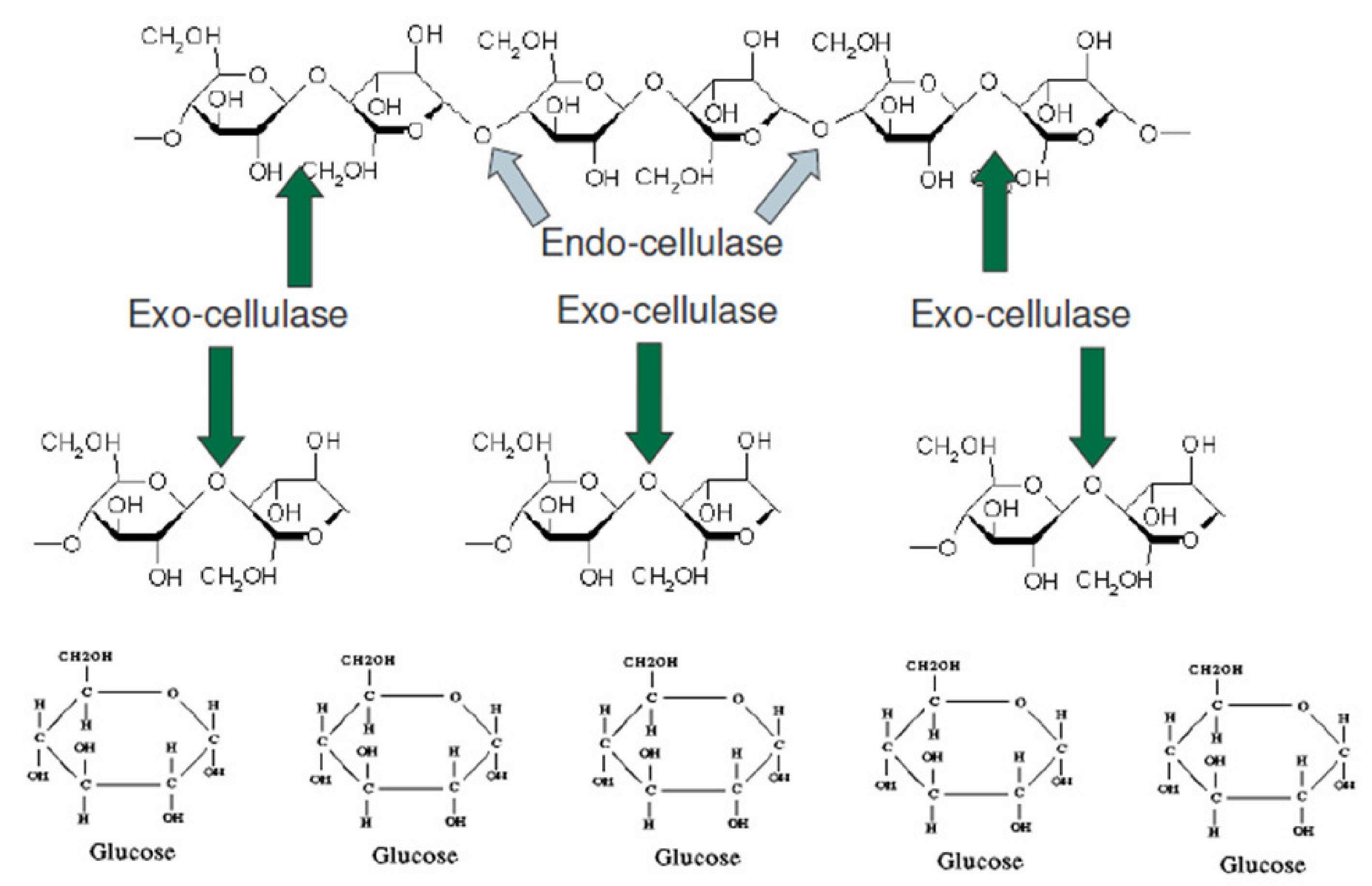
- <u>soft-rot species </u>(mostly soil-inhabiting Ascomycota) have rather intermediate capabilities, being able to degrade cellulose and hemicellulose rapidly, but lignin only slowly.

These differences in behaviour are a reflection of the different enzymes produced by these organisms and serve to emphasise that the organisms must digest complexes of potential nutrient sources and assemble panels of different enzymes to do so.

WHITE-ROT AND BROWN-ROT MUSHROOM FUNGI

CELLULOSE BIODEGRADATION

Breakdown of cellulose is chemically straightforward, but is complicated by its physical form. Mild acid hydrolysis of cellulose releases soluble sugars, but does not go to completion; oligomers of 100-300 glucose residues remain.

