

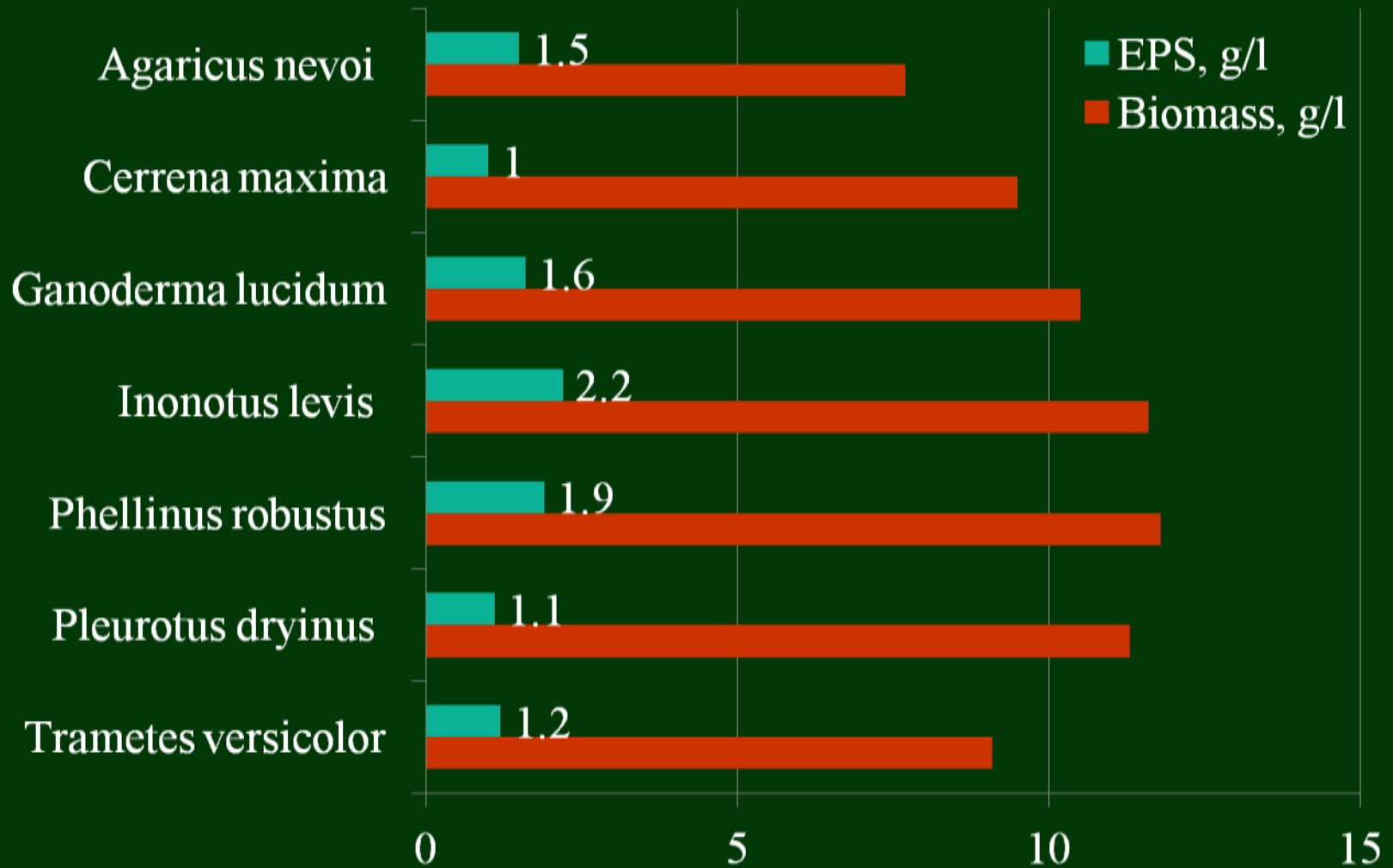
Biotechnological potential of wood-rotting basidiomycetes

