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Heptamaloxyloglucan

Volume 3 – B.8 (AS)

Rapporteur Member State: France
Co-Rapporteur Member State: Spain

Version History

When	What
2020-09	Initial RAR

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Table of contents

B.8. ENVIRONMENTAL FATE AND BEHAVIOUR

Technical Heptamaloxyloglucan (coded EL101GV) is a branched xyloglucan molecule, part of hemicellulose extracted from apple pomace by enzymatic hydrolysis and deacetylation/reduction after fractioning and purification. Samples are then purified and conditioned by lyophilisation.

Xyloglucan is the principal hemicellulosic component of primary cell walls of dicotyledonous and non-graminaceous monocotyledonous plants. Xyloglucan plays a physiological key role in maintaining cell wall integrity by cross-linking individual cellulose microfibrils in the primary cell wall. Specific oligosaccharides such as heptamaloxyloglucan can be produced naturally from xyloglucan by partial hydrolysis with cellulase (β-1,4-D-glucanase) and various other enzymes which are present in plants and soil micro-organisms. It has been demonstrated that these specific oligosaccharides accumulate extracellularly in plants and act at very low concentrations as signaling molecules that participate in cell-cell and wall-nucleus communication (Fry et al., 1993[1]; Buchanan et al., 2000[2]).

The active substance heptamaloxyloglucan (MW = 1079 g/mol, CAS number [870721-81-6], minimum purity of 78%) is a xyloglucan-derived oligosaccharide made of 7 glycosidic monomer units (polymerisation degree = 7). There are β-1,4 linkages on the main chain between the two D-glucopyranosyl units and terminal D-glucitol, and α-1,2, β-1,2 and α-1,6 linkages between the various monomer units present in side chains. The latter side chain-monomers are D-xylopyranosyl (α-1,6-linked to D-glucopyranosyl), D-galactopyranosyl (β-1,2-linked to D-xylopyranosyl) and L-fucopyranosyl (α-1,2-linked to D-galactopyranosyl) (Table B.8 (AS) - 1).
All these hexose and hexol residues are natural components of the apple and of other dicotyledonous plants, where they are major constituents of cellulose and hemicellulose molecules, which are the principal components of cell walls.

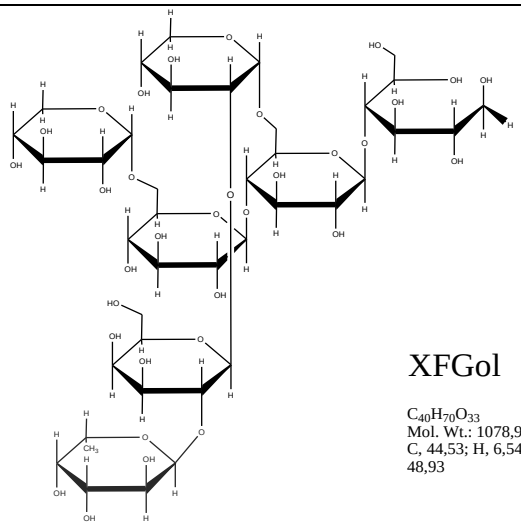
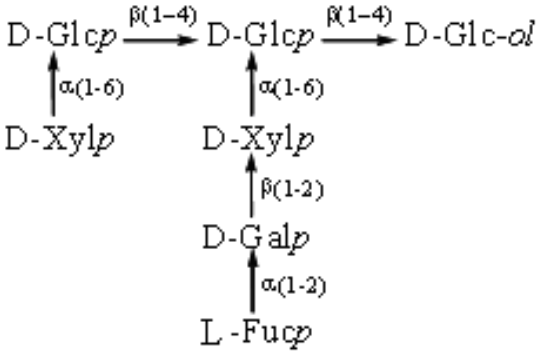
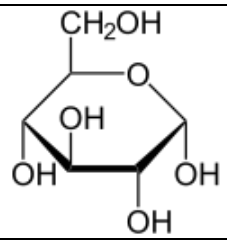
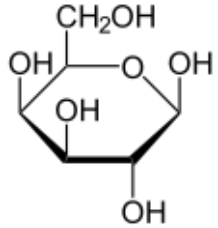
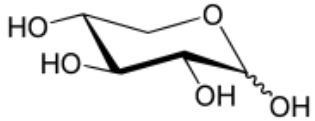
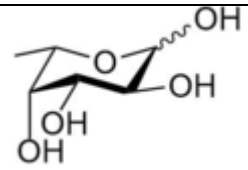
Heptamaloxyloglucan acts as a stimulator of plant defence natural mechanisms (“elicitor”) in order to increase the cold resistance of the grapevine.
As heptamaloxyloglucan occurs in plants and soil at very low levels, the manufacturing process mimics natural phenomenon in order to accelerate the rate of natural biochemical processes and thus increase heptamaloxyloglucan yields.

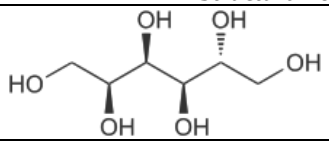
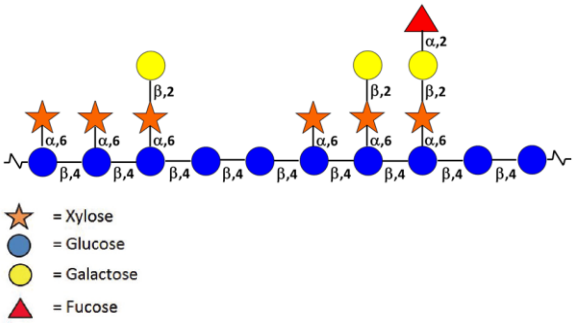
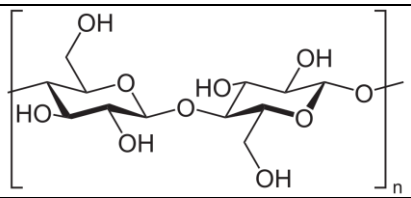
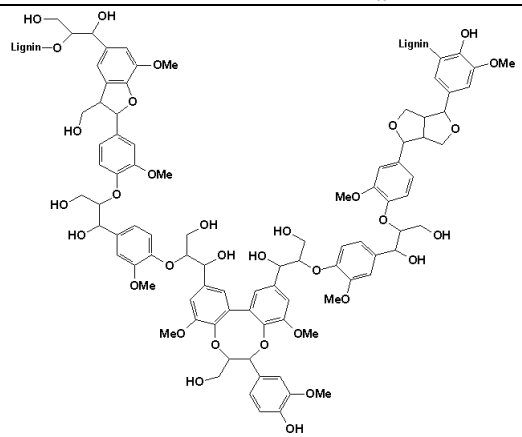
The representative formulation (PEL101GV) is equivalent to the technical active substance (EL101GV).

A summary of the structures of heptamaloxyloglucan, its monomer units and other carbohydrates mentioned in the literature studies reported in this section is presented in the following table.

[1] Fry. S.C., Aldington S., Hetherington P.R., Aitken J., 1993. “Oligosaccharides as Signals and Substrates in the Plant Cell Wall“.Plant Physiol., Vol. 103 (1993), pp. 1-5.
[2] Buchanan B.B. et al, 2000. “Chapter 2: The Cell Wall. Biochemistry and molecular Biology of Plants”. B. Buchanan, W. Gruissem, R. Jones, Eds. 2000, pp 52-89.

Table B.8 (AS) - 1: Comparison of sugar compounds and heptamaloxyloglucan

Name	Structural formula	Comments
Heptamaloxylogulcan	 XFGol $C_{40}H_{70}O_{33}$ Mol. Wt.: 1078,96 C, 44,53; H, 6,54; O, 48,93 	Following the IUPAC nomenclature, heptamaloxyloglucan is part of the oligosaccharides within the carbohydrates family. The IUPAC name of heptamaloxyloglucan is: *{[α-D-Xyl p-*(1→6)]-*β-D-Glc p-*(1→4)}{[*α-L- Fuc p-*(1→2)-*β-D-Gal p-*(1→2)-*α-D-Xyl p-*(1→6)]-*β-D-Glc p-*(1→4)}-*D-Glc-ol* Xyl p: xylopyranosyl Glc p: glucopyranosyl Fuc p: fucopyranosyl Gal p: galactopyranosyl Glc-ol: glucitol
Hexose	-	a hexose is a monosaccharide with six carbon atoms
Glucose (glucopyranose)		Most abundant carbohydrate Present in heptamaloxyloglucan
Galactose (galactopyranose)		Present in heptamaloxyloglucan
Xylose (xylopyranose)		Present in heptamaloxyloglucan
Fucose (fucopyranose)		Present in heptamaloxyloglucan

Name	Structural formula	Comments
Glucitol (sorbitol)		Present in heptamaloxyloglucan
Hemicellulose	-	Hemicelluloses are a heterogeneous group of branched polysaccharides composed of a linear backbone of a β-1,4-linked homopolymer of a sugar (e.g., glucose), from which short side chains of other sugars (e.g., xylose, galactose, fucose) protrude.[3]
Xyloglucan		Xyloglucan is a generic name of linear polysaccharides consisting of (β1→4)-linked d-glucan substituted with xylose, and generally can be found in plant cell walls.[4] Xyloses can be substituted with galactose and fucose.
Cellulose		Cellulose is a polysaccharide consisting of a linear chain of several hundred to many thousands of β(1→4) linked D-glucose units.
Lignin		Lignin is a complex biopolymer composed of different amounts of three monolignols, namely p-coumarylalcohol, coniferyl alcohol, and sinapyl alcohol.[5]
Xylan	-	Xylans are a diverse group of polysaccharides belonging to the hemicellulose group of the cell-wall biopolymers of vegetables with the common feature of a β-(1→4)-linked L-xylose backbone.[6]

[3] Plant Cell Biology, From Astronomy to Zoology, Randy Wayne, 2010, Pages 339-356
[4] Comprehensive Glycoscience, From Chemistry to Systems Biology, Volume 2, K. Nishinari, M. Takemasa, H. Zhang, R. Takahashi, 2007, Pages 613-652
[5] Lignin in Polymer Composites, 2016, Pages 195-206
[6] Nutraceutical and Functional Food Components, Effects of Innovative Processing Techniques, 2017, Pages 39-101

B.8.1. FATE AND BEHAVIOUR IN SOIL

B.8.1.1. Route and rate of degradation in soil

No specific study on the rate and route of degradation was submitted. Instead, the applicant provided studies from literature to show that heptamaloxyloglucan, as other polysaccharides, could be degraded under natural conditions in soil.
The literature data was considered acceptable when it was possible to derive useful information (even general or qualitative) on how xyloglucans, hemicellulose or carbohydrates could be degraded, assimilated or used by the different organisms. Summaries of these publications are reported below. A general conclusion on the route of degradation is also provided in point B.8.1.1.1.5.

Data point	CA 7.1.1.1/01
Report author	Warren R.A.J.
Report year	1996
Report title	Microbial hydrolysis of polysaccharides
Journal	Annu. Rev. Microbiol. 1996. 50:183–212
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

This review refers to the systems of enzymes produced by microorganisms for the hydrolysis of polysaccharides to metabolisable products.

The naturally occurring substrates are insoluble and microorganisms utilising them must use extracellular enzymes, free or associated with the cell surface, to convert the polysaccharides to soluble products that are transportable into the cells.

Microorganisms efficiently degrade starch, chitin and polysaccharides in plant cell walls. The diversity of enzymes involved in cellulose hydrolysis must be a consequence of the different sugars and linkages present in plant cell walls. Among polysaccharides, cellulose is more tightly associated with plant cell wall than mannans or xylans. Plant cell walls comprise also minor components such as xyloglucans, galactomannans, pectins, and some glucans.

The microbial degradation of polysaccharides entails diverse glycoside hydrolases with different specificities and modes of action. The enzyme systems involved are complex as many of the individual enzymes are modular proteins comprising one or more catalytic domains linked to ancillary domains that often include one or more substrate-binding domains, and as the systems comprise from a few to 20 or more enzymes, all of which hydrolyze a particular substrate. Systems for the hydrolysis of plant cell walls usually contain more components than systems for the hydrolysis of starch and chitin because the cell walls contain several polysaccharides. As organisms that degrade cellulose usually degrade hemicelluloses also, cellulases and hemicellulases are considered as components of systems for the hydrolysis of plant cell walls. The bacteria *Cellulomonas fimi* and *Thermomonospora fusca* (a mesophilic aerobic soil bacterium and a thermophilic actinomycete common in compost, respectively) for example have similar plant cell wall hydrolysing systems.

Microorganisms degrading polysaccharides produce multi-component enzyme systems of varying degrees of complexity (depending on the substrate). These systems have a number of characteristics in common. Plant cell wall hydrolysis requires enzymes hydrolyzing β–1,4 and β–1,3 glycosidic bonds; β–1,4 xylosidic bonds and – β-1,4 mannosidic bonds; and perhaps others. Two basic types of systems are involved in the hydrolysis of plant cell walls: complexed systems like the cellulosome and non-associated systems like those in aerobic

microorganisms. Both types of system contain multiple endoglucanases and multiple xylanases. Cell wall hydrolyzing systems appear to be more complex than those hydrolyzing starch and chitin.

The hydrolysis of plant cell walls is a relatively slow process. This is compensated by the high efficiency of the enzymes involved in hydrolysis of glycosidic bonds.

Among β-1,4 glucanases, the most striking accessory domains are the dockerins of the catalytic components of the multienzyme complexes, or cellulosomes, produced by some anaerobic cellulolytic microorganisms, especially certain *Clostridia* species.

Comments (RMS)

This study provides supporting information on the microbial hydrolysis of polysaccharides such as xyloglucans by soil microorganisms. This supports that heptamaloxyloglucan is degraded by microorganisms in soil.

Data point	CA 7.1.1.1/02
Report author	De Vries R. & Visser J.
Report year	2001
Report title	Aspergillus enzymes involved in degradation of plant cell wall polysaccharides
Journal	Microbiology and Molecular Biology Reviews, Vol. 65, No. 4, p. 497-522
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

This review summarises current knowledge on the different classes of enzymes involved in plant cell wall polysaccharide degradation produced by *Aspergilli*, the genes encoding these enzymes, and the regulation of these genes. It also provides some general information on the role of xyloglucans and enzymes involved in their degradation.

Plant cell wall polysaccharides are the most abundant organic compounds found in the environment. They make up 90% of the plant cell wall and can be divided into three groups: cellulose, hemicellulose, and pectin. Cellulose represents the major constituent of cell wall polysaccharides and consists of a linear polymer of β-1,4-linked D-glucose residues. The cellulose polymers are present as ordered structures (fibers), and their main function is to ensure the rigidity of the plant cell wall. Hemicelluloses are more heterogeneous polysaccharides and are the second most abundant organic structure in the plant cell wall.
Xyloglucans are present in the cell walls of dicotyledonae and some monocotylodonae (e.g., onion). Xyloglucans consist of a β-1,4- linked D-glucose backbone substituted by D-xylose. L-Arabinose and D-galactose residues can be attached to the xylose residues, and L-fucose has been detected attached to galactose residues in xyloglucan. Xyloglucans interact with cellulose microfibrils by the formation of hydrogen bonds, thus contributing to the structural integrity of the cellulose network.
Four classes of enzymes are involved in the biodegradation of cellulose: endoglucanases hydrolyze cellulose to glucooligosaccharides; cellobiohydrolases release cellobiose from crystalline cellulose; β-Glucosidases degrade the oligosaccharides to glucose and exoglucanases release glucose from cellulose and glucooligosaccharides. All four classes of enzymes have been identified in *Aspergilli*. Endoglucanases and β-glucosidases are also able to degrade the backbone of xyloglucan. Some accessory enzymes act on the substituents or the side chains of the plant cell wall component structures. Some of these enzymes act on linkages between a main-chain residue and a substituent, whereas other enzymes cleave internal or terminal linkages of side chains.
Aspergilli produce different classes of these accessory enzymes that act on plant cell wall polysaccharides. For example, α-D-xylosidases can release α-linked xylose residues from xyloglucan. The removal of D-galactose residues from plant cell wall polysaccharides requires the action of α-galactosidases and β-galactosidases.

Comments (RMS)

Part of this review is related to the enzymatic degradation of plant cell wall polysaccharides (such as xyloglucans) by enzymes produced by *Aspergilli*, a group of filamentous fungi with a large number of species. This supports that heptamaloxyloglucan is degraded by microorganisms in soil.

Data point	CA 7.1.1.1/03
Report author	Wershaw R.
Report year	2004
Report title	Evaluation of conceptual models of natural organic matter (humus) from a consideration of the chemical and biochemical processess of humification
Journal	Scientific investigations report 2004-5121, US Geological Survey, Reston (Virginia)
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

In this report, the degradation pathways of the components of plant tissue and the interactions of the resulting degradation products in soil and natural water are discussed in order to develop a compartmental model of natural organic matter.

