读书报告

吉伟利

2017-08-19

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

A xylanase-aided enzymatic pretreatment facilitates cellulose nanofibrillation

Lingfeng Long [a,b], Dong Tian [b,c], Jinguang Hu [b,*], Fei Wang [a], Jack Saddler [b]

[a] Jiangsu Key Lab of Biomass-based Green Fuel & Chemicals, College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, PR China
[b] Department of Wood Science, University of British Columbia, Vancouver V6T 1Z4, Canada
[c] Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, PR China

IF:5.94

1 Introduction

2 Materials and methods

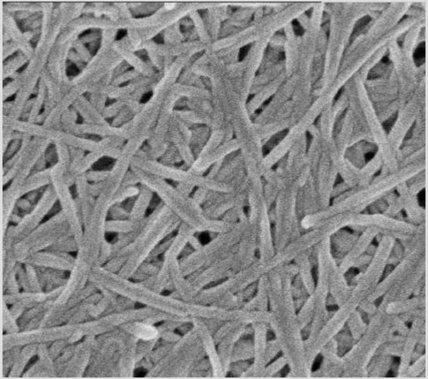
3 Results and discussion

Introduction

纳米纤维化纤维素
（Nanofibrillated cellulose， NFC）

用物理机械方法制备出的纳米纤维素

化学预处理高成本！



纳米纤维素晶体
（Nanocrystalline cellulose， NCC）

用酸水解或酶解的方法制备出的纳米纤维素

难以回收再利用！

透明生物材料，超级电容器，生物传感器，药物……

- 机械精制 → 过程中的高能量投入
  纳米纤维素产品的异质性

  高压均质化
  微流化
  研磨……

- 冷冻干燥
- 超声波处理
- 微波

  ……

"OPEN UP/ LOOSENING/ SOFTENING"

TEMPO氧化
羧甲基化
磺化等

酶反应的高特异性和环境友好的反应条件，基于生物学的预处理方法可能更有吸引力

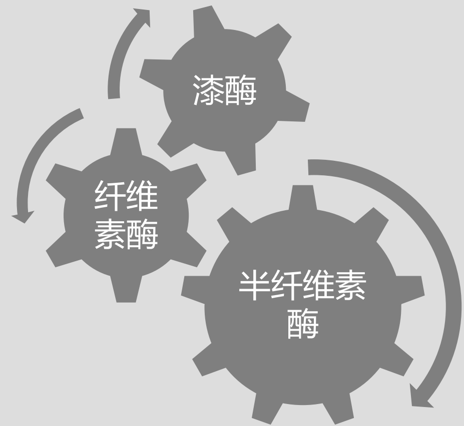
典型的纤维素酶

内切葡聚糖酶（EG）

外切葡聚糖酶（Exo）

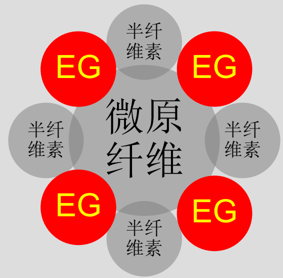
改善有限！

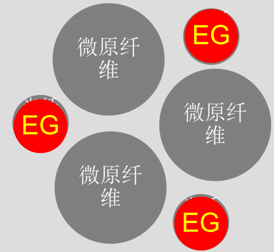
纤维素酶 “辅助酶”



Introduction

bleached Kraft pulp (BKP)
漂白牛皮纸浆





存在一些半纤维素，可以增强生物复合材料的热稳定性和强度

因此，通过半纤维素酶选择性去除/切割某些半纤维素对于成功生产和应用来自BKP的纳米纤维素是至关重要的。

Introduction

内切葡聚糖酶

+

木聚糖酶

→ 四种

→ 漂白牛皮纸浆BKP

→ 硬木 软木

→

- 碳水化合物含量
- 总纤维形态
- 纤维素聚合度和结晶度

Materials and methods

Materials and methods

纤维素原始原料

- 软木牛皮纸浆(BSK)
- 硬木牛皮纸浆(BHK)

大约30g未干燥的纸浆底物

↓

1%（v/v）乙酸+5%（w/v）$NaClO_2$=300ml溶液

↓

室温下黑暗中过夜孵育

↓

漏斗过滤并用水充分洗涤

EG：Novozym 476

四种商业木聚糖酶

Biofeed（Xyn1，Novo Nordisk，Baagsvaend Denmark）
Biobrite（Xyn2，Novozymes，Franklington，NC）
Multifect（Xyn3，Genencor US，Inc.，Palo Alto CA）
Htec（Xyn4，Novozymes Franklington，NC）