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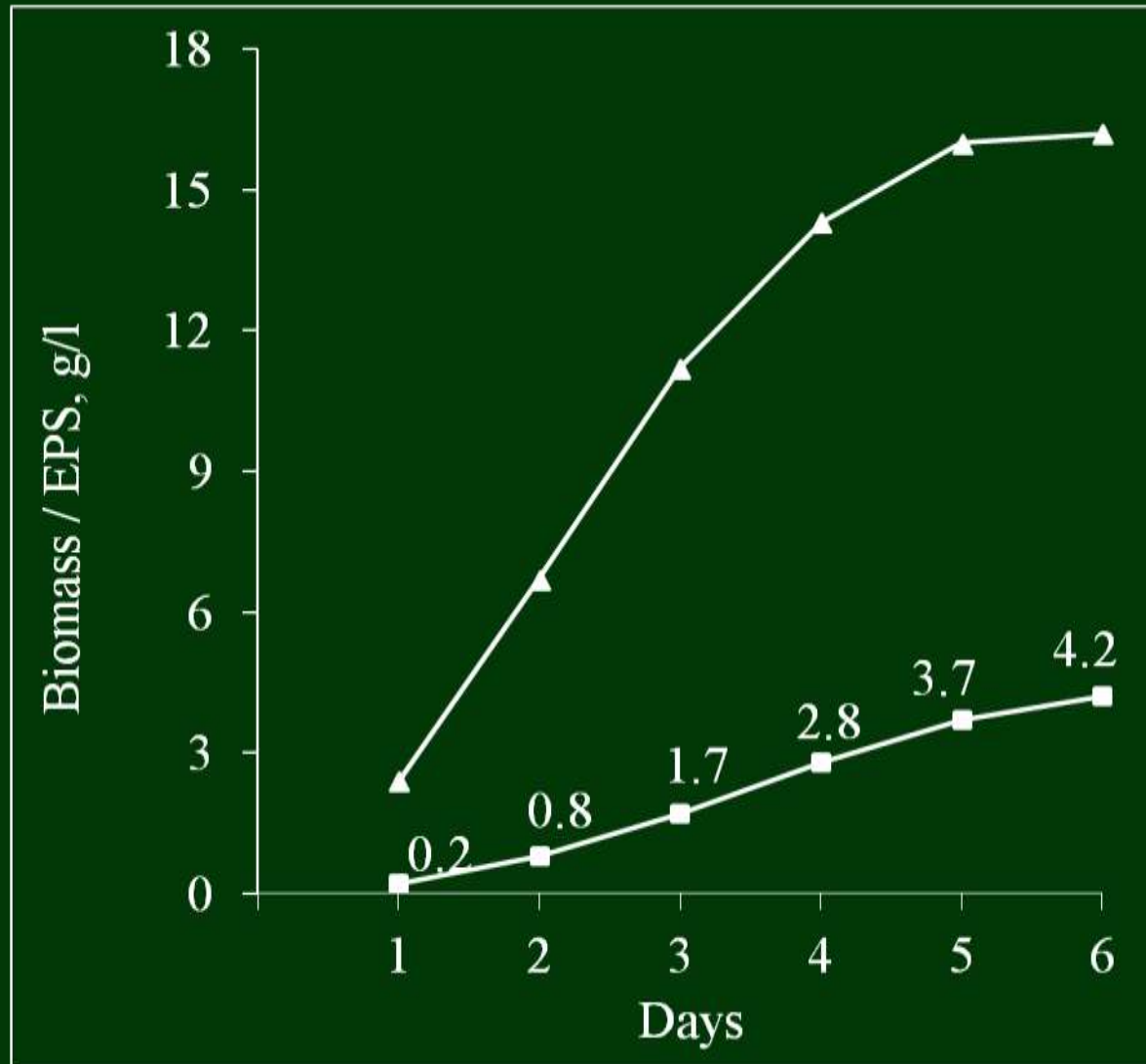
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Biomass and EPS production in basidiomycetes submerged cultivation (2% glucose)



Profiles of *Inonotus levis* biomass (▲) and EPS (■) in laboratory fermenter



Basidiomycetes lectin activity in submerged and solid-state fermentation of wheat bran

Fungi	HA titer (T ⁻¹)		Specific HA (U mg ⁻¹)	
	SSF	SF	SSF	SF
<i>C. unicolor</i> 301	512	64	4761	1123
<i>F. fomentarius</i> 9	32	32	769	361
<i>F. trogii</i> 146	128	2	323	23
<i>G. applanatum</i> 25	16384	16	166667	286
<i>P. ostreatus</i> 108	256	32	2734	332
<i>P. coccineus</i> 310	4096	16	4462	57
<i>T. versicolor</i> 5	64	2	521	43

Effect of lignocellulosic substrates on the *C. unicolor* biomass and culture liquid lectin activity