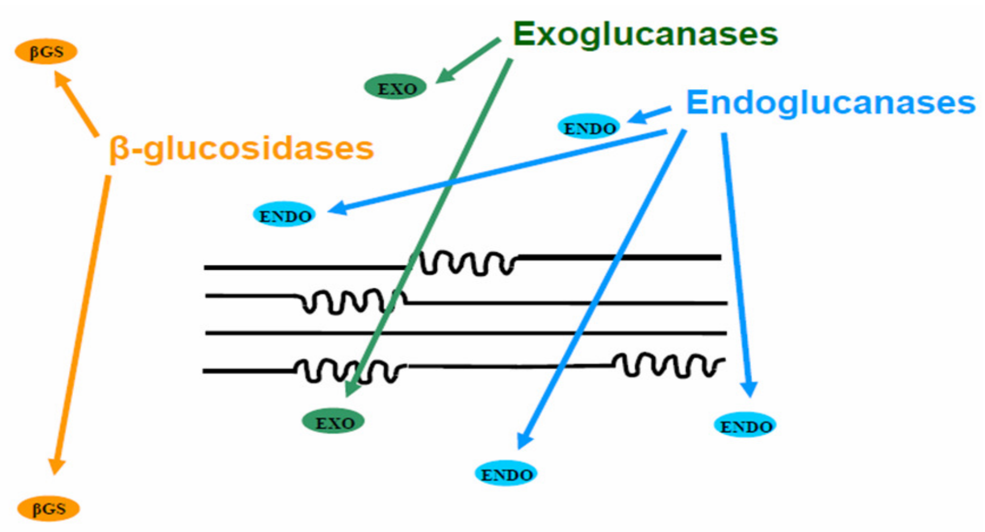


The cellulolytic enzyme (cellulase) complex of white-rot Basidiomycota like *Phanerochaete chrysosporium* and Ascomycota like *Trichoderma reesei* consists of a number of hydrolytic enzymes: Endoglucanase, exoglucanase and cellobiase (which is a β-glucosidase) which work synergistically and, in both bacteria and fungi, are organised into an extracellular multienzyme complex called a cellulosome (Bégum & Lemaire, 1996).

- Endoglucanase attacks cellulose at random, producing glucose, cellobiose (a disaccharide made up of two glucose molecules) and some cellotriose (a trisaccharide).

- Exoglucanase attacks from the non-reducing end of the cellulose molecule, removing glucose units; it may also include a cellobiohydrolase activity which produces cellobiose by attacking the non-reducing end of the polymer.

- Cellobiase is responsible for hydrolysing cellobiose to glucose. Glucose is, therefore, the readily-metabolised end-product of cellulose breakdown by enzymatic hydrolysis.



HEMICELLULOSE BIODEGRADATION

Enzymes responsible for hemicellulose degradation are named according to their substrate specificity; for example, mannanases degrade mannans, xylanases degrade xylans, etc. As xylans predominate in plant walls, more is known about xylanases.

Xylanases can be induced by their substrate, the response being for the fungus to produce a complex of enzymes rather than a single one.

The complex consists of at least two endoxylanases and a β-xylosidase.

The endoxylanases degrade xylan to xylobiose and other oligosaccharides while the xylosidase degrades these smaller sugars to xylose. Some arabinose is also formed, showing that the xylanase complex is able to hydrolyse the branch points in xylan.

LIGNIN BIODEGRADATION

This extreme resistance to microbial degradation is part of the principal function of lignin as it can protect other polymers from attack.

We are used to hydrolases cleaving polymers (polysaccharides, proteins, lipids), but the carbon-carbon and ether bonds that join subunits together in lignin must be cleaved by an *oxidative* process and a range of enzymes are needed for lignin to be degraded.

Although ability to digest simple synthetic lignins has been reported for a few bacteria, ability to degrade natural lignins is generally considered to be limited to a very few fungi. The list includes a range of Basidiomycota and Ascomycota, and the fungi involved are generally known as 'white-rots' because the lignin they digest away provides the main pigmentation of wood.

Oxidative lignin breakdown depends on a panel of enzymes including:

- lignin peroxidase, (a haem [Fe]-containing protein) that catalyses H_2O_2-dependent oxidation of lignin.

- manganese peroxidase, which also catalyses H_2O_2-dependent oxidation of lignin.

- laccase (a copper-containing protein) that catalyses demethylation of lignin components.

These are the key enzymes, and are described in a little more detail below. In addition,

- glyoxal oxidase, an extracellular peroxide-generating enzyme, and
- veratryl alcohol, which is a degradation product of lignin, also have important functions in lignin break down.

The process of catabolic lignin degradation involves:

- cleavage of ether bonds between monomers;
- oxidative cleavage of the propane side chain;
- demethylation;
- benzene ring cleavage to ketoadipic acid which is fed into the tricarboxylic acid cycle as a fatty acid.