四种商业木聚糖酶活性和性质的测定

木聚糖酶（0.03mL）

1%白桦木聚糖溶解在50mM柠檬酸磷酸盐缓冲液（0.07mL）

反应混合物在50℃温育15分钟

加入0.2mL 3，5-二硝基水杨酸（DNS），然后煮沸5分钟

550nm处测量吸光度

pH：3-8

温度：45-65℃

测定商业木聚糖酶的最适pH和温度。所有实验一式三份进行

Materials and methods

EG

EG+Xyn1

EG+Xyn2

BSK BHK

EG+Xyn4

EG+Xyn3

使用牛血清白蛋白（BSA）作为蛋白质标准，通过茚三酮测定法测定蛋白质浓度

混合物，50℃，pH 5，摇床2小时

100℃下加热15分钟以使酶变性，离心13,000g，10min，以收集上清液

酶水解后，用去离子水洗涤纤维五次，并在8mL去离子水中稀释（0.01g），振幅超声40分钟，不用酶处理的纸浆做对照组。

分析方法

组成分析

- BSK、BHK的化学组成

保水值（wrv）

保水值可以说明纤维的润胀程度，从而反映出细纤维化程度，说明了纤维之间结合力的大小。

西蒙斯染色（SS）

评估比表面积的变化

扫描电子显微镜观察

超声前后BSK、BHK纤维变化

粘度平均分子量测量

平均聚合度（DP）

$DP^{0.905} = 0.75[\eta]$

X射线衍射分析

结晶度指数（CrI）

$CrI\ (\%) = (1 - I_{am}/I_{200}) \times 100$

FTIR

傅里叶变换红外光谱

紫外可见光谱

Results and discussion

来自硬木（BHK）和软木（BSK）的漂白牛皮纸浆的化学成分

Table 1
Chemical composition (dry material basis) of bleached Kraft pulps from hardwood (BHK) and softwood (BSK).

Substrates	Sugar and lignin composition of water insoluble component of the substrates						Abbreviation
	Ara	Gal	Glu	Xyl	Man	AIL	
Hardwood pulp	0.5	0.1	80.3	17.2	1.4	2.6	BHK
Softwood pulp	0.3	0.6	83.5	8.4	8.9	1.9	BSK

Ara, Arabinan; Gal, Galactan; Glu, Glucan; Xyl, Xylan; Man, Mannan; AIL, Acid Insoluble Lignin. Values shown were the mean of the average of three experiments.

Ara: 阿拉伯糖
Gal: 半乳糖
Glu: 葡聚糖
Xyl: 木聚糖
Man:甘露聚糖
AIL: 酸不溶性木质素

四种商业木聚糖的特征

Table 2
Characteristics of commercial xylanases.

Xylanase preparation	Protein content (mg/mL) 蛋白质含量	Specific activity (U/mg) 比活度	Optimum pH 最佳pH	Optimum temperature (°C) 最佳温度	Abbreviation
Biofeed	4.2	1312.6	6	55	Xyn1
Biobrite	123.8	388.7	6	50	Xyn2
Multifect	36.8	251.2	5	50	Xyn3
HTec	35.2	148.4	5	60	Xyn4

Specific activities of commercial xylanases were assayed at pH 5, 50 °C. Values shown were the mean of the average of three experiments, and the variation about the mean was below 5%.

由于本研究中使用的主要内切葡聚糖酶制剂Novozym 476在50℃和pH5.0下还能最佳地降低纤维素DP（聚合度），所以在所有下列酶处理过程中都选择了这一条件，以简化实验设计中的变量。

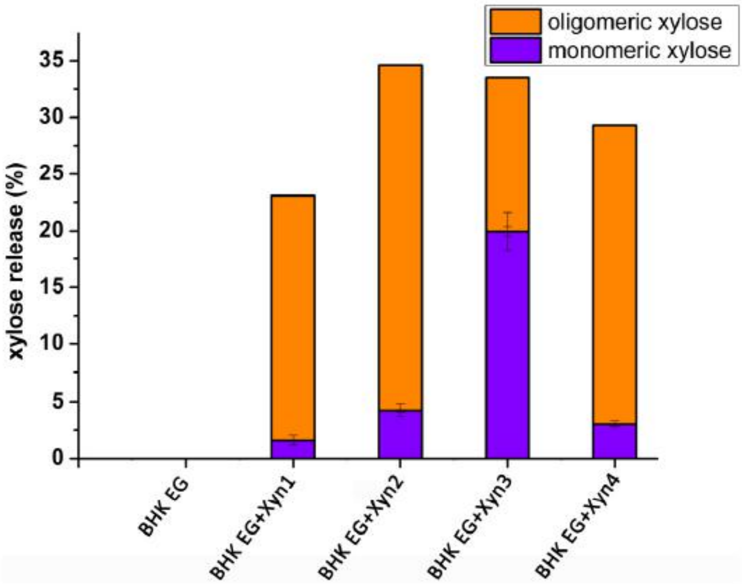


Fig. 1. Xylose (wt% of g/g xylan) released from enzymatic treatments of bleached hardwood Kraft pulp (BHK). Oligomeric xylose, the difference between xylose after 4% sulfuric acid hydrolysis at 121 °C for 60 min and monomeric xylose; Monomeric xylose, xylose released after enzymatic pretreatment. EG, Xyn1, Xyn2, Xyn3, Xyn4 are assigned to endoglucanase, Biofeed, Biobrite, Multifect, HTec, respectively.

