



读书报告

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A xylanase-aided enzymatic pretreatment facilitates cellulose nanofibrillation



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1 Introduction

2 Materials and methods

3 Results and discussion



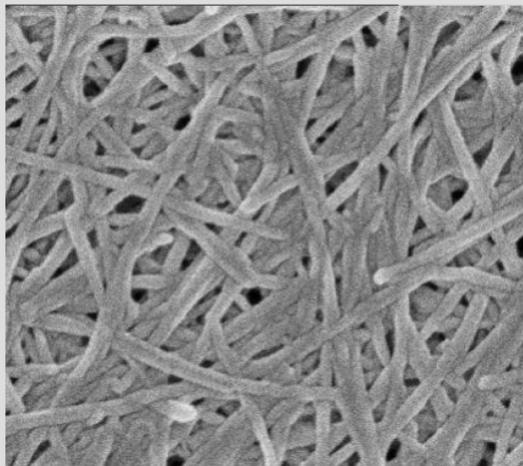
Introduction

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

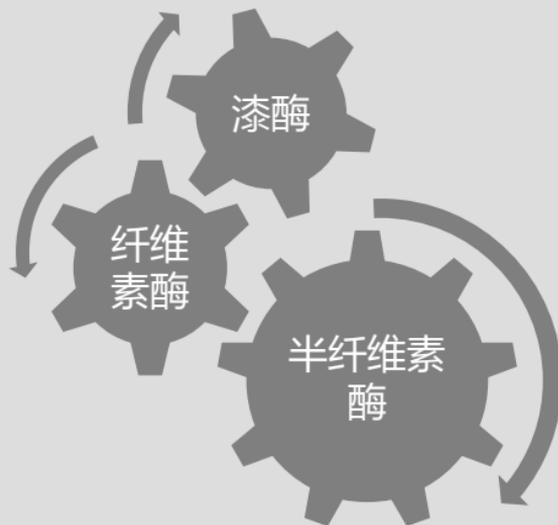
典型的纤维
素酶

内切葡聚糖酶
(EG)

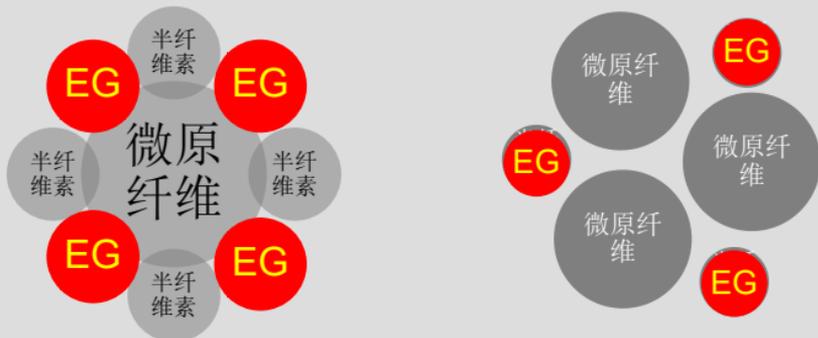
外切葡聚糖酶
(Exo)

改善有限！

纤维素酶 “辅助酶”

