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香港城市大學
City University of Hong Kong

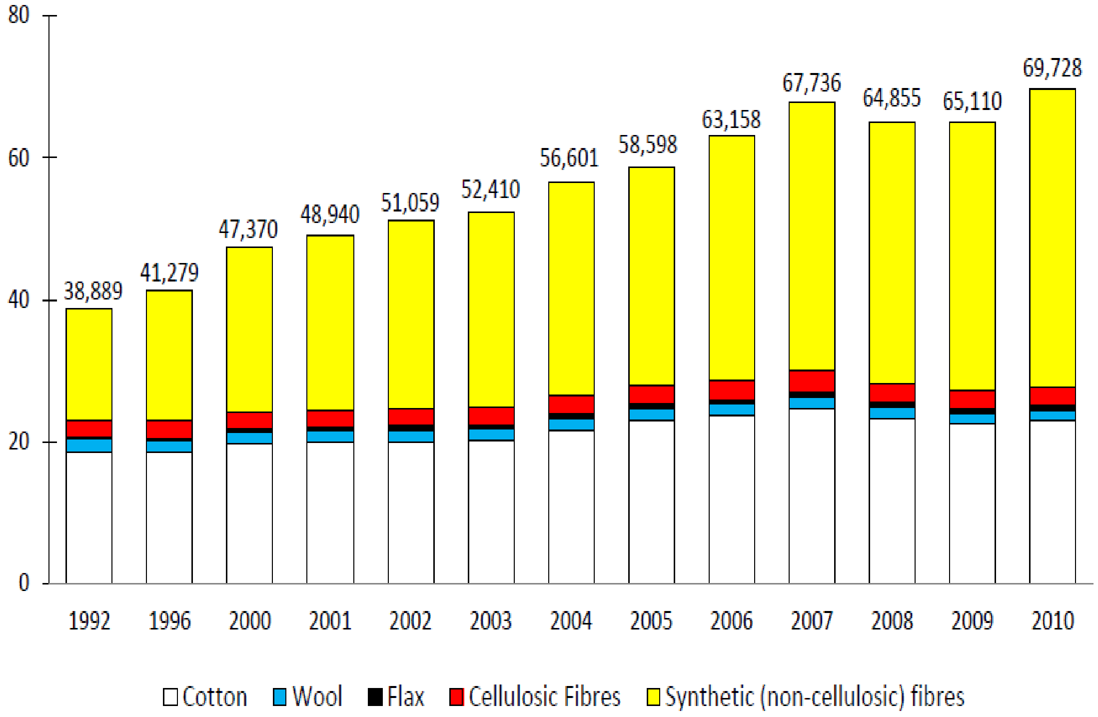
Cellulase Production via Solid State Fermentation on Textile Waste

HU Yunzi

5th International Conference on Sustainable Solid Waste Management
Athens, 2017

Project background-Textile consumption

- In the decade of 2001-2010, the textile consumption expanded by **42 - 43%**
- In 2011-2014, annual textile consumption reached **80 - 90 million tonnes**



Textile wastes: 10-20% of all textile products

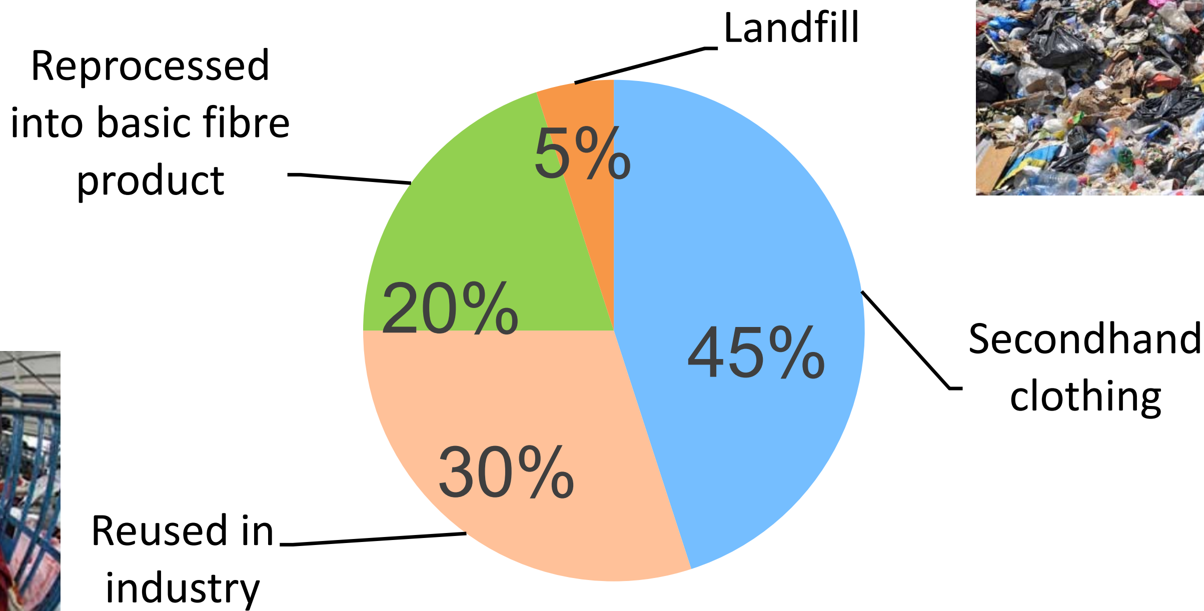
Generation of textile waste (million tonnes / year)		Textile waste per capita (kg/year)
China	26.0	19.2
UK	1.0	15.6
US	12.4	32.0
HK	0.17	23.7

Source: China Association of Resource Comprehensive Utilization; Waste & Resources Action Programme (UK); SMART textile recycling (US); Department EP of Hong Kong

Where did they go?

(ITP/109/15TP)

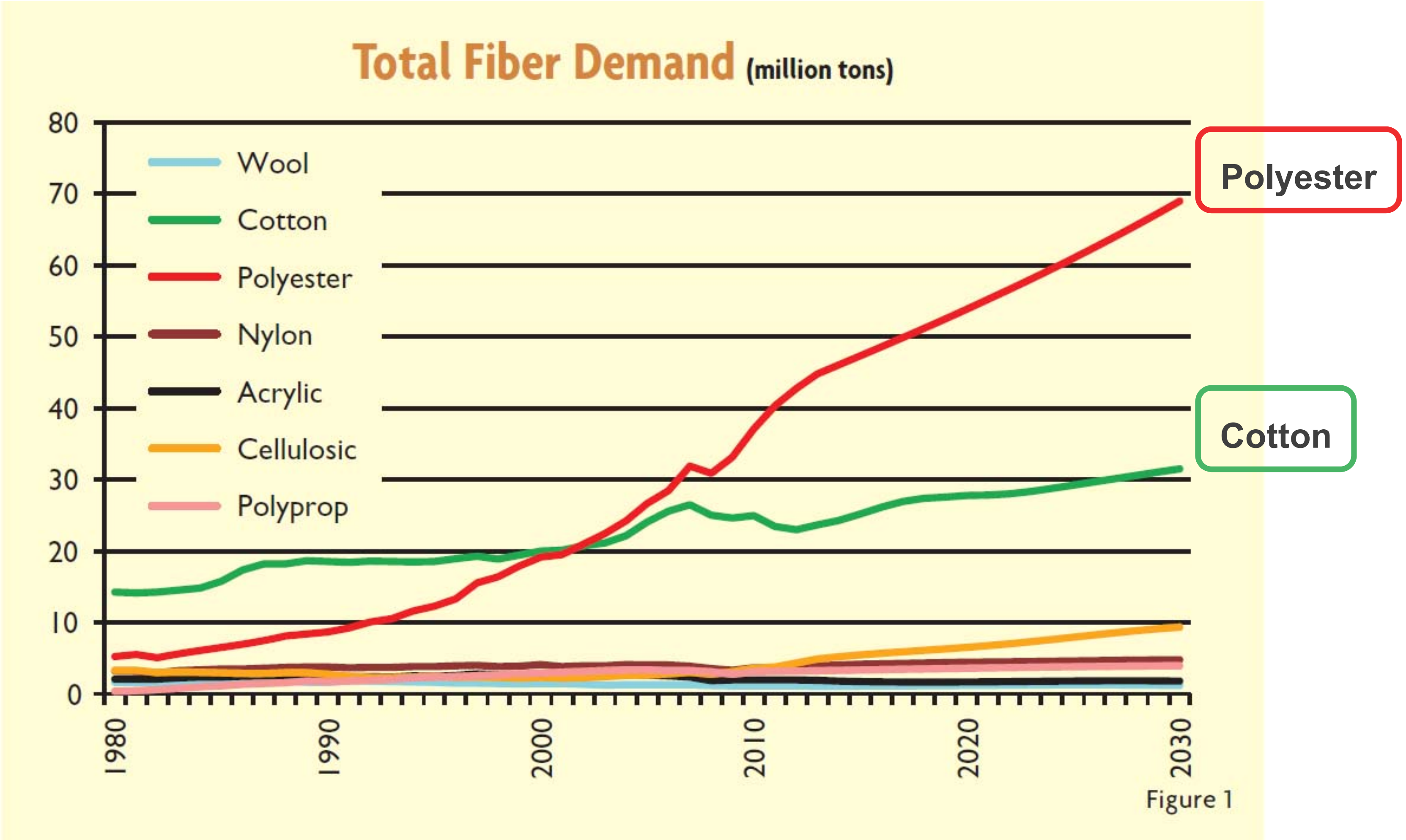
- Landfill: **85%** of the total textile solid waste
- Recyclable percentage: **95%** of landfilled textile waste
- Currently recycling percentage: **14-15%**



Source: U.S. Environmental Protection Agency and SMART Association, British Material Recycling Association