ΤΟ ΠΡΟΒΛΗΜΑ ΒΡΙΣΚΕΙ ΛΥΣΕΙΣ (!)

και οι λύσεις αξιοποιούνται …

BIOTECHNOLOGICAL IMPLICATIONS AND APPLICATIONS

Lignin-degrading enzymes have considerable promise in several areas of biotechnology.

Biopulping has already been mentioned as an industry where ligninolytic enzymes can improve the quality of pulp by releasing and purifying the cellulose (Breen & Singleton, 1999).

Laccases have potential, too, for:

- pulp bleaching,
- detoxification (particularly of pulp mill effluents),
- removal of phenolics from wines,
- chemical transformation of pharmaceuticals.

Further, many pesticidal treatments depend on benzene rings for their effectiveness; chemicals like pentachlorophenol (PCP) and polychlorinated biphenyls (PCBs). These persist in the environment because there are so few organisms able to catabolise them.

But organisms that can catabolise lignin have all the tools necessary to destroy persistent pesticides as well (Reddy, 1995).

Bioconversion of lignocellulose into useful, higher value, products normally requires multi-step processes, the steps including:

- collection and mechanical, chemical or biological pretreatment;
- hydrolysis of polymers to produce readily metabolised (usually sugar) molecules;
- fermentation of the sugars to produce a microbial or chemical end-product;
- separation,
- purification,
- packaging and marketing.

Note that Table 14 shows official estimates of the *annual* production of these agricultural wastes.

The amounts are staggering; the total of the entries shown in Table 14 is 1.2 *billion* metric tons, and does not include municipal solid wastes like waste paper or garden refuse collected for recycling.
Several uses have been suggested for bioprocessed lignocellulosic wastes, for example: use as raw material for the production of ethanol, in the hope that an alternative fuel manufactured using biological methods will have environmental benefits.