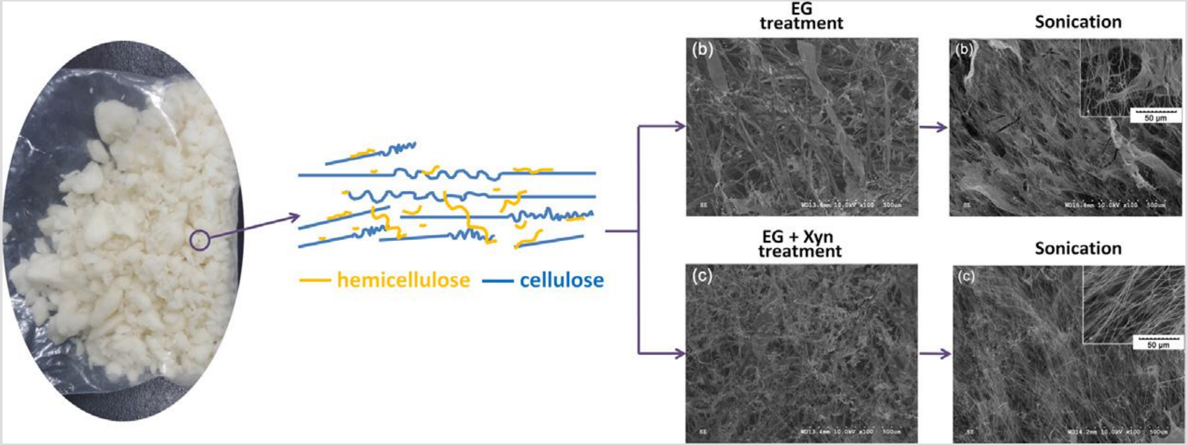
硬木　酶水解的特征

Table 3
Characteristics of enzymatic hydrolyzed BHKs.

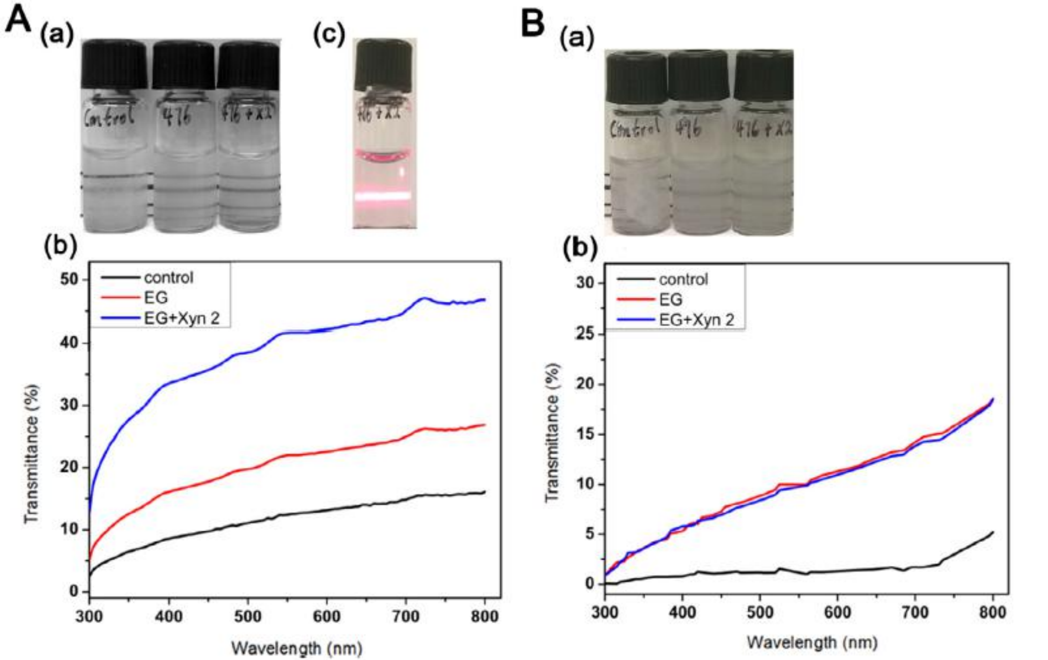
Samples	聚合度 DP	结晶度指数 CrI	微晶尺寸 Sc (nm)	保水值 WRV (%)	西蒙斯指数 SS (mg/g pulp)
Control	1320 ± 4.1	54.3%	2.6	331.5 ± 0.1	45.5
EG	878 ± 4.7	76.3%	3.6	317.3 ± 0.1	53.4
EG+Xyn1	854 ± 7.1	77.0%	3.8	310.3 ± 0.1	55.9
EG+Xyn2	834 ± 4.3	77.1%	4.0	309.5 ± 0.1	59.2
EG+Xyn3	815 ± 2.5	76.5%	3.9	315.7 ± 0.0	49.8
EG+Xyn4	827 ± 4.3	77.7%	3.9	313.6 ± 0.1	54.6