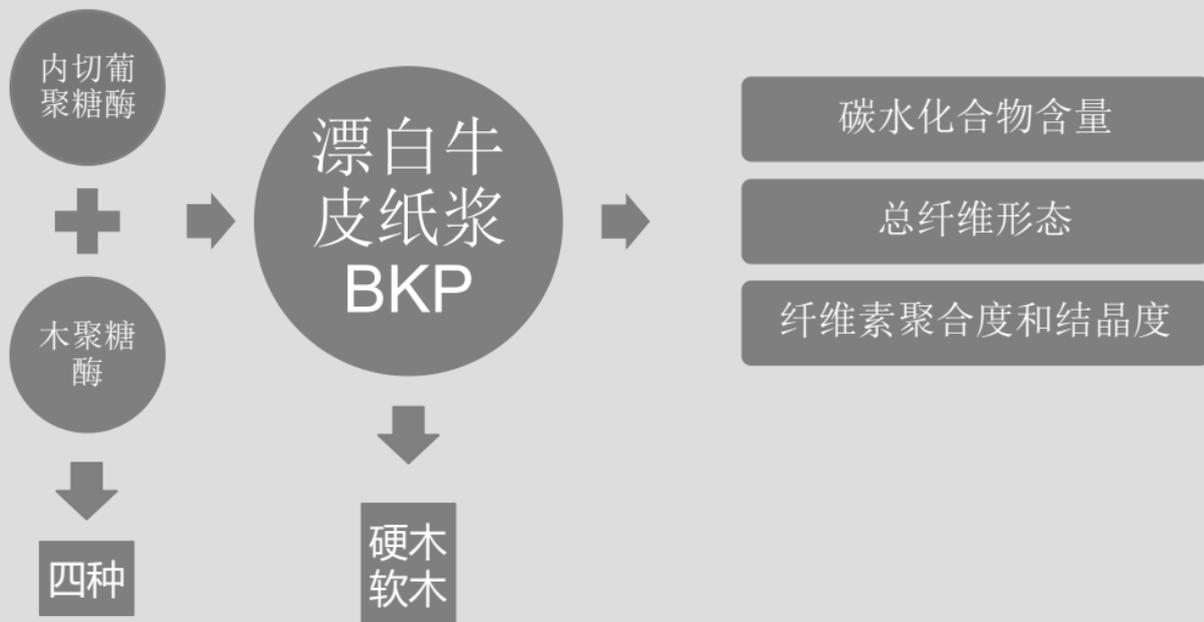


bleached Kraft
pulp (BKP)
漂白牛皮纸浆



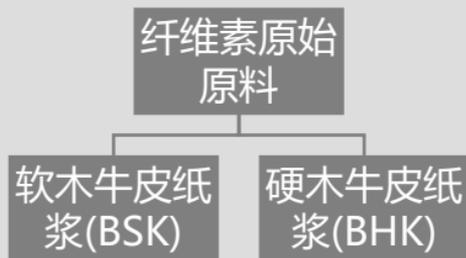
存在一些半纤维素，可以增强生物复合材料的热稳定性和强度

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。





Materials and methods



大约30g未干燥的纸浆底物

1% (v/v) 乙酸+5% (w/v)
NaClO₂=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, Franklinton, NC)

Multifect (Xyn3, Genencor US, Inc., Palo Alto CA)

Htec (Xyn4, Novozymes Franklinton, 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°C温育
15分钟