Project background-Textile consumption

- Cotton and Polyester: Top 2 in Fibre Demand



Source: Textile World, Man-Made Fibers Continue To Grow

Project introduction

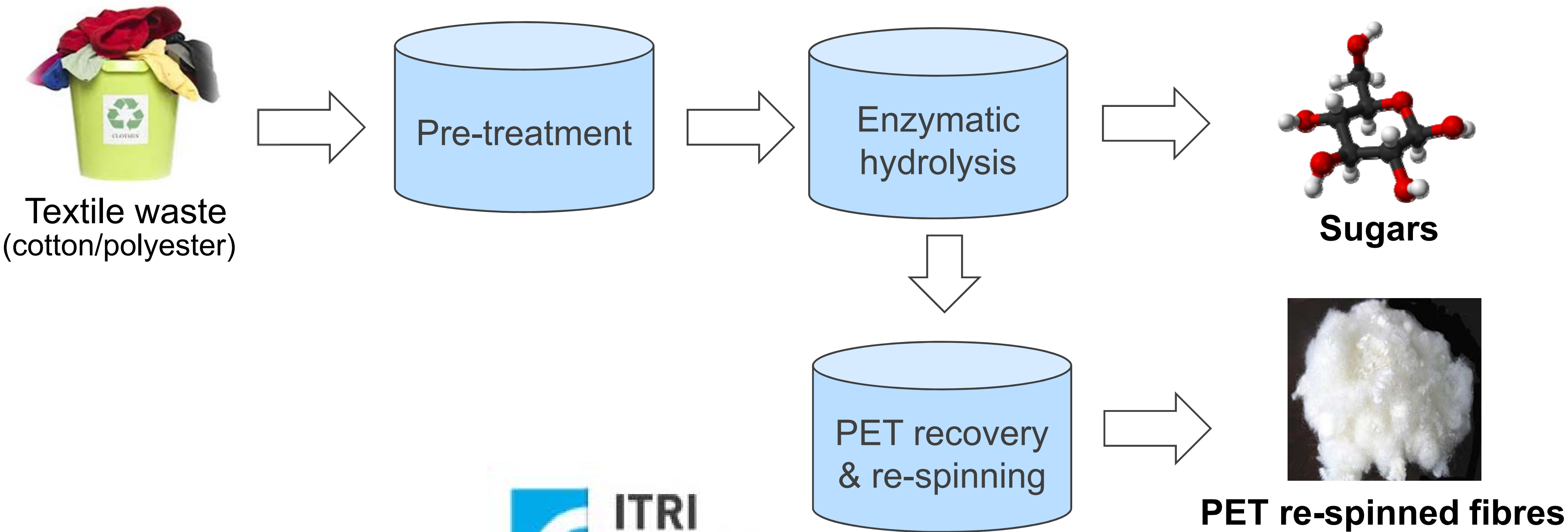
(ITP/109/15TP)

We propose a sustainable textile waste recycling strategy in HK:

1. A novel approach of textile waste treatment via biological method

2. Recovery of glucose from textile wastes through enzymatic hydrolysis

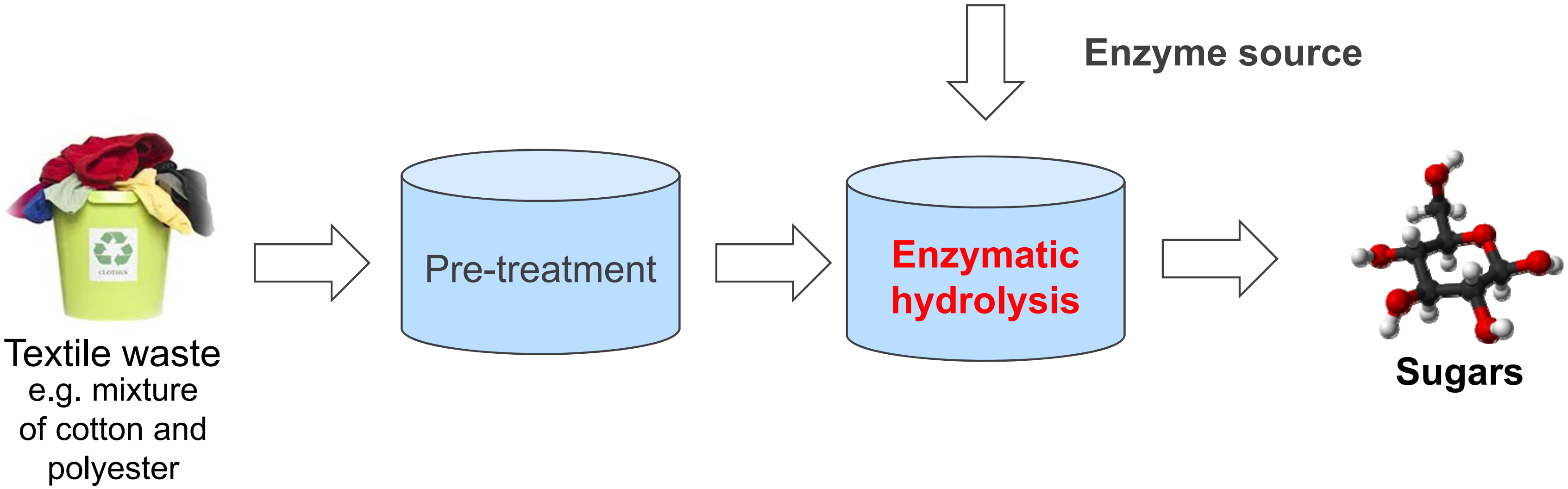
3. Separate the PET fiber from textile waste and reuse it in textile industry



Research target

Fungal cellulase production on textile waste

(Conducted by Dr. Carol Lin from CityU and Dr. Du Chenyu from the University of Huddersfield)



Materials	Dye	Pre-treatment	Source of enzymes
• 100% Cotton • 100% Polyester • Cotton/PET blend (80/20, 60/40, 40/60) • Jean	• Reactive • Disperse • Indigo	• Alkaline • Milling • Autoclave	• **Fungal enzymes (solid state fermentation: SSF)**

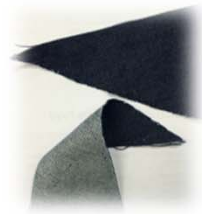
Cellulase production via SSF on textile waste

(ITP/109/15TP)

Substrate: different types of textile fabrics (from H&M)





Component	Cotton 100%	Cotton 80% PET 20%	Cotton 60% PET 40%	Cotton 40% PET 60%	PET 100%	Jeans (Cotton 99%, Elastane 1%)
Dye	Reactive dyestuff	Reactive dyestuff	Reactive dyestuff	Reactive dyestuff	Disperse dyestuff	Indigo dye

Cellulase production via SSF on textile waste

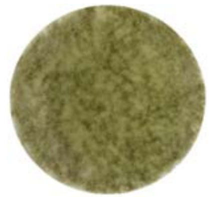
(ITP/109/15TP)

Cellulase producing fungal strains

A. niger CKB

A. niger N402

T. reesei



T. longibrachiatum

A. oryzae

R. variabilis

Aspergillus niger **CKB*:* Isolated from natural environment (provided by Dr. Diannan Lu, Tsinghua University)**

Aspergillus niger **N402: From Prof. David Archer in the University of Nottingham in the United Kingdom**

T. reesei: Trichoderma reesei **ATCC 24449**

T. longibrachiatum: Trichoderma longibrachiatum (**Prof. Colin Webb, The University of Manchester, United Kingdom)**

R. variabilis: Rhizomucor variabilis **(provided by Tsinghua University)**

Cellulase production via SSF on textile waste

(ITP/109/15TP)

1. Screen of fungal strains

Substrate: 100% cotton fabric

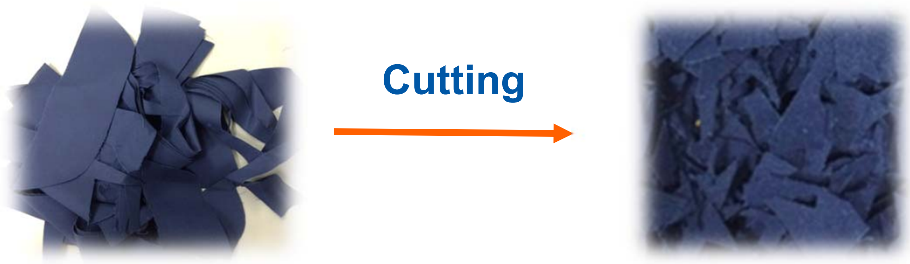
Moisture: 65-85%

Duration: 7 days

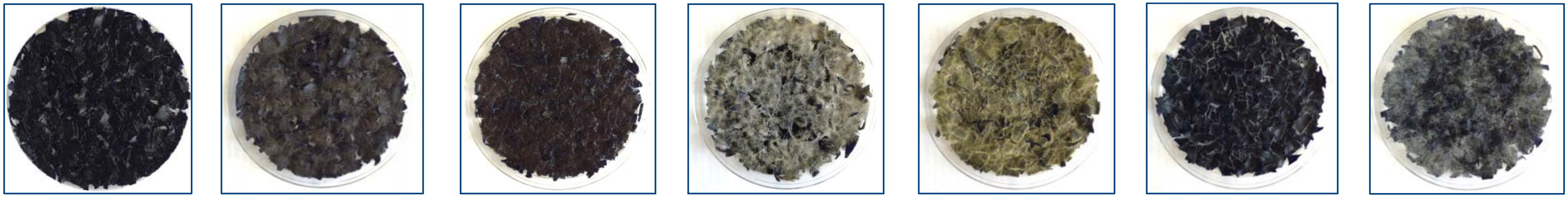
Temperature: 28°C

Supplemented nutrient: yeast extract 2.5% (w/w)

$0.8 \times 0.8\ cm^2$



Day 0 | Day 7 | Day 7 | Day 7 | Day 7 | Day 7 | Day 7



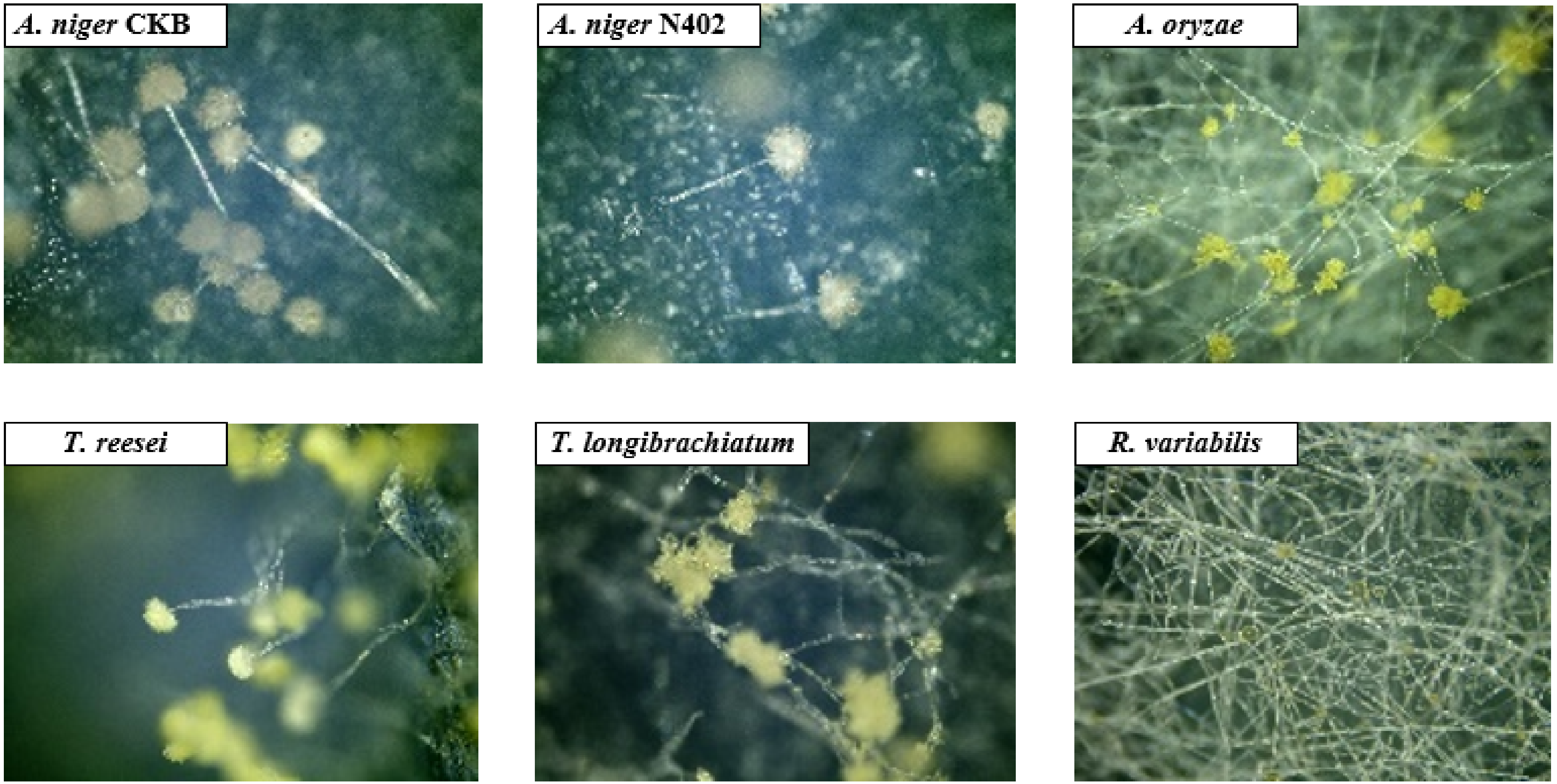
Before SSF | *A. niger* CKB | *A. niger* N402 | *A. oryzae* | *T. longibrachiatum* | *T. reesei* | *R. variabilis*