Natural organic matter is depicted as being composed of molecular aggregates (supramolecular aggregates) of plant degradation products held together by non-covalent bonds. The most commonly used name of natural organic matter fractions are: humus, humic substances, humic acid, fulvic acid, and humin.

Biodegradation of primary metabolites is carried out both by the organisms that originally produced the metabolites and by exogenous organisms. For example, carbohydrates and proteins that are produced in plant leaves are degraded during senescence by endogenous enzyme systems to monomeric species that can be stored for use in the following growing season. In addition, heterotrophic, exogenous microorganisms are dependent on primary metabolites produced by plants in their metabolic processes. The enzyme systems that catalyse these endogenous and exogenous reactions are often very similar. The biodegradation of primary metabolite polymers generally involves hydrolytic depolymerization reactions that ultimately produce monomeric species. As these reactions proceed, the average size of the molecules continually decreases until only monomers are left. Primary metabolites also undergo oxidative degradation during cell respiration to produce energy (catabolism). Organic acids produced during catabolism ultimately are utilised in one of the cell respiration cycles such as the citric acid cycle. Size reduction also occurs during oxidative degradation.

The hemicellulose components are mainly composed of β-1-4-linked pentoses and hexoses; however, some β-1-3-gycosidic bonds may also be present. Some branching of the chains is present in most of the hemicellulose components. The monomeric units of hemicelluloses are: D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methylglucuronic acid, D-galacturonic acid, and D-glucuronic acid.
Cellulose is degraded aerobically by eubacteria and fungi to carbon dioxide and water, and is degraded anaerobically by protozoa and slime molds to methane and water. These organisms secrete a variety of enzymes that attack cellulose in different ways.
The hydrolytic degradation of hemicelluloses by hemicellulases produces monomeric saccharides and acetic acid. Enzymes specific for the different saccharides and bonds in a given type of hemicellulose are required for the degradation of the particular hemicellulose. Four different enzymes are required to degrade the common hemicellulose O-acetyl-4-O-methylglucuronxylan: endo-1-4-β-xylanase, acetyl esterase, α-glucuronidase, and β-xylosidase.

Comments (RMS)

This study provides supporting information on the biodegradation of plant polymers such as cellulose and hemicellulose. This supports that heptamaloxyloglucan is degraded by microorganisms in soil.

Data point	CA 7.1.1.1/04
Report author	Karroum M. et al.
Report year	2004
Report title	Importance et devenir des biopolymères (lignines et polysaccharides) dans les sols d'une chronoséquence de hêtraies (Fagus sylvatica) en forêt de Fougères (France) / Importance and fate of biopolymers (lignins and polysaccharides) in soils of Fagus sylvatica stands of various ages in Fougères forest (Britany - France)
Journal	Ann. For. Sci 61 (2004) 211-233
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

The humification process rests on the transformation of initial components of microbial or phyto-inherited organic matter comprising mainly lignin, polysaccharides (pectins, hemicelluloses and cellulose) and polypeptids. The aim of this study was to precise the route of transformation of lignins and polysaccharides, which are major biopolymers reaching the soil via plants falls. In soil, these polymers are transformed and degraded according to different rates due to the diversity of humus type and depending on the stability of the associations formed with other components such as polyphenols, oligopeptids and amino acids. The inventory and quantification of polysaccharides by gaz chromatography was based on an acid hydrolysis technic in 2 steps, which allows distinguishing the structural entities (cellulose and hemicelluloses) that are destroyed and the microbial exo-polysaccharides produced by the microflora.

In this study, four beech stands of various ages were selected in 1997 to study the evolution of lignins and structural polysaccharides (cellulose, hemicelluloses) in the soil cover. The 10-year-old station has a mull type humus. In the 27-year-old stand there is a mull-moder mosaic whereas in the 87 and 145-year-old stands humus is a moder. Twenty-one soil profiles were sampled by separating the different humus layers, *i.e.*, OL and OF, present in mull and moder, and the OH layer, only present in the moder. Organo-mineral A11 and A12 horizons were also sampled with some A13 horizons in the mull stations.
Lignins are abruptly degraded in mull where they represent 52‰ of the total organic carbon (TOC) in OL and only 12‰ in A1 horizons. Similarly, polysaccharides undergo degradation from 236‰ (OL) to 105‰ (A1) of TOC. The fast decrease in the concentration of these components over a small depth interval is indicative of a strong biological activity. The lignin alteration is evidenced by the decrease of the ratio of syringic compounds over vanillic compounds that reveal methoxyl group losses and the increase of vanillic acid over vanillic aldehyde which indicates oxidative depolymerization of lignins.
Regardless of the age of the plot, the OL, OF and OH organic layer present a high level of total sugar with a prevalence of hemicellulosic sugars over cellulose sugars. The structural polysaccharides (cellulose and hemicelluloses) of ligno-cellulosic material of plants undergo a degradation depending on the depth. However, at the same time, there is a neosynthesis of a new microbial polysaccharides phase dominated by glucose, mannose and galactose in *Fagus sylvatica* stands. All these polysaccharides which are unstable and soluble in the surface layer disappear mainly in the organo-mineral major horizons.
In old stands (87, 145-y.) where humus was of a uniform moder type, the decrease of the xylose/mannose ratio in the OF and OH layers reveals the production of microbial sugars at the expense of the phyto-inherited polysaccharides, like cellulose and hemicelluloses which decrease, whereas lignins are strongly degraded. The decrease of the structural polysaccharides continues in the underlying A1 horizon, similar to the evolution observed in the transition from mull to moder although at a slower degradation rate.

Comments (RMS)

This study provides supporting information on humification and fate of hemicellulose under forest conditions. This supports that heptamaloxyloglucan is degraded by microorganisms in soil.

Data point	CA 7.1.1.1/05
Report author	Pérez J. & Muñoz-Dorado J.
Report year	2002
Report title	Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview
Journal	Int Microbiol (2002) 5: 53-63
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

The aim of this review is to provide an overview of the degradation of cellulose, hemicellulose, and lignin and the enzymatic systems involved, with first a brief description of the structure of the cell wall and its components.

In the environment, lignocellulose derives from wood, grass, agricultural residues, forestry wastes and municipal solid wastes. It is the major component of biomass. It consists of three types of polymers: cellulose, hemicellulose, and lignin. These polymers are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkages. A great variety of fungi and bacteria can fragment these macromolecules by using a battery of hydrolytic or oxidative enzymes.

The interactions between cellulotic and non-cellulotic microorganism populations (synergistic relationship) lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic conditions, and carbon dioxide, methane and water under anaerobic conditions. Microorganisms capable of degrading cellulose produce a battery of enzymes with different specificities, working together. Cellulases hydrolyze the β-1,4-glycosidic linkages of cellulose. Traditionally, they are divided into two classes referred to as endoglucanases and cellobiohydrolases.
Endoglucanases (endo-1,4-β-glucanases) can hydrolyze internal bonds (preferably in cellulose amorphous regions) releasing new terminal ends. Cellobiohydrolases (exo-1,4-β-glucanases) act on the existing or endoglucanase-generated chain ends. An effective hydrolysis of cellulose also requires β-glucosidases, which break down cellobiose releasing two glucose molecules. Products of cellulose hydrolysis are available as carbon and energy sources for cellulolytic microorganisms or other microbes living in the environment where cellulose is being degraded.

Hemicelluloses are biodegraded to monomeric sugars and acetic acid. Hemicellulases are frequently classified according to their action on distinct substrates. Xylan is the main carbohydrate found in hemicellulose. Its complete degradation requires the cooperative action of a variety of hydrolytic enzymes. An important distinction should be made between endo-1,4-β-xylanase and xylan 1,4-β-xylosidase. The former generates oligosaccharides from the cleavage of xylan; the latter works on xylan oligosaccharides, producing xylose. In addition, hemicellulose biodegradation needs accessory enzymes such as xylan esterases, ferulic and p-coumaric esterases, α-l-arabinofuranosidases, and α-4-O-methyl glucuronosidases acting synergistically to efficiently hydrolyze wood xylans and mannans.
In the case of O-acetyl-4-O-methylglucuronxylan, one of the most common hemicelluloses, four different enzymes are required for degradation: endo-1,4-β-xylanase (endoxylanase), acetyl esterase, α-glucuronidase and β-xylosidase.

In conclusion, cellulose, lignocellulose and lignin, are major sources of plant biomass in the environment; therefore, their recycling is indispensable for the carbon cycle. Each polymer is degraded by a variety of microorganisms (such as *Trichoderma*, *Pseudomanas*, *Streptomyces*, *Thermonospora* [...] naturally occurring in soil) which produce a battery of enzymes that work synergically.

Comments (RMS)

Although the section describing the degradation of hemicellulose is dedicated to the degradation of xylan only, it is regarded as complementary information on the degradation of molecules that are similar to xyloglucans. This supports that heptamaloxyloglucan is degraded by microorganisms in soil.

Data point	CA 7.1.1.1/06
Report author	Aro N. et al.
Report year	2005
Report title	Transcriptional regulation of plant cell wall degradation by filamentous fungi
Journal	FEMS Microbiology Reviews 29 (2005) 719-739
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

This review summarises current knowledge on transcriptional regulation of genes coding for enzymes involved in the breakdown of plant cell wall biopolymers. It provides general information on plant cell wall composition and hydrolysis.
Plant cell wall consists mainly of the large biopolymers cellulose, hemicellulose, lignin and pectin. These biopolymers are degraded by many microorganisms, in particular filamentous fungi (such as the brown-rot fungi *Trichoderma reesei*, *Aspergillus niger* and *Penicillium* and the ligninolytic white-rot fungus *Phanerochaete*), with the aid of extracellular enzymes acting synergistically. Filamentous fungi have a key role in degradation of the most abundant biopolymers found in the environment, cellulose and hemicelluloses, and therefore are essential for the maintenance of the global carbon cycle. The production of plant cell wall degrading enzymes, cellulases, hemicellulases, ligninases and pectinases, is regulated mainly at the transcriptional level in filamentous fungi.
Hemicelluloses are the second most abundant polysaccharide in the environment, and have a heterogeneous composition of various sugar units. Their hydrolysis occurs by the concerted action of endo-enzymes cleaving internally the main chain, exo-enzymes liberating monomeric sugars and ancillary enzymes cleaving the side chains of the polymers or oligosaccharides leading to the release of various mono- and disaccharides depending on the hemicellulose type. From the *Aspergillus* species nearly 20 genes encoding endoxylanases and β-xylosidases have been cloned. Xylanase genes have been cloned from a number of other fungi as well, e.g. *Penicillium*, *A. bisporus*, and the rice blast fungus *Magnaporthe grisea*. Numerous other genes encoding hemicellulose backbone and side chain cleaving enzymes have been characterised, most from the species of *Aspergillus*. Hemicelluloses are chemically highly variable and their hydrolysis leads to the formation of a variety of pentose and hexose sugars and acids.

Comments (RMS)

This review provides supporting information on the biodegradation of hemicellulose by fungi. This supports that heptamaloxyloglucan is degraded by microorganisms in soil.

Data point	CA 7.1.1.1/07
Report author	Tuomela M. et al.
Report year	2000
Report title	Biodegradation of lignin in a compost environment: a review
Journal	Bioressource Technology 72 (2000) 169-183
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

This literature survey reviews knowledge on biodegradability and compostability of lignocellulosic materials and briefly the microbial activity during composting. The main emphasis is made on thermophilic fungi because of their occurrence in compost and other fungi such as white-rot fungi as it is the most important group of lignin biodegraders in the environment.

Composting is nowadays a general treatment method for municipal solid waste. Organic material is converted to carbon dioxide, humus and heat by compost microorganisms (such as *Bacillus*, *Aspergillus*, *Pseudomonas*...). It is assumed that humus is formed mainly from lignin. Thus, lignin is not totally mineralized during composting. The elevated temperatures found during the thermophilic phase are essential for rapid degradation of lignocellulose. After the easily degradable carbon sources have been consumed, more resistant compounds such as cellulose, hemicellulose and lignin are degraded and partly transformed into humus. Humus is the end product of the humification process, in which compounds of natural origin are partially transformed into relatively inert humic substances. Complex organic compounds like lignin are mainly degraded by thermophilic microfungi and actinomycetes.
In the environment, lignocellulose accounts for the major part of biomass and, consequently, its degradation is essential for the operation of the global carbon cycle (Figure B.8 (AS) - 1).

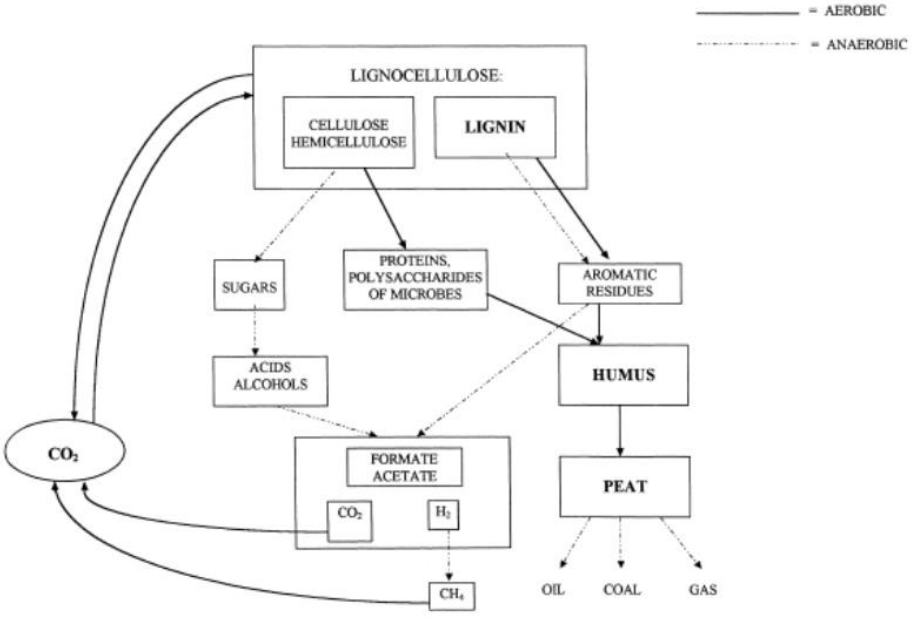


Figure B.8 (AS) - 1: Global carbon cycle (Brown, 1985; Colberg, 1988)

In mushroom compost, thermophilic fungi are responsible for the degradation of lignocellulose, which is a prerequisite for the growth of the edible fungus. However, most of them are known to be able to degrade wood or other lignocellulose, cellulose or hemicelluloses. The ability of fungi to hydrolyse hemicelluloses is probably more common than cellulose hydrolyzation.

Comments (RMS)

This literature survey provides qualitative information on degradation of lignin in compost by microorganisms that naturally occurred in soil or plant litter. Hemicellulose is also degraded during the composting process. This supports that heptamaloxyloglucan is degraded by microorganisms in soil.

Data point	CA 7.1.1.1/08
Report author	Kelker N.E. & Anderson R.L.
Report year	1971
Report title	Sorbitol metabolism in Aerobacter aerogenes
Journal	Journal of Bacteriology, Vol. 105, No. 1, p 160-164
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

In this study, sorbitol (D-glucitol) metabolism in *Aerobacter aerogenes* PRL-R3 is shown to proceed via the pathway: sorbitol → sorbitol 6-phosphate → D-fructose 6-phosphate.
Sorbitol phosphorylation is mediated by a phosphoenolpyruvate (PEP): sorbitol 6-phosphotransferase system, and sorbitol 6-phosphate oxidation by a pyridinenucleotide- linked dehydrogenase. Mutants deficient in sorbitol 6-phosphate dehydrogenase or a component (enzyme I) of the phosphotransferase system did not grow on sorbitol, whereas revertants, which had regained these enzymatic activities, grew normally.
The pathway of sorbitol metabolism in *A. aerogenes* PRL-R3 thus is distinct from the pathway(s) occurring in *Acetobacter suboxydans*, *Pseudomonas fluorescens*, *Celivibrio polyoltrophicus*, *Bacillus subtilis*, and *Rhizobium meliloti*, in which sorbitol is oxidized to D-fructose or L-sorbose.

Comments (RMS)

This study provides information on possible degradation of glucitol (D-glucitol is one of the monomer units of heptamaloxyloglucan) by *Aerobacter aerogenes* which could be found in soil. This supports that heptamaloxyloglucan and its monomers are degraded by microorganisms in soil.