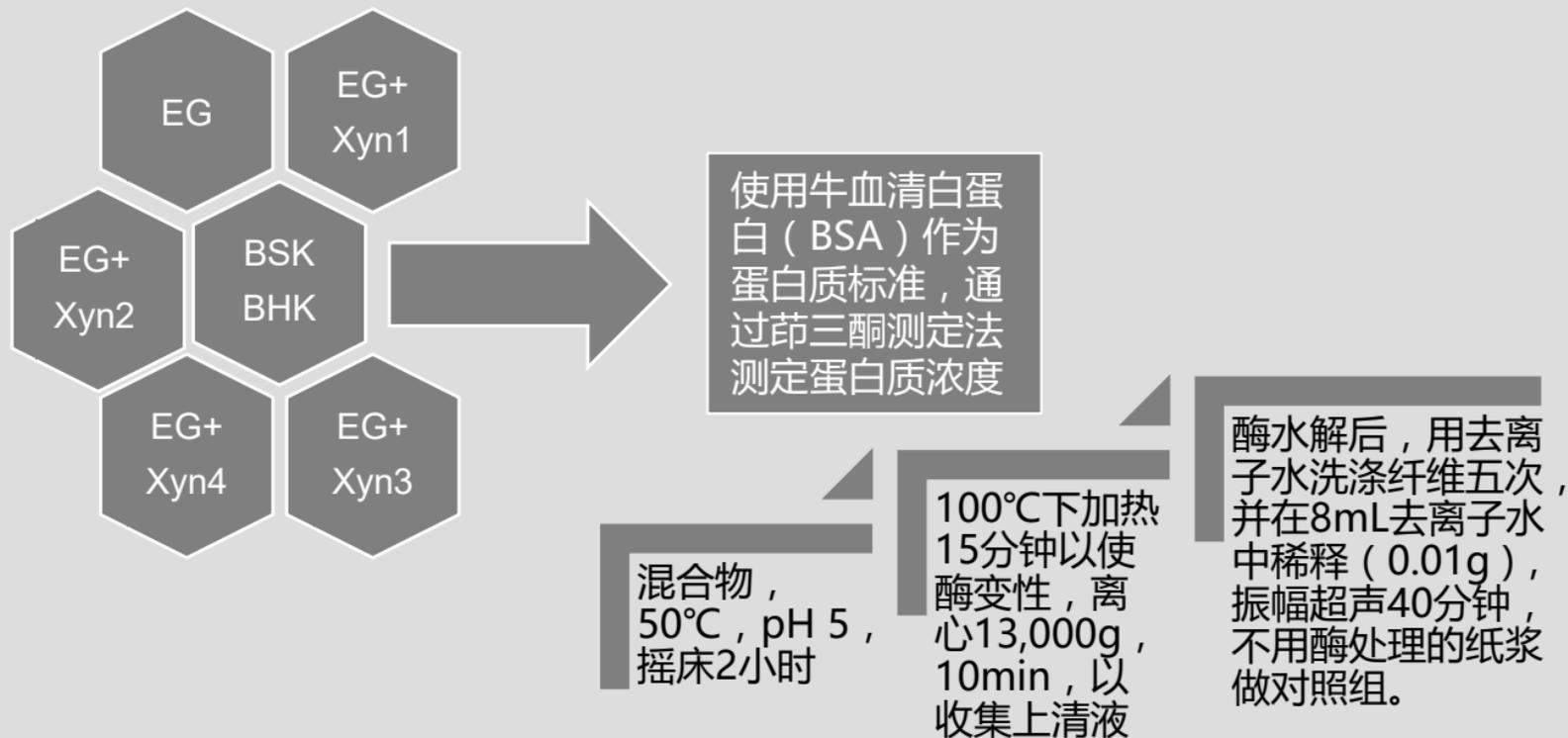
加入0.2mL
3, 5-二硝
基水杨酸
(DNS),
然后煮沸5
分钟

550nm处测
量吸光度

pH : 3-8

温度 : 45-65°C

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



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 (Crl)

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

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	蛋白质含量 Protein content (mg/mL)	比活度 Specific activity (U/mg)	最佳pH Optimum pH	最佳温度 Optimum temperature (°C)	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°C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。

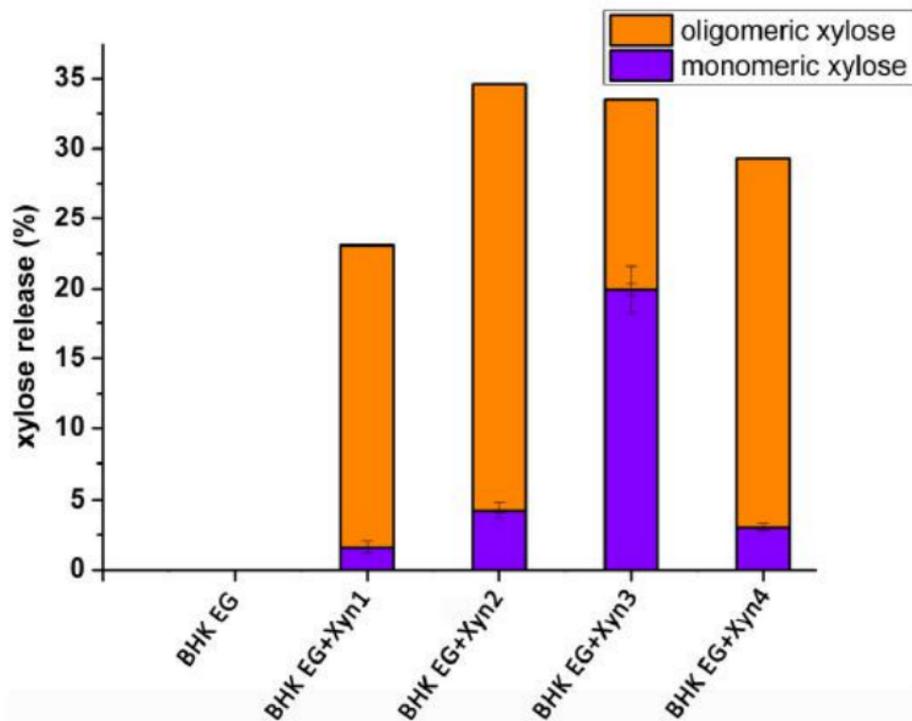


Fig. 1. Xylose (wt% of g/g xylan) released from enzymatic treatments of bleached hardwood Kraft pulp (BHK). Oligomeric xylose, the difference between xylose after 4% sulfuric acid hydrolysis at 121 °C for 60 min and monomeric xylose; Monomeric xylose, xylose released after enzymatic pretreatment. EG,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