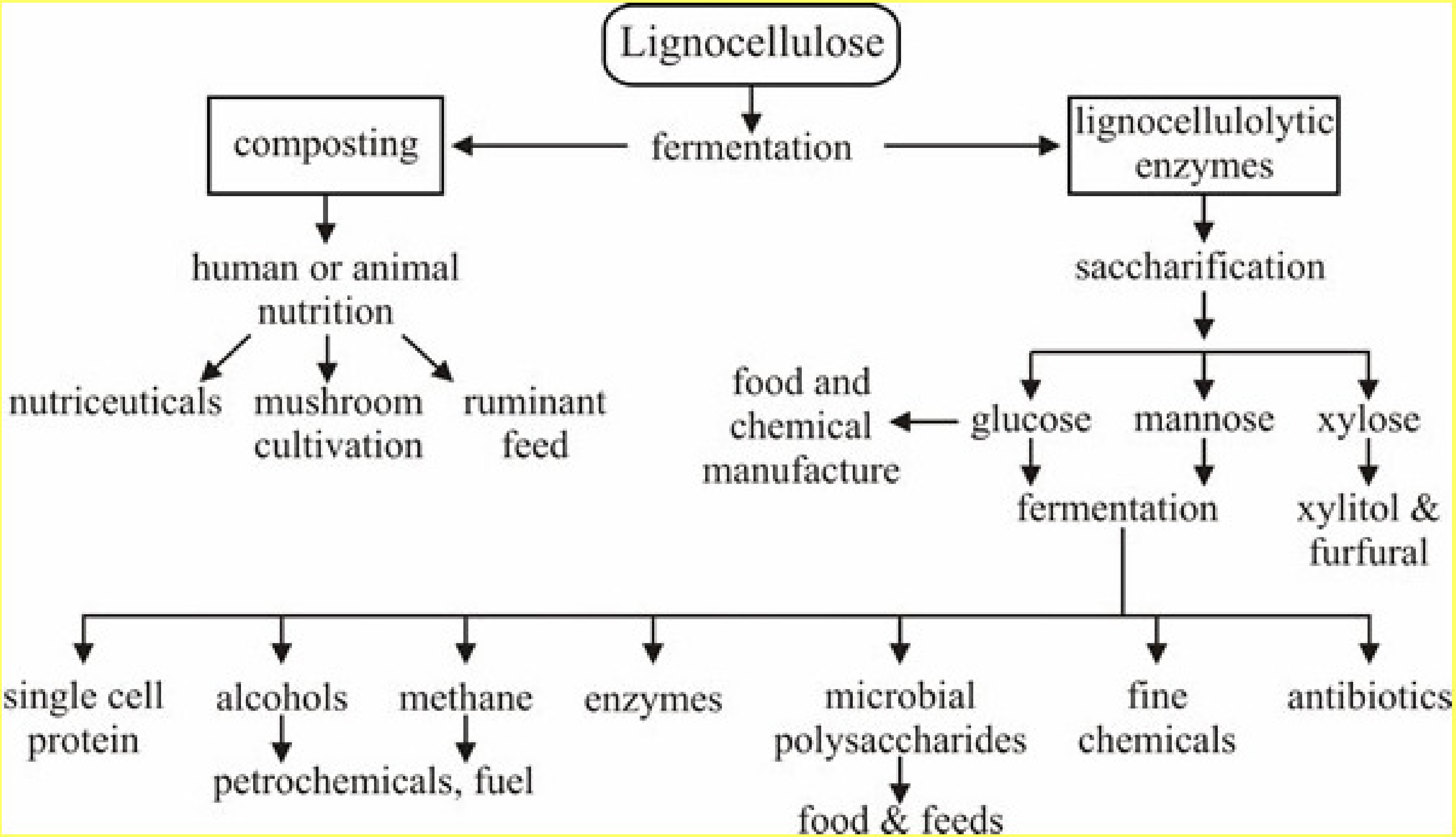


Fig. 39. Generalised process stages in lignocellulose waste bioconversion and the range of potential products. Adapted from Sanchez, 2009.

ΑΠΟΤΟΞΙΚΟΠΟΙΗΣΗ ΚΑΙ ΑΞΙΟΠΟΙΗΣΗ ΑΠΟΒΛΗΤΩΝ ΜΕΣΩ ΕΠΙΛΕΓΜΕΝΩΝ ΒΙΟΜΕΤΑΤΡΟΠΩΝ ΚΑΙ ΧΡΗΣΗ ΜΥΚΗΤΩΝ

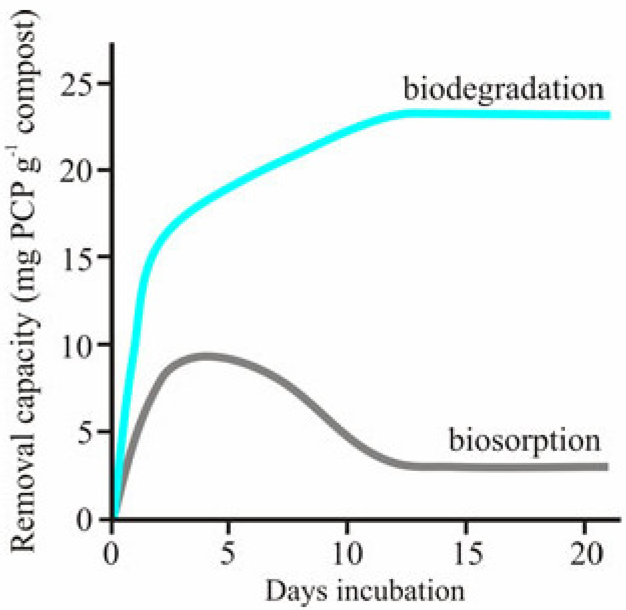
Κυριότερες εφαρμογές προστιθέμενης αξίας που σχετίζονται με την εκμετάλλευση των μακρομυκήτων:

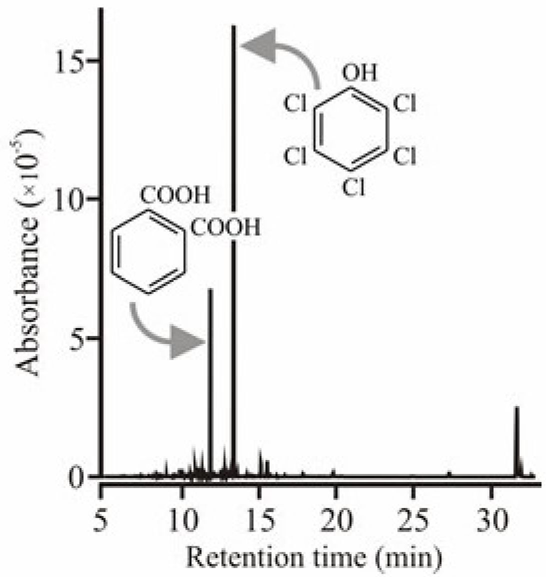
- ✓ Καλλιέργεια εδώδιμων μανιταριών
- ✓ Παραγωγή ενώσεων με φαρμακευτικές ιδιότητες
- ✓ Γονιμότητα και βελτίωση δομής εδαφών
- ✓ Εξυγίανση εδαφών
- ✓ Ανάπτυξη φυτών
- ✓ Επισχετικότητα κατά των εδαφογενών ασθενειών φυτών
- ✓ Παραγωγή ζωοτροφών
- ✓ Αποδόμηση ξενοβιοτικών ενώσεων
- ✓ Προσρόφηση-εξουδετέρωση τοξικών ενώσεων
- ✓ Αποχρωματισμός οργανικών ρυπαντών
- ✓ Μετατροπή-αξιοποίηση αποβλήτων από γεωργικές, δασικές και αγροβιομηχανικές δραστηριότητες