Data point	CA 7.1.1.1/09
Report author	Brechtel E. et al.
Report year	2002
Report title	L-Glucitol catabolism in Stenotrophomonas maltophilia Ac
Journal	Applied and environmental microbiology, Vol. 68, No. 2, p582-587
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

The purpose of this study is to characterize a new pathway through which *Stenotrophomonas maltophilia* is capable of utilizing rare and unnatural carbohydrate substrates.

Stenotrophomonas maltophilia Ac (previously named *Pseudomonas* sp. strain Ac, or also *Xanthomanas maltophilia*) was isolated from soil using a mineral medium and the unnatural polyol L-glucitol as the selective carbon source. This bacterium is known to convert the unnatural polyol L-glucitol to D-sorbose during growth on the former as the sole source of carbon and energy. All enzymes operating in a pathway that channels L-glucitol via D-sorbose into compounds of the intermediary metabolism were demonstrated, and for some prominent reactions the products of conversion were identified. D-Sorbose was converted by C-3 epimerization to D-tagatose, which, in turn, was isomerized to D-galactose. D-Galactose was the initial substrate of the De Ley-Doudoroff pathway, involving reactions of NAD-dependent oxidation of D-galactose to D-galactonate, its dehydration to 2-keto-3-deoxy-D-galactonate, and its phosphorylation to 2-keto-3-deoxy-D-galactonate 6-phosphate. Finally, aldol cleavage yielded pyruvate and D-glycerate 3-phosphate as the central metabolic intermediates.

Comments (RMS)

This study provides information on possible degradation of D-galactose (one of the monomer units of heptamaloxyloglucan). This supports that heptamaloxyloglucan and its monomers are degraded by microorganisms in soil.

Data point	CA 7.1.1.1/10
Report author	Galloway A. et al.
Report year	2018
Report title	Xyloglucan is released by plants and promotes soil particle aggregation
Journal	New Phytologist (2018) 217: 1128–1136 - DOI: 10.1111/nph.14897
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

This study examined the potential role of xyloglucan in the formation of soils. Xyloglucan released from a range of plants (including angiosperms: wheat, maize, barley, pea, tomato, rapeseed, Arabidopsis thaliana and bryophytes: Marchantia polymorpha L. and Blasia pusilla L., Lunularia cruciata, Physcomitrella patens) has been quantified using monoclonal antibody detecting xyloglucan and enzyme-linked immunosorbent assays (ELISA) system. The influence of xyloglucan on soil particle aggregation has been analyzed by scanning electron microscopy.

Findings

Significant secretions of xyloglucan were detected in all plants involved in the study, angiosperms and bryophytes, with higher secretions for wheat grown hydroponically, and Lunalaria and Marchantia grown on agar. It was found that wheat grown hydroponically for 14 d released 20 µg xyloglucan/g FW (fresh weight) and that M. polymorpha gemma cultured for 28 d on solid agar media released >50 µg xyloglucan/g FW.
Xyloglucan is found to be effective in promoting the aggregation of soil particles. A significant increase in aggregate volume > 2000 μm^3 is observed in presence of xyloglucan (0.1% (w/w)).
Xyloglucan has been extracted from pasture soil and glacial forefield soils and was detected at maximum concentrations of 0.8 g/kg soil and >20mg/kg soil respectively.

Conclusion

This study shows that xyloglucan is released by a wide range of plants. This polysaccharide can be extracted in significant quantities from soils (grassland and glacial forefield soils in this study). Finally, this work supports that xyloglucan is an effective promoter of soil aggregation and formation.

Comments (RMS)

This study provides useful information on the natural occurrence of xyloglucan in soils.

Data point	CA 7.1.1.1/11
Report author	Valinhas et al.
Report year	2018
Report title	Xylose fermentation to ethanol by new Galactomyces geotrichum and Candida akabanensis strains.
Journal	PeerJ. 2018 Apr 27;6:e4673. doi: 10.7717/peerj.4673. eCollection 2018.
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

This study examined the performance of naturally occurring yeast strains to produce ethanol from xylose. The fungi were isolated from fruits and roots and selected for their respective ability to use xylose as sole carbon source. The selected strains were morphologically and biochemically characterized, and genetically identified by homology analysis of 5.8S and 26S ribosomal RNA gene sequences. Assays of alcoholic fermentation enabled the evaluation of their performance to produce ethanol.

Findings

Three strains (over 202) were selected on the ability to use xylose a sole source of carbon to produce gas. These strains were capable to convert xylose into ethanol. Ethanol yield as a function of substrate consumption ($Y_{P/S}$, g/g) values for the production of ethanol ranging between 0.29 and 0.35 g/g were observed under non-optimized conditions. Two of the strains were identified as belonging to Galactomyces geotrichum species. Another strain was identified as Candida akabanensis.

Conclusion

Several yeast species have already been identified as being capable of converting xylose to ethanol, including Kluyveromyces cellobiovorus, Pachysolen tannophilus, Spathaspora passalidarum, Spathaspora arborariae, Scheffersomyces shehatae and Scheffersomyces stipitis. In this study three new fungi strains with this metabolic feature have been identified. Two of the strains were identified as belonging to Galactomyces geotrichum species. Another strain was identified as Candida akabanensis.

Comments (RMS)

This study provides supportive information on possible degradation of xylose (one of the monomer units of heptamaloxyloglucan) by naturally occurring yeast strains to produce ethanol. This supports that heptamaloxyloglucan and its monomers are degraded by microorganisms in soil.

Data point	CA 7.1.1.1/12
Report author	Veras H.C. et al.
Report year	2017
Report title	Comparative assessment of fermentative capacity of different xylose-consuming yeasts.
Journal	Microb Cell Fact (2017) 16:153 - DOI 10.1186/s12934-017-0766-x
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

In this work, xylose fermentative capacity of four naturally-xylose fermenting yeast strains (*Scheffersomyces stipitis, Spathaspora passalidarum, Spathaspora arborariae and Candida tenuis*) was compared under aerobic, oxygen-limited and anaerobic conditions to understand the effects of oxygen levels on yeast xylose metabolism for ethanol production. Xylose fermentation was evaluated in defined mineral medium by the determination of ethanol production from xylose. Xylose reductase (XR) and xylitol dehydrogenase (XDH) specific activities were determined for each strain in crude-cell extracts.

Findings

Performances of the four yeasts were greatly influenced by oxygen availability. S. stipitis and S. passalidarum showed the highest ethanol yields (above 0.44 $g.g^{-1}$) under oxygen limitation. However, S. passalidarum produced 1.5 times more ethanol than S. stipitis under anaerobiosis. While C. tenuis showed the lowest xylose consumption rate and incapacity to produce ethanol, S. arborariae showed an intermediate fermentative performance among the yeasts. NAD(P)H xylose reductase (XR) activity in crude cell extracts correlated with xylose consumption rates and ethanol production.

Conclusion

This study shows that the four yeasts were able to use xylose as substrate of fermentation to produce ethanol and that the performances of this activity of the four yeasts were greatly influenced by oxygen availability.

Comments (RMS)

This study provides supporting information on possible degradation of xylose (one of the monomer units of heptamaloxyloglucan) by naturally occurring yeast strains to produce ethanol. This supports that heptamaloxyloglucan and its monomers are degraded by microorganisms in the soil.

Data point	CA 7.1.1.1/13
Report author	Yamaguchi et al.
Report year	2000
Report title	Differences in the recognition of glucan elicitor signals between rice and soybean: b-glucan fragments from the rice blast disease fungus pyricularia oryzae that elicit phytoalexin biosynthesis in suspension-cultured rice cells
Journal	The Plant Cell, Vol. 12, 817–826
Report No	-
Document No	-
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

When attacked by pathogens such as fungi, bacteria, and viruses, higher plants initiate various defense responses, including the following: production of phytoalexins, enzymes such as chitinase and b-glucanase, proteinase inhibitors, and hydroxyproline-rich glycoproteins; generation of reactive oxygen species; and lignification. These responses can be triggered by the elicitor molecules derived from the cell surfaces of various fungi, bacteria, and host plants or from the proteins/glycoproteins secreted by microorganisms.

Partial acid/enzymatic hydrolysis of the β-(1→3, 1→6)-glucan from the cell walls of the rice blast disease fungus Pyricularia oryzae (*Magnaporthe grisea*) released elicitor-active fragments that induced phytoalexin biosynthesis in suspension-cultured rice cells. From the digestion of the glucan by an endo-β-(1→3)-glucanase, one highly elicitor-active glucopentaose was purified as a reduced compound, tetraglucosyl glucitol. The structure of this tetraglucosyl glucitol as well as two other related tetraglucosyl glucitols was elucidated as follows:

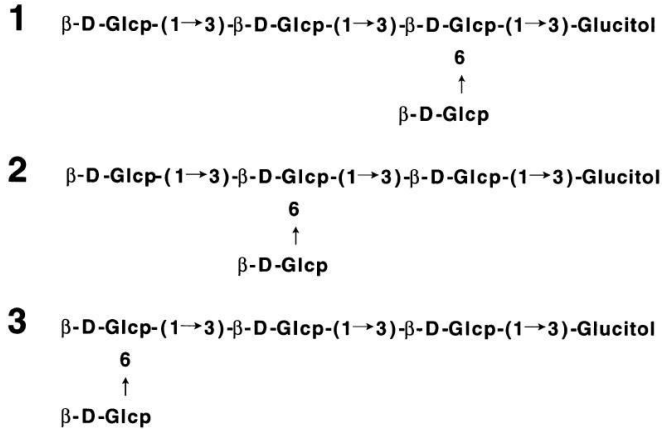


Figure B.8 (AS) - 2: Structure of the Elicitor-Active Tetraglucosyl Glucitol Obtained from the β-Glucan of P. Oryzae.

Comments (RMS)

This study shows that enzymes such as endo-β-(1→3)-glucanase can degrade polysaccharides into oligosaccharides with a terminal glucitol. This confirms that oligosaccharides with a terminal glucitol exist in nature. Please note that in heptamaloxyloglucan, the bond between the glucopyranosyl and glucitol is β-(1→4) while it is β-(1→3) in the tetraglucosyl glucitol. However, it can be assumed that endo-β-(1→4)-glucanase or exo-β-(1→4)-glucanase could hydrolyse xyloglucan to form D-Glcp-β-(1→4)-Glucitol.

B.8.1.1.1. Route of degradation in soil

B.8.1.1.1.1. Aerobic degradation

The hydrolysis of hemicelluloses (including xyloglucans) occurs by the concerted action of endo-enzymes cleaving internally the main chain, exo-enzymes liberating monomeric sugars and ancillary enzymes cleaving the side chains of polymers or oligosaccharides leading to the release of various mono- or disaccharides (CA 7.1.1.1/06, Aro et al., 2005; CA 7.1.1.1/03, Wershaw, 2004).

The sugar residues take part to the microorganisms metabolism and are finally incorporated into microorganisms cellular structures or mineralized to CO_2 (CA 7.1.1.1/03, Wershaw, 2004).

Degradation products of heptamaloxyloglucan are monomeric sugars and shorter xyloglucans that are metabolised by a wide array of microorganisms (CA 7.1.1.1/03, Wershaw, 2004).

B.8.1.1.1.2. Anaerobic degradation

According to literature studies provided by the applicant, oligosaccharides may be degraded by fermentation under anaerobic conditions (CA 7.1.1.1/01, Warren, 1996 and CA 7.1.1.1/03, Wershaw R., 2004).

It may be noticed that anaerobic conditions may be considered as not relevant regarding the timing of application of heptamaloxyloglucan, i.e. early spring to prevent damage from frost.

B.8.1.1.1.3. Soil photolysis

Some publications are available on cellulose and hemicellulose. In the study by Baker (1996, CA 7.1.1.3/01), UV appears to be a significant driver of litter mass loss. It supports a synergistic interaction between UV and microorganisms for litter decomposition where hemicellulose is a significant component. This is confirmed by the study of Brandt et al. (2010, CA 7.1.1.3/02) which concludes that hemicelluloses are naturally degraded, and that UV radiation contributes to this phenomenon. Lin et al. (2015, CA 7.1.1.3/03) conclude that UV radiation has a small but positive effect on hemicellulose decomposition.

Data point	CA 7.1.1.3/01
Report author	Baker, N. R., & Allison, S. D.
Report year	1996
Report title	Ultraviolet photodegradation facilitates microbial litter decomposition in a Mediterranean climate
Journal	Ecology, 96(7), 1994–2003. doi:10.1890/14-1482.1
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

This study aims at evaluating the influence of ultraviolet radiation (UV) on lignin, cellulose and hemicellulose photodegradation and on the microorganism activity in litter decomposition in a semiarid climate (Mediterranean ecosystem).

Twelve 1m^2 plots were paired into six split-plots at the University of California, Irvine Arboretum in Irvine, California, USA (33°90' N, 117°51' W). Each set of paired plots consisted of one ambient plot (hereafter referred to as the UV-pass treatment) and one plot covered with polyester UV-blocking film supported by a PVC frame (hereafter referred to as the UV-block treatment). This film blocked 68% of all UV while allowing 90% transmittance of visible light, as measured by a UV photometer on-site. PVC frames were 1 m on each side and set up 40 cm above the soil surface, with strips of UV-blocking film 20 cm wide used to cover the plot area under the frame.

Within each paired plot, two types of litterbags were deployed: four containing litter of Avena species (A. barbata and A. fatua) with 7.38% (60.05%; mean 6 SE) lignin by mass, and four containing litter of Elymus condensatus (Giant wild rye), a grass species with 13.05% (60.08%) lignin by mass. Avena litter contained 4.79% (60.10%) crude protein and 4.08% (60.03%) ethanol soluble carbohydrates, while Elymus litter contained 3.81% (60.09) and 2.44 % (60.19), respectively.
Both litter types were more similar in cellulose and hemicellulose content than they were in lignin content (Table B.8 (AS) - 1). Hereafter, Avena litter is referred to as low lignin and Elymus litter is referred to as high lignin.

Table B.8 (AS) - 2: Carbon fraction content across all treatments

	Lignin (g)		Cellulose (g)		Hemicellulose (g)	
Treatment	Initial	June 2013	Initial	June 2013	Initial	June 2013
L–, UV+	0.139 ± 0.001	0.167 ± 0.011	0.738 ± 0.006	0.587 ± 0.025	0.587 ± 0.005	0.284 ± 0.016
L–, UV–	0.139 ± 0.001	0.181 ± 0.015	0.738 ± 0.006	**0.670 ± 0.035**	0.587 ± 0.005	**0.338 ± 0.012**
L+, UV+	0.244 ± 0.001	0.230 ± 0.013	0.827 ± 0.003	0.678 ± 0.019	0.484 ± 0.004	0.267 ± 0.023
L+, UV–	0.244 ± 0.001	0.263 ± 0.023	0.827 ± 0.003	**0.803 ± 0.028**	0.484 ± 0.004	**0.348 ± 0.018**

Notes: Bold values indicate when means under UV-block were significantly different ($P < 0.05$, Tukey test) from means of the same litter type under UV-pass. Low-lignin samples are L–, and high-lignin samples are L+. UV block samples are UV–, and UV pass samples are UV+.

Litter of each type was collected by clipping standing litter at least 20 cm above the soil surface to minimize prior soil contact, then homogenized by clipping to <5 cm lengths and mixing.

Four litterbags of both litter types were deployed into each of the six paired plots, resulting in 96 total litterbags (= 4 × 2 × 6 × 2). One litterbag of each litter type was then collected randomly from each of the paired plots at the end of the first dry season (2 October 2012), the middle of the wet season (18 January 2013), the end of the wet season (4 June 2013), and the end of the second dry season (17 September 2013), for a total of five time points (including the initial deployment) over a period of 15 months.

Collected litter was weighed to determine mass loss before being ground into fragments ,0.5 cm in length and subsampled for extracellular enzyme assays, a bacterial cell count assay, and a fungal hyphae staining assay. The remainder of the litter was weighed and ovendried to determine moisture content before being sent off for near-infrared (or near-IR) analysis of litter chemistry.

Findings

Litter mass loss was affected by both litter type and by UV treatment. Low-lignin litter lost the most mass across both UV treatments, with an average of 23.2% mass loss by June 2013. High-lignin litter lost an average of 11.0% of original mass across both UV treatments over the same time period. There was no significant interaction between litter type and UV treatment. High-lignin litter lost 16.2% of original mass by June 2013 under UV-pass, but exhibited negligible (<1%) mass loss under UV-block. Low-lignin samples showed a similar pattern (29.0% mass loss in UV-pass samples vs. 17.4% mass loss in UV-block samples), but post hoc tests within dates were only marginally significant (Fig. 1).