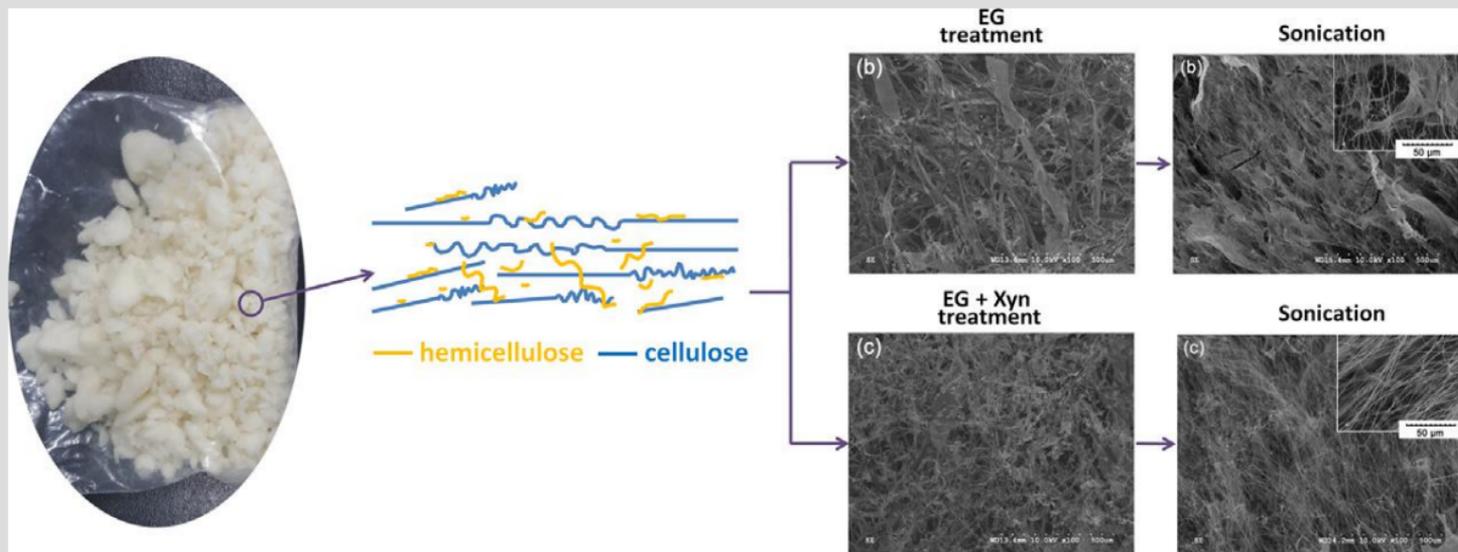
硬木 酶水解的特征

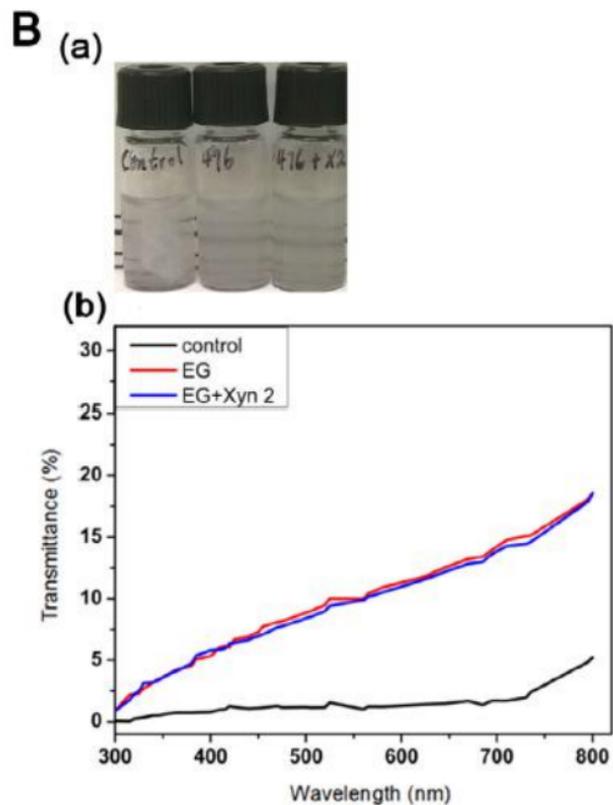
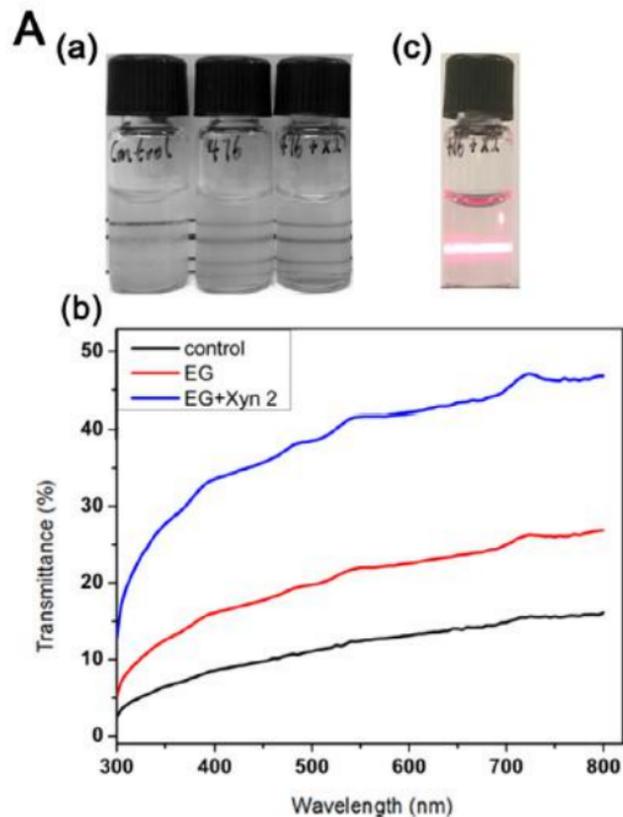
Table 3
Characteristics of enzymatic hydrolyzed BHKs.