Substrate	Specific hemagglutinating activity (U mg ⁻¹)				
	Biomass		Culture liquid		
Strains	301	302	301	302	
Wheat bran	1123	4000	3333	274	
EPR	847	303	455	0	
Mandarin peels	1785	625	1667	196	
Banana peels	641	714	3846	222	
Walnut leaves	7692	2500	10000	34	
Walnut pericarp	5882	3030	217	31	

Basidiomycetes submerged biomass lectins sugar specificity

Fungi	Specificity to sugars
<i>Cerrena unicolor</i>	Gal, GalNAc, Mal
<i>Daedalea quercina</i>	Lac, Arab
<i>Ganoderma applanatum</i>	Mal, Arab, Man
<i>Omphalotus olearius</i>	Rham, Arab, GalNAc
<i>Trametes versicolor</i>	Gal, GalNAc, Rham

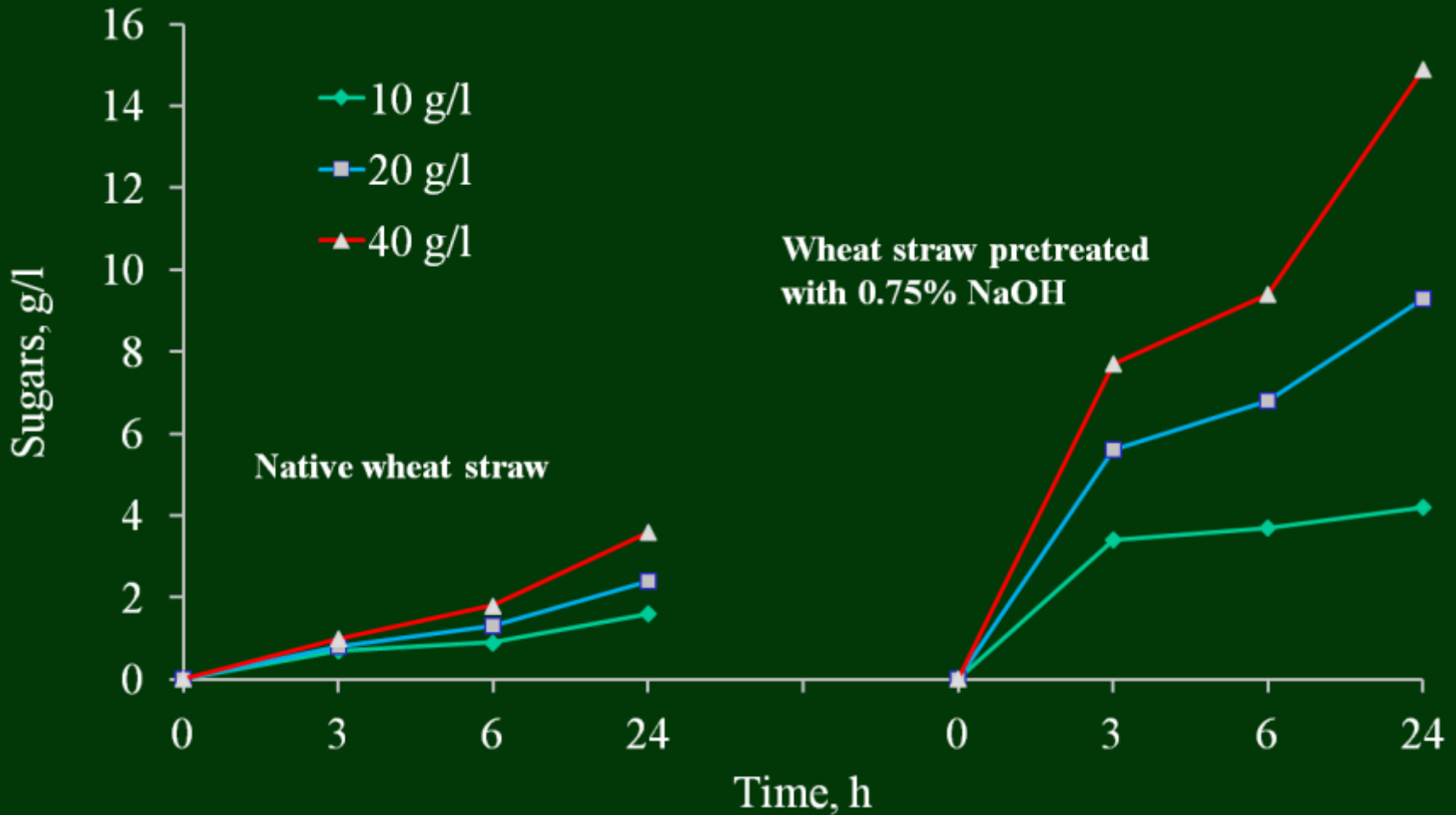
Antioxidant activity of extracts from submerged mycelia (2 mg/ml)