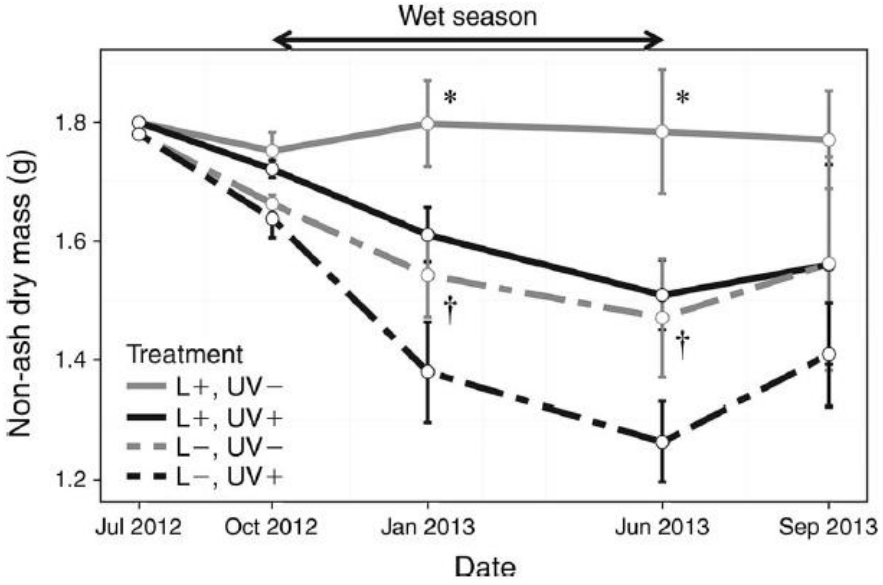


Figure B.8 (AS) - 3: Dry mass of the non-ash component of litter

A positive effect of UV on both fungal abundance and the potential activities of several assayed extracellular enzymes was observed.

Additionally, under ambient UV only, a significant correlation between potential activities of cellulase and oxidase enzymes and both the concentrations and degradation rates of their target compounds is shown.

Conclusion

This study shows that UV exposure has a positive effect on both litter decomposition rates and microbial decomposer activity.
UV-blocking reduces litter mass loss, but does not have a significant direct effect on litter lignin content. Instead, UV-blocking significantly reduces the degradation of cellulose and hemicellulose, potentially by limiting the direct or indirect photolysis of cellulose or the lignocellulose matrix that would otherwise occur under ambient UV. UV-blocking does not appear to increase bacterial or fungal abundance, and may in fact be detrimental for microbial decomposition, as extracellular enzymes produced by the microbial decomposer community were more effective at degrading their target substrates under ambient UV. These results indicate that UV photodegradation is an important driver of litter decomposition through its effects on nonlignin compounds and facilitation of microbial activity.

Comments (RMS)

This study shows that UV radiation has a positive effect on hemicellulose (including heptamaloxyloglucan) decomposition.

Data point	CA 7.1.1.3/02
Report author	L. A. Brandt, J. Y. King, S. E. Hobbie, D. G. Milchunas and R. L. Sinsabaugh
Report year	2010
Report title	The Role of Photodegradation in Surface Litter Decomposition Across a Grassland Ecosystem Precipitation Gradient
Journal	Ecosystems (2010) 13: 765–781. DOI: 10.1007/s10021-010-9353-2
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-

Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

This study aims at evaluating the role of ultraviolet radiation (UV) on litter photodegradation in different ecosystems (Cedar Creek, Minnesota; Central Plains, Colorado; and Sevilleta, New Mexico represent mesic, semiarid, and arid grasslands, respectively). In a 2-year field study, the fiber composition of litter of three grassland sites has been studied (% of hemicellulose, cellulose, lignin). In parallel, UV radiation reaching the litter has been manipulated (using UV-blocking and UV-passing plastic screens). Two dominant grasses have been used in this study: *A. gerardii* and *B. gracilis*.

Findings

UV radiation did not affect cellulose loss but did increase hemicellulose loss.
At Cedar Creek and Central Plains, UV exposure significantly increased loss of the hemicellulose fraction an average of 14% in both species (Table 3). At Sevilleta, UV exposure significantly increased loss of the hemicellulose fraction by 63% in A. gerardii at the fourth collection date. UV exposure did not affect loss of the hemicellulose fraction in B. gracilis at Sevilleta, although sample sizes were too small to statistically analyse for the final collection date.

Table B.8 (AS) - 3: Fibre fraction remaining

Species	Site	Time (years)	% Hemicellulose remaining		% Cellulose remaining		% Lignin remaining	
			UV block	UV pass	UV block	UV pass	UV block	UV pass
A. gerardii	Cedar Creek	0.2	93.8 (0.9)	91.1 (0.9)	97 (1.3)	101.2 (1)	100.8 (3.5)	106.1 (3.2)
		0.5	96.1 (4.1)	86.2 (0.6)	94.1 (3.8)	93.1 (2.6)	126.8 (13.0)	115.0 (5.1)
		1.2	80.2 (4.8)	70.4 (1.4)	80.3 (4.8)	76.2 (2.3)	109.5 (4.8)	97.5 (2.7)
		2.1	59.6 (1.0)	56.5 (0.9)	65.5 (3.2)	64.5 (2.1)	96.2 (12.0)	100.0 (4.2)
	Central Plains	0.2	99.7 (1.9)	93.1 (2.3)	93.7 (2.0)	92.0 (2.4)	138.4 (8.4)	120.8 (10.6)
		0.5	91.4 (2.8)	86.2 (1.8)	88.8 (2.0)	87.8 (2.2)	87.5 (3.7)	87.3 (3.8)
		1.2	82.8 (2.3)	80.4 (2.1)	84.1 (2.0)	85.1 (2.5)	95.7 (4.6)	100.1 (11.9)
		2.1	67.8 (4.6)	64.4 (3.2)	67.8 (4.1)	73.3 (4.4)	85.5 (7.5)	92.3 (11.4)
	Sevilleta	0.2	90.4 (1.1)	93.0 (2.1)	94.7 (1.1)	91.8 (0.7)	120.2 (4.9)	118.7 (8.2)
		0.5	83.0 (3.2)	76.3 (3.2)	83.7 (2.1)	70.9 (6.7)	80.7 (5.2)	70.4 (6.4)
		1.0	89.6 (1.0)	84.1 (2.7)	98.5 (2.5)	96.8 (3.1)	73.2 (9.3)	61.3 (10.7)
		2.0	65.1 (4.5)	42.9 (6.9)	75.7 (4.5)	59.8 (10.9)	81.3 (5.3)	54.9 (8.9)
B. gracilis	Cedar Creek	0.2	78.8 (0.8)	76.9 (0.7)	85.5 (2.2)	85.1 (1.4)	101.2 (2.5)	85.1 (2.7)
		0.5	66.5 (0.6)	60.2 (2.5)	65.3 (1.7)	64.7 (3.8)	102.6 (3.6)	96.8 (2.7)
		1.2	54.6 (1.0)	47.3 (0.9)	50.2 (0.6)	50.2 (1.0)	92.0 (2.0)	82.9 (2.2)
		2.1	38.2 (1.4)	33.4 (1.1)	41.0 (2.9)	35.6 (3.3)	82.9 (3.0)	75.1 (9.1)
	Central Plains	0.2	90.8 (1.8)	87.5 (1.3)	90.6 (1.0)	89.0 (1.1)	111.0 (4.5)	122.6 (5.8)
		0.5	78.5 (1.3)	74.2 (1.7)	75.6 (1.7)	78.2 (1.7)	77.6 (3.4)	62.5 (7.7)
		1.2	65.9 (2.1)	62.2 (1.9)	65.2 (2.3)	69.4 (1.4)	86.3 (2.9)	78.0 (3.2)
		2.1	47.0 (4.4)	48.4 (3.1)	46.0 (4.5)	49.3 (3.4)	68.1 (5.0)	64.3 (4.9)
	Sevilleta	0.2	83.4 (2.2)	77.2 (2.7)	88.6 (4.0)	85.0 (3.0)	108.7 (13.7)	119.8 (9.9)
		0.5	53.5 (3.9)	40.9 (8.6)	51.0 (5.2)	40.0 (10.4)	62.5 (14.9)	57.9 (23.3)
		1.0	40.5 (5.4)	41.6 (9.0)	45.4 (5.2)	50.0 (11.1)	57.0 (9.4)	32.0 (9.7)
		2.0	NA	NA	NA	NA	NA	NA

Mean (n = 10) and standard error shown. Lignin values over 100% indicate a net increase in lignin-like compounds, potentially from a buildup of microbial by-products.

Conclusion

UV exposure tended to increase loss of the hemicellulose fraction.
Hemicellulose makes up a much larger proportion of the initial litter mass (32% in A. gerardii, 38% in B. gracilis) than lignin does; therefore, it has a much greater potential to influence total litter mass loss if it is

photochemically susceptible. Photodegradation could weaken the lignocellulose matrix, making hemicellulose more accessible to hydrolytic enzymatic decay through an increase in binding site availability. Alternatively, hemicellulose could be broken down through indirect photolysis, in which the absorption of UV radiation by lignin leads to the production of free radicals, which break bonds in other compounds, such as hemicellulose, in the lignocellulose matrix.

Comments (RMS)

This study confirms that UV radiation has a positive effect on hemicellulose (including heptamaloxyloglucan) decomposition.

Data point	CA 7.1.1.3/03
Report author	Yang Lin, Jennifer Y. King, Steven D. Karlen, John Ralph
Report year	2015
Report title	Using 2D NMR spectroscopy to assess effects of UV radiation on cell wall chemistry during litter decomposition
Journal	Biogeochemistry - DOI 10.1007/s10533-015-0132-1
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

This study aims at evaluating the contribution of photodegradation in litter degradation and in particular in hemicellulose and linin degradation. The molecular-level changes in litter chemistry associated with photodegradation was studied using two-dimensional nuclear magnetic resonance (2D NMR) spectroscopic methods. For this study, litter of *Bromus diandrus* was exposed in the field to two levels of radiation [with and without ultraviolet (UV) wavelengths] and two durations of exposure (2.5 months during summer, and 1 year).

Findings

The litter hemicellulose fraction decreased significantly from 31.6 to 23.8 and 25.9 % after 1 year of decomposition in the UV pass and the UV block treatment respectively.

Conclusion

These data suggest that UV radiation has a small but positive effect on degradation of hemicellulose compared to other decomposition processes.

Comments (RMS)

This study confirms that UV radiation has a small but positive effect on hemicellulose decomposition.

B.8.1.1.1.4. Additional information: natural occurrence

Xyloglucans are present in the cell walls of dicotyledonae and some monocotylodonae (e.g., onion) and consist of a β-1,4- linked D-glucose backbone substituted by D-xylose (De Vries R. et al. 2001).

Xyloglucans are thoroughly degraded into monosaccharides by cellulases and hemicellulases of soil microorganisms (CA 7.1.1.1/06, Aro et al., 2004; CA 7.1.1.1/03, Wershaw, 2004). Therefore, heptamaloxyloglucan appears to be an intermediate of biodegradation in a transitory state.

Carbohydrate reduction is a natural plant process. For instance, β-glucanase is capable of cleaving fungi cell walls in order to release tetraglucosyl glucitol that will be recognized by rice cells as an elicitor signal (CA 7.1.1.1/13 Yamagushi et al., 2000).

In a study provided by Elicityl (reported below), a sample of a batch of heptamaloxyloglucan manufacturing process was taken after solid/liquid separation (centrifugation) which follows pomace enzymatic hydrolysis by natural enzymes from *Aspergillus*. Some heptamaloxyloglucan was detected after solid-phase-extraction chromatography, strengthening the fact that this molecule does occur in nature by natural processes (Havet and Salvador, 2007).

The natural concentration of heptamaloxyloglucan in the soil of an apple orchard can be estimated considering that:

- 2.7 g of heptamaloxyloglucan are obtained from 1.2 tons of apple (Havet et al. 2007);
- the yield of an apple orchard is approximately 50 tons / ha;
- there is a 1 % loss at maturity (natural fall, harvest losses).

The natural contribution is therefore:
2.7 * 50 * 0.01 / 1.2 = **1.1 g/ha**

This value is similar to the intended application rate on vines.

Reference	CA 7.1.1/additional information
Report author	Havet and Salvador
Report year	2007
Report title	Heptamaloxyloglucan quantification in pomace enzymatic hydrolysis solution
Report No	05-905012-003, 2006-01-13
Document No	-
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes

Test objective

The main aim is to detect and/or quantify Heptamaloxyloglucan from two different timing in batch AND0506 EL101GV of ELICITYL manufacturing process (ref. HEPTAMALOXYLOGLUCAN_A II K 01 08 01 manufacturing process for further details) by taking samples after pomace enzymatic hydrolysis (Timing 1) and after the first purification stage (Solid-Phase-Extraction Chromatography also called Low chromatography, Timing 2). Remembering our process, it is composed of 12 steps; the samples are taken after the step 3 and step 5 (see page 5 HEPTAMALOXYLOGLUCAN _A II K 01 08 01 manufacturing process).

Analytical method

The technical test item is analysed by HPLC using an anionic exchange technology. The oligosaccharide is detected by a pulse amperometric electrochemical technique.

The quantification is made by external standardisation. The calculation of Heptamaloxyloglucan concentration is done by measuring the area peak (expressed as counts).
The calibration curve is calculated with the individual value obtained from the analysis of the reference item solutions. The calibration is carried out by the pilot software of the chromatographic chains (Dionex Chromeleon). Standards are regularly positioned in the analytical sequence. The software is using in calibration mode “bracketed” to adjust the calibration during the analysis sequence.

Results

A- Heptamaloxyloglucan analysis Timing 1: after enzymatic hydrolysis.

The sample is taken after solid/liquid separation (centrifugation) which follows pomace enzymatic hydrolysis from batch AND0506 using natural enzymes from Aspergillus (used for the manufacturing process).
Analysis show an important number of impurities in which Heptamaloxyloglucan was found to be at very low level. As a consequence, all the molecules show a very poor resolution during chromatography analysis not permitting quantification of Heptamaloxyloglucan. Nevertheless, overlaying chromatographic traces with Heptamaloxyloglucan standard enables to identify the molecule in the analysed sample with a strong confidence.

B- Heptamaloxyloglucan analysis Timing 2: after SPE pre-purification.

Heptamaloxyloglucan quantification is rendered possible at this pre-purification step thanks to low pressure chromatography (equal to Solid Phase chromatography). This technique excludes an important part of molecules facilitating Heptamaloxyloglucan detection and analysis. Heptamaloxyloglucan is analysed from several concentration samples (dry weight references). The results are given in the following table:

Dry weight (mg/L)	**Heptamaloxyloglucan (mg/L)**	**% w/w (weight/weight)**
40	0.093	0.233
100	0.194	0.194
200	0.695	0.348
400	1.191	0.298
% w/w Standard mean		**0.268**

C- Heptamaloxyloglucan quantity calculation from timing 2

The batch specifications and measurements were as following:

Pomace weight at the batch start	120 kg
Total volume at the batch start	1200 L

Volume after SPE (collection)	75 L
SPE concentration (dry weight)	13.4 L

According to these indications, dry matter is equal to about 1000 g (75L*13.4 g/L) in low pressure chromatography collection (SPE).
As a consequence, Heptamaloxyloglucan total weight is 1000 g * 0.268%= 2.7 g.
As the start total volume is 1200 L, Heptamaloxyloglucan concentration is equal to 2.7 g / 1200 L = 2.25 mg/L.

Taking into account that apple pomace is 10 % fresh weight of apple, and knowing that at the start of the batch there was 120 kg of apple pomace, there are 2.7 g of Heptamaloxyloglucan from 1200 kg fresh apples. In conclusion, final Heptamaloxyloglucan concentration is given at 2.25 mg/kg (apple).

Conclusion

Pomace enzymatic hydrolysis analysis of Heptamaloxyloglucan was found to be impossible because of poor resolution among all the analytes. On the other hand, analysis of SPE collection (low pressure chromatography collection) enables to quantify eptamaloxyloglucan which has a concentration equal to 2.25 mg/L or equal to 2.25 mg/kg of fresh apple.
As a conclusion Heptamaloxyloglucan was found (detect and quantify) in the timing called timing 2 (SPE collection or low pressure chromatography collection) of the manufacturing process.

Comments (RMS)

This study provides supporting information regarding natural occurrence of heptamaloxyloglucan in apple.

B.8.1.1.1.5. Summary on route of degradation in soil

Considering that heptamaloxyloglucan, which is a xyloglucan-derived oligosaccharide, could be an intermediate compound of natural organic matter decomposition process, which could undergo degradation by endogenous soil microorganisms naturally occurring in soil. No specific study on the rate and route of degradation was submitted and none are deemed necessary since scientific literature studies provide adequate information in this specific case.