DP, degree of polymerization; CrI, crystallinity index; Sc, crystallite size; WRV, water retention value; SS, Simons' stain. Control, substrates were incubated at the same condition without the addition of enzymes. BHK, EG, Xyn1, Xyn2, Xyn3, Xyn4 are assigned to the bleached hardwood Kraft pulp, endoglucanase, Biofeed, Biobrite, Multifect, HTec, respectively.

Results and discussion



Results and discussion



内切葡聚糖酶　　木聚糖酶

底物：硬木纸浆

木聚糖酶：具有较低的β-木糖苷酶

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Unravelling the capability of *Pyrenophora phaeocomes* S-1 for the production of ligno-hemicellulolytic enzyme cocktail and simultaneous bio-delignification of rice straw for enhanced enzymatic saccharification

Shubhangi Rastogi [a], Raman Soni [b], Jaspreet Kaur [c], Sanjeev Kumar Soni [a,*]

[a] *Department of Microbiology, Panjab University, Chandigarh 160014, India*
[b] *Department of Biotechnology, D.A.V. College, Chandigarh 160011, India*
[c] *Department of Biotechnology, University Institute of Engineering and Technology, Panjab University, Chandigarh 160014, India*

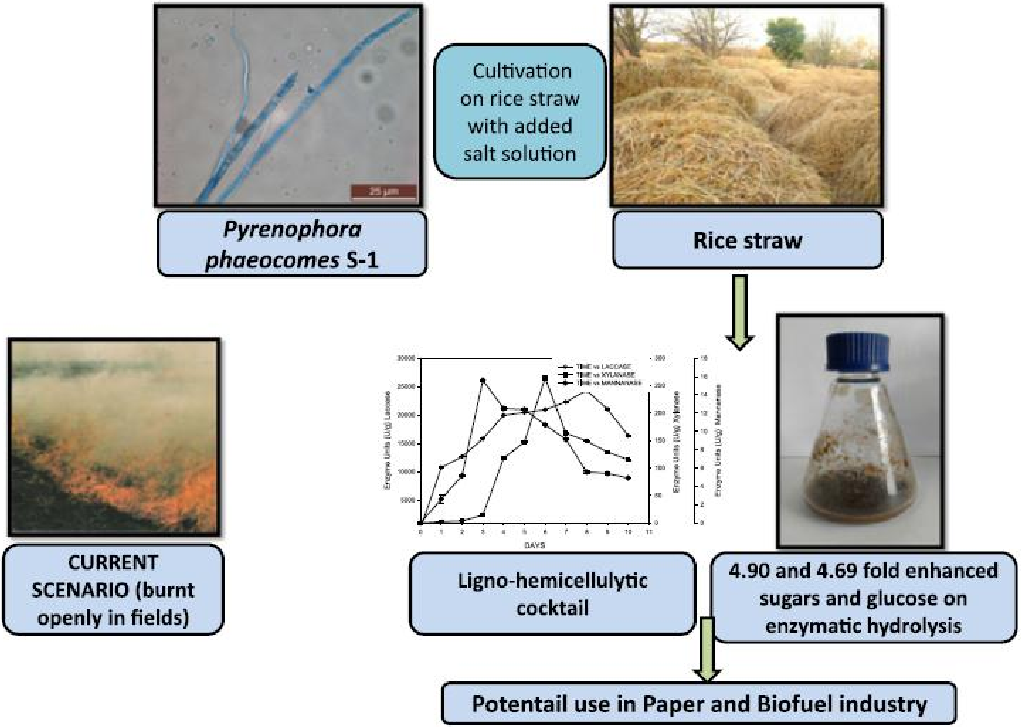

Pyrenophora phaeocomes S-1
Cultivation on rice straw with added salt solution
Rice straw
CURRENT SCENARIO (burnt openly in fields)
DAYS
Ligno-hemicellulytic cocktail
4.90 and 4.69 fold enhanced sugars and glucose on enzymatic hydrolysis
Potentail use in Paper and Biofuel industry

Table 1
Enzyme activities of the selected fungal strain on different lignocellulosic residues moistened with distilled water.