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





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



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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



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Pyrenophora phaeocomes S-1

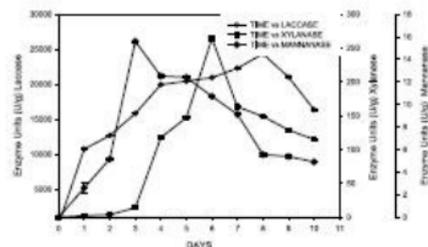
Cultivation
on rice straw
with added
salt solution



Rice straw



**CURRENT
SCENARIO** (burnt
openly in fields)



**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		Potato Peels	1669.42 ± 46.75	39.87 ± 1.00	25.94 ± 4.72
11	10土豆皮	Dried Grasses	16669.42 ± 58.43	45.66 ± 0.57	4.54 ± 0.08
12	11干草	Corn Stover	22305.79 ± 128.55	41.80 ± 1.14	11.42 ± 0.04
13	12玉米秸秆	Ground Nut Shells	578.51 ± 46.75	22.01 ± 1.00	2.42 ± 0.014
14	13地螺母壳	Pineapple Extracts	429.75 ± 70.12	32.77 ± 1.00	49.57 ± 0.71
15	14菠萝提取物	Mausami Peels	396.69 ± 70.12	35.81 ± 0.71	6.37 ± 0.46
16	15 Mausami	Saw Dust	90.90 ± 81.81	26.48 ± 0.43	4.98 ± 1.03
17	16木屑	Banana Stalks	1123.96 ± 93.5	31.76 ± 1.29	46.70 ± 0.04
18	17香蕉茎	Kitchen Waste	479.33 ± 116.87	33.07 ± 4.30	57.31 ± 1.38
19	18厨房垃圾	Sarkanda	264.46 ± 70.12	19.58 ± 1.00	0.89 ± 0.04
20	19Sarkanda	Pulp (Wheat straw alkali pretreated)	595.04 ± 70.12	24.35 ± 0.57	4.22 ± 0.19