The literature data confirm that xyloglucans belong to the hemicellulose family, one of the principal components of plant cell wall, along with cellulose, pectin and lignin (CA 7.1.1.1/01, Warren, 1996, CA 7.1.1.1/06, Aro N. et al., 2005).
The monosaccharide units of heptamaloxyloglucan are the same as in xyloglucans, except for D-glucitol (CA 7.1.1.1/02, De Vries & Visser, 2001). These monomers (or hemicellulosic sugars) are part of natural organic matter found in soils (CA 7.1.1.1/04, Karroum et al., 2004).

Xyloglucans are released by a wide range of plants. These polysaccharides can be extracted in relatively abundant quantities from soils (grassland and glacial forefiel soils, for example). In addition, xyloglucans are effective promoters of soil aggregation and formation (CA 7.1.1.1/10, Galloway et al., 2018).
As microorganisms that degrade cellulose usually also degrade hemicellulose (and thus xyloglucans), cellulases and hemicellulases are considered as components of systems for the enzymatic cleavage/hydrolysis of plant cell walls (CA 7.1.1.1/01, Warren, 1996).
Cellulases hydrolyse the β-1,4-glycosidic linkages of cellulose. An effective hydrolysis of cellulose also requires β-glucosidases (CA 7.1.1.1/05, Pérez & Muñoz-Dorado, 2002). The hydrolysis of hemicelluloses occurs by the concerted action of endo-enzymes cleaving internally the main chain, exo-enzymes liberating monomeric sugars and ancillary enzymes cleaving the side chains of polymers or oligosaccharides leading to the release of various mono-or disaccharides (CA 7.1.1.1/06, Aro et al., 2004).
In this complex system of biodegradation, heptamaloxyloglucan could be considered as an intermediate compound which will be further degraded into monosaccharides.
During chemical and biochemical processes involved in the degradation of natural organic matter (humification), cellulolytic microorganisms transform organic matter into smaller molecules (CA 7.1.1.1/07, Tuomela et al., 2000). The structural polysaccharides (cellulose and hemicelluloses) of ligno-cellulosic material of plants undergo a degradation depending on the depth (CA 7.1.1.1/04, Karroum et al., 2004). Hemicellulose recycling from plant biomass is indispensable for the carbon cycle (CA 7.1.1.1/05, Perez, 2002). Heptamaloxyloglucan as an intermediate of biodegradation should be fully part of the carbon cycle.

Different classes of enzymes are involved in plant cell wall polysaccharide degradation. They are produced by several saprophytic[7] organisms such as bacteria, archea and fungi (for example Aspergilli, Cellulomonas fimi, a mesophilic aerobic soil bacterium and Thermomonospora fusca, a thermophilic actinomycete common in compost) (CA 7.1.1.1/02, De Vries & Visser, 2001; CA 7.1.1.1/01, Warren, 1996). Plant cell wall hydrolysis requires particular enzymes hydrolyzing β–1,4 and β–1,3 glycosidic bonds; β–1,4 xylosidic bonds and β–1,4 mannosidic bonds (CA 7.1.1.1/01, Warren, 1996). Four different enzymes are required to degrade the common hemicellulose O-acetyl-4-O-methylglucuronxylan: endo-1-4-β-xylanase, acetyl esterase, -glucuronidase, and -xylosidase (CA 7.1.1.1/03, Wershaw, 2004). *Aspergilli* produce different classes of accessory enzymes that act on plant cell wall polysaccharides: for example, α-D-Xylosidases, α-galactosidases and β–galactosidases (CA 7.1.1.1/02, De Vries & Visser, 2001).

Some yeast species have been identified as being capable of converting xylose (a monomer unit of heptamaloxyloglucan) to ethanol, including *Kluyveromyces cellobiovorus, Pachysolen tannophilus, Spathaspora passalidarum, Spathaspora arborariae, Scheffersomyces shehatae, Scheffersomyces stipitis and Candida tenuis* (CA 7.1.1.1/12, Veras et al., 2017 and CA 7.1.1.1/11, Valinhas et al.2018). The performance of this activity is greatly influenced by oxygen availability (CA 7.1.1.1/12, Veras et al., 2017).
Three additional fungi strains with this metabolic feature have been identified in the study by Valinhas et al. (2018, CA 7.1.1.1/11). Two of the strains were identified as belonging to *Galactomyces geotrichum* species. Another strain was identified as *Candida akabanensis* (CA 7.1.1.1/11, Valinhas et al.2018).

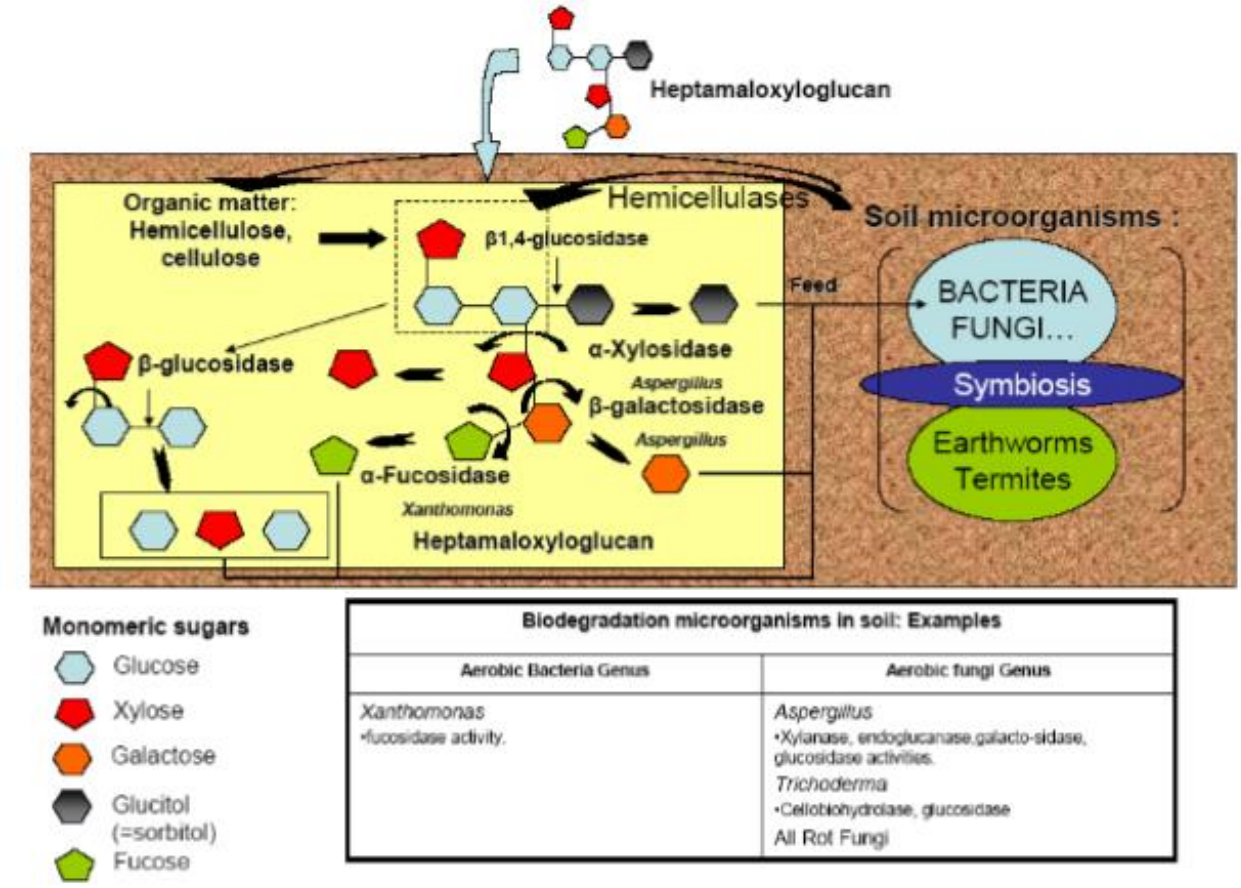
[7] organisms that survive by decomposing dead or decaying organic matter

Regarding the glucitol residue, soil microorganisms thoroughly catabolize this sugar. The pathway of glucitol (sorbitol) metabolism described in *Pseudomonas*, *Aerobacter*, *Cellvibiro*, *Rhizobium* oxydize glucitol as a carbon source for organism growth use (CA 7.1.1.1/08, Kelker, 1971).

Many microorganisms can utilize the galactose monomer as a substrate for growth by virtue of two alternative metabolic routes (KCA 7.1/09, Brechtel, 2002).

Literature studies confirm that UV radiation has a small but positive effect on hemicellulose degradation compared to other decomposition processes.

Taking into account of all information given by the cited literature, the applicant proposed a pathway of biodegradation in Figure B.8 (AS) - 4. The representation of heptamaloxyloglucan molecule and monomeric sugars are schematic. Real spatial formula illustration of heptamaloxyloglucan can be found under Vol. 3 CA B.1. To facilitate the review of the scheme, the notifier used the same symbols for the glucidic monomer units of heptamaloxyloglucan and its expected degradation products (monomeric sugars). Therefore in heptamaloxyloglucan representation, D-glucopyranosyl, D-glucitol, D-xylopyranosyl, D-galactopyranosyl and L-fucopyranosyl are represented by the glucose, glucitol, xylose, galactose and fucose monomeric sugar symbols, respectively.



Biodegradation microorganisms in soil: Examples	
Aerobic Bacteria Genus	**Aerobic fungi Genus**
Xanthomonas •fucosidase activity.	*Aspergillus* •Xylanase, endoglucanase,galacto-sidase, glucosidase activities. *Trichoderma* •Cellobiohydrolase, glucosidase All Rot Fungi

Figure B.8 (AS) - 4: Fate and behaviour of heptamaloxyloglucan in soil

Comments (RMS)

Using available literature, the applicant provided general information on the way oligosaccharides such as heptamaloxyloglucan, which is a xyloglucan-derived oligosaccharide, could be degraded in soil by enzymatic action of the microorganisms. These data are useful to understand how heptamaloxyloglucan could be degraded in soil. Moreover, the assimilation and degradation of xyloglucan-like molecules by soil macro-organisms such as earthworms is facilitated by soil microorganisms that they ingest together with soil (see Vol. 3 CA B.9.7). Therefore, the fate and behaviour of heptamaloxyloglucan in soil and possible assimilation and degradation by soil micro- and macro-organisms is considered as well described.

B.8.1.1.2. Rate of degradation in soil

The release of xyloglucans from the cell wall is due to leaves and plant debris fractionation and to enzymatic cleavage of short-chain oligosaccharides.
The rate of degradation of xyloglucans is directly correlated to the soil biomass (which produces the various enzymes necessary to the degradation) and to the soil temperature, which controls the activity of the enzymes. In addition, short chain soluble xyloglucans, like heptamaloxyloglucan are more easily degraded than long-chain insoluble molecules, so the half-life of the active substance in soil is expected to be short. Furthermore heptamaloxyloglucan is readily biodegradable as shown in L'Haridon J. (2006, CA 7.2.2.1/01).

No experimental DT_{50} in soil value was made available. However, heptamaloxyloglucan has a negative log (K_{ow}) value and is readily biodegradable. Therefore, according to ECHA guidance on the Biocidal Products Regulation, vol. IV[8] (10/2017), a DT_{50} in soil of 30 days can be used by default.

B.8.1.1.2.1. Laboratory studies

B.8.1.1.2.1.1. Rate of degradation of heptamaloxyloglucan

No study has been performed since the active substance could be produced under natural conditions by enzymatic degradation of xyloglucans by soil microorganisms. In addition, it is expected to be rapidly degraded in soil and is intended to be used at very low rates (max. 4 × 0.56 g a.s./ha).

B.8.1.1.2.1.2. Rate of degradation of the metabolites

No data, not required. Heptamaloxyloglucan is a polysaccharide which leads to smaller-sized oligosaccharides and monosaccharides after degradation. No other relevant metabolites, degradation or reaction products are expected.

B.8.1.1.2.2. Field studies

According to the nature of the active substance, no study is required.

B.8.1.1.2.3. Soil accumulation studies

According to the nature of the active substance, no study is required.

B.8.1.2. Adsorption and desorption in soil

B.8.1.2.1. Adsorption and desorption of the active substance

According to the nature of the active substance, no study is required.
Indeed, heptamaloxyloglucan is part of the organic matter of the soil and therefore calculation of a Koc is not considered relevant.

RMS would like to underline that the MCI method used in QSAR KOCWIN version 2.00 may not be reliable for heptamaloxyloglucan. Indeed, it is expected that log Koc estimates are less accurate for compounds outside the molecular weight range of the training set compounds, and/or that have more instances of a given fragment than the maximum for all training set compounds. Molecular weight of heptamaloxyloglucan is 1079 g/mol, which is outside the ranges of both training set molecular weights (32.04 - 665.02 g/mol) and validation molecular weights (73.14 - 504.12 g/mol).
For information, the following Koc values are obtained with KOCWIN version 2.00:

- MCI method: 6.6×10^{9} mL/g;
- Log Kow method: 3.4×10^{-10} mL/g.

[8] Guidance on Biocidal Products Regulation: Volume IV Environment - Assessment and Evaluation (Parts B+C); Reference: ECHA-17-G-23-EN; Cat. Number: ED-01-17-897-EN-N ISBN: 978-92-9020-151-9 DoI: 10.2823/033935 Publ.date: October 2017

Overall, RMS considers that QSAR estimations of Koc value for heptamaloxyloglucan are not reliable.

For modelling, RMS recommends using the following worst-case values:
- Kfoc of 0 mL/g for PECgw and PECsw;
- Kfoc of 10000 mL/g for PECsed;
- 1/n of 1.

B.8.1.2.1. Adsorption and desorption of degradation products

No data, not required. Heptamaloxyloglucan is a polysaccharide which leads to smaller-sized oligosaccharides and monosaccharides after degradation. No other relevant metabolites, degradation or reaction products are expected to appear.

B.8.1.3. Mobility in soil

B.8.1.3.1. Column leaching studies

No data submitted, none required (please refer to point B.8.1.2.1).

B.8.1.3.2. Aged column leaching studies

No data submitted, none required (please refer to point B.8.1.2.1).

B.8.1.3.3. Lysimeter studies

No data submitted, none required (please refer to point B.8.1.2.1).

B.8.2. FATE AND BEHAVIOUR IN WATER AND SEDIMENT

B.8.2.1. Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

B.8.2.1.1. Hydrolysis

Data point	CA 7.2.1.1/01
Report author	Ricau H.
Report year	2005
Report title	Abiotic degradation on the technical heptamaloxyloglucan, pH dependent hydrolysis (Test C7)
Report No	05-905012-003, 2006-01-13
Document No	-
Guidelines followed in study	Directive 92 / 69 / EEC paragraph C7 of July 31st, 1992
Deviations from current test guideline	pH values of 5, 7 and 9 were used in the test instead of 4, 7 and 9 No temperature recording is available although temperature of water-bath was followed during the study to be at 50±0.5°C. These minor deviations are not expected to modify study conclusions
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes

Methods

Hydrolysis of heptamaloxyloglucan was investigated in buffer solutions with pH adjusted to 5, 7 and 9.

For each pH, test solutions of 12.4 g technical heptamaloxyloglucan/L (batch AND0205, purity 88%) was prepared by adding 310 mg of technical heptamaloxyloglucan into 25 mL of buffer (pH 5, 7 and 9).
At test initiation, and after 2.4 hours and 5 days, 2 mL of the test solution was diluted 25 times with water before analysis, except for pH 5 and 7 after 5 days where dilution was 20 times. Test was conducted with duplicate flasks for each of the three pH. Heptamaloxyloglucan was analysed by HPLC and detected by pulsed amperometric electrochemical technique using the method described in study report 05-905012-007).

Results

The pH values at test initiation and after 5 days at 20°C were 4.96, 7.01, 8.89 and 5.02, 6.98, 8.72, respectively. The mean temperature was 50.0±0.5°C. Results are summarized in the following table:

Table B.8 (AS) - 4: Concentration of heptamaloxyloglucan and percentage of hydrolysis depending on pH

	Concentration (mean value, g/L)			% of hydrolysis		
Time	0	2.4 h	5 d	0 (1)	2.4 h	5 d
pH 5	11.54	11.77	11.77	0	ns	ns
pH 7	11.66	11.56	12.18	0	ns	ns
pH 9	11.46	11.67	12.37	0	ns	ns
(1) notifier assumed that at t = 0 the hydrolysis was 0% ns: not significant						

No significant change in heptamaloxyloglucan concentration was observed during the study.

Conclusion

No significant hydrolysis of heptamaloxyloglucan was observed at pH 5, 7 and 9 after 5 days. PEL101GV is hydrolytically stable.