Sr. No.		Lignocellulosic residues	Enzyme activities (IU gds^{-1})		
			Laccase[a]	Xylanase[b]	Mannanase[c]
1	1米糠	Rice Bran	301.65 ± 15.08	32.21 ± 0.32	11.57 ± 0.57
2	2脱油米糠	De-oiled Rice bran	9.09 ± 0.45	5.05 ± 0.05	51.04 ± 2.55
3	3小麦麸	Wheat Bran	631.40 ± 31.57	56.38 ± 0.07	4.16 ± 0.20
4	4甘蔗渣	Sugarcane baggase	5.78 ± 0.028	27.33 ± 0.03	3.67 ± 0.18
5	5二手茶叶	Used Tea leaves	10.74 ± 0.53	25.90 ± 0.03	4.44 ± 0.22
6	6小麦秸秆	Wheat Straw	19.83 ± 0.99	40.64 ± 0.03	1.93 ± 0.09
7	7稻草	Rice Straw	102.47 ± 5.12	31.84 ± 0.06	1.97 ± 0.09
8	8稻壳	Rice husk	0	26.73 ± 0.08	1.26 ± 0.06
9	9干树叶	Dried Tree Leaves	604.13 ± 30.20	316.93 ± 0.42	1.00 ± 0.05
10	10土豆皮	Potato Peels	241.32 ± 12.06	173.38 ± 0.42	0
11	11干草	Dried Grasses	563.63 ± 28.18	51.33 ± 0.16	6.38 ± 0.31
12	12玉米秸秆	Corn Stover	238.01 ± 11.90	28.99 ± 0.06	3.72 ± 0.18
13	13地螺母壳	Ground Nut Shells	387.60 ± 19.38	29.88 ± 0.05	2.53 ± 0.12
14	14菠萝提取物	Pineapple Extracts	42.14 ± 2.10	310.04 ± 0.89	3.59 ± 0.17
15	15 Mausami	Mausami Peels	106.61 ± 5.33	6.06 ± 0.08	44.23 ± 2.21
16	16木屑	Saw Dust	47.10 ± 2.35	0	6.37 ± 0.31
17	17香蕉茎	Banana Stalks	5.78 ± 0.28	272.72 ± 0.78	3.78 ± 0.18
18	18厨房垃圾	Kitchen Waste	316.52 ± 15.82	5.52 ± 0.05	37.36 ± 1.86
19	19Sarkanda	Sarkanda	1336.36 ± 66.81	303.14 ± 0.67	40.94 ± 2.04
20	20纸浆	Pulp (Wheat straw alkali pretreated)	26.44 ± 1.32	0	0.89 ± 0.04

ANOVA results – [a,b,c]p-value P = <0.001; F_L-value 1516.438; F_X-value 9112.506; F_M-value 30839.759.
Overall significance level = 0.05.

Table 2
Enzyme activities of the selected fungal strain on different lignocellulosic residues moistened with stajic medium.

Sr. No.		Lignocellulosic residues	Enzyme activities (IU gds^{-1})		
			Laccase[a]	Xylanase[b]	Mannanase[c]
1	1米糠	Rice Bran	330.57 ± 23.37	13.39 ± 3.87	11.57 ± 0.57
2	2脱油米糠	De-oiled Rice bran	4008.26 ± 58.43	212.27 ± 0.57	38.60 ± 1.56
3	3小麦麸	Wheat Bran	14652.89 ± 58.43	83.61 ± 0.57	20.93 ± 0.17
4	4甘蔗渣	Sugarcane baggase	3123.96 ± 350.63	22.42 ± 0.43	1.17 ± 0.15
5	5二手茶叶	Used Tea leaves	74.38 ± 58.43	24.25 ± 0.71	10.67 ± 0.21
6	6小麦秸秆	Wheat Straw	25413.23 ± 35.06	38.76 ± 0.28	8.66 ± 0.07
7	7稻草	Rice Straw	10859.51 ± 46.74	22.01 ± 1.00	10.36 ± 0.25
8	8稻壳	Rice husk	669.42 ± 35.06	16.13 ± 0.71	2.48 ± 0.01
9	9干树叶	Dried Tree Leaves	1396.69 ± 35.06	38.96 ± 10.04	42.16 ± 1.33
10	10土豆皮	Potato Peels	1669.42 ± 46.75	39.87 ± 1.00	25.94 ± 4.72
11	11干草	Dried Grasses	16669.42 ± 58.43	45.66 ± 0.57	4.54 ± 0.08
12	12玉米秸秆	Corn Stover	22305.79 ± 128.55	41.80 ± 1.14	11.42 ± 0.04
13	13地螺母壳	Ground Nut Shells	578.51 ± 46.75	22.01 ± 1.00	2.42 ± 0.014
14	14菠萝提取物	Pineapple Extracts	429.75 ± 70.12	32.77 ± 1.00	49.57 ± 0.71
15	15 Mausami	Mausami Peels	396.69 ± 70.12	35.81 ± 0.71	6.37 ± 0.46
16	16木屑	Saw Dust	90.90 ± 81.81	26.48 ± 0.43	4.98 ± 1.03
17	17香蕉茎	Banana Stalks	1123.96 ± 93.5	31.76 ± 1.29	46.70 ± 0.04
18	18厨房垃圾	Kitchen Waste	479.33 ± 116.87	33.07 ± 4.30	57.31 ± 1.38
19	19Sarkanda	Sarkanda	264.46 ± 70.12	19.58 ± 1.00	0.89 ± 0.04
20	20纸浆	Pulp (Wheat straw alkali pretreated)	595.04 ± 70.12	24.35 ± 0.57	4.22 ± 0.19