ΠΑΡΑΔΕΙΓΜΑ 1. ΑΠΟΔΟΜΗΣΗ ΔΥΣΤΡΟΠΩΝ (“RECALCITRANT”) ΡΥΠΑΝΤΩΝ

Επώαση για διάστημα λίγων εβδομάδων του εξαντλημένου υποστρώματος από την καλλιέργεια του εδώδιμου μανιταριού *Pleurotus ostreatus* παρουσία πενταχλωροφαινόλης οδηγεί στην αποδόμηση του ρυπαντή μέσω μιας καταβολικής διαδικασίας κατά την οποία απομακρύνονται τα χλωριωμένα τμήματα για να ακολουθήσει διάσπαση του δακτυλίου του βενζενίου (Chiu et al. 1998).

P.ostreatus LPB 09 on coffee spent ground

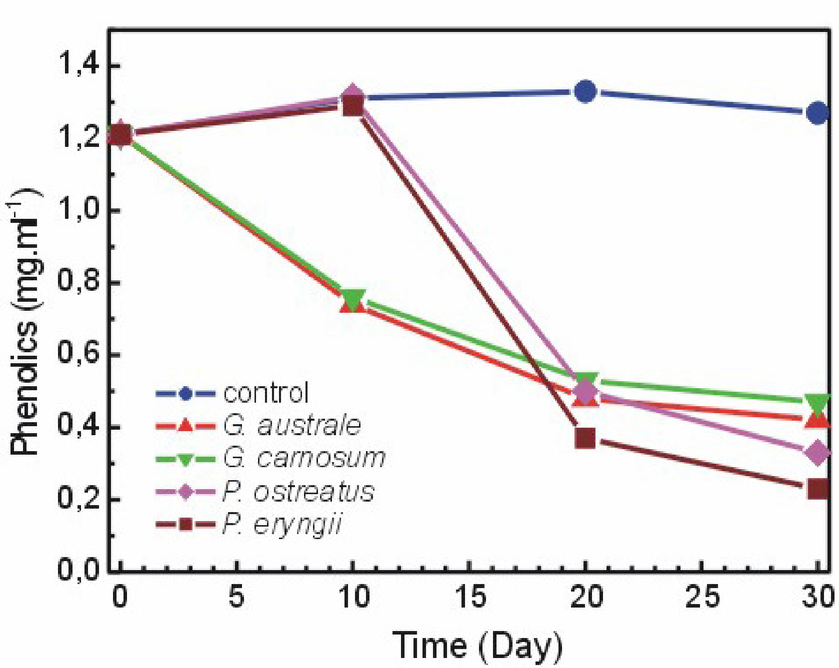


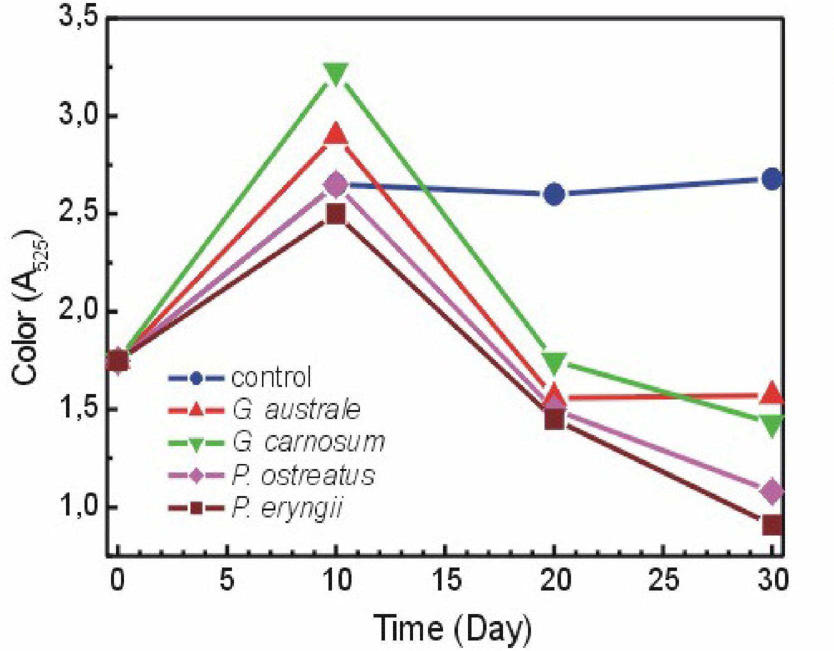


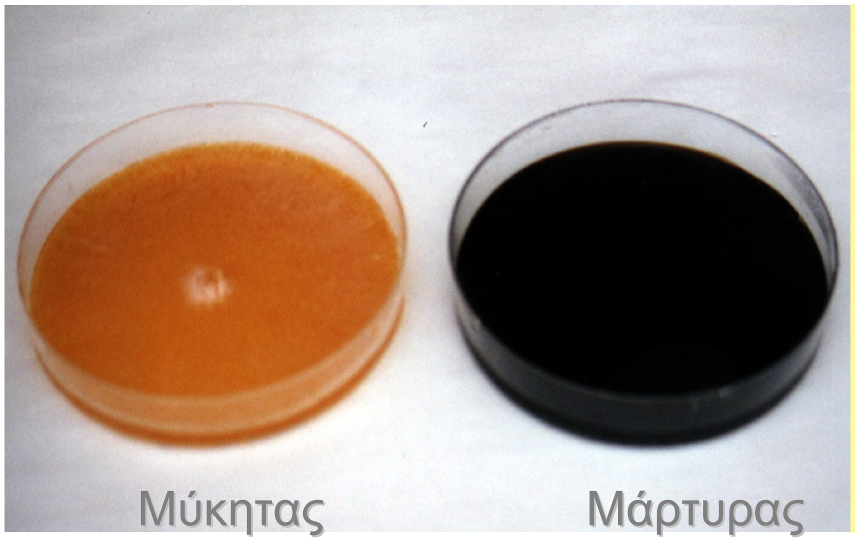
Βασικά χαρακτηριστικά των υγρών αποβλήτων ελαιουργείων (ΥΑΕ)