ANOVA results - ^{abc}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

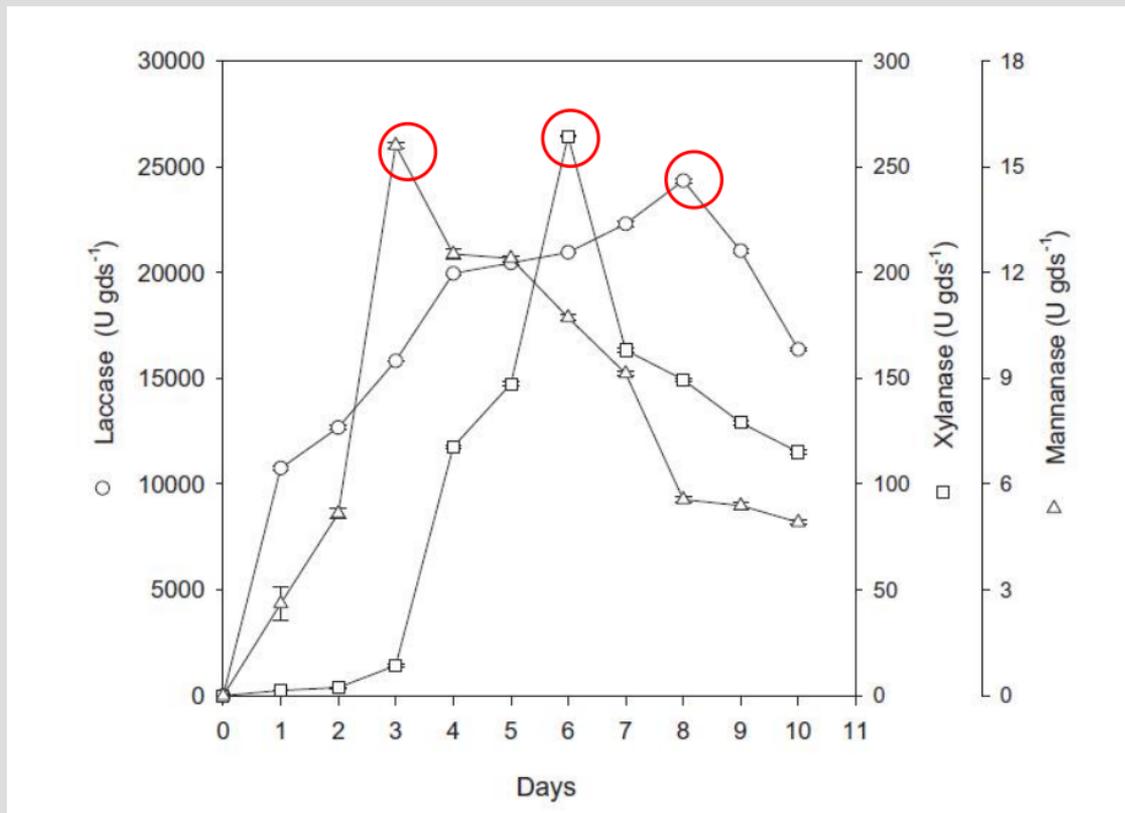
通过不同底物上的各种真菌的固态培养物比较木质纤维素酶的产率

Table 3

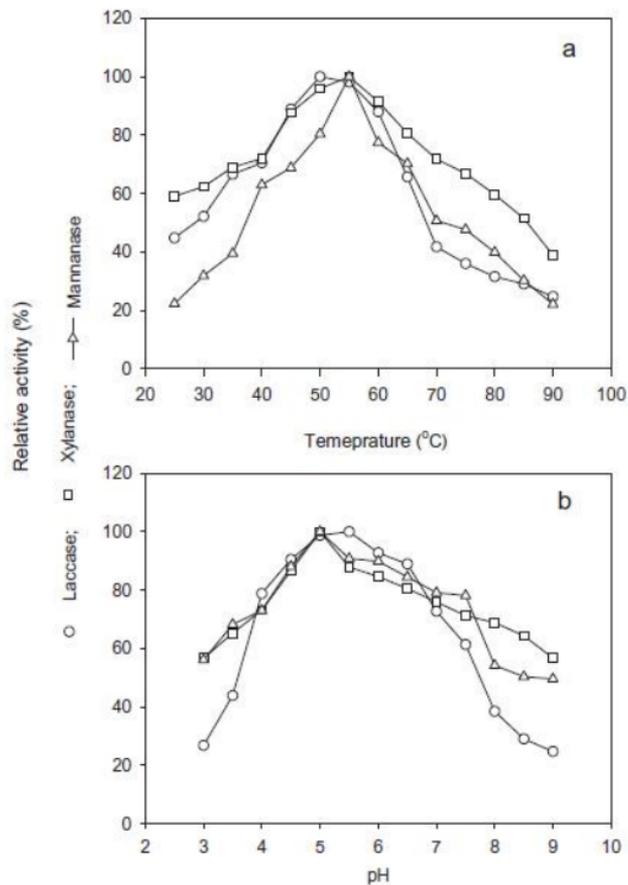
Comparison of lingo-hemicellulytic enzyme yields by solid state cultures of various fungi on different substrates.