ANOVA results – [a,b,c]p-value P = <0.001; F_L-value 12,464.922; F_X-value 531.059; F_M-value 437.167.
Overall significance level = 0.05.

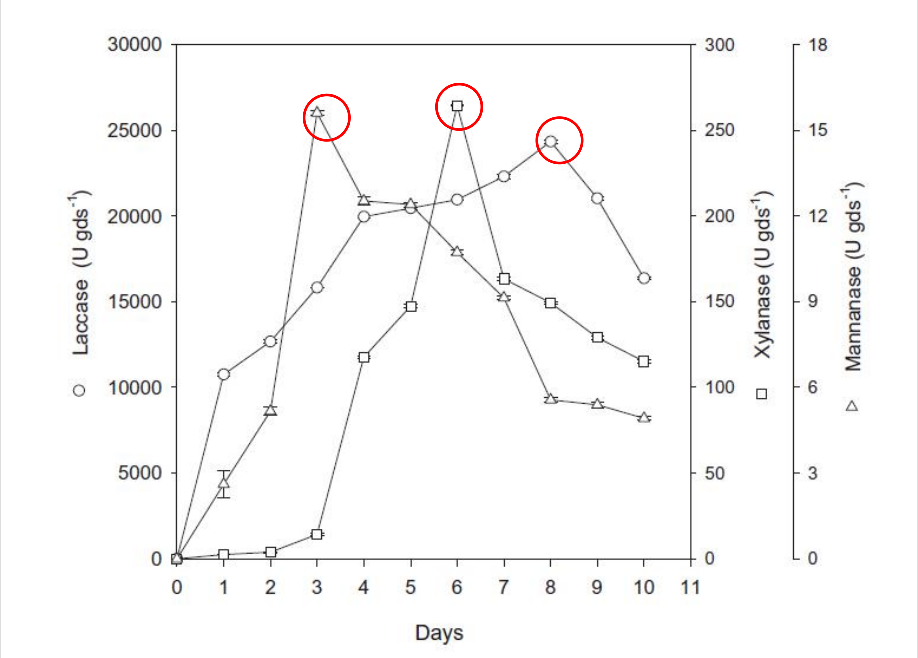
漆酶：小麦秸秆、玉米秸秆、干草、稻草
木聚糖酶：脱油米糠、麦麸、干草、玉米秸秆
甘露聚糖酶：厨房垃圾、菠萝提取物、香蕉茎、干树叶

通过不同底物上的各种真菌的固态培养物比较木质纤维素酶的产率

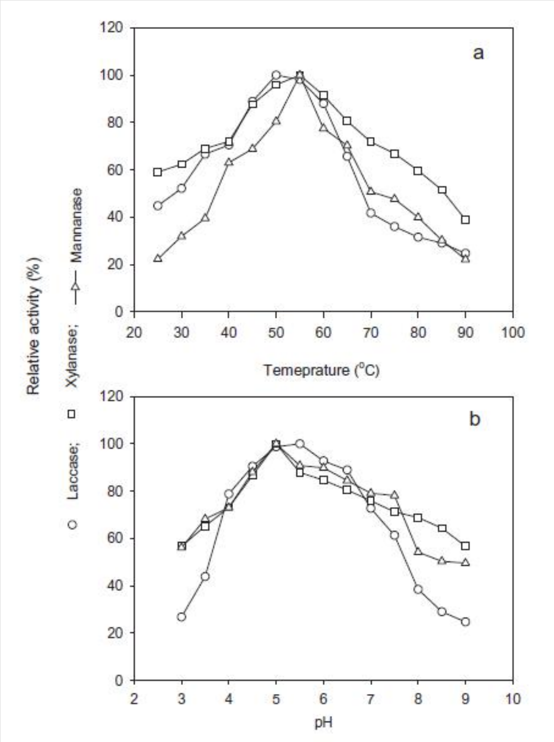
Table 3
Comparison of lingo-hemicellulytic enzyme yields by solid state cultures of various fungi on different substrates.