Comments (RMS)

According to the information summarised in the report, the study was performed according to OECD Guideline 111. Although it was not precised if the test was performed in the dark to avoid photolysis and in anaerobic conditions, this has no impact on the conclusion since no degradation of the substance was observed.
In the preliminary test, hydrolysis of heptamaloxyloglucan is lower than 10% after 5 days. Therefore, according to OECD Guideline 111, heptamaloxyloglucan is considered hydrolytically stable.

B.8.2.1.2. Photolysis

According to the nature of the active substance, no study was performed. In Volume 3 – B2 (CA), heptamaloxyloglucan is photochemically stable as it has no peak absorption with molecular absorption coefficient higher than 10 L/mol/cm at wavelength > 290nm.

In point B.8.1.1.1.3, it was shown that UV radiation has a small but positive effect on degradation of hemicellulose compared to other decomposition processes.

B.8.2.2. Route and rate of biological degradation in aquatic systems

B.8.2.2.1. Ready biodegradability

Data point	CA 7.2.2.1/01
Report author	L'Haridon, J.
Report year	2006
Report title	Determination of the ready biodegradability CO2 evolution test
Report No	31237ECS

Document No	-
Guidelines followed in study	Directive 92/69/EEC paragraph C.4-C of July 31st, 1992 - Directive 93/21/EEC (27th April 1993) - OECD guideline No. 301B (17th July 1992)
Deviations from current test guideline	pH of the mineral medium was not controlled (and adjusted at 7.4 ± 0.2) before the start of the test, but since the test medium was prepared according to test guideline, the study was considered to be valid.
Previous evaluation	Yes, evaluated and accepted in the DAR (2009)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes

Methods

Technical heptamaloxyloglucan (EL101GV, batch ANN0304, purity 78.2%w/w) was dissolved in reconstituted water (OECD mineral medium) prepared from deionized water with conductivity < 10 µS/cm.
Five flasks were used to determine the quantity of carbon dioxide evolved by the degradation of the test item:

- two flasks containing the inoculum (inoculum blanks),
- two flasks containing the test item (at 10.0 mg/L of total organic carbon (TOC)) and inoculum (test solutions),
- one flask containing the reference item (sodium acetate at 10.0 mg/L of TOC) and inoculum (procedure control).

The inoculum consisted of sewage sludge sampled from the aeration tank of a sewage treatment plant and then aerated for 6 days. Inoculum concentration was 20.0 mg/L (dry weight) in all test vessels. CO_2 scrubbed air was bubbled through the flasks for the 28-day test period.

Results

Environmental parameters were recorded as: pH: 7.48 to 7.74, room temperature: 20°C to 23°C. The reference item degraded normally under the test conditions.
The percentage of degradation is reported in the following table:

Table B.8 (AS) - 5: Degradation of heptamaloxyloglucan (% of initial concentration)

Day	Test item			Reference item
	flask 1	flask 2	average	
1	0.00	0.00	0.00	5.30
4	29.02	22.23	25.63	37.02
6	52.70	44.91	48.81	57.50
8	68.29	64.39	66.34	68.19
11	77.33	72.43	74.88	72.63
14	80.48	76.28	78.38	74.58
18	82.18	78.38	80.28	75.38
22	82.18	77.78	79.98	75.88
25	81.18	78.18	79.68	75.78
28	79.33	76.33	77.83	76.03

Conclusion

The biodegradation of heptamaloxyloglucan reached 76% at the end of the 10-day window (the 10 days immediately following reaching of 10% biodegradation) and 78% at the end of the test. Under the experimental conditions, the test item heptamaloxyloglucan was therefore readily biodegradable in the 28-day modified Sturm test.

Comments (RMS)

As the test item has a minimum purity of only 78.2% it could be questionable whether readily biodegradability observed in this 28-day modified Sturm test was totally provided by heptamaloxyloglucan. However as the impurities have a structure close to heptamaloxyloglucan structure, results of the study are considered acceptable, i.e. EL101GV is set as a readily biodegradable compound.

B.8.2.2.2. Aerobic mineralisation

Heptamaloxyloglucan is a natural plant cell wall component and follows the degradation pathway of xyloglucans.
Heptamaloxyloglucan is a short-chain oligosaccharide and is freely soluble (558 g/L at 20°C). It is therefore directly accessible to enzymatic degradation and its degradation rate in water is likely to be quite rapid. Furthermore it has been shown that heptamaloxyloglucan is readily biodegradable (CA 7.2.2.1/01, L'Haridon, J. 2006) demonstrating a very rapid biodegradation through bacterial metabolism.
Therefore, according to the nature of the active substance and its ready biodegradability, no studies are deemed necessary.

No experimental DT_{50} in water value was made available. However, heptamaloxyloglucan is readily biodegradable. Therefore, according to ECHA guidance on the Biocidal Products Regulation, vol. IV[9] (10/2017), a DT_{50} in water of 15 days can be used by default.

B.8.2.2.3. Water / sediment systems

Some publications are available on cellulose and hemicellulose degradation in water/sediment systems.

Freixa et al. (2016; CA 7.2.2.3/01) shows that hemicellulose-degrading enzymes are present in rivers water and, thus that hemicellulose can naturally be degraded. This is further confirmed in a review done by Romani et al. (2012, CA 7.2.2.3/02). In particular, enzyme activity shows preferential use of cellulose and hemicellulose in autumn as a response to an increased allochthonous input. The study by Younes et al. (2015, CA 7.2.2.3/03) shows that the degradation of hemicellulose occurs in peatland. In particular, hemicellulose components (glucose, arabinose, xylose, and galactose) are degraded at the interface between mesotelm and catotelm.

It should also be noted that the additional amount of hemicellulose brought to surface water and sediment that will originate from the use of heptamaloxyloglucan, compared to other sources of hemicellulose, is not expected to be significant.

Data point	CA 7.2.2.3/01
Report author	A. Freixa, E. Ejarque,S. Crognale, S. Amalfitano, S. Fazi, A. Butturini, A. M. Romani
Report year	2016
Report title	Sediment microbial communities rely on different dissolved organic matter sources along a Mediterranean river continuum
Journal	Limnol. Oceanogr. 00, 2016, 00–00 - doi: 10.1002/lno.10308
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

This study reports the description the longitudinal patterns of sediment bacterial community composition and dissolved organic matter utilization under base flow and drought conditions in a Mediterranean river. Among the

[9] Guidance on Biocidal Products Regulation: Volume IV Environment - Assessment and Evaluation (Parts B+C); Reference: ECHA-17-G-23-EN; Cat. Number: ED-01-17-897-EN-N ISBN: 978-92-9020-151-9 DoI: 10.2823/033935 Publ.date: October 2017

different parameter studied, the total prokaryotic abundance and bacterial community composition and the extracellular enzyme activities have been investigated.

Findings

The results indicated that sediment microbial communities were affected by dissolved organic matter quality and origin along the river continuum. In headwaters the potential degradation of cellulose and hemicellulose was greater (i.e., higher b-glucosidase and b-xylosidase activities), suggesting higher microbial utilization of allochthonous detritus from terrestrial origin.

Conclusion

This study supports that hemicellulose-degrading enzymes are present in rivers water.

Comments (RMS)

This study supports the fact that microorganisms able to degrade hemicellulose (including heptamaloxyloglucan) are present in rivers water.

Data point	CA 7.2.2.3/02
Report author	Anna M. Romani, Stefano Amalfitano, Joan Artigas, Stefano Fazi, Sergi Sabater, Xisca Timoner, Irene Ylla, Annamaria Zoppini
Report year	2012
Report title	Microbial biofilm structure and organic matter use in Mediterranean streams
Journal	Hydrobiologia · October 2012; DOI: 10.1007/s10750-012-1302-y
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

This paper is a literature review on microbial biofilm structure and organic matter use in Mediterranean streams. River and stream biofilms are microbial assemblages of autotrophic and heterotrophic microorganisms that are attached to organic and inorganic surfaces (rocks, cobbles, sediment grains, leaf litter, wood).

Dissolved organic matter (DOM) comprises most of the carbon in streams and rivers, which is continuously supplied both from terrestrial input (allochthonous material) as well as from in-stream processes (autochthonous material). Allochthonous DOM sources mainly come from terrestrial plant materials (leachates from surrounding soils, grasses, and inputs from riparian trees). This allochthonous material is more recalcitrant, more resistant to biological degradation, and encompasses heterogeneous refractory organic substances of high molecular weight. In autumn, the benthic heterotrophs (bacteria and fungi) became more relevant and there is a greater importance of plant material (cellulose, hemicellulose, lignin) in sustaining stream system functioning as indicated by the increase of cellulolytic and ligninolytic enzyme activities.

When river flow becomes fragmented in pools, a decrease in the use of peptides but increase in polysaccharide decomposition by the microbial biofilm has been reported, indicating a decrease in available fresh C and N organic matter sources as drought progresses. During this period, processing rates of deposited material are likely to differ from those in flowing water, and periodic hypoxia in pools causes microbial metabolism to fluctuate between aerobic and anaerobic pathways.

Flooding episodes also induce changes in the metabolic profile of microbial communities, probably as a result of the increase of allochthonous organic carbon and nutrients in the flowing water. Zoppini et al. (2010) observed that b-glucosidase and lipase activities contribute substantially in metabolizing allochthonous materials during these periods.

Hydrology appears as one of the main factors affecting the biofilm structure and functioning. The fluctuations in microbial biomass and in enzyme activities suggest that efficiencies are also fluctuating, because in the rewetting there is a biomass decrease but some activities are increased.

Conclusion

Mediterranean stream biofilms show higher use of peptides during the favorable period for epilithic algae development (spring), and preferential use of cellulose and hemicellulose in autumn as a response to allochthonous input.

Comments (RMS)

This review supports that microorganisms able to degrade hemicellulose (including heptamaloxyloglucan) are present in rivers water.

Data point	CA 7.2.2.3/03
Report author	Younes K., Abdelli G., Araji N., Grasset L.
Report year	2015
Report title	Comparison of thermochemical and chemical methods for the analysis of carbohydrates in an ombrotrophic peatland
Journal	27th International Meeting on Organic Geochemistry September 13-18 2015, Prague, Czech - DOI: 10.13140/RG.2.2.22570.00965
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

This study aims at comparing complementary methods of analysis of carbohydrates in soil and sediment. Glucose, arabinose, xylose, mannose, galactose, rhamnose, ribose, and cellulose were assessed in the three different layers of peatland: catotelm (depth 0 to to -30 cm), mesotelm (depth -30 to to -60 cm) and acrotelm (depth -60 to to -100 cm).
The method involving hexamethyldisilazane (HMDS) enables the analysis of thermolabile monosaccharides and/or occurring initially in polymers susceptible to be cracked by HMDS, and the method involving Tetramethylammonium hydroxide (TMAH) enables the analysis of free and terminal monosaccharides. The methods based on acid hydrolysis (HCl and THFa) enable the analysis of free and polymerized carbohydrates (excluding cellulose).

Findings

The analyses show that carbohydrates amounts decrease with depth. Two slight relative increases in the upper part of the acrotelm (fresh organic matter) and at the interface between mesotelm and catotelm (lowest limit of the water table) are observed. Glucose appears to be in a thermolabile form only at the interface between mesotelm and catotelm (corresponding to depolymerisation of hemicellulose).

Conclusion

This study shows that all the assessed carbohydrates are present in the peatland. A particular increase of hemicellulose components: glucose, xylose, and galactose at the interface between mesotelm and catotelm, supports that the degradation of hemicellulose occurs in this area.

Comments (RMS)

This study supports that hemicellulose (including heptamaloxyloglucan) is degraded by microorganisms present in peatland.

B.8.2.3. Degradation in the saturated zone

Investigations on the degradation in the saturated zone are considered not to be necessary.

B.8.2.4. Impact of water treatment procedure

Heptamaloxyloglucan is neither a fungicide nor a bactericide and according to its structure, no degradation product of concern is expected. Consequently, no specific experimental data on the impact of heptamaloxyloglucan on water treatment procedures is deemed necessary. The applicant provided the following studies from the literature.

Data point	Impact of water treatment procedure/01
Report author	Wang W., Zhang C., Tong S., Cui Z., Liu P
Report year	2018
Report title	Enhanced enzymatic hydrolysis and structural features of corn stover by NAOH and ozone combined pretreatment
Journal	Molecules 2018, 23, 1300; doi:10.3390/molecules23061300
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

In this work, a two-step pretreatment using NaOH and ozone was performed on corn stover to improve its enzymatic hydrolysis, compositions and structural characteristics compared to unpretreated.

Findings

A pretreatment with 2% (w/w) NaOH at 80°C for 2 h followed by ozone treatment for 25 min with an initial pH 9 was found to be the optimal procedure and the maximum efficiency (91.73%) of cellulose enzymatic hydrolysis was achieved. Furthermore, microscopic observation of changes in the surface structure of the samples showed that holes were formed, and lignin and hemicellulose were partially dissolved and removed. Disruption of hydrogen bonds in cellulose and disruption of ester bonds in hemicellulose; cleavage of bonds linkage in lignin-carbohydrate complexes; removal of methoxy in lignin and hemicellulose were also observed.

Conclusion

NaOH and ozone are efficient treatments to alter cellulose and hemicellulose structures and promoted subsequent enzymatic hydrolysis of cellulose.

Comments (RMS)

This study is acceptable and shows that a pretreatment with NaOH and ozone promotes degradation of hemicellulose (including heptamaloxyloglucan).

Reference	Impact of water treatment procedure/02
Report author	Yao S., Wang C., Gao C., Shi L. Nie S. Qin C.
Report year	2018
Report title	Molecular simulation of reaction mechanism for hemicellulose model compound during chlorine dioxine bleaching
Journal	BioResources 13(2), 3763-3777
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No
GLP/Officially recognised testing facilities	No (Literature study)
Acceptability/Reliability:	Yes

Material and Methods

The aim of the present work was to appraise the reaction mechanism of the reaction of hemicellulose with chlorine dioxide in order to provide a theoretical basis for reducing adsorbable organic halogens (AOX) formation based on the perspective of hemicellulose.

Solutions of D-xylose were prepared and added to a three-neck flask. A sulfuric acid solution and sodium hydroxide were used to adjust the pH of the solution. The reactions were initiated by mixing D-xylose and chlorine dioxide solutions directly in a three-neck flask.

The chemical composition of the reaction products and content of D-xylose in the reaction solution were quantified by GC-MS.

Findings

The AOX formation at various temperatures (20°C, 30°C, 40°C, 50°C, and 60°C) with time is shown in Fig. 4, and was divided into two stages. One stage was a rapid growth stage within 45 min, and the other was the saturation concentration stage after 45 min. The equilibrium concentration of AOX was 1.35 kg.tp-1 at 20°C. It increased as the temperature increased, and was 1.46 kg.tp-1, 1.56 kg.tp-1, 1.66 kg.tp-1, and 1.75 kg.tp-1 at 30°C, 40°C, 50°C, and 60°C, respectively. The growth rate of the equilibrium concentration was stable. There is a linear relationship between the temperature and the formation of AOX.

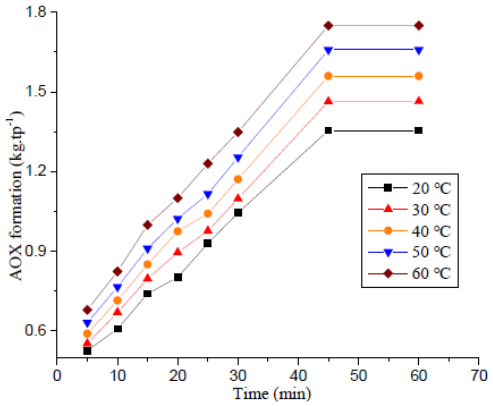


Fig. 4. Temperature and time on influence of AOX formation

The only chloride in the water samples after the reaction between D-xylose and chlorine dioxide was chloroacetic acid. The chemical composition of chlorine dioxide bleaching wastewater was detected by GC-MS, and chloroacetic acid was detected, which indicated that the main reaction product was chloroacetic acid. The GC-MS analysis of the samples showed that they contained organic acids and alkalis, such as propanoic acid, formic acid, acetic acid, ethanol, erythritol, and so on. These organic acids and alkalis were produced by D-xylose hydrolysis.

Conclusion

The final product of the reaction between hemicellulose and chlorine dioxide during the bleaching process was chloroacetic acid.
Xylitol was generated by D-xylose degradation, and then chloroacetic acid was generated by a series of oxidation, fracture, and substitution reactions on xylitol. The results provide a new way to resolve the biggest environmental pollution problem during the ECF bleaching process.

Comments (RMS)

This study shows that D-xylose reacts with ClO_2 to form adsorbable organic halogens (AOX) such as chloroacetic acid.
However, the potential risk from the additional amount of haloacetic acids that will originate from the use of heptamaloxyloglucan, compared to other naturally occurring sources of xylose and other sugars, is expected to be negligible.