- Παραγωγή ιδιαίτερα μεγάλων ποσοτήτων (περί τα 10 εκ. κ.μ. ετησίως στις χώρες της Μεσογείου) μέσα σε περιορισμένο χρονικά διάστημα (3-4 μήνες)
- Σκούρο καφέ – μαύρο χρώμα
- Εκπομπή δυσάρεστων οσμών
- Υψηλή περιεκτικότητα σε οργανικό φορτίο (BOD_5: 35-110 g/L, COD: 60-120 g/l)
- Όξινο pH : 3 - 5.5
- Υψηλή περιεκτικότητα σε πολυφαινολικές ενώσεις (έως 80g/L) και σε αιωρούμενα στερεά (ολικά στερεά έως 20g/L)

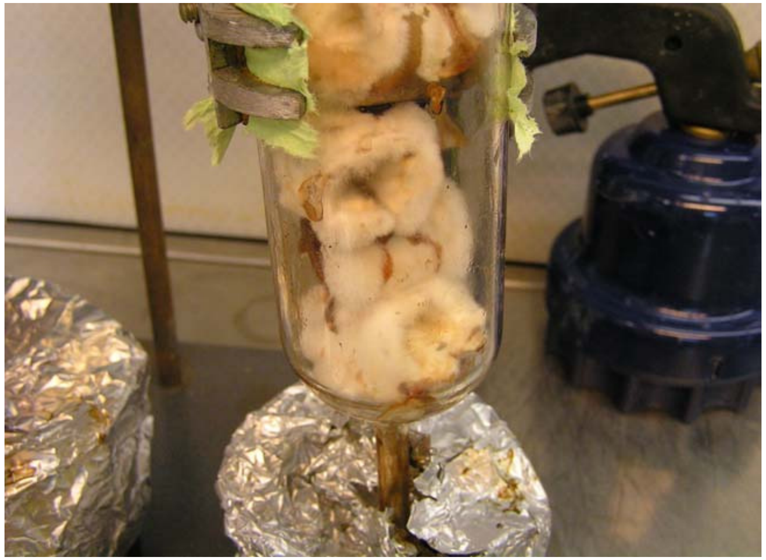
ΠΑΡΑΔΕΙΓΜΑ 2. Αποδόμηση – αποτοξικοποίηση ρυπαντών (ΥΑΕ) με χρήση μακρομυκήτων (Antoniou et al. 2003, Baldrian et al. 2005, Ζερβάκης και συνεργάτες, αδημοσίευτα)