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

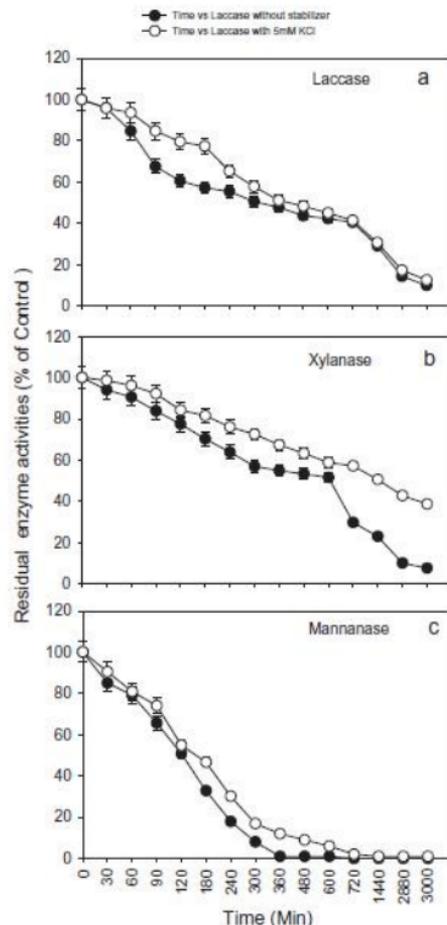
在稻秆固态发酵过程中由*P. phaeocomes* S-1生产木质素 - 半纤维素混合物的各种酶的时间过程。



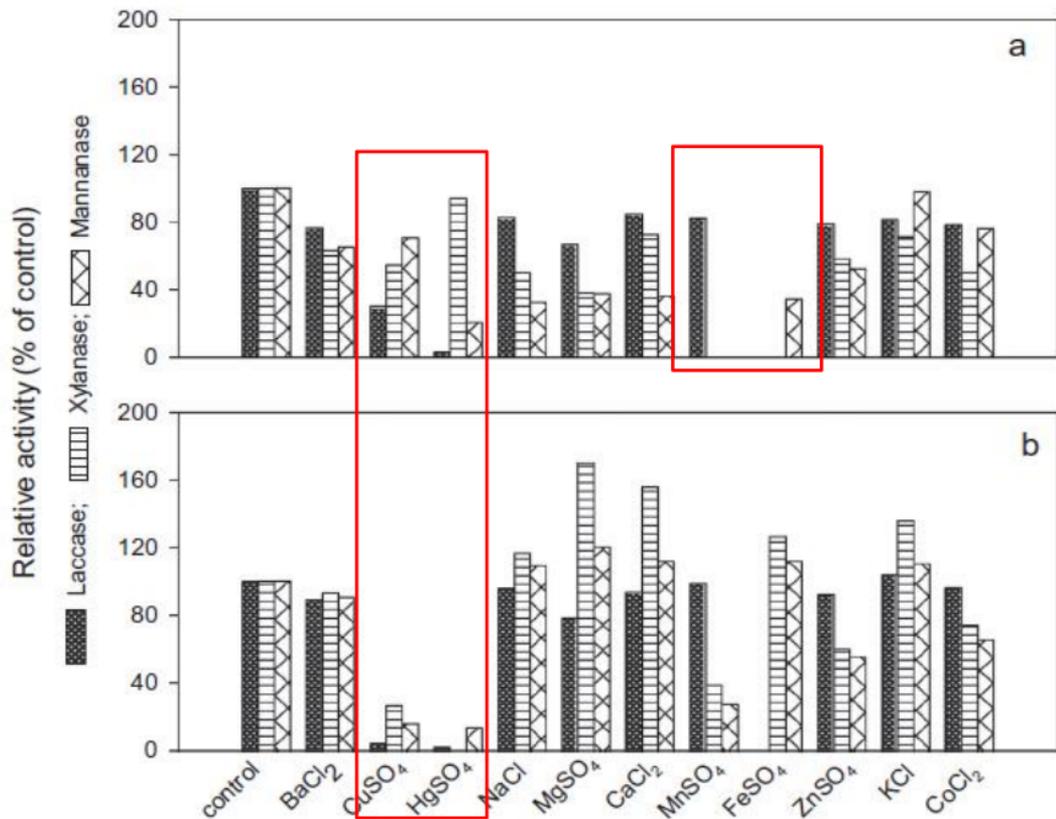
温度 (a) 和pH (b) 对比由P. phaeocomes S-1生产的木质素 - 半纤维素混合物的各种酶的活性曲线。