Cellulase production via SSF on textile waste

(ITP/109/15TP)

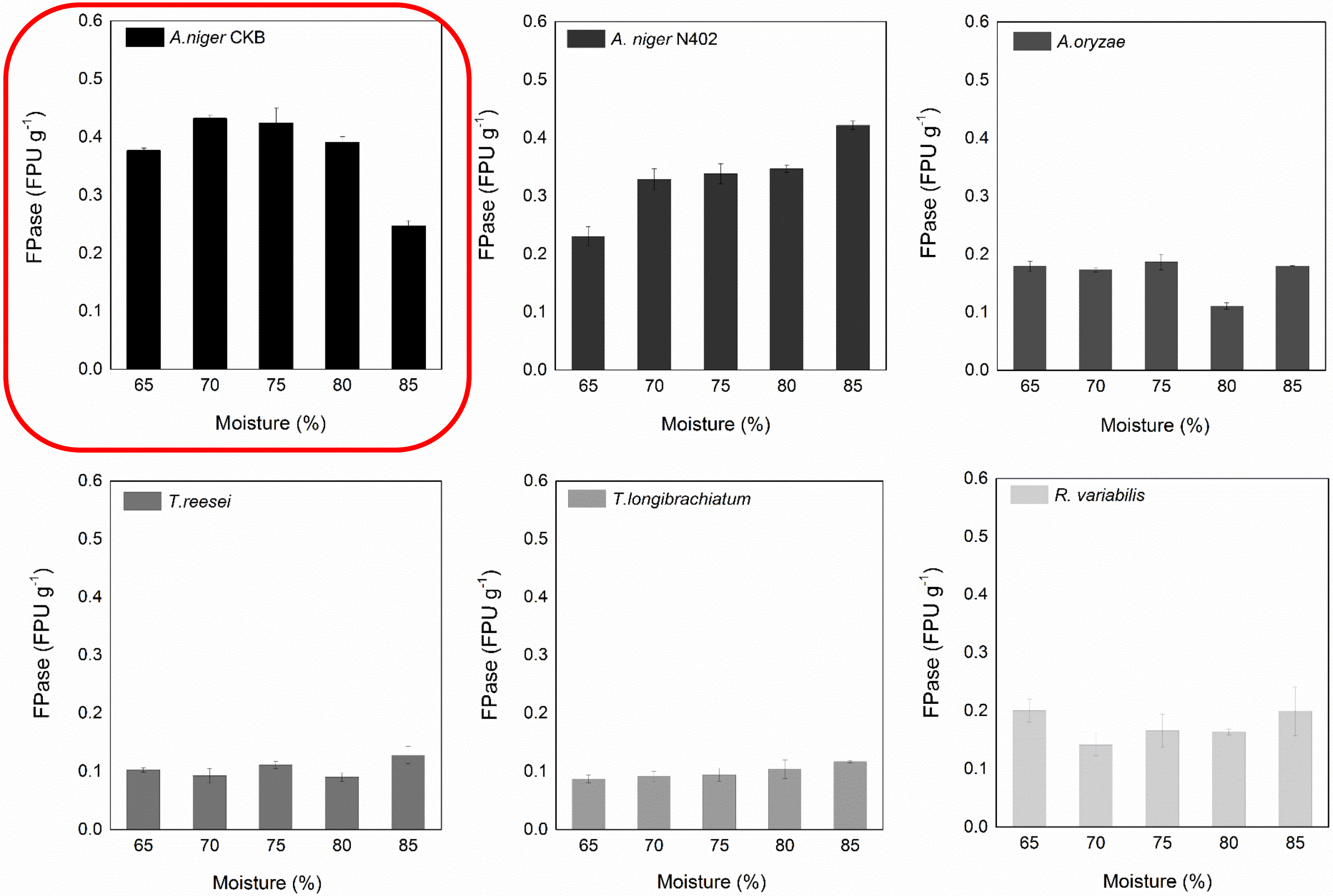
1. Screen of fungal strains

Microscope detection



Cellulase production via SSF on textile waste

1. Screen of fungal strains



Cellulase production via SSF on textile waste

2. Comparison of different textile fabrics

Substrate: 6 types of textile fabrics; Moisture: 75%; Temperature: 28°C; Yeast extract: 2.5 w/w%

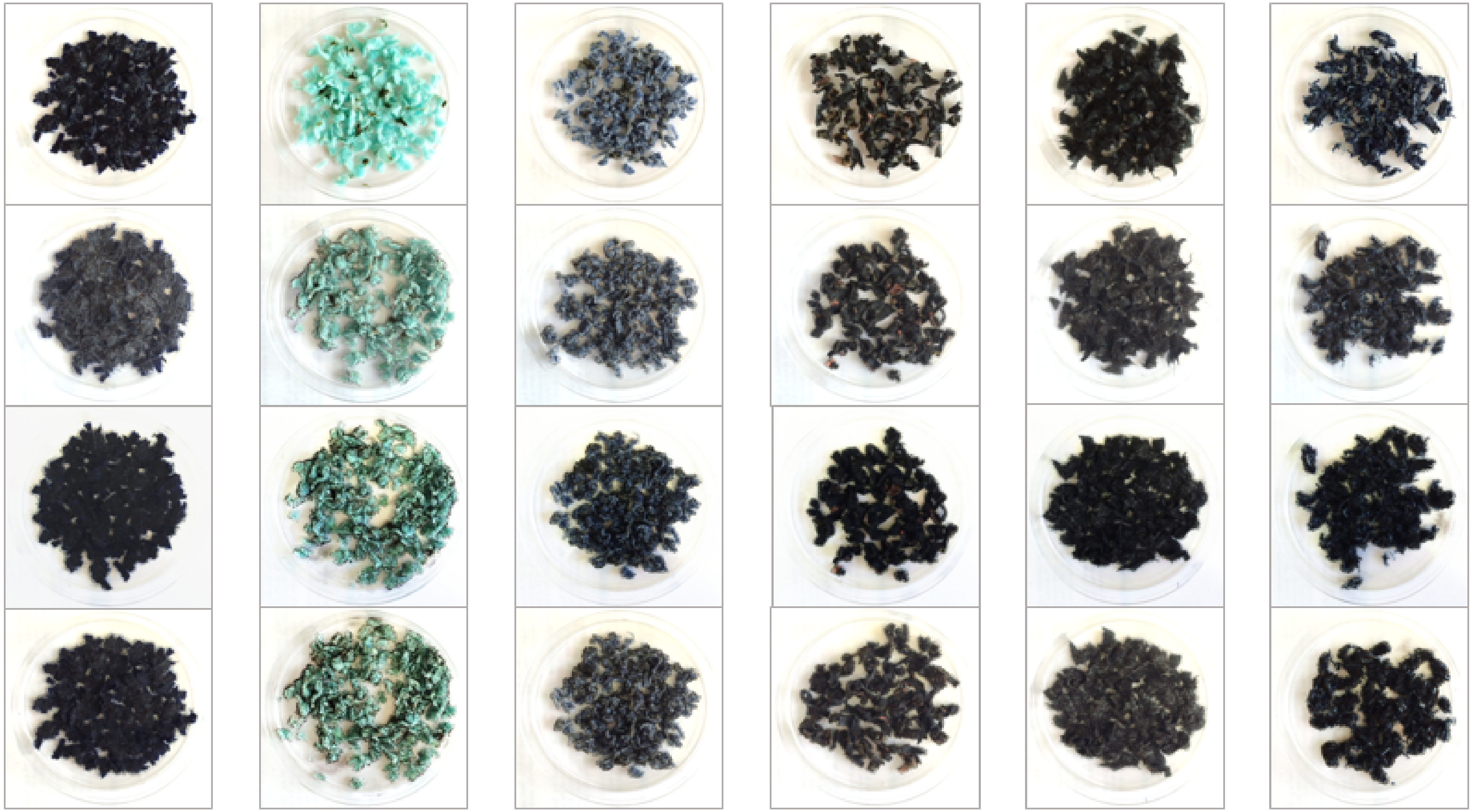
Cotton 100% | Cotton 80% PET 20% | Cotton 60% PET 40% | Cotton 40% PET 60% | PET 100% | Jeans