B.8.3. FATE AND BEHAVIOUR IN AIR

B.8.3.1. Route and rate of degradation in air

The calculated vapour pressure of heptamaloxyloglucan is $1.1*10^{-11}$ Pa at 20°C and therefore, heptamaloxyloglucan is not expected to volatilize from plant and soil surface in the air compartment.
Thus, no study regarding the route and rate of degradation of heptamaloxyloglucan in air is deemed necessary.

The Atkinson half-life of heptamaloxyloglucan in air was calculated using AOPWIN v.1.92 (EPI Suite), a 12-hour day and a hydroxyl radical concentration of 1.5×10^6 OH/cm^{-3} (default). A half-life in the upper atmosphere was calculated to be 0.037 days, or 27 min, based on a 12 hours day. Based on the results, the persistence of heptamaloxyloglucan in the air is not expected to be a point of concern.

B.8.3.2. Transport via air

No data provided and none required (see point B.8.3.1)

B.8.3.3. Local and global effects

No data provided and none required (see point B.8.3.1)

B.8.4. RESIDUE DEFINITION

B.8.4.1. Residue definition for risk assessment

Soil:	Heptamaloxyloglucan
Groundwater:	Heptamaloxyloglucan
Surface water and sediment:	Heptamaloxyloglucan
Air:	Heptamaloxyloglucan

B.8.4.2. Residue definition for monitoring:

According to the B.6 and B.9 points of the RAR, there is no toxicologically or eco-toxicologically relevant residue identified.

B.8.5. MONITORING DATA CONCERNING FATE AND BEHAVIOUR OF THE ACTIVE SUBSTANCE, METABOLITES, DEGRADATION AND REACTION PRODUCTS

There is no toxicologically or eco-toxicologically relevant residue of heptamaloxyloglucan in any environmental compartment identified. Therefore, monitoring is not relevant.

No data was submitted or deemed necessary by the RMS.

B.8.6. REVIEW OF LITERATURE

B.8.6.1. Summary

Article 8(5) of Regulation (EC) No 1107/2009 requires applicants submitting dossiers for approval of active substances to provide relevant scientific peer reviewed open literature. This summary of scientific peer reviewed open literature conforms to EFSA guidance "Submission of scientific peer-reviewed open literature under Regulation (EC) No 1107/2009, EFSA Journal 2011; 9(2):2092".

A total of 35 references were identified for heptamaloxyloglucan for the fate and behaviour in environment and evaluated for potential relevance.
22 references were determined to be not relevant based on rapid assessment. 10 were determined to be not relevant based on detailed assessment. 3 studies were considered relevant and selected for inclusion in the dossier.

B.8.6.2. Search methods

B.8.6.2.1. Bibliographic database selection

Aim is to find scientific peer-reviewed open literature, as required by Article 8(5) of Regulation (EC) No 1107/2009, on heptamaloxyloglucan and its relevant metabolites dealing with toxicological and toxicokinetic studies, residues, fate and behaviour in the environment and ecotoxicological studies which are published within the last ten years from various data sources.

The literature review has been performed using a broad collection of relevant databases for the literature search (see Table B.8 (AS) - 6). The following databases have been consulted to complete the scientific peer-reviewed open literature on heptamaloxyloglucan and its degradation products:
1. Pubmed, 2. ScienceDirect, 3. Europe PMC, 4. Agricola

The search was analysed manually for each relevant reference without using dedicated software. Any removal of ambiguity or de-duplication was done manually based on the literature unique ID (e.g., PMID) or title of the literatures.

Table B.8 (AS) - 6: List of databases for the literature search of heptamaloxyloglucan

Database	Date of the Latest Database Update Included in the Search
1. Pubmed	2018/09
2. ScienceDirect	2018/09
3. Europe PMC	2018/09
4. Agricola	2018/09

Justifications for choosing these databases as literature sources are detailed in the following table.

Table B.8 (AS) - 7: List of databases / Justification for Choosing the Source

Database	Justification for Choosing the Source
1. Pubmed	PubMed comprises over 26 million citations for biomedical literature from MEDLINE, life science journals, and online books. PubMed citations and abstracts include the fields of biomedicine and health, covering portions of the life sciences, behavioral sciences, chemical sciences, and bioengineering. PubMed also provides access to additional relevant web sites and links to the other NCBI molecular biology resources.
2. ScienceDirect	ScienceDirect is a website providing subscription-based access to a large database of scientific and medical research. It hosts over 12 million pieces of content from 3,500 academic journals and 34,000 e-books. The journals are grouped into four main sections: Physical Sciences and Engineering, Life Sciences, Health Sciences, and Social Sciences and Humanities. Article abstracts are freely available, but access to their full texts (in PDF and, for newer publications, also HTML) generally require a subscription or pay-per-view purchase.
3. Europe PMC	Europe PMC is a repository, providing access to worldwide life sciences articles, books, patents and clinical guidelines. Europe PMC provides links to relevant records in databases such as Uniprot, European Nucleotide Archive (ENA), Protein Data Bank Europe (PDBE) and BioStudies.
4. Agricola (National Agricultural Library + National Agricultural Library-Articles)	AGRICOLA records describe publications and resources encompassing all aspects of agriculture and allied disciplines, including animal and veterinary sciences, entomology, plant sciences, forestry, aquaculture and fisheries, farming and farming systems, agricultural economics, extension and education, food and human nutrition, and earth and environmental sciences.

B.8.6.2.2. Search strategy

The main parameters that allow the characterization of the literature search are listed below. Trade names were not considered in the literature search as they are covered by the search on heptamaloxyloglucan: any trade name should be found without reference to the active substance.

The search identified scientific peer-reviewed open literatures published in last 10 years (≥ 2008).

Table B.8 (AS) - 8 presents the Section Specific Search terms for the database search on heptamaloxyloglucan. The information used for screening the selected databases to identify all relevant publications consists of common names, as far as available.
Active substance EL101GV is an oligosaccharide made of 7 glucidic monomer units. There are β-1,4 linkages on the main chain between the two D-glucopyranosyl units and terminal D-glucitol, and α-1,2, β -1,2 and α -1,6 linkages between the various monomer units present in side chains. The latter side chain-monomers are D-xylopyranosyl (α -1,6-linked to D-glucopyranosyl), D-galactopyranosyl (β-1,2-linked to D-xylopyranosyl) and L-fucopyranosyl (α -1,2-linked to D-galactopyranosyl). Therefore these terms have been used for the literature search.

Table B.8 (AS) - 8: Section Specific Search terms for the compilation of the database

Database	Specific Search terms
1_Pubmed Hepta-sorbi	In abstract: Heptamaloxyloglucan Oligoxyloglucan Heteroglycan Xyloglucan In title: Saccharide Oligosaccharide Monosaccharide Sorbitol
2_Pubmed glucitolxylose	In title: Xylose Glucitol
3_Pubmed Pyran	In title Glucopyranosyl Glucopyranose Xylopyranosyl Xylopyranose Galactopyranosyl Galactopyranose Fucopyranosyl Fucopyranose
4_ScienceDirect Xyloglyca	In title Heptamaloxyloglucan OR Oligoxyloglucan OR Heteroglycan OR Xyloglucan

Database	Specific Search terms
5_ScienceDirect Sacch	Saccharide OR Oligosaccharide OR Monosaccharide OR Sorbitol OR Xylose OR Glucopyranosyl OR Glucopyranose OR Glucitol
6_ScienceDirect pyran	Xylopyranosyl OR Xylopyranose OR Galactopyranosyl OR Galactopyranose OR Fucopyranosyl OR Fucopyranose
7_EuropePMC hepta	(ABSTRACT:"oligoxyloglucan" OR ABSTRACT:"heptamaloxyloglucan" OR ABSTRACT:"heteroglycan" OR ABSTRACT:"xyloglucan" OR TITLE:"saccharide" OR TITLE:"oligosaccharide" OR TITLE:"monosaccharide") AND (FIRST_PDATE:[2008-01-01 TO 2018-09-24])
8_EuropePMC xylose	(TITLE:"sorbitol" OR TITLE:"xylose" OR TITLE:"glucopyranosyl" OR TITLE:"glucopyranose" OR TITLE:"glucitol" OR TITLE:"xylopyranosyl" OR TITLE:"xylopyranose" OR TITLE:"galactopyranosyl" OR TITLE:"galactopyranose" OR TITLE:"fucopyranosyl" OR TITLE:"fucopyranose") AND (FIRST_PDATE:[2008-01-01 TO 2018-09-24])
9_NAL hept	In any field: Heptamaloxyloglucan Oligoxyloglucan Heteroglycan Xyloglucan In title: Saccharide Oligosaccharide Monosaccharide Sorbitol
10_NAL pyran	In title Xylose glucitol Glucopyranosyl Glucopyranose Xylopyranosyl Xylopyranose Galactopyranosyl Galactopyranose Fucopyranosyl Fucopyranose
11_NAL articles hepta	In any field: Heptamaloxyloglucan Oligoxyloglucan Heteroglycan Xyloglucan
12_NAL articles sacc	In title: Saccharide Oligosaccharide Monosaccharide Sorbitol glucitol
13_NAL articles pyran	In title Xylose Glucopyranosyl Glucopyranose Xylopyranosyl Xylopyranose Galactopyranosyl Galactopyranose Fucopyranosyl Fucopyranose

B.8.6.3. Results

A total of 17 611 summary records was retrieved before removing duplicates, divided as:

Table B.8 (AS) - 9: List of publications after first search and compilation of all databases

Database	Specific Search terms	First search
1_Pubmed Hepta-sorbi	In abstract: Heptamaloxyloglucan OR Oligoxyloglucan OR Heteroglycan OR Xyloglucan OR In title: Saccharide OR Oligosaccharide OR Monosaccharide OR Sorbitol AND 01/01/2008 – 23/10/2018	2 731
2_Pubmed glucitolxylose	In title: Xylose OR Glucitol AND 01/01/2008 – 23/09/2018	1 136
3_Pubmed Pyran	In title Glucopyranosyl OR Glucopyranose OR Xylopyranosyl OR Xylopyranose OR Galactopyranosyl OR Galactopyranose OR Fucopyranosyl OR Fucopyranose AND 01/01/2008 – 07/09/2018	352
4_ScienceDirect Xyloglyca	In title, abstract, or author-specified keywords Heptamaloxyloglucan OR Oligoxyloglucan OR Heteroglycan OR Xyloglucan AND 2008-2018	368

Database	Specific Search terms	First search
5_ScienceDirect Sacch	In title Saccharide OR Oligosaccharide OR Monosaccharide OR Sorbitol OR Xylose OR Glucopyranosyl OR Glucopyranose OR Glucitol AND 2008-2018	3 032
6_ScienceDirect pyran	In title Xylopyranosyl OR Xylopyranose OR Galactopyranosyl OR Galactopyranose OR Fucopyranosyl OR Fucopyranose AND 2008-2018	56
7_EuropePMC hepta	(ABSTRACT:"oligoxyloglucan" OR ABSTRACT:"heptamaloxyloglucan" OR ABSTRACT:"heteroglycan" OR ABSTRACT:"xyloglucan" OR TITLE:"saccharide" OR TITLE:"oligosaccharide" OR TITLE:"monosaccharide") AND (SRC:"AGR" OR SRC:"CTX" OR SRC:"PAT" OR SRC:"PPR" OR SRC:"MED")[10] AND (FIRST_PDATE:[2008-01-01 TO 2018-09-24])	2 949
8_EuropePMC xylose	(TITLE:"sorbitol" OR TITLE:"xylose" OR TITLE:"glucopyranosyl" OR TITLE:"glucopyranose" OR TITLE:"glucitol" OR TITLE:"xylopyranosyl" OR TITLE:"xylopyranose" OR TITLE:"galactopyranosyl" OR TITLE:"galactopyranose" OR TITLE:"fucopyranosyl" OR TITLE:"fucopyranose") AND (FIRST_PDATE:[2008-01-01 TO 2018-09-24])	2 136
9_NAL hept	In any field: Heptamaloxyloglucan Oligoxyloglucan Heteroglycan Xyloglucan In title: Saccharide Oligosaccharide Monosaccharide Sorbitol (search done 09/2018)	23
10_NAL pyran	In title Xylose glucitol Glucopyranosyl Glucopyranose Xylopyranosyl Xylopyranose Galactopyranosyl Galactopyranose Fucopyranosyl Fucopyranose (search done 09/2018)	12
11_NAL articles hepta	In any field: Heptamaloxyloglucan Oligoxyloglucan Heteroglycan Xyloglucan (search done 09/2018)	1 073
12_NAL articles sacc	In title: Saccharide Oligosaccharide Monosaccharide Sorbitol glucitol (search done 09/2018)	1 986
13_NAL articles pyran	In title Xylose Glucopyranosyl Glucopyranose Xylopyranosyl Xylopyranose Galactopyranosyl Galactopyranose Fucopyranosyl Fucopyranose (search done 09/2018)	1 757
Total	**(Total search in Excel sheet KCA 9.3/01)**	**17 611**

The total of publications before removing duplicates was summarised in the following table:

[10] AND Sources: Agricola (USDA/NAL), CiteXplore records, Patents, Preprint records, PubMed/MEDLINE (NKM)

Table B.8 (AS) - 10: List of publications after removing of too old literature (before 2007)

	Heptamaloxyloglucan
Total number of publications retrieved (with duplicates) (global search results)	17 611
Total number of too old publications (Before 2008 in Excel sheet KCA 9.3/01)	2 670
Total number of publications retrieved removing too old literature	**14 941**

After removing duplicates, the total number of publications is presented below:

Table B.8 (AS) - 11: List of publications after removing duplicates/triplicates

	Heptamaloxyloglucan
Total number of publications retrieved removing too old literature	14 941
Total removed publications (duplicates/triplicates – Duplicate title in Excel sheet KCA 9.3/01)	8 977
Total number of publications retrieved after removing of duplicates	**5 964**

B.8.6.4. Evaluation

B.8.6.4.1. Rapid assessment on the literature review

A rapid assessment based on the reading of the titles allows performing a first selection.
The number of obviously irrelevant publications appears in Table B.8 (AS) - 12, documenting the study selection process.

The literature search was done on all criteria referred to fate and behaviour in the environment (OECD II 7.1 to 7.13). Indeed, all publications were reviewed one by one according to the title. If a publication was considered as relevant according to the title for environmental fate purpose (soil, water, sediment, air), then its abstract and after its full text were reviewed. Within the relevant studies according to the title, only the publications which could have an interest in term of risk assessment (according to the abstract and then the full-text) have been kept.

Table B.8 (AS) - 12: List of publications after rapid assessment

	HEPTAMALOXYLOGLUCAN			
Study selection process	Toxicology	Residues	E-Fate	Ecotoxicology
Number of publications excluded after rapid assessment for relevance according to title (not relevant according to title in Excel sheet KCA 9.3/01)	5 731			
Number of publications further assessed in detail (possible relevant literature for at least one section according to title – Relevant according to title)	**233**			

B.8.6.4.2. Detailed assessment of the literature review

Those publications, which have passed the rapid assessment, have been evaluated based on abstract and full text versions.

Table B.8 (AS) - 13: Results of the study selection process for environmental fate section

Assessment according to title	Number of possible relevant publications (title relevant for at least one section)	35
Assessment "section"	Number of publications further assessed according to abstract for respective section (possible relevant literature for this section)	35
	Number of publications excluded according to irrelevance of abstract for respective section (excluded literature for this section)	22
	Number of publications further assessed according to full-text for respective section (possible relevant literature for this section)	13
	Number of publications excluded according to irrelevance of full-text for respective section (excluded literature for this section)	10
	Number of publications not excluded for relevance after detailed assessment (i.e. relevant publications) (included literature)	3

The results of the detailed assessment are shown in Table B.8 (AS) - 14 to Table B.8 (AS) - 16.

The list of references relevant according to title but excluded after detailed assessment of abstract is presented in the following table.