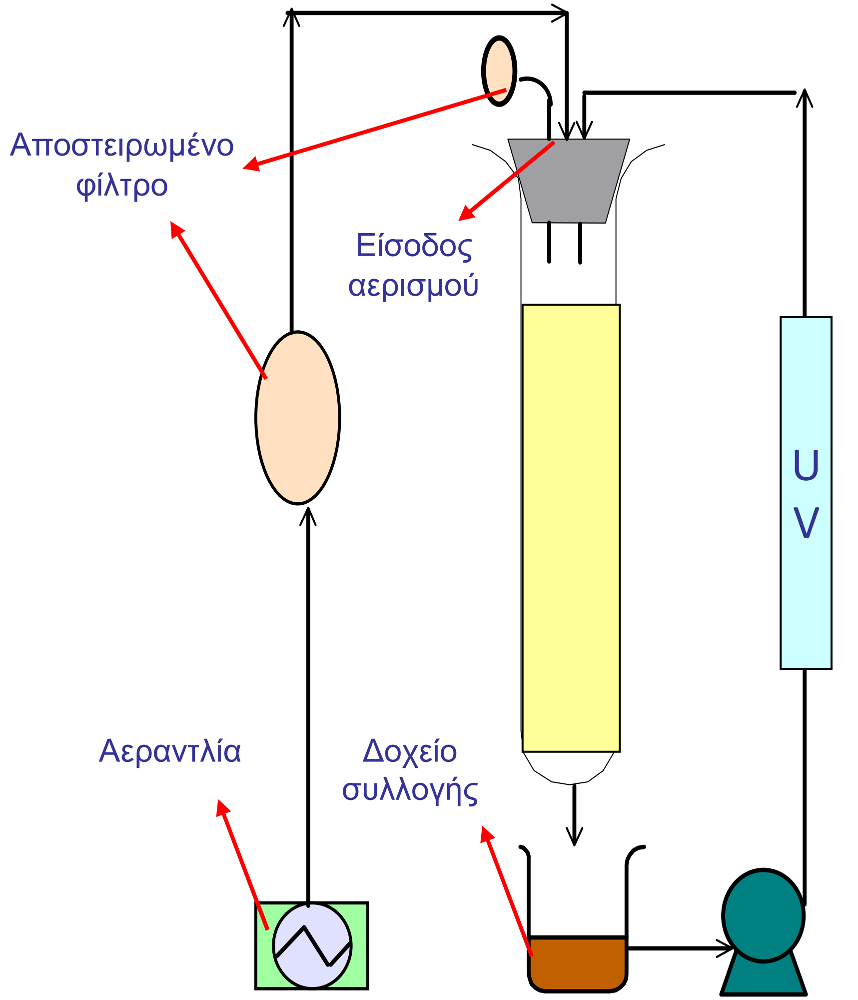




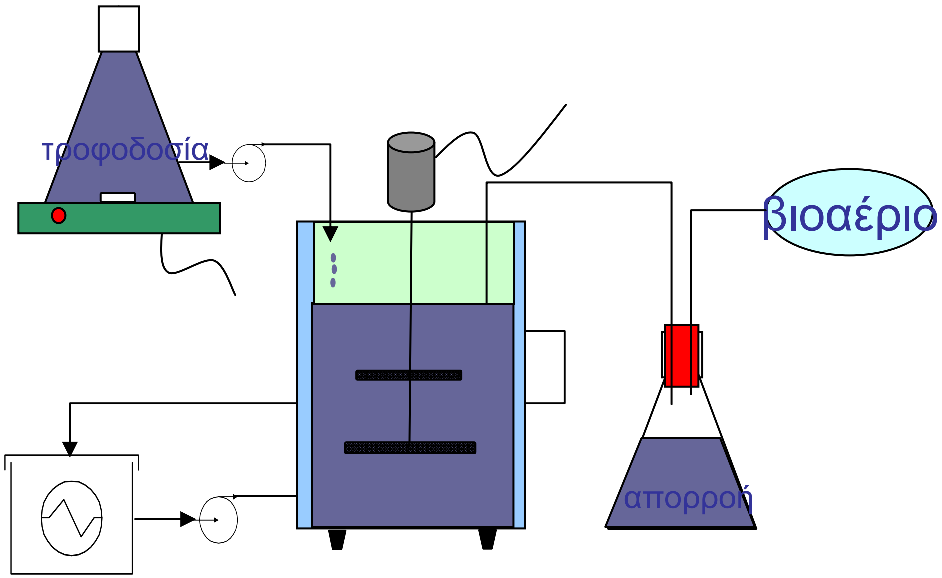


Λειτουργία αντιδραστήρα με ανακυκλοφορία, τύπου διαβρεχόμενης κλίνης (Λυμπεράτος και συνεργάτες)