Day 1

Day 3

Day 5

Day 7



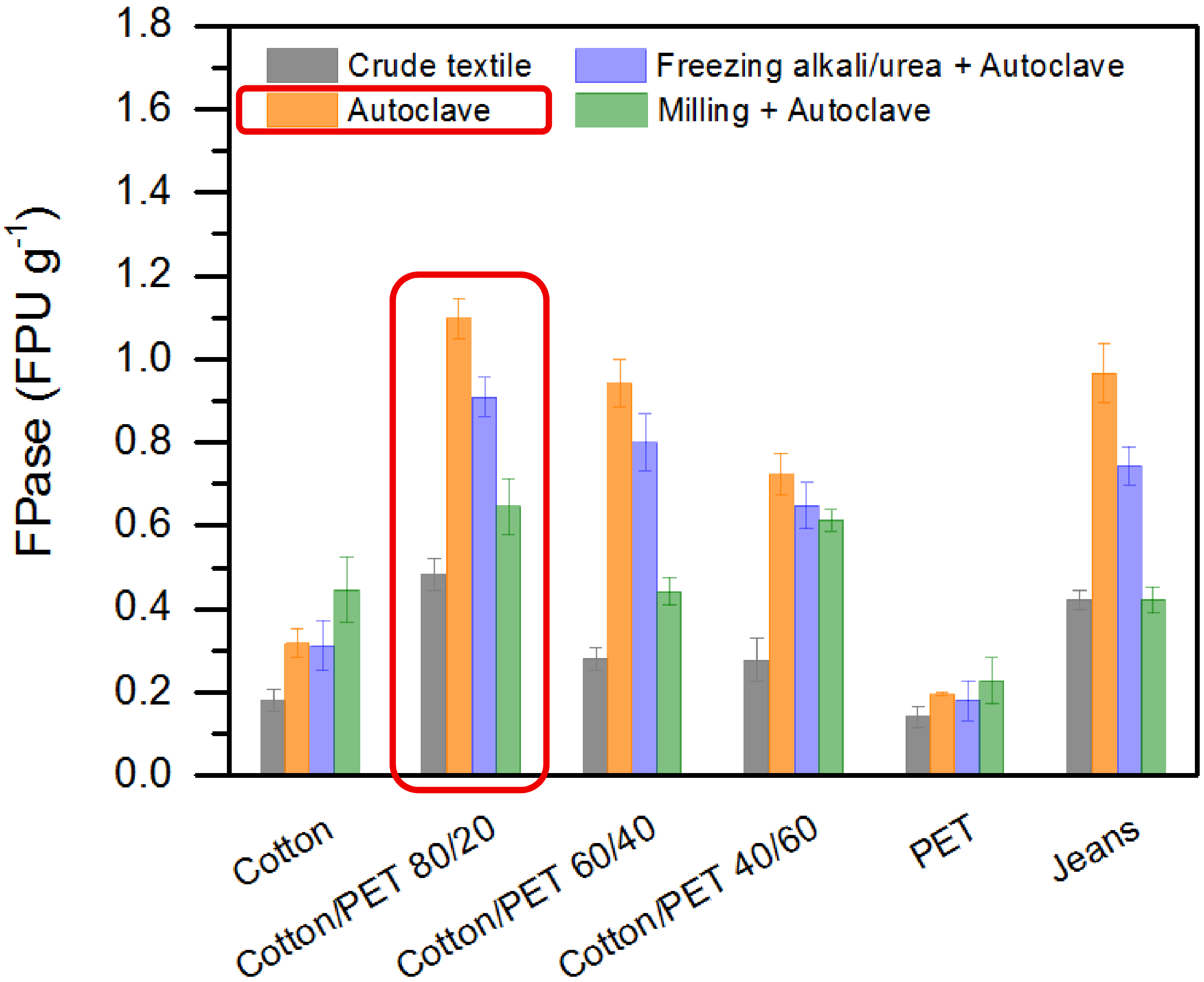
Cellulase production via SSF on textile waste

(ITP/109/15TP)

2. Comparison of different textile fabrics

Pretreatments

1. **Autoclave (121˚C for 15 min)**
2. **Freezing alkali/urea**
   (Soaking at -20 ˚C for 6 h)
3. **Milling (powder form, < 1 mm^2)**



THE HONG KONG POLYTECHNIC UNIVERSITY 香港理工大學

Pretreatments were conducted by our collaborators Dr. Shao-Yuan Leu and Dr. Hao Liu in Hong Kong Polytechnic University

Cellulase production via SSF on textile waste

4. Optimization of fermentation condition by Response Surface Methodology

Response: Cellulase activity (FPU g^{-1})

Numeric factor	Unit	Low value	High value	-alpha	+alpha
A. pH	-	5	7	4	8
B. Yeast extract	w/w %	1	4	0	5.5
C. Inoculum size	Spores g^{-1} textile	1.6E+007	4.6E+007	1E+006	6.1E+007
D. Moisture	%	60	80	50	90

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.72	14	0.12	12.25	< 0.0001	significant
A-pH	*4.778E-006*	*1*	*4.778E-006*	*4.755E-004*	*0.9829*	
B-YE	*4.431E-003*	*1*	*4.431E-003*	*0.44*	*0.5167*	
C-Inoculum size	*0.14*	*1*	*0.14*	*13.68*	*0.0021*	
D-Moisture	*0.60*	*1*	*0.60*	*59.30*	*< 0.0001*	

Conclusions

- Model: significant
- Importance of factors:

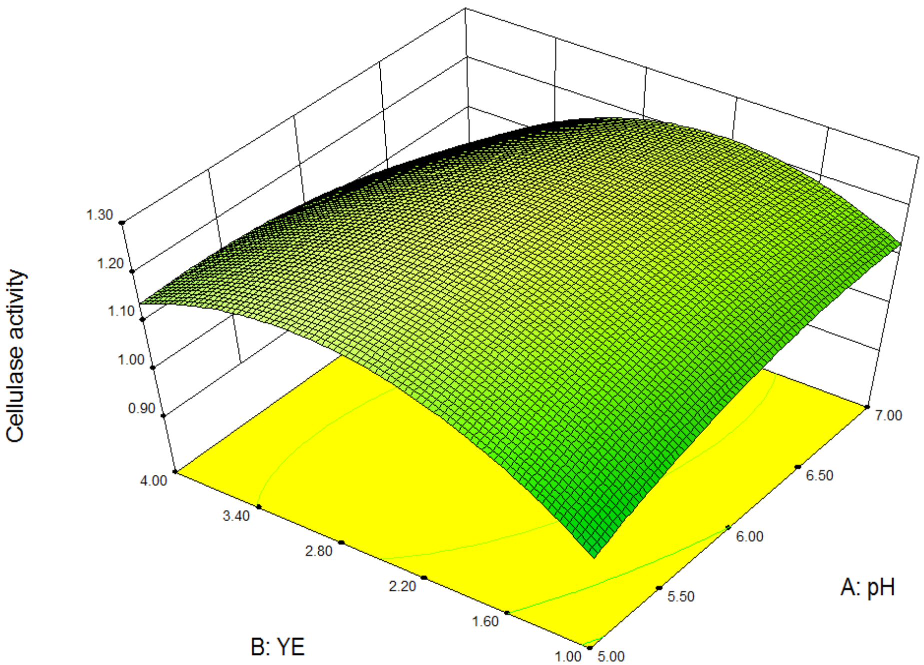
Moisture > Inoculum size

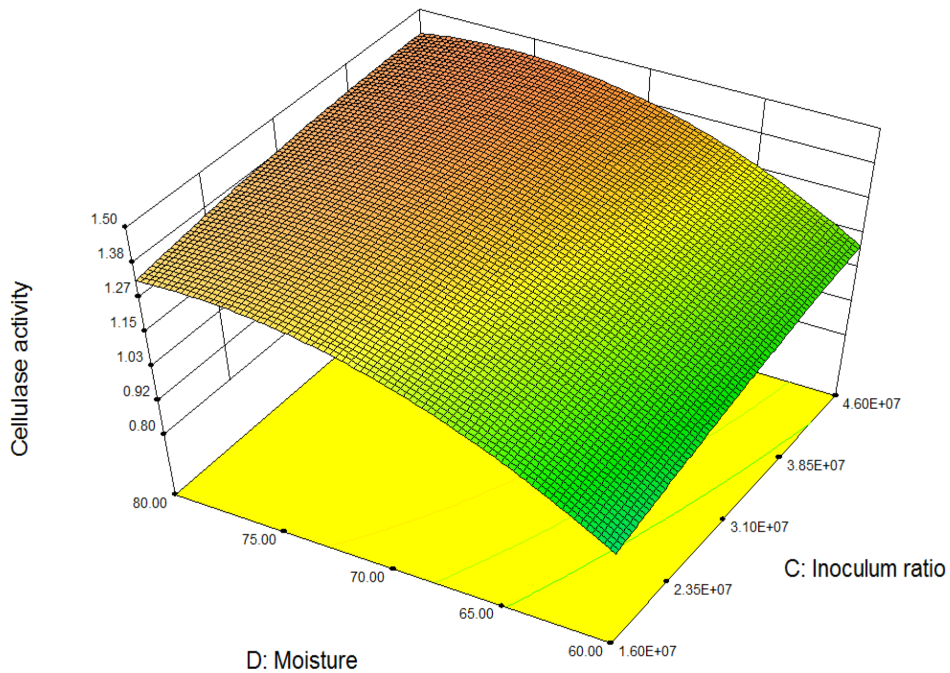
> > pH > Yeast extract

Cellulase production via SSF on textile waste

(ITP/109/15TP)

4. Optimization of fermentation condition by Response Surface Methodology





Optimal condition

Moisture 77-78%, inoculum size 3.1 x 10^7 spores g^{-1} textile, pH 6-7, yeast extract 2.3 w/w%

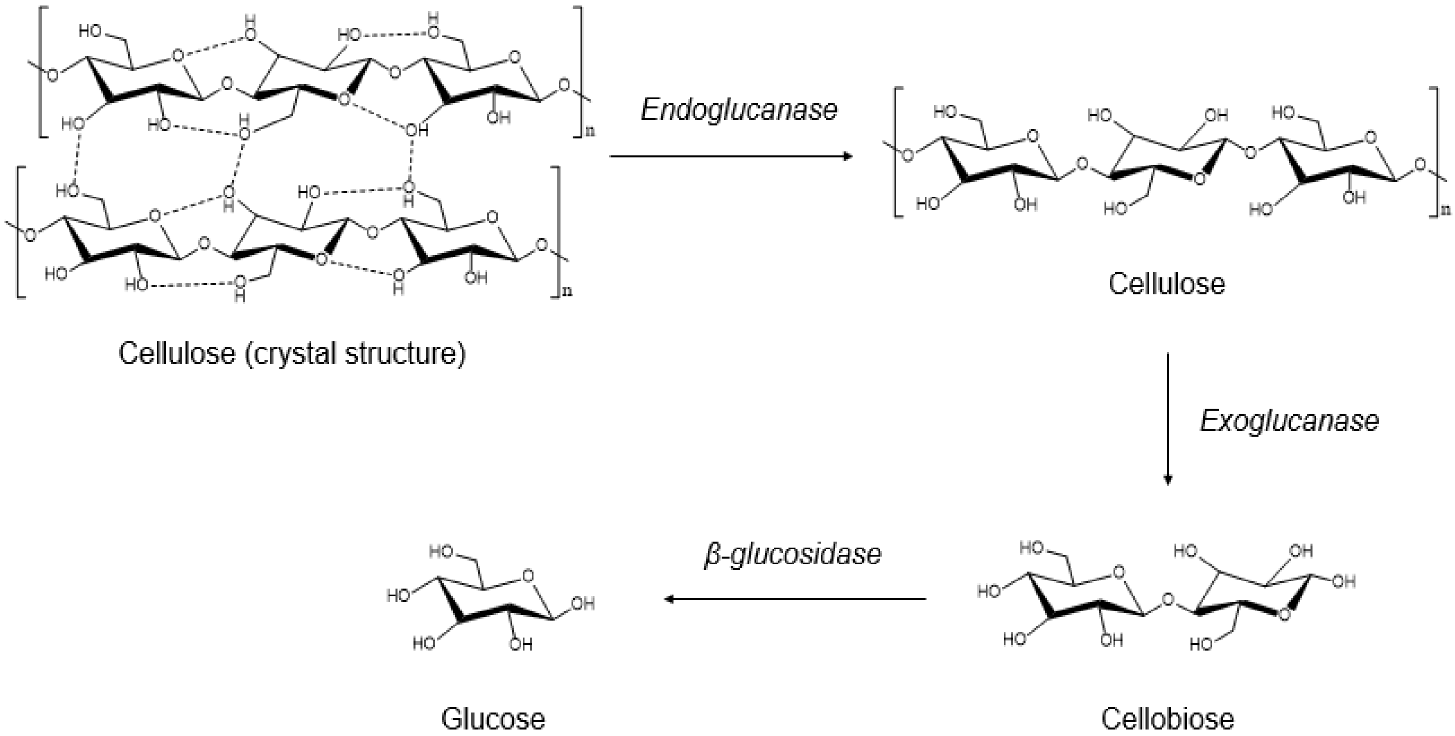
High point

1.44 FPU g^{-1} from cotton/PET 80/20

(17% increase from 1.24 FPU g^{-1})