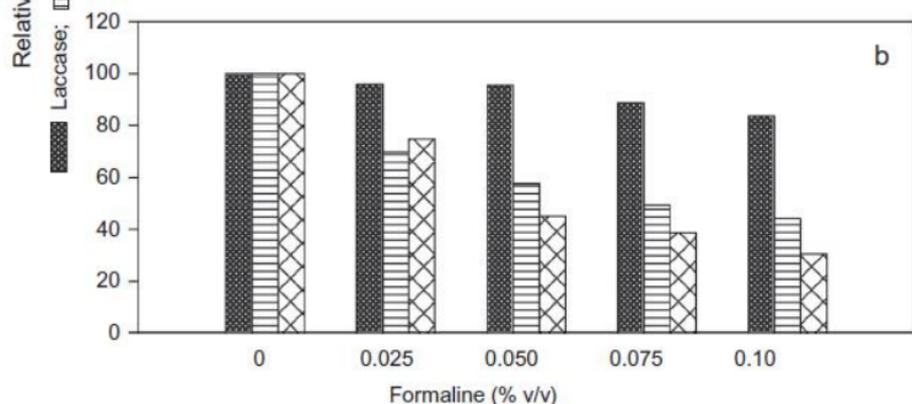
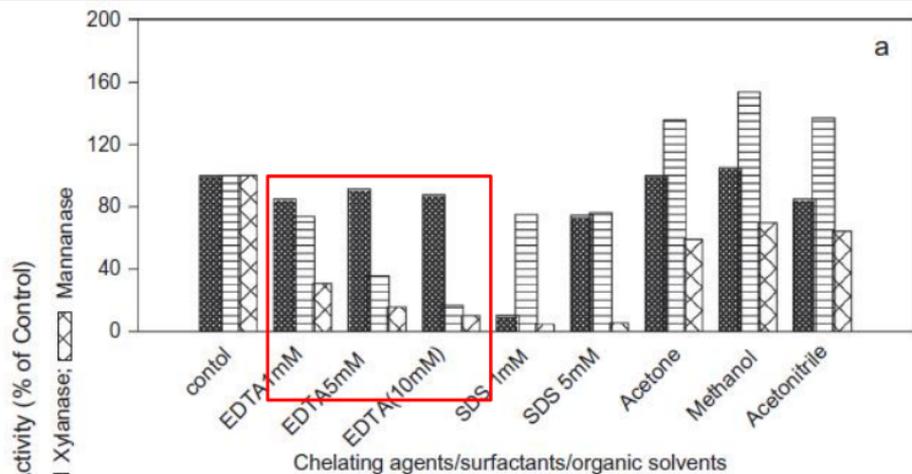
在50°C下没有和与5mM KCl的来自P.Phaeocomes S-1的漆酶 (a) , 木聚糖酶 (b) 和甘露聚糖酶 (c) 的热稳定性曲线。 开放符号表示含有5mM KCl的酶 , 封闭符号代表不含KCl的酶。



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。

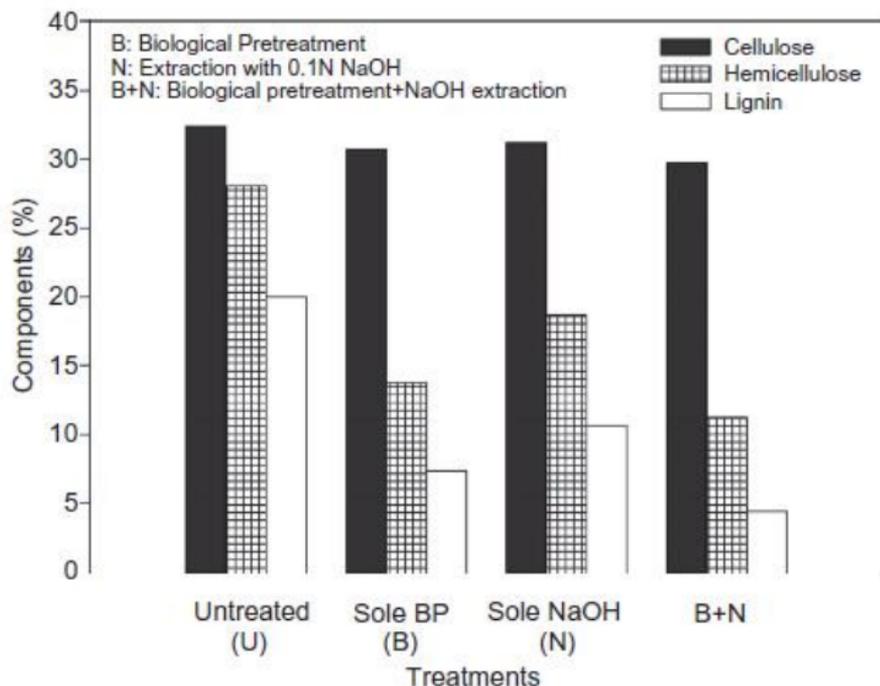


Fig. 6. Changes in percentage composition of rice straw after sole biological treatment (40 d), sole extraction with 0.1 N NaOH (30 min; room temperature) and the Biological pretreatment followed by extraction with 0.1 N NaOH.



THANKS