Fungi	Water extract	Ethanol extract
<i>Agrocybe aegerita</i>	65.8	46.6
<i>Armillaria mellea</i>	77.0	71.3
<i>Agaricus nevoi</i>	86.9	92.1
<i>Coprinus comatus</i>	26.0	0
<i>Flammulina velutipes</i>	85.3	77.3
<i>Ganoderma lucidum</i>	81.2	77.0
<i>Pleurotus eyingii</i>	62.5	71.5
<i>Phellinus robustus</i>	38.8	17.6
<i>Trametes versicolor</i>	29.5	72.8
BHA, 1 mg/ml		95

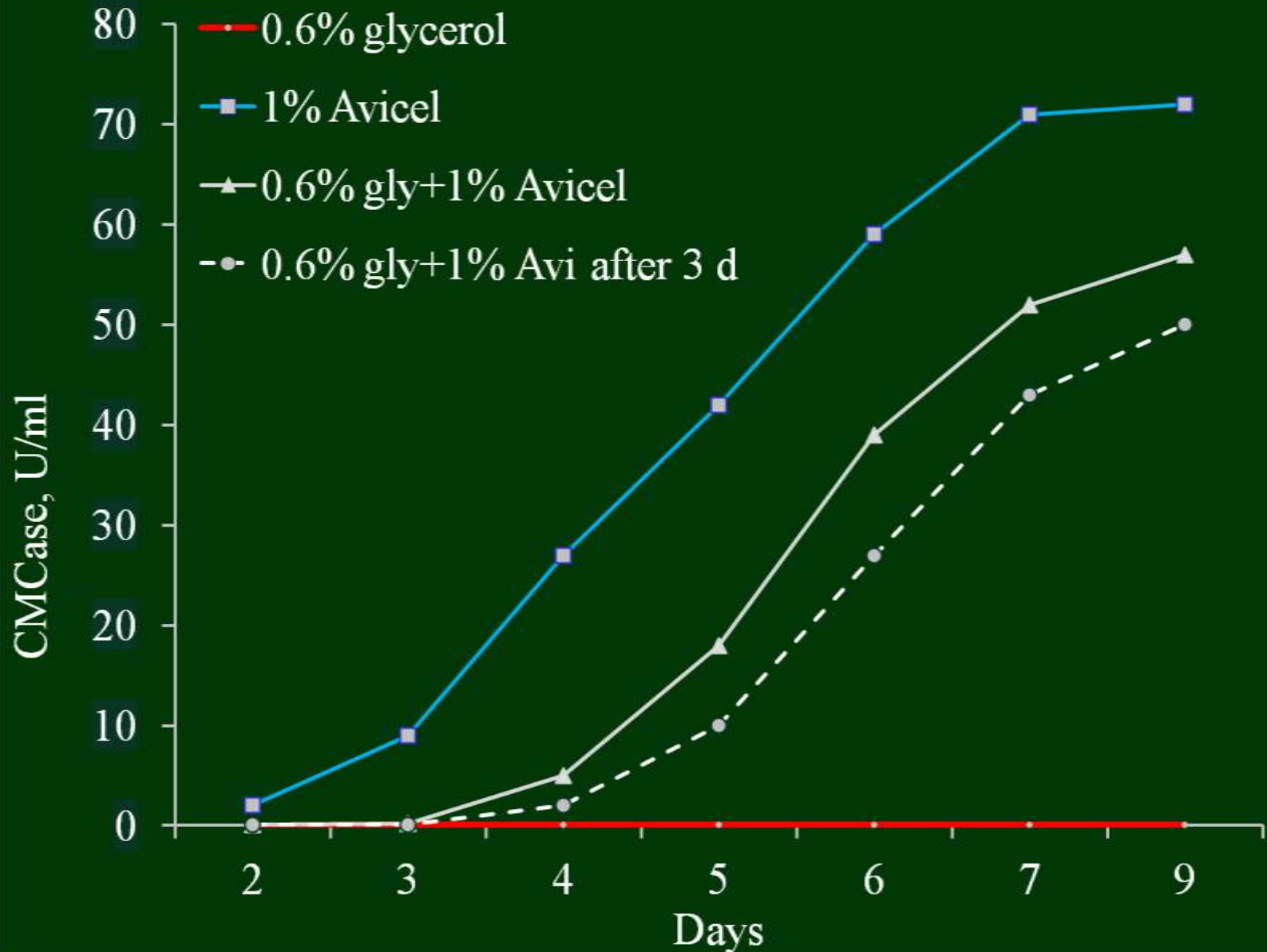
Basidiomycetes hydrolytic enzymes activity