Cellulase production via SSF on textile waste

5. Time courses of total cellulase activity and individual cellulase activities



Synergistic effect of a complete cellulase system

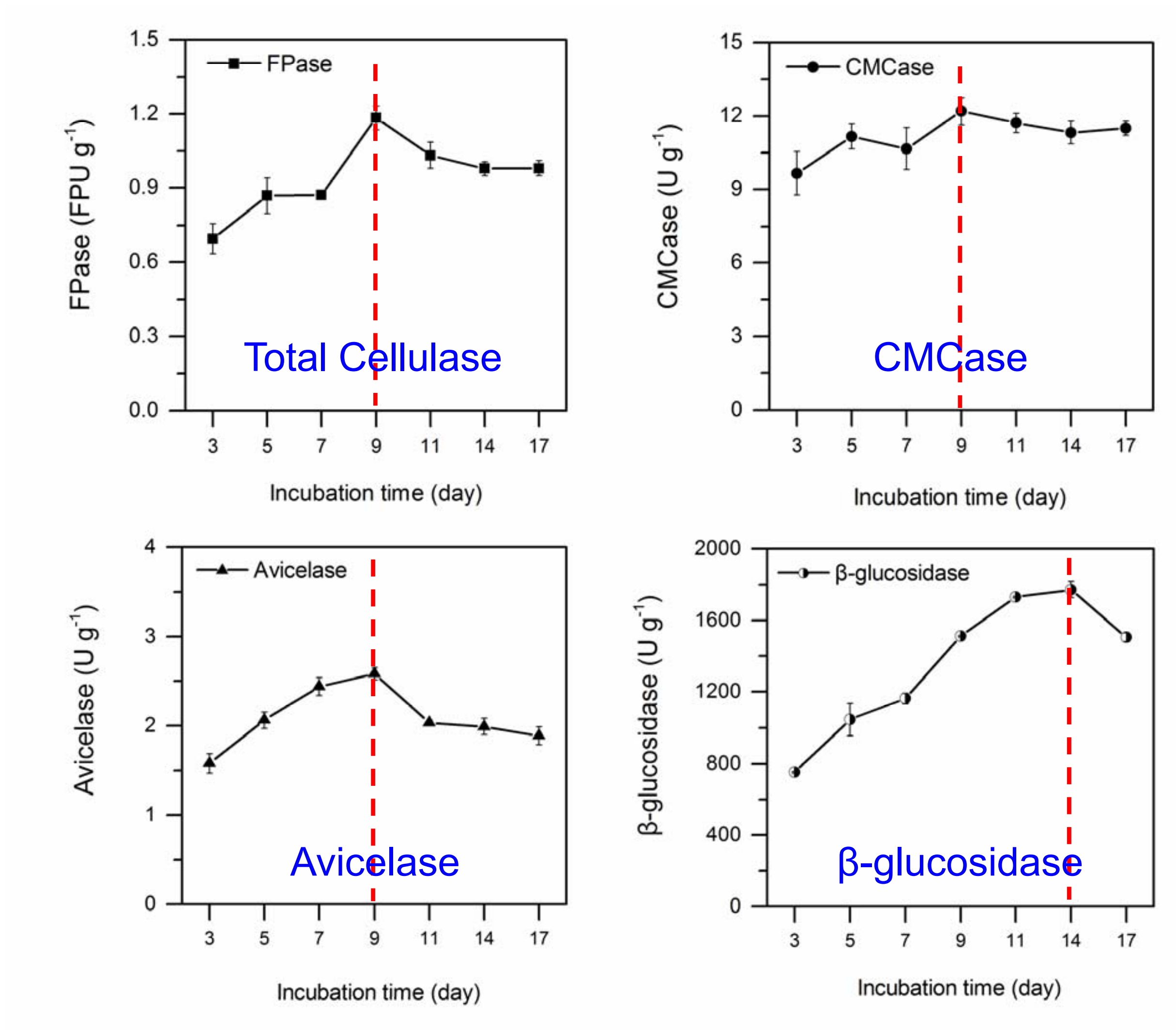
Cellulase	Substrate for activity test
Total cellulase	Filter paper
Endoglucanase	CMC
Exoglucanase	Avicel
β-glucosidase	pGPN

CMC: carboxymethyl cellulose
pGPN: *p*-nitrophenyl-β-D-glucopyranoside

Cellulase production via SSF on textile waste

(ITP/109/15TP)

5. Time courses of total cellulase activity and individual cellulase activities



Cellulase production via SSF on textile waste

(ITP/109/15TP)