Fungi	Enzyme yields (U gds^{-1})			Time (d)	Substrate used	References
	Laccase	Xylanase	Mannanase			
P.phaeocomes S-1	10859.51 ± 46.74	22.01 ± 1.00	10.45 ± 0.128	8	Rice straw	Present study
Panus tigrinus	1090	–	–	10	Rice straw	Ruqayyah et al. (2013)
Aspergillus heteromorphus	8.2	160.8	–	12/6	Microwave-alkaline pretreated rice straw	Singh et al. (2011)
Phlebia floridensis	1.46	–	–	20	Rice straw	Sharma and Arora 2011
T.versicolor	72.9 ± 1.4	98.9 ± 6.4	35.5 ± 3.9	21	Wheat straw	Valaskova and Baldrian (2006)
Trametes taogii	900	413.15		14	Saw dust	Levin et al. (2008)
Pleurotus ostreatus	1360.5 ± 22.2	–	33.9 ± 0.8	21	Wheat straw	-do-
P. citrinopileatus	3.73 ± 0.55	0.12 ± 0.04	–	30	Wheat straw	Carabajal et al. (2012)
P. ostreatus	8.22 ± 0.79	0.14 ± 0.05	–	30	-do-	-do-
L. edodes	57 ± 4.7	200 ± 14	–	7	Tree leaves	Elisashvili et al. (2008)
P. ostreatus	15	9	–	3/2	Tomato pomace	Iandolo et al. (2011)
Trametes versicolar	35	50	–	16/13	-do-	-do-
P. ostreatus	161.3	–	–	5	Sugar cane baggase	Karp et al. (2015)

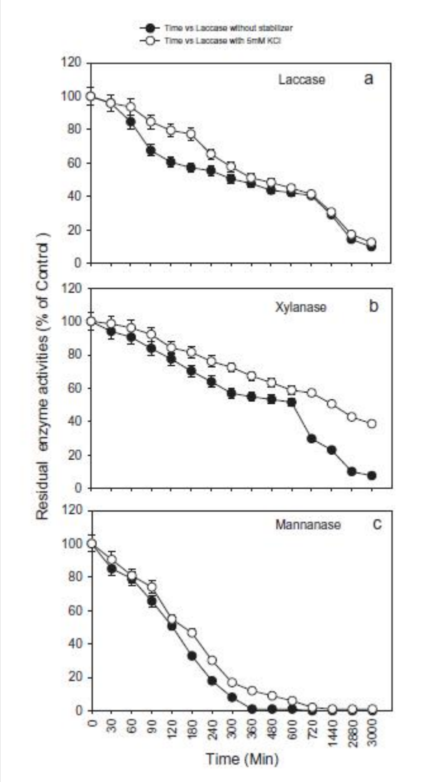
在稻秆固态发酵过程中由P. phaeocomes S-1生产木质素 - 半纤维素混合物的各种酶的时间过程。



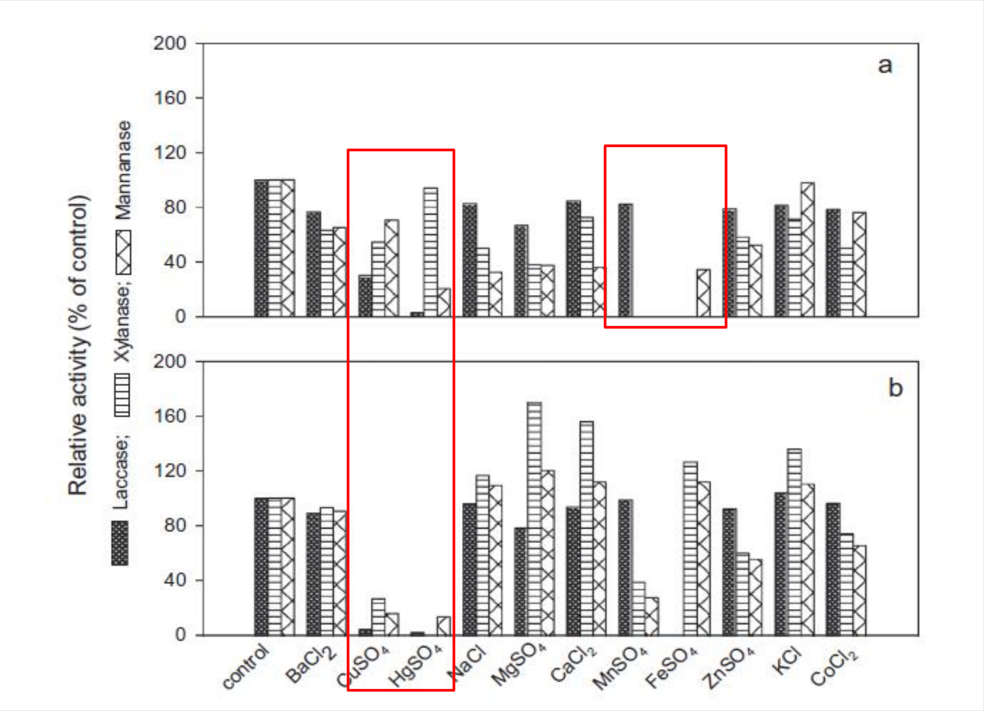
温度（a）和pH（b）对比由P. phaeocomes S-1生产的木质素－半纤维素混合物的各种酶的活性曲线。