Fungi	CMCase (U ml ⁻¹)	Xylanase (U ml ⁻¹)	FPA (U ml ⁻¹)
<i>I. lacteus</i> (1.5% Avicel)	86	96	4.9
(1.5% Avicel +4% MP)	187	137	6.5
<i>P. coccineus</i> (1.5% A)	79	109	6.1
(1.5% Avicel +4% MP)	90	79	8.9
<i>S. commune</i> (1.5% A)	53	678	5.4
(1.5% Avicel +4% MP)	71	280	7.3

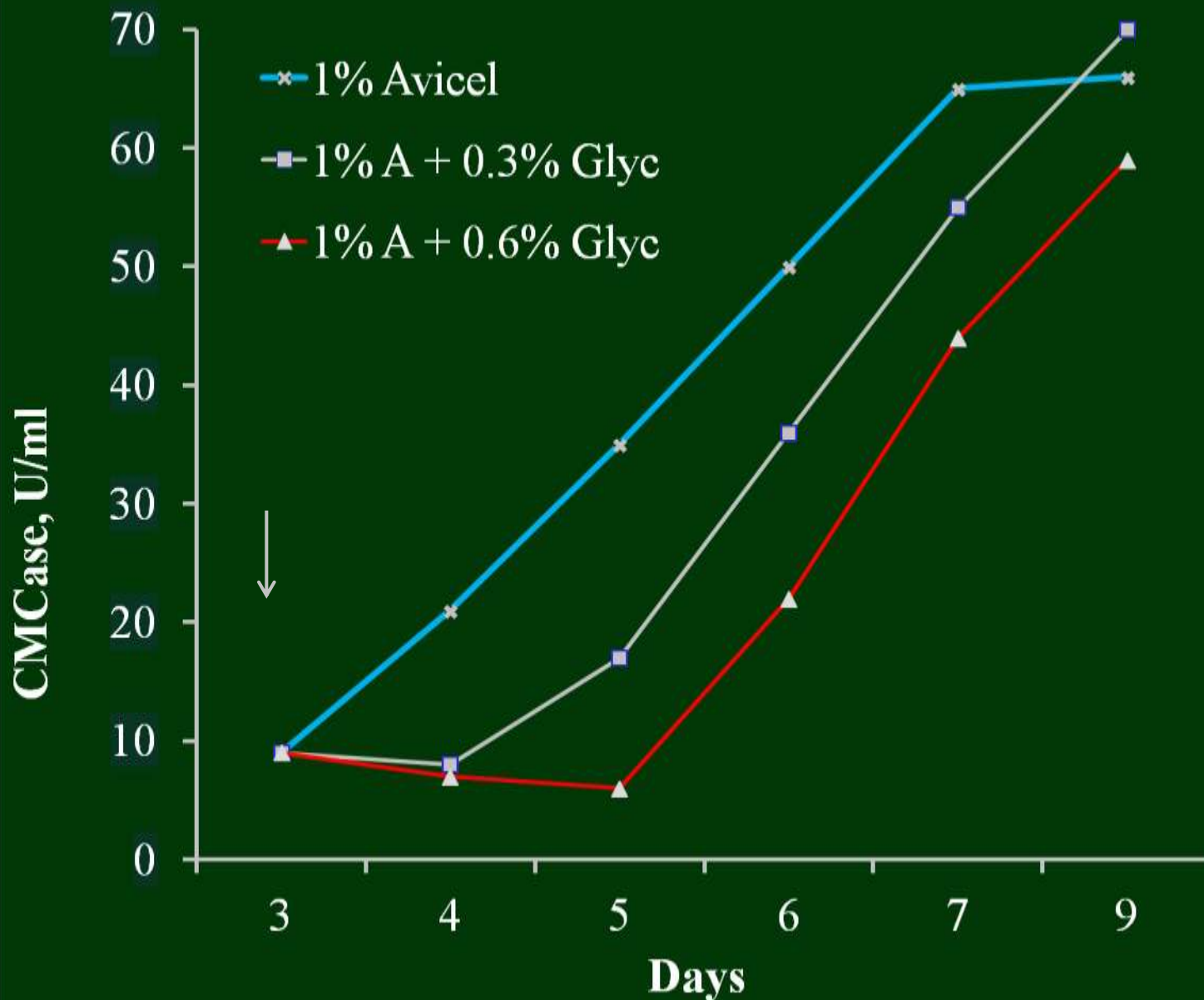
Saccharification of native and pretreated wheat straw (Enzyme load - 10 FPU/g)