5. Time courses of total cellulase activity and individual cellulase activities

Effect of avicel (1 w/w%) on cellulase time profile

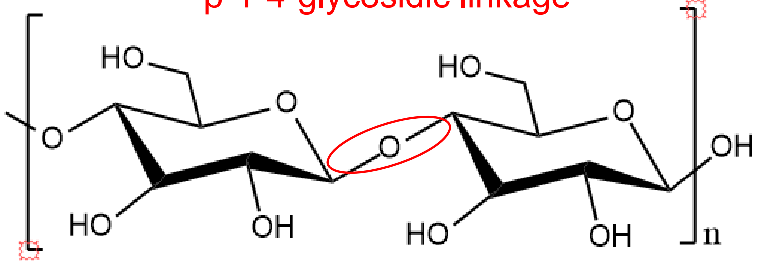
Substrate: Cotton/PET 80/20

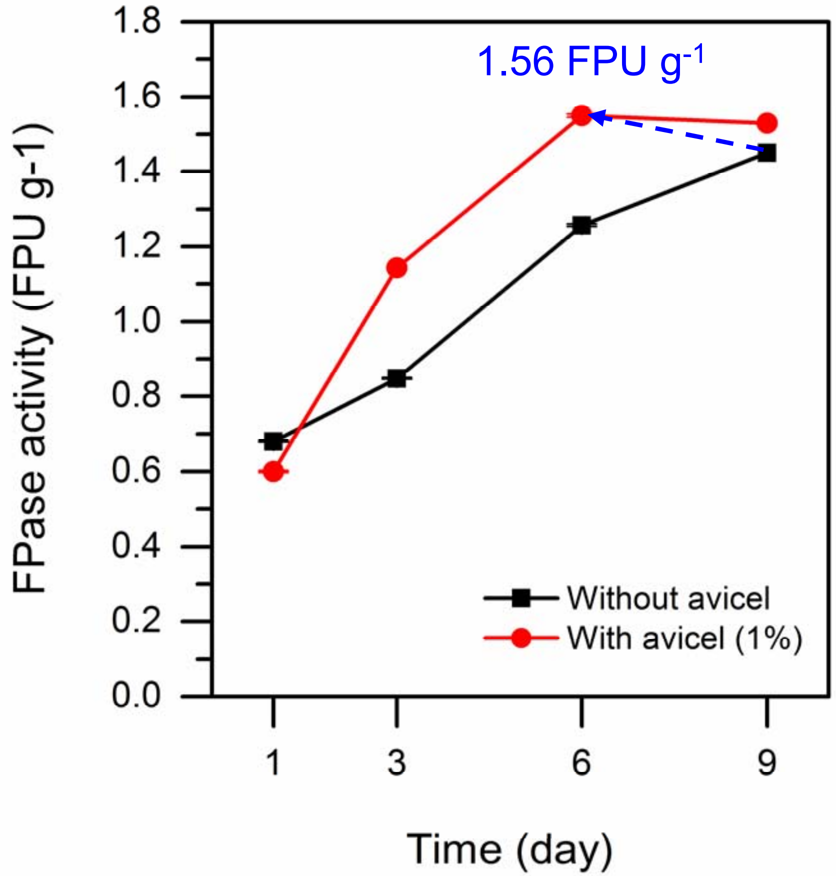
Moisture: 78%

Inoculum size: 3.1E+7 spores g^{-1}

Temperature: 28°C

Duration : 9 days



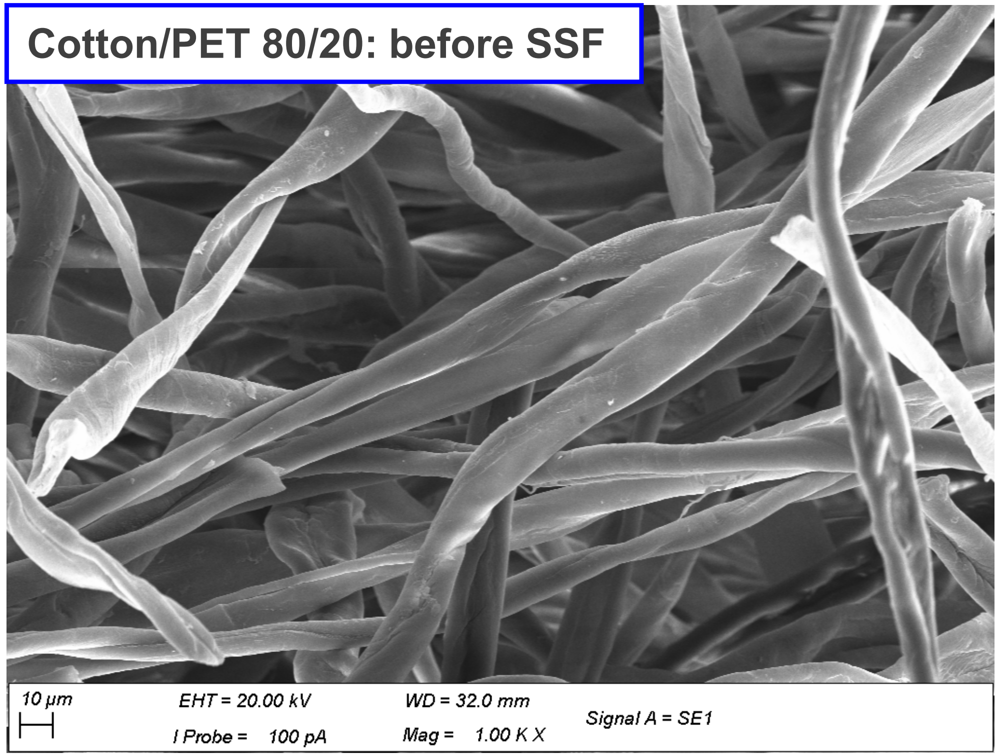


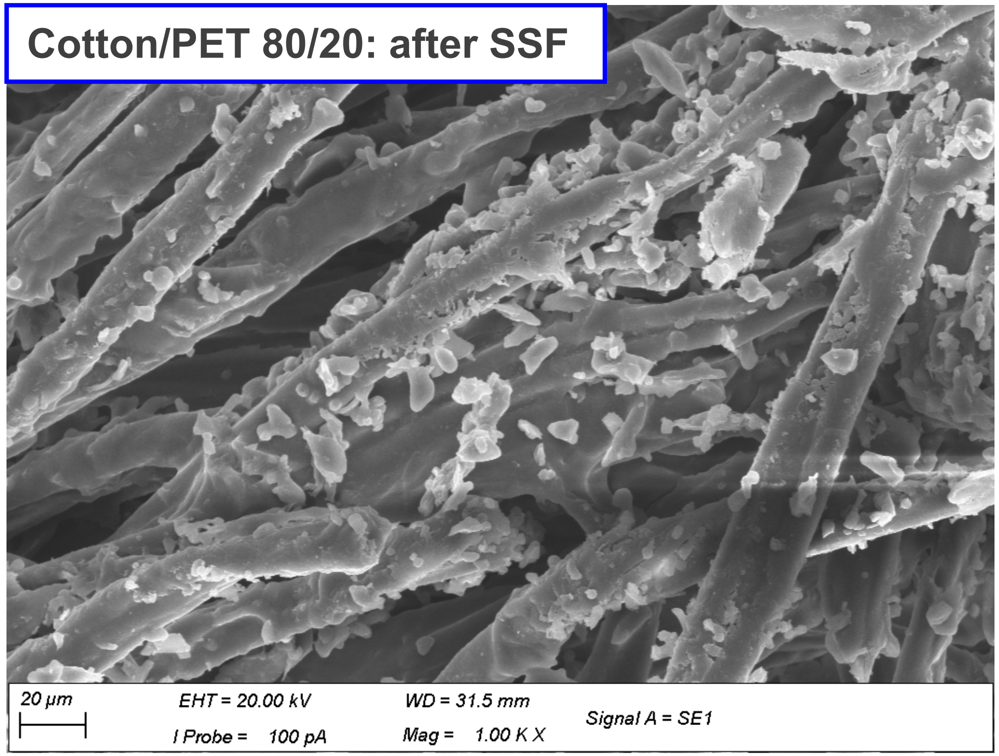
Cellulase production via SSF on textile waste

(ITP/109/15TP)

6. Scanning Electron Microscope (SEM) detection

Magnification of 1000x

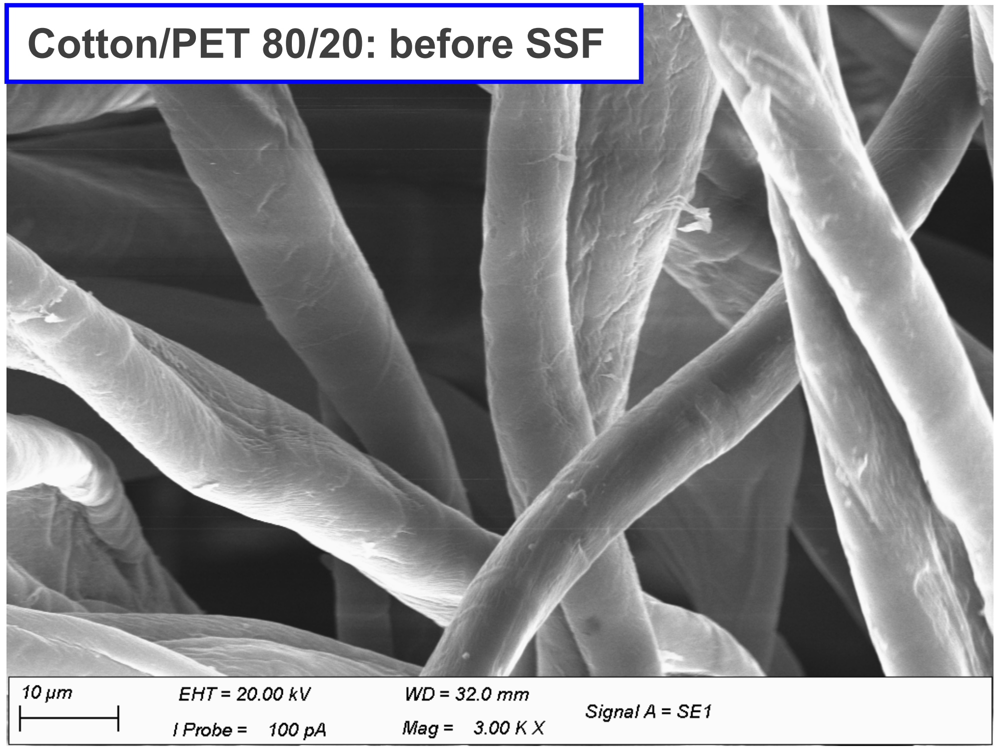




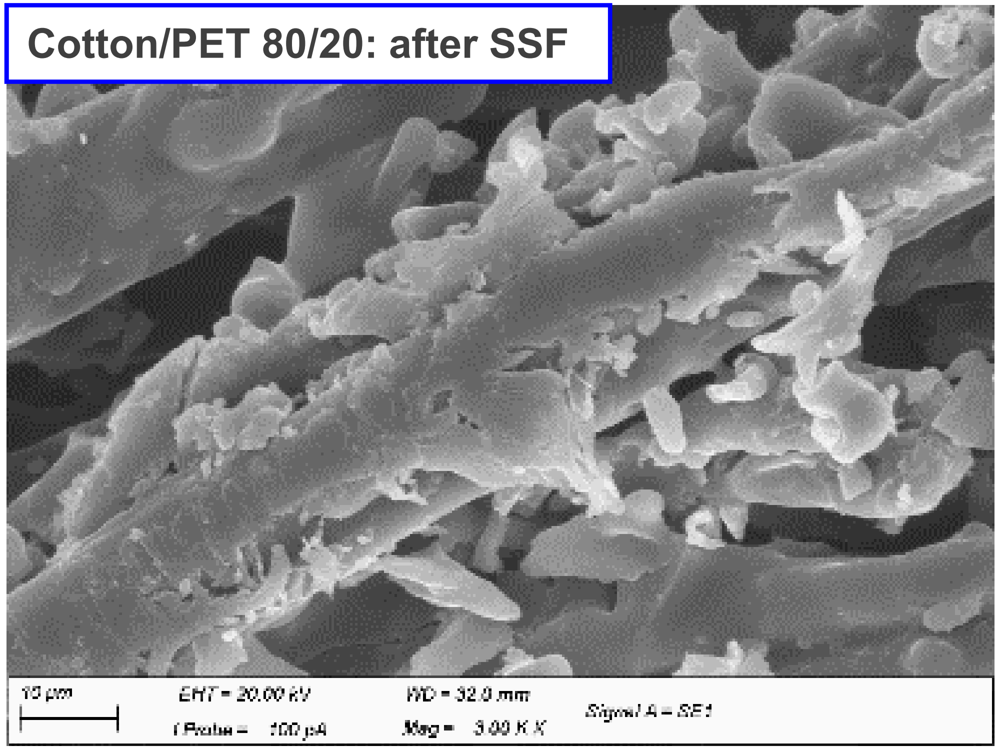
Cellulase production via SSF on textile waste

6. Scanning Electron Microscope (SEM) detection

Magnification of 3000x



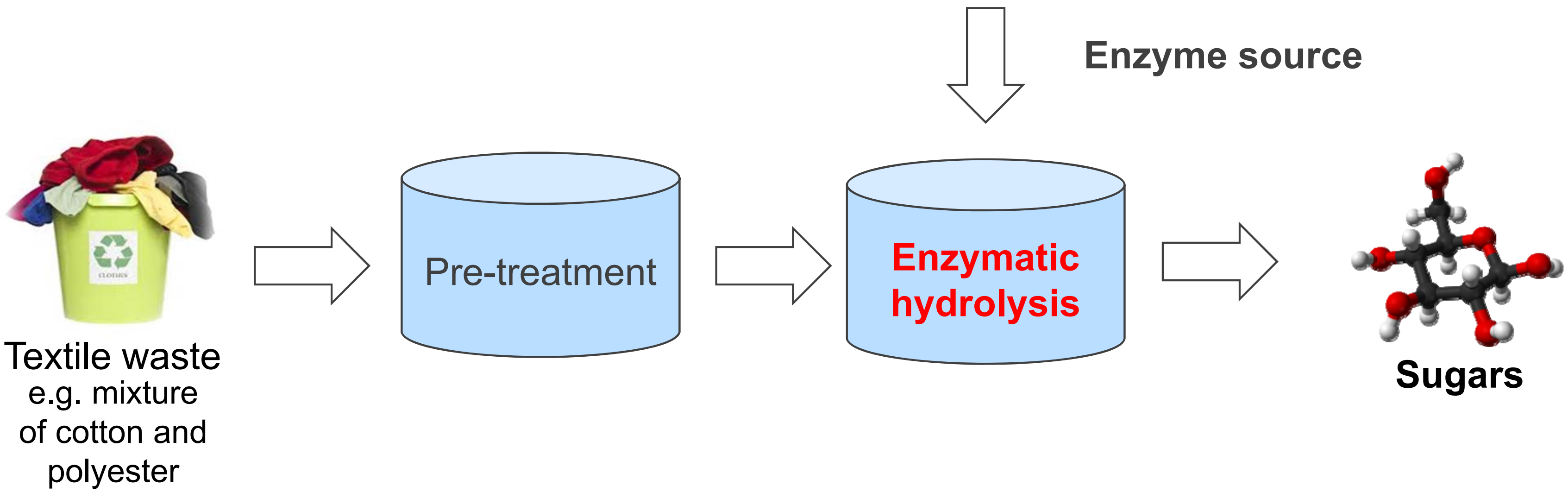
Cotton/PET 80/20: before SSF


Cotton/PET 80/20: after SSF

Research target

Fungal cellulase production on textile waste

(Conducted by Dr. Carol Lin from CityU and Dr. Du Chenyu from the University of Huddersfield)