Table B.8 (AS) - 14: Report of studies relevant according to title but excluded after detailed assessment of abstract

Author(s)	Year	Title	Source	Reason for not including in dossier
ENVIRONMENT				
-	2016	Fructose and sorbitol	Elsevier 460-461	information from a book, not fully available
Buckeridge, M. S.	2010	Seed cell wall storage polysaccharides: models to understand cell wall biosynthesis and degradation	Plant Physiol 154 1017-23	non relevant according to abstract (biochemical mechanisms in seeds)
Cheng, Jun; Song, Wenlu; Xia, Ao; Su, Huibo; Zhou, Junhu; Cen, Kefa	2012	Sequential generation of hydrogen and methane from xylose by two-stage anaerobic fermentation	International Journal of Hydrogen Energy 37 13323-13329	non relevant according to abstract (industrial production of hydrogen and methane)
Combo, Agnan Marie Michel; Aguedo, Mario; Quiévy, Nicolas; Danthine, Sabine; Goffin, Dorothée; Jacquet, Nicolas; Blecker, Christophe; Devaux, Jacques; Paquot, Michel	2013	Characterization of sugar beet pectic-derived oligosaccharides obtained by enzymatic hydrolysis	International Journal of Biological Macromolecules 52 148-156	non relevant according to abstract (only characterization)
Constantin, Julian Gelman; Schneider, Matthias; Corti, Horacio R.	2016	Glass Transition Temperature of Saccharide Aqueous Solutions Estimated with the Free Volume/Percolation Model	Journal of physical chemistry 120 5047-5055	non relevant according to abstract (chemical parameters)
Forgo, Peter; Kiss, Attila; Korózs, Marietta; Rapi, Sándor	2013	Thermal degradation and consequent fragmentation of widely applied oligosaccharides	Microchemical Journal 107 37-46	non relevant according to abstract (temperature of degradation are too high compared to natural ones)
Galvão, Alessandro Cazonatto; da Silva Robazza, Weber; Arce, Pedro Felipe; Mocelin, Adriane; Paludo, Ananda Regina	2017	Experimental study and thermodynamic modeling of xylitol and sorbitol solubility in mixtures of methanol and ethanol at different temperatures	Journal of Molecular Liquids 248 509-514	non relevant according to abstract (alcohol solutions of xylose and glucitol)

Author(s)	Year	Title	Source	Reason for not including in dossier
Hou, X.	2012	Anaerobic xylose fermentation by Spathaspora passalidarum	Applied microbiology and biotechnology 94 205-214	non relevant according to abstract (industrial strain of yeasts)
Joe Shaw, A.; Jenney, Francis E.; Adams, Michael W. W.; Lynd, Lee R.	2008	End-product pathways in the xylose fermenting bacterium, Thermoanaerobacterium saccharolyticum	Enzyme and Microbial Technology 42 453-458	non relevant according to abstract (industrial production)
Le Gall, H.; Philippe, F.; Domon, J. M.; Gillet, F.; Pelloux, J.; Rayon, C.	2015	Cell Wall Metabolism in Response to Abiotic Stress	Plants (Basel) 4 112-66	non relevant according to abstract (data on biochemical mechanisms)
Meijnen, J. P.; de Winde, J. H.; Ruijssenaars, H. J.	2009	Establishment of oxidative D-xylose metabolism in Pseudomonas putida S12	Appl Environ Microbiol 75 2784-91	non relevant according to abstract (modified bacteria strain)
Mouro, Adriane; Cadete, Raquel M.; Santos, Renata O.; Rosa, Carlos A.; Stambuk, Boris U.	2014	Xylose and cellobiose fermentation by yeasts isolated from the Brazilian biodiversity	BMC proceedings 8 P202-P202	non relevant according to abstract (fuel ethanol bioproduction)
Nishinari, K.; Takemasa, M.; Yamatoya, K.; Shirakawa, M.	2009	19 - Xyloglucan	Woodhead Publishing 535-566	non relevant according to abstract (data not relevant for efate purpose)
Ren, Nanqi; Cao, Guangli; Wang, Aijie; Lee, Duu-Jong; Guo, Wanqian; Zhu, Yuhong	2008	Dark fermentation of xylose and glucose mix using isolated Thermoanaerobacterium thermosaccharolyticum W16	International Journal of Hydrogen Energy 33 6124-6132	non relevant according to abstract (thermophilic strain)
Sparkman, O. David; Penton, Zelda E.; Kitson, Fulton G.	2011	Chapter 35 - Sugars (Monosaccharides)	Academic Press 407-410	non relevant according to abstract (analytical method review)
Takahashi, Machiko; Yamamoto, Ryoichi; Sakurai, Naoki; Nakano, Yuki; Takeda, Takumi	2015	Fungal hemicellulose-degrading enzymes cause physical property changes concomitant with solubilization of cell wall polysaccharides	Planta 241 359-370	non relevant according to abstract (biochemical mechanisms in wheat)
Tani, Tatsunori; Taguchi, Hisataka; Akamatsu, Takashi	2017	Analysis of metabolisms and transports of xylitol using xylose- and xylitol-assimilating Saccharomyces cerevisiae	Journal of bioscience and bioengineering	non relevant according to abstract (recombined strain of yeasts)
Wang, Yan; Guo, Qingbin; Douglas Goff, H.; LaPointe, Gisèle	2018	Oligosaccharides: Structure, Function and Application	Elsevier	information from a book, not fully available
Wasserstrom, L.; Portugal-Nunes, D.; Almqvist, H.; Sandstrom, A. G.; Liden, G.; Gorwa-Grauslund, M. F.	2018	Exploring D-xylose oxidation in Saccharomyces cerevisiae through the Weimberg pathway	AMB Express 8 33	non relevant according to abstract (biochemical pathway in modified genetic yeast strain)
Zabotina, O. A.	2012	Xyloglucan and its biosynthesis	Front Plant Sci 3 134	non relevant according to abstract (only biosynthesis)

Author(s)	Year	Title	Source	Reason for not including in dossier
Zhang, Y.	2008	Étude des interactions entre oligosaccharides impliqués dans l'adhésion cellulaire. Exemple du trisaccharide Lewisx	Annales Pharmaceutiques Françaises 66 319-324	non relevant according to abstract (biochemical mechanism)
Zhi, Wenbiao; Hu, Yonghong; Yang, Wenge; Kai, Yumei; Cao, Zheng	2013	Measurement and correlation of solubility of D-sorbitol in different solvents	Journal of Molecular Liquids 187 201-205	non relevant according to abstract (solubility in organic solvents)

The list of references excluded after detailed assessment of full-text is presented in the following table.

Table B.8 (AS) - 15: Report of studies relevant according to abstract but excluded after detailed assessment of full-text

Author(s)	Year	Title	Source	Reason for not including in dossier
ENVIRONMENT				
Ait Lahmidi, N.; Courty, P. E.; Brule, D.; Chatagnier, O.; Arnould, C.; Doidy, J.; Berta, G.; Lingua, G.; Wipf, D.; Bonneau, L.	2016	Sugar exchanges in arbuscular mycorrhiza: RiMST5 and RiMST6, two novel Rhizophagus irregularis monosaccharide transporters, are involved in both sugar uptake from the soil and from the plant partner	Plant Physiol Biochem Vol 107 Pages 354-363	not relevant: No interesting information on active substance or a derivated active substance.
Attia, Mohamed; Stepper, Judith; Davies, Gideon J.; Brumer, Harry	2016	Functional and structural characterization of a potent GH74 endo-xyloglucanase from the soil saprophyte Cellvibrio japonicus unravels the first step of xyloglucan degradation	FEBS journal Vol 283 Pages 1701-1719	not relevant - Biochemical study. No interest for efate part.
Pepe-Ranney, C.; Campbell, A. N.; Koechli, C. N.; Berthrong, S.; Buckley, D. H.	2016	Unearthing the Ecology of Soil Microorganisms Using a High Resolution DNA-SIP Approach to Explore Cellulose and Xylose Metabolism in Soil	Front Microbiol Vol 7 Page 703	not relevant - Biochemical study. No interest for efate part.
Procentese, Alessandra; Raganati, Francesca; Olivieri, Giuseppe; Russo, Maria Elena; Salatino, Piero; Marzocchella, Antonio	2015	Continuous xylose fermentation by Clostridium acetobutylicum – Assessment of solventogenic kinetics	Bioresource Technology Vol 192 Pages 142-148	not relevant - No information on the degradation of xylose.
Rushdi, Ahmed I.; Oros, Daniel R.; Al-Mutlaq, Khalid F.; He, Ding; Medeiros, Patricia M.; Simoneit, Bernd R. T.	2016	Lipid, sterol and saccharide sources and dynamics in surface soils during an annual cycle in a temperate climate region	Applied Geochemistry Vol 66	not relevant - The saccharides studied are not interesting for this dossier
Sack, E. L.; van der Wielen, P. W.; van der Kooij, D.	2011	Flavobacterium johnsoniae as a model organism for characterizing biopolymer utilization in oligotrophic freshwater environments	Appl Environ Microbiol Vol 77 Pages 6931-8	not relevant - No interest for environmental fate.
Stick, Robert V.; Williams, Spencer J.	2009	Chapter 6 - Monosaccharide Metabolism	Elsevie	not relevant - only description of monosaccharide metabolism
Stick, Robert V.; Williams, Spencer J.	2009	Chapter 9 - Disaccharides, Oligosaccharides and Polysaccharides	Elsevier Pages 321-341	not relevant - only description of the different disaccharides, oligosaccharides and polysaccharides such as cellulose, starch, glycogen, sucrose, fructans, mannans, etc. but no specific data linked to apple or grape

Author(s)	Year	Title	Source	Reason for not including in dossier
Temudo, M. F.; Mato, T.; Kleerebezem, R.; van Loosdrecht, M. C.	2008	Xylose anaerobic conversion by open-mixed cultures	Appl Microbiol Biotechnol Vol 82 Pages 231-9	not relevant - Biochemical study. No interest for efate part.
Werner, Kajsa; Pommer, Linda; Broström, Markus	2014	Thermal decomposition of hemicelluloses	Journal of Analytical and Applied Pyrolysis Vol 110 Pages 130-137	not relevant - The temperature of degradation is not compatible with an real environemental degradation.

The list of relevant studies after detailed assessment of full-text documents is presented in the following table.

Table B.8 (AS) - 16: Report of all relevant studies after detailed assessment of full-text documents

KCA - SANCO Data Point	Author(s)	Year	Title	Source	Classification of study
ENVIRONMENT					
KCA 7.1	Galloway, Andrew F.; Pedersen, Martin J.; Merry, Beverley; Marcus, Susan E.; Blacker, Joshua; Benning, Liane G.; Field, Katie J.; Knox, J. Paul	2018	Xyloglucan is released by plants and promotes soil particle aggregation	new phytologist Vol 217 Pages 1128-1136	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 7.1	Valinhas, R. V.; Pantoja, L. A.; Maia, A. C. F.; Miguel, Mgcp; Vanzela, Apfc; Nelson, D. L.; Santos, A. S.	2018	Xylose fermentation to ethanol by new Galactomyces geotrichum and Candida akabanensis strains	PeerJ Vol 6 Page e4673	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 7.1	Veras, H. C. T.; Parachin, N. S.; Almeida, J. R. M.	2017	Comparative assessment of fermentative capacity of different xylose-consuming yeasts	Microb Cell Fact Vol 16 Page 153	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.

B.8.6.5. Conclusion

In the frame of this literature search for the active substance heptamaloxyloglucan, 5 964 references were identified and evaluated for their potential relevances for the data requirements of all sections.
In conclusion, 3 studies were considered relevant for the environmental fate and behaviour section.

Comments (RMS)

RMS notes that the literature studies Brandt et al. (2010) and Lin et al. (2015) reported in point B.8.1.1.1.3 as well as studies Freixa et al. (2016), Romani et al. (2012) and Younes et al. (2015) reported in point B.8.2.2.3 are not listed among the total 17 611 results of the search. These studies were provided after submission of the initial RAR dossier, in response to questions from the RMS regarding the photodegradation and fate and behaviour in water and sediment. Therefore, it cannot be excluded that other relevant studies were not retrieved. However, the information already collected on heptamaloxyloglucan is deemed sufficient and no additional literature search is deemed necessary.

B.8.7. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used Y/N If yes, for which data point?
CA 7.1.1.1/01	Warren R.A.J.	1996	Microbial hydrolysis of polysaccharides GLP: No Published: Yes	N	N	-	Literature study	Y, same data point
CA 7.1.1.1/02	De Vries R. & Visser J.	2001	Aspergillus enzymes involved in degradation of plant cell wall polysaccharides GLP: No Published: Yes	N	N	-	Literature study	Y, same data point
CA 7.1.1.1/03	Wershaw R.	2004	Evaluation of conceptual models of natural organic matter (humus) from a consideration of the chemical and biochemical processess of humification GLP: No Published: Yes	N	N	-	Literature study	Y, same data point
CA 7.1.1.1/04	Karroum M. et al.	2004	Importance and fate of biopolymers (lignins and polysaccharides) in soils of Fagus sylvatica stands of various ages in Fougères forest GLP: No Published: Yes	N	N	-	Literature study	Y, same data point
CA 7.1.1.1/05	Pérez J. & Muñoz-Dorado J.	2002	Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview GLP: No Published: Yes	N	N	-	Literature study	Y, same data point
CA 7.1.1.1/06	Aro N. et al.	2005	Transcriptional regulation of plant cell wall degradation by filamentous fungi GLP: No Published: Yes	N	N	-	Literature study	Y, same data point
CA 7.1.1.1/07	Tuomela M. et al.	2000	Biodegradation of lignin in a compost environment: a review GLP: No Published: Yes	N	N	-	Literature study	Y, same data point
CA 7.1.1.1/08	Kelker N.E. & Anderson R.L.	1971	Sorbitol metabolism in Aerobacter aerogenes GLP: No Published: Yes	N	N	-	Literature study	Y, same data point
CA 7.1.1.1/09	Brechtel E. et al.	2002	L-Glucitol catabolism in Stenotrophomonas maltophilia Ac GLP: No	N	N	-	Literature study	Y, same data point

			Published: Yes					
CA 7.1.1.1/10	Galloway A. et al.	2018	Xyloglucan is released by plants and promotes soil particle aggregation GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.1.1.1/11	Valinhas et al.	2018	Xylose fermentation to ethanol by new Galactomyces geotrichum and Candida akabanensis strains. GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.1.1.1/12	Veras H.C. et al.	2017	Comparative assessment of fermentative capacity of different xylose-consuming yeasts. GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.1.1.1/13	Yamaguchi et al.	2000	Differences in the recognition of glucan elicitor signals between rice and soybean: b-glucan fragments from the rice blast disease fungus pyricularia oryzae that elicit phytoalexin biosynthesis in suspension-cultured rice cells GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.1.1.3/01	Baker, N. R., & Allison, S. D.	1996	Ultraviolet photodegradation facilitates microbial litter decomposition in a Mediterranean climate GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.1.1.3/02	L. A. Brandt, J. Y. King, S. E. Hobbie, D. G. Milchunas and R. L. Sinsabaugh	2010	The Role of Photodegradation in Surface Litter Decomposition Across a Grassland Ecosystem Precipitation Gradient GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.1.1.3/03	Yang Lin . Jennifer Y. King . Steven D. Karlen . John Ralph	2015	Using 2D NMR spectroscopy to assess effects of UV radiation on cell wall chemistry during litter decomposition GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.1.1/ additional information	Havet and Salvador	2007	Heptamaloxyloglucan quantification in pomace enzymatic hydrolysis solution GLP: No Published: No	N	N	-	Elicityl	N
CA 7.2.1.1/01	Ricau H.	2005	Abiotic degradation on the technical heptamaloxyloglucan, pH dependent hydrolysis (Test C7) GLP: Yes	N	N	-	Elicityl	Y, same data point

			Published: No					
CA 7.2.2.1/01	L'Haridon, J.	2006	Determination of the ready biodegradability CO2 evolution test GLP: Yes Published: No	N	N	-	Elicityl	Y, same data point
CA 7.2.2.3/01	A. Freixa, E. Ejarque,S. Crognale, S. Amalfitano, S. Fazi, A. Butturini, A. M. Romani	2016	Sediment microbial communities rely on different dissolved organic matter sources along a Mediterranean river continuum GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.2.2.3/02	Anna M. Romani, Stefano Amalfitano, Joan Artigas, Stefano Fazi, Sergi Sabater, Xisca Timoner, Irene Ylla, Annamaria Zoppini	2012	Microbial biofilm structure and organic matter use in Mediterranean streams GLP: No Published: Yes	N	N	-	Literature study	N
CA 7.2.2.3/03	Younes K., Abdelli G., Araji N., Grasset L.	2015	Comparison of thermochemical and chemical methods for the analysis of carbohydrates in an ombrotrophic peatland GLP: No Published: Yes	N	N	-	Literature study	N
Impact of water treatment procedure/01	Wang W., Zhang C., Tong S., Cui Z., Liu P	2018	Enhanced enzymatic hydrolysis and structural features of corn stover by NAOH and ozone combined pretreatment GLP: No Published: Yes	N	N	-	Literature study	N
Impact of water treatment procedure/02	Yao S., Wang C., Gao C., Shi L. Nie S. Qin C.	2018	Molecular simulation of reaction mechanism for hemicellulose model compound during chlorine dioxine bleaching GLP: No Published: Yes	N	N	-	Literature study	N