Induction of *P. coccineus* cellulase synthesis



P. coccineus cellulase synthesis catabolite repression



Basidiomycetes enzyme activity in submerged fermentation of ethanol production residue

Fungi	Laccase (U ml ⁻¹)	MnP (U ml ⁻¹)
<i>Cerrena unicolor</i> 300	93.1	2.41
<i>Fomes fomentarius</i> 9	1.0	0.08
<i>Ganoderma lucidum</i> 447	61.5	0.10
<i>Omphalotus olearius</i> 174	1.3	0.12
<i>Phellinus robustus</i> 250	0.9	4.40
<i>Trametes versicolor</i> 13	19.0	0.90
<i>Trametes zonata</i> 540	0.4	0.10

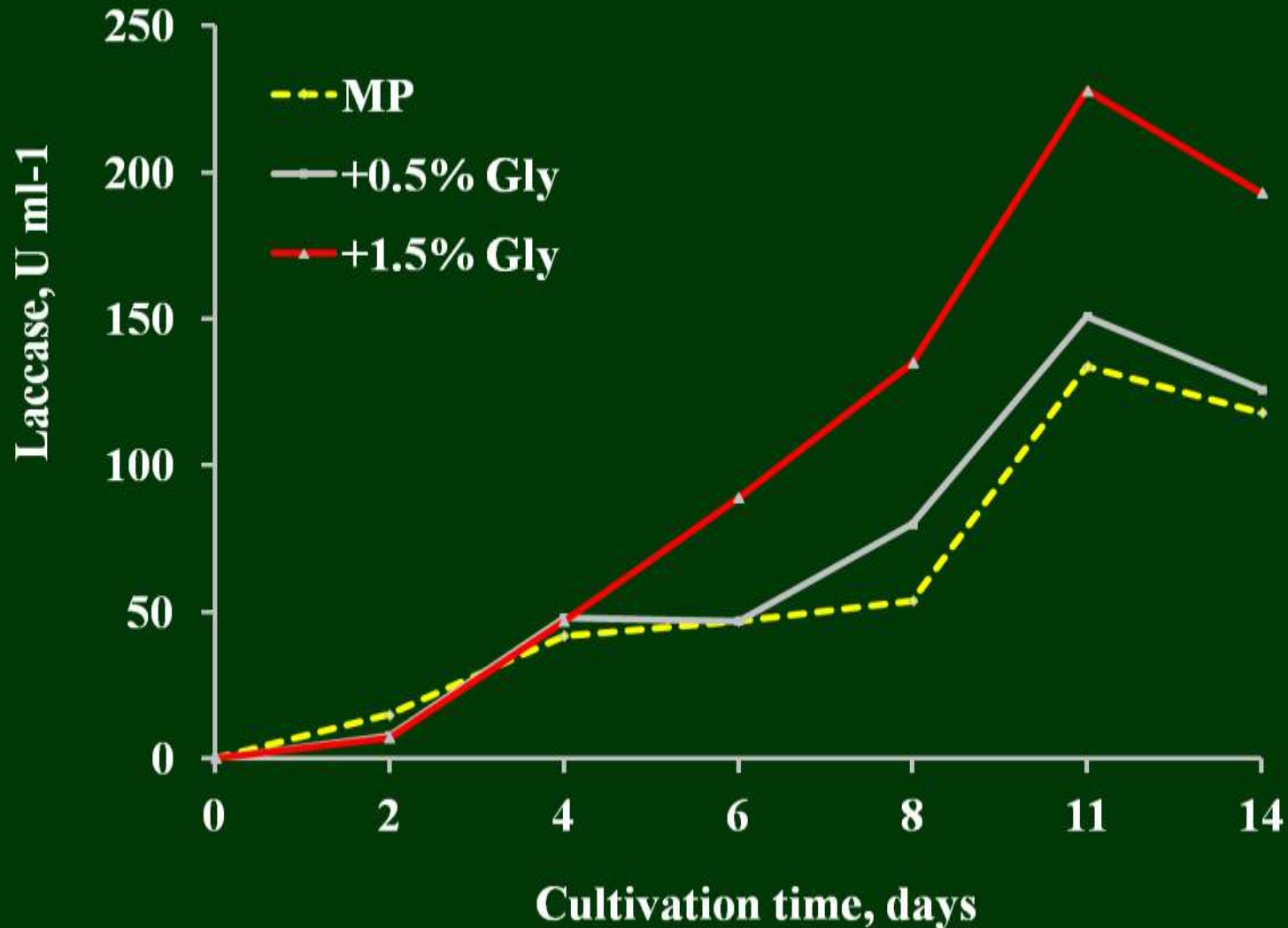
Fungi diversity in laccase activity (U ml⁻¹)