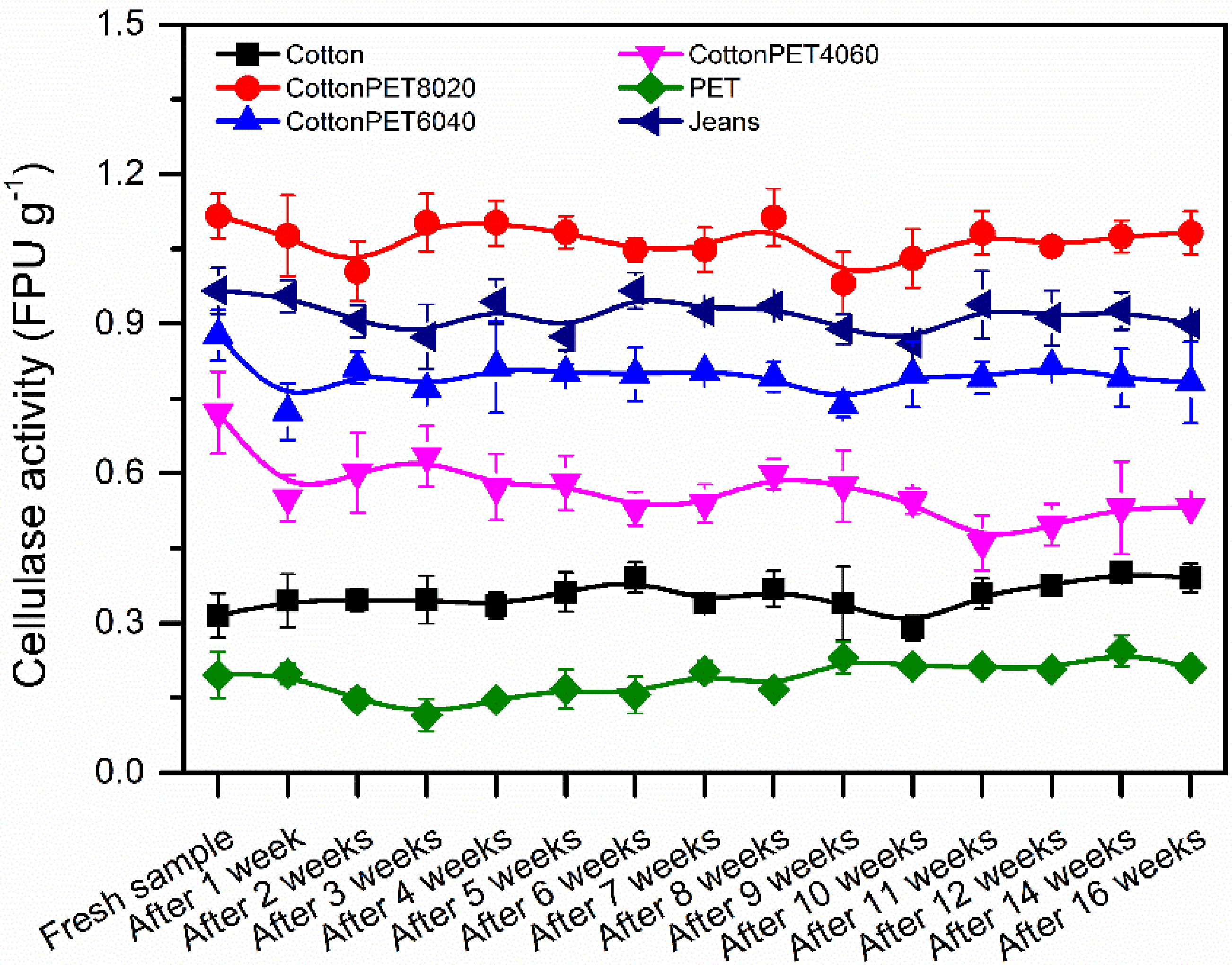


Materials	Dye	Pre-treatment	Source of enzymes
• 100% Cotton • 100% Polyester • Cotton/PET blend (80/20, 60/40, 40/60) • Jean	• Reactive • Disperse • Indigo	• Alkaline • Milling • Autoclave	• **Fungal enzymes** **(solid state fermentation: SSF)**

Cellulase production via SSF on textile waste

(ITP/109/15TP)

7. Retention of fungal cellulase extract (in -20˚C freezer)



	Cellulase activity ($FPU\ g^{-1}$)	
	Fresh sample	After 4 months
Cotton	0.31	0.39
Cotton/PET 80/20	1.12	1.08
Cotton/PET 60/40	0.88	0.78
Cotton/PET 40/60	0.72	0.53
PET	0.20	0.21
Jeans	0.97	0.90

Cellulase activity maintained stably for 4 months

(ITP/109/15TP)

Conclusions

- Cellulase was successfully produced on textile waste by solid state fermentation;
- The fungal strain ***A. niger* CKB** was selected (provided by Prof. Diannan Lu in Tsinghua University);
- Moisture and inoculum size are important factors;
- The highest cellulase activity was around **1.56 FPU g^{-1}**, obtained on textile of cotton 80% and PET 20% within **6 days** (80% moisture, 3.1E+7 spores g^{-1} textile);
- Crystalline structure of textile substrate was partially disrupted by cellulase digestion;
- Retention of crude fungal cellulase activity by freezing storage for application in textile hydrolysis.

Acknowledgements

(ITP/109/15TP)

- **The Hong Kong Research Institute of Textiles and Apparel (HKRITA)**
- **Sponsor:** H&M Hennes & Mauritz (Far East) Ltd.
- **Supervisor:**
  - Dr. Carol Lin
- **Collaborators:**
  - Dr. Chenyu Du (University of Huddersfield, United Kingdom)
  - Dr. Shao-Yuan Leu (Hong Kong Polytechnic University)
  - Dr. Hao Liu (South China University of Technology, China)
  - Dr. Diannan Lu (Tsinghua University, China)
- **Research team**

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Thank you for attention !

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Appendix

(ITP/109/15TP)

Literature review

Materials	Treatment			Fermentation		Polyester separation Efficiency (%)	Ref.
	Pre-treatment	Enzyme treatment	Glucose yield (%)	process	yield		
white 40/60 polyester/cotton blend	12 wt% NaOH -20 ~ 0 °C for 1h (5% solid load)	30 FPU cellulase 60 IU β-glucosidase per gram of cellulose	82	*S. cerevisiae* SSF	70% theoretical Ethanol	98	{Gholamzad, 2014 #1}
100% cotton linters; red T-shirt ; blue polyester/cotton (40/60) blended shirt	5 g/L Na2S2O4 and Na2CO3 solution 100 °C for 1 h 85% phosphoric acid 50 °C , 100 rpm for 2 h	10 FPU/g Cellulase AP3 50 °C 250 rpm 48 h	90 80 60	*Z. mobilis* SSF	50 g/L Ethanol from 75g/L waste textile		{Kuo, 2014 #2}
jeans	85% phosphoric acid 50 °C , 130 rpm for 24 h	7.5 FPU/g cellulase 50 °C 130 rpm 96 h	79.2			100	{Shen, 2013 #4}
100% cotton T-shirts	[AMIM]Cl (ionic liquid) 110 °C for 90 min	66 U/g cellulase 50 °C, 80 rpm for 96 h	94				{Hong, 2012 #5}
orange 50/50 polyester/ cotton blend; blue 40/60 polyester/viscose blend	85% N-methylmorpholine-N-oxide 120 °C for 2h	20 FPU/g cellulase 30 IU/g β-glucosidase 48 h	92				{Jeihanipour , 2010 #6}
blue jeans textiles	18% (w/v) NaOH 23 °C for 3 h	20 FPU/g cellulase 30 IU β-glucosidase 45 °C for 48 h.	99	*S. cerevisiae* SSF	85–86%		{Jeihanipour , 2009 #7}

Appendix

Analysis of Variance (F-test)

$$F = \frac{\text{Varience between treatment (MSTreatment)}}{\text{Varience within treatment (MSError)}}$$

$$MS_{Treatment} = \frac{\sum_i n_i(\bar{X}_i - \bar{X})^2}{K - 1}$$

$$MS_{Error} = \frac{\sum_{ij} n_i(X_{ij} - \bar{X}_i)^2}{N - K}$$

N: number of all results obtained;

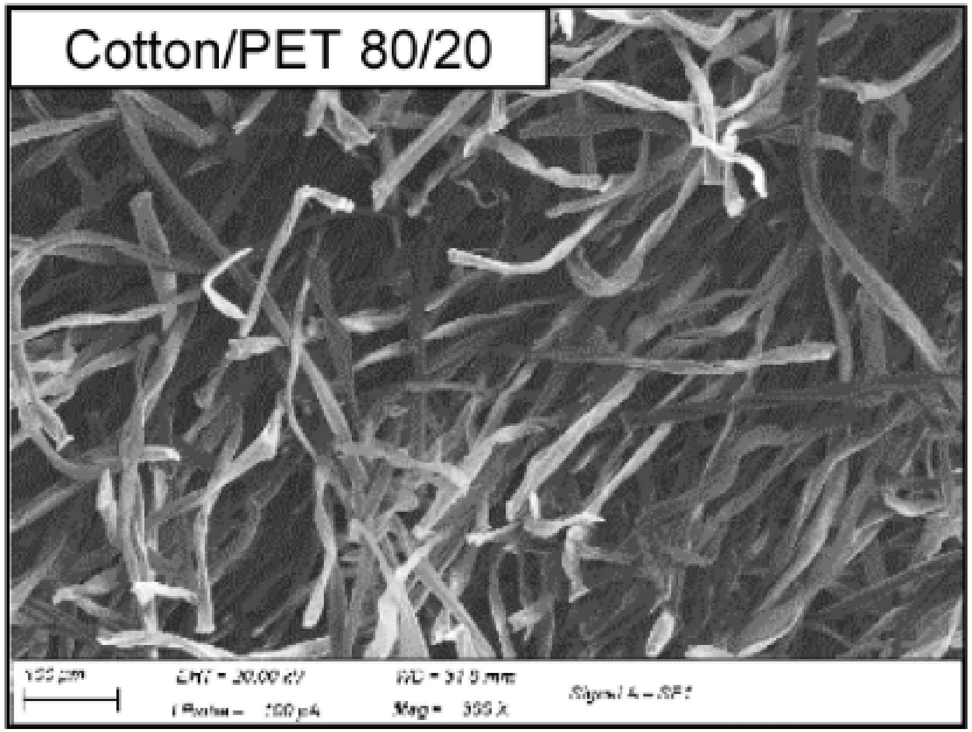
K: number of treatment;