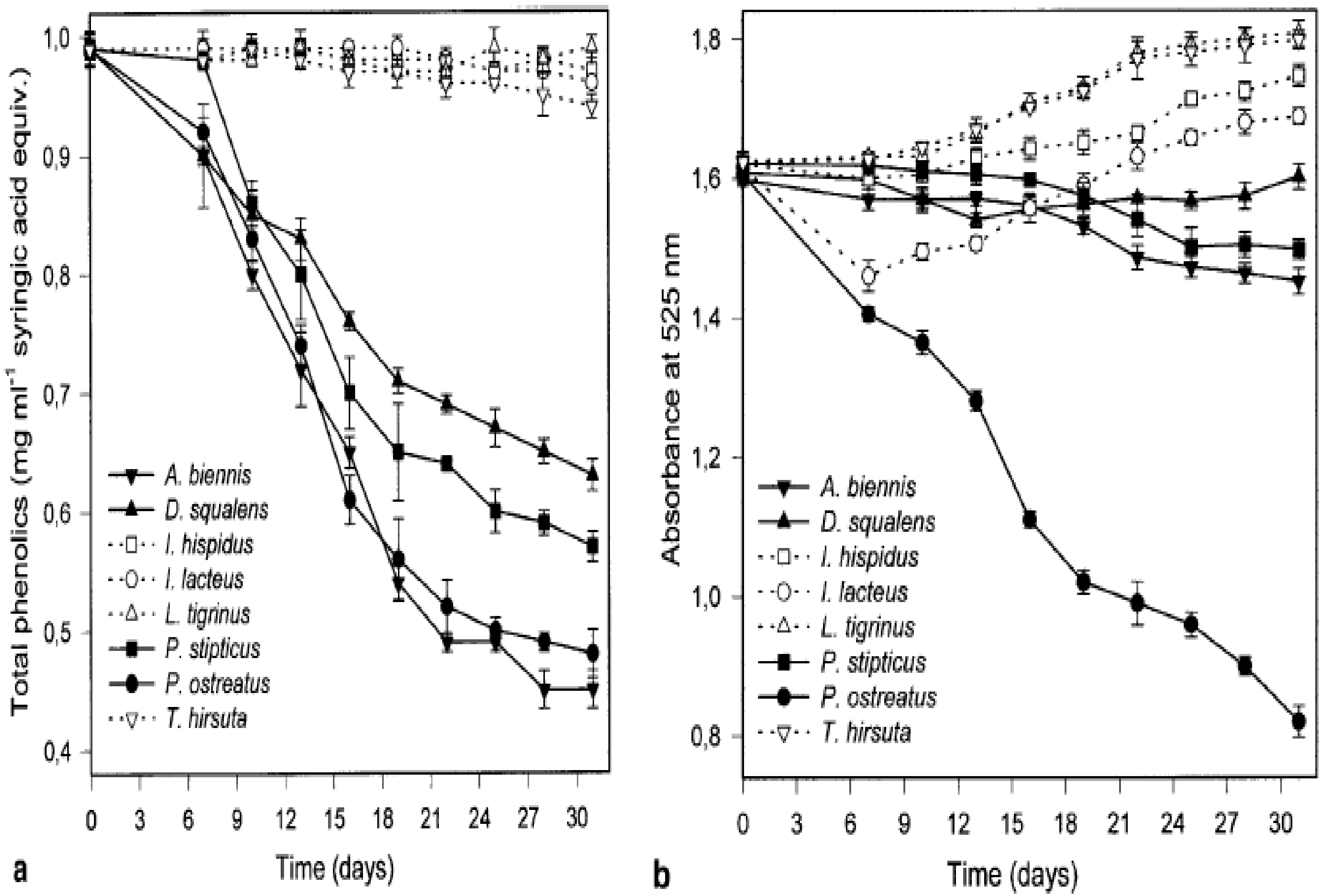
Αναερόβιος αντιδραστήρας (Λυμπεράτος και συνεργάτες)



CSTR - ενεργός όγκος: 3L, θερμοκρασία: 35 C

Η επεξεργασία των ΥΑΕ με τη χρήση μυκήτων λευκής σήψης έχει εφαρμοστεί με ιδιαίτερα ικανοποιητικά αποτελέσματα και μπορεί να αποτελέσει μέθοδο προεπεξεργασίας του αποβλήτου για τη μετέπειτα αναερόβια χώνευσή του με στόχο την παραγωγή βιοαερίου

ΠΑΡΑΔΕΙΓΜΑ 3. Αποδόμηση – αποτοξικοποίηση ρυπαντών (ΑΕΕ) με χρήση μακρομυκήτων (Aggelis et al. 2002)



ΠΑΡΑΔΕΙΓΜΑ 4. Παραγωγή εδώδιμης βιομάζας από απόβλητα ελαιουργείων

Treatment of olive mill wastes (OMWW and two-phase sludge by-product) by *Pleurotus* spp. and *Agrocybe cylindracea*
(Zervakis 2005, Ζερβάκης, αδημοσίευτα στοιχεία)