Fungi	Glycerol	Mandarin peels
<i>Cerrena unicolor</i> 300	28.5	86.5
<i>Coriolopsis gallica</i> 1184	51.5	95.9
<i>Fomes fomentarius</i> 38	6.1	17.5
<i>Ganoderma adspersum</i> 845	0.3	22.5
<i>Ganoderma lucidum</i> 246	0.1	75.4
<i>Ganoderma tsugae</i> 1032	0.3	21.3
<i>Panus lecometei</i> 159	46.5	38.3
<i>Pleurotus nebrodensis</i> 1019	0.7	2.8
<i>Trametes versicolor</i> 775	14.8	7.3

Diversity toward lignocellulosic substrate (U ml⁻¹)

Growth substrate	<i>C. unicolor</i>		<i>P. lecometei</i>	
	Laccase	MnP	Laccase	MnP
Wheat bran	151.6	0.7	6.3	1.5
Wheat straw	1.7	0.8	2.0	0.7
Mandarin peels	86.5	2.6	38.3	0.5
Banana peels	74.3	3.1	7.6	0.2
Walnut pericarp	15.7	8.3	76.7	0.2
OMW	78.6	2.8	2.1	0.6

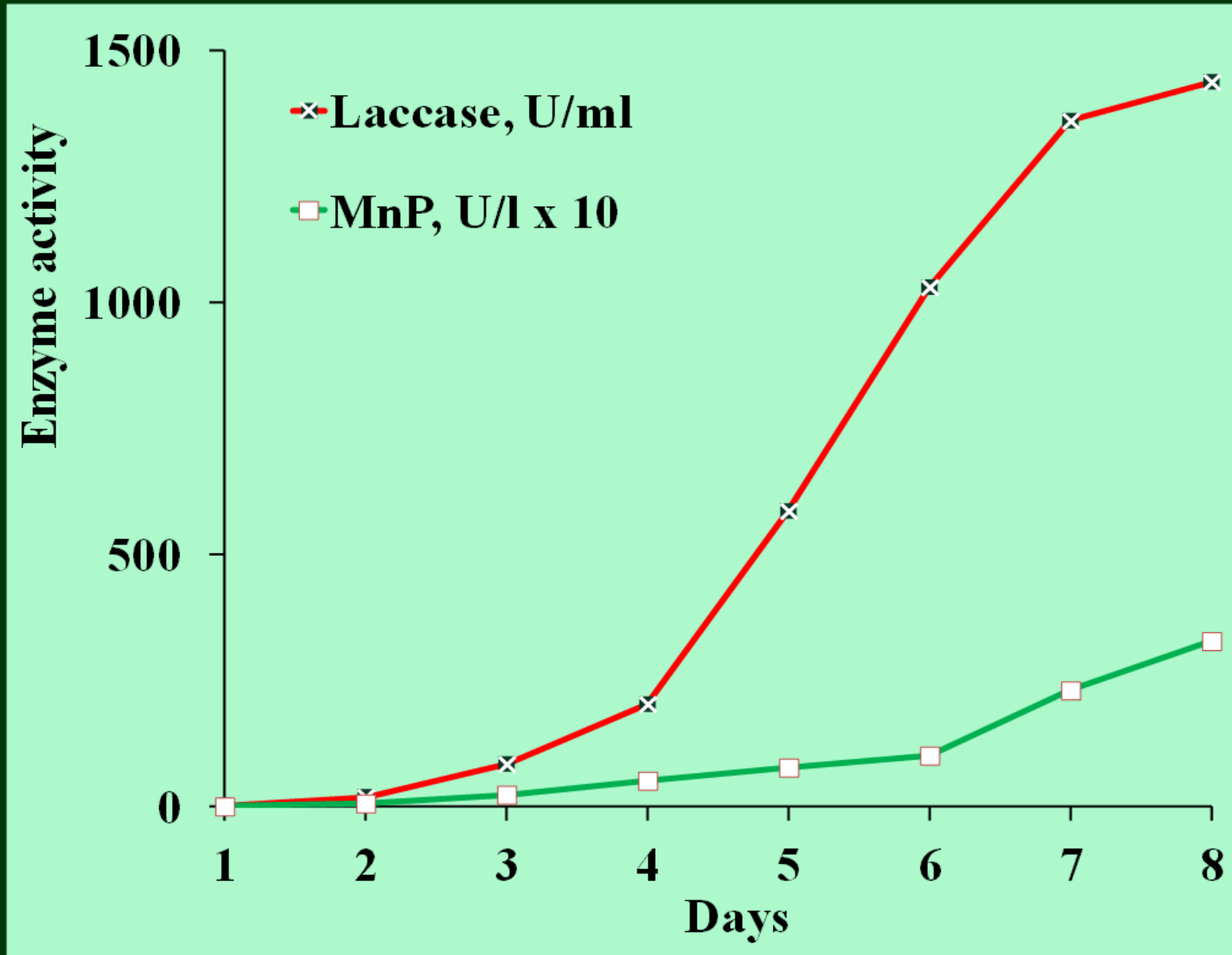
Effect of additional carbon source on the *C. unicolor* laccase production