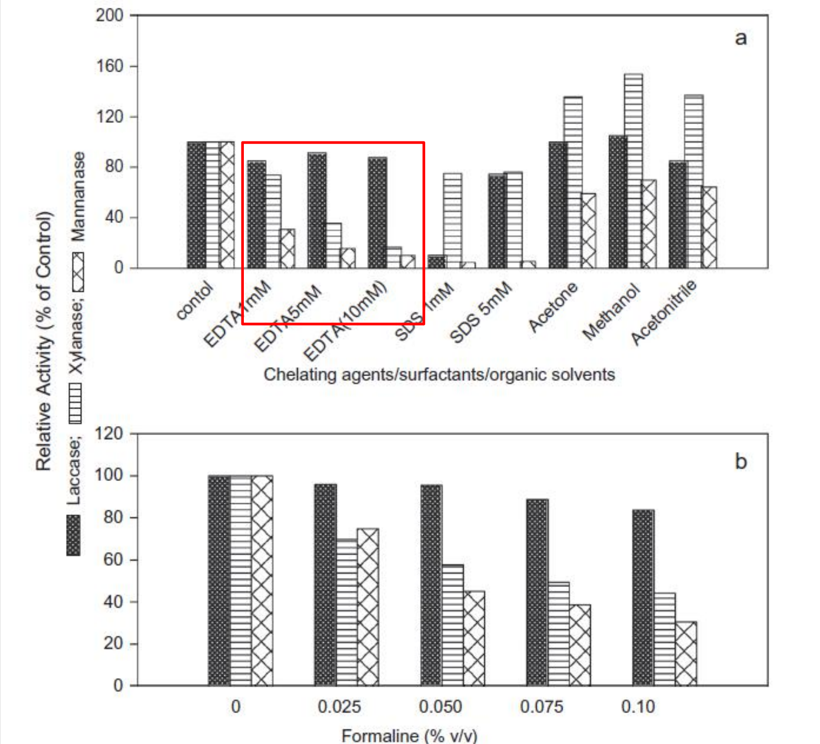
在50℃下没有和与5mM KCl的来自P.Phaeocomes S-1的漆酶（a），木聚糖酶（b）和甘露聚糖酶（c））的热稳定性曲线。 开放符号表示含有5mM KCl的酶，封闭符号代表不含KCl的酶。



1mM（a）和5mM（b）的各种金属盐对由P.Phaeocomes S-1生产的木质素 - 半纤维素混合物的各种酶的活性的影响。



EDTA，抑制剂，表面活性剂和有机溶剂对由P. phaeocomes S-1生产的木质素 - 半纤维素混合物的各种酶的活性的影响。



单独生物处理后的稻草组成百分比变化（40 d），用0.1N NaOH（30分钟，室温）单独提取，生物预处理，然后用0.1N NaOH萃取。

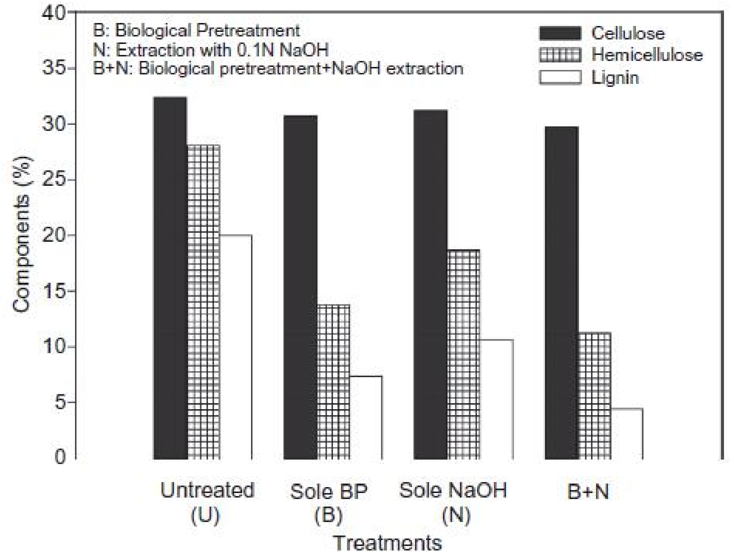


Fig. 6. Changes in percentage composition of rice straw after sole biological treatment (40 d), sole extraction with 0.1 N NaOH (30 min; room temperature) and the Biological pretreatment followed by extraction with 0.1 N NaOH.

THANKS