Effect of aromatic compounds on the *C. unicolor* enzyme activity in mandarin peels-containing medium

Compounds	mM	Laccase, U ml ⁻¹	MnP, U ml ⁻¹
Control	0	107.7	2.4
Ferulic acid	1.0	93.5	2.8
Hydroquinone	0.3	62.2	1.5
Pirogallol	1.0	179.4	3.2
Veratryl alcohol	1.0	97.4	4.1
Xylidine	1.0	184.7	4.2
TNT	0.3	238.7	3.2

C. unicolor laccase and MnP production in fermenter





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(12) **United States Patent**
Elsashvili et al.(10) **Patent No.:** US 8,753,844 B2
(45) **Date of Patent:** Jun. 17, 2014(54) **OVERPRODUCTION OF LIGNINOLYTIC ENZYMES**(75) **Inventors:** Vladimir Elsashvili, Thilisi (GE); Evg Kachlishvili, Thilisi (GE); Tamas Torek, Richmond, CA (US)(73) **Assignee:** The Regents of The University of California, Oakland, CA (US)(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 39 days.(21) **Appl. No.:** 13/464,801(22) **Filed:** May 4, 2012(65) **Prior Publication Data**
US 2012/0295324 A1 Nov. 22, 2012**Related U.S. Application Data**

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(51) **Int. Cl.**
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C12N 1/34 (2006.01)
C12N 9/02 (2006.01)(52) **U.S. Cl.**
USPC 435/71.1; 435/171; 435/189(58) **Field of Classification Search**
None

See application file for complete search history.

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Presentation slides presented by Vladimir Elsashvili at Pacific Rim Summit on Industrial Biotechnology and Biorenergy (Honolulu, HI) on Nov. 15, 2009.

Presentation by Vladimir Elsashvili at Pacific Rim Summit on Industrial Biotechnology and Biorenergy (Honolulu, HI) on Nov. 10, 2009.

Primary Examiner — Suzanne M Nauken**Assistant Examiner** — Ine W Lee(74) **Attorney, Agent, or Firm** — Knobbe, Martin, Olson & Bear LLP(57) **ABSTRACT**Methods, compositions, and systems for overproducing ligninolytic enzymes from the basidiomycetous fungus are described herein. As described, the method can include incubating a fungal strain of *Cerrena unicolor* IBS 305 in a fermentation system having growth medium which includes lignocellulosic material and then cultivating the fungal strain in the fermentation system under conditions wherein the fungus expresses the ligninolytic enzymes. In some cases, the lignocellulosic material is mandarin peel, ethanol production residue, walnut pericarp, wheat bran, wheat straw, or banana peel.**14 Claims, 3 Drawing Sheets**

Thank you for your attention!