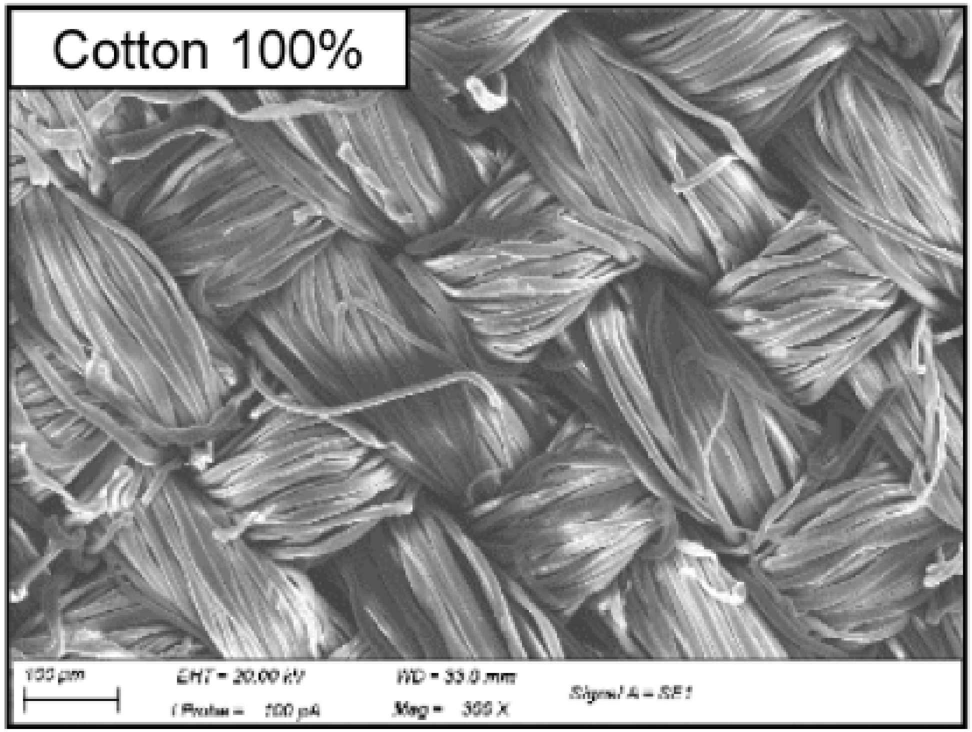
n: number of result in a treatment;

$\bar{X}$: average value of all results;

$\bar{X}_i$: average value of results in a treatment

Appendix





Cotton/PET blended textile (furry surface structure)

Larger surface area

Better aerobic condition

Appendix

4. Optimization of fermentation condition by Response Surface Methodology

Suggested optimal solution

Moisture 78%, inoculum size 4.6 x 10^7 spores g^{-1} textile, pH 7.2-7.3, yeast extract 2.3 w/w%

Predicted response

1.48 FPU g^{-1} (from cotton/PET 80/20)

Test

1) Inoculum size 4.6 x 10^7 spores g^{-1} textile:
2) Inoculum size 3.1 x 10^7 spores g^{-1} textile:
3) Inoculum size 1.6 x 10^7 spores g^{-1} textile:

Actual response

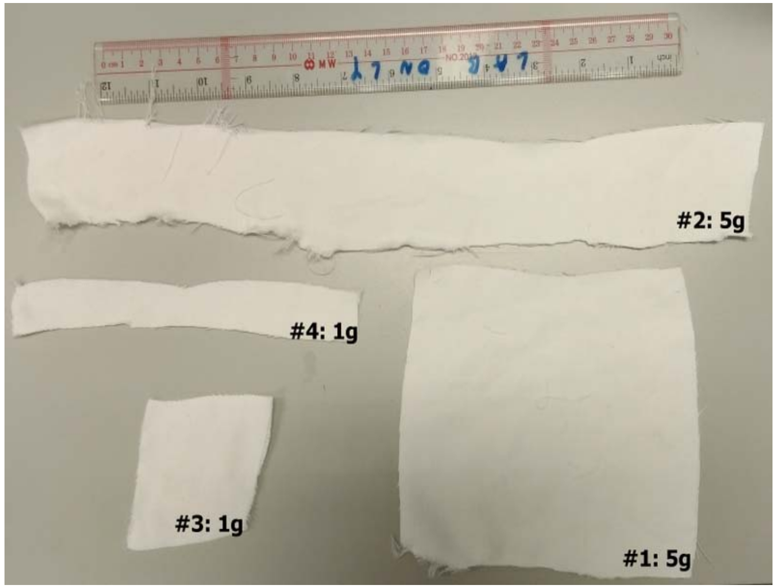
1.46 FPU g^{-1}

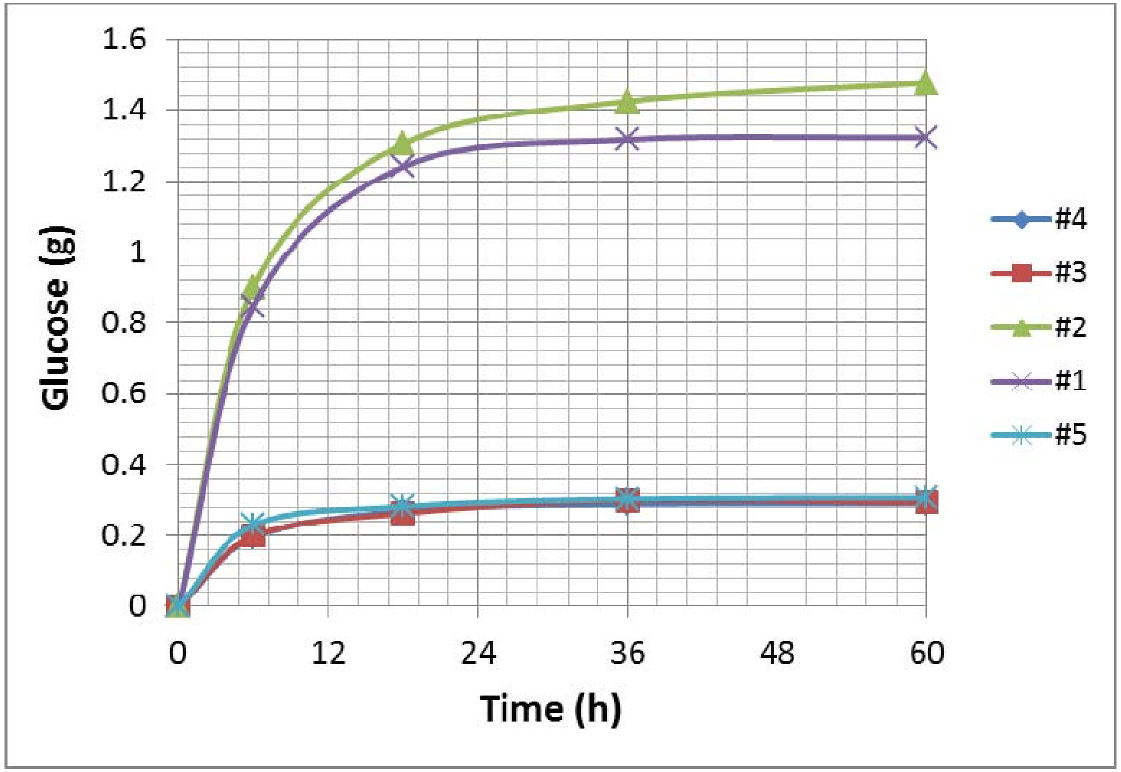
1.44 FPU g^{-1}

1.13 FPU g^{-1}

Appendix

- **Effect of fabric size on the enzymatic hydrolysis**





No.	Shape	Initial weigh (g)	Final weigh (g)	Glucose (g)	Glucose recovery (g/kg)
1	Square (14×14 cm^2)	5.00	3.54	1.33	266
2	Rectangle (40×5 cm^2)	5.00	3.44	1.48	296
3	Square (6.3×6.3 cm^2)	1.00	0.66	0.29	290
4	Rectangle (18×2.2 cm^2)	1.00	0.70	0.29	290
5	Pieces (0.3×0.3 cm^2)	1.00	0.67	0.31	310

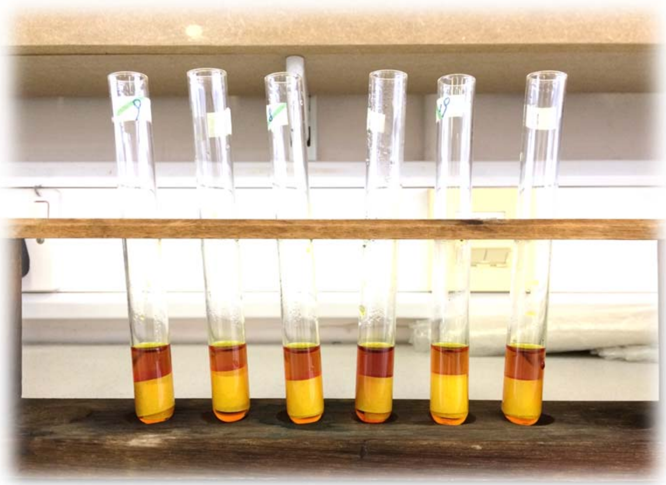
Appendix

Cellulase activity measurement

Enzyme extraction

(N. Pensupa, 2013)

1) Dissolved in 5 mM citric acid buffer
2) Blending and centrifuge
3) Collect the enzyme solution



Cellulase activity detection

(Filter paper activity, B. Adney, 1996)

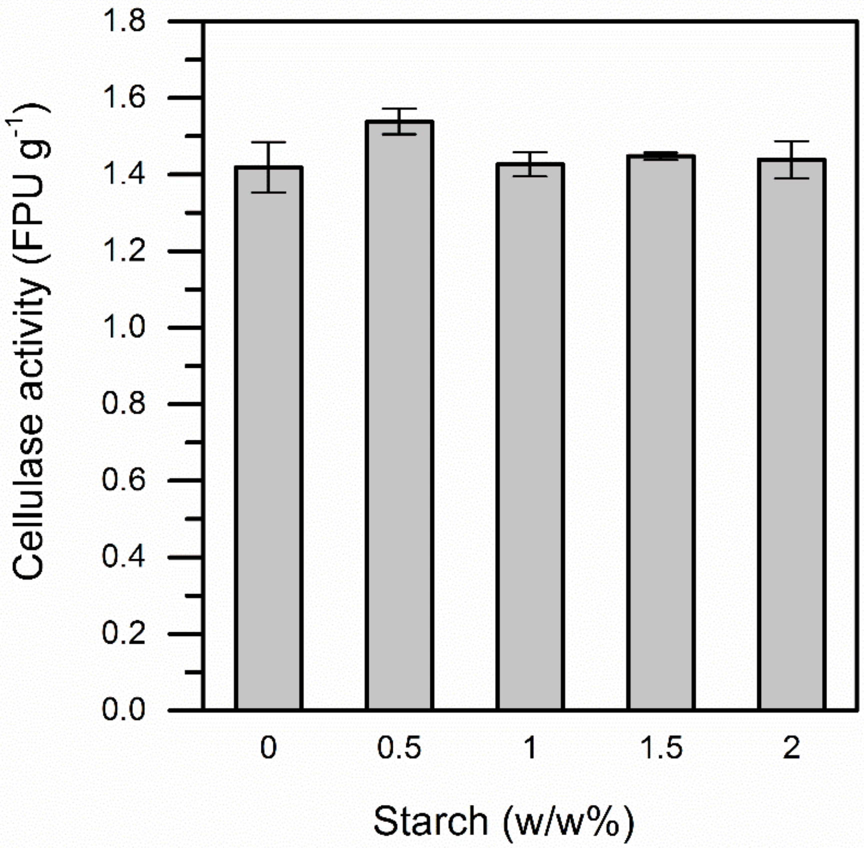
1) 0.5 ml enzyme solution + 1 ml citric acid buffer + 50 mg filter paper roll
2) Water bath 50 ℃ for 1 h
3) + 3 ml DNS solution and boiling for 5 minutes
4) UV detection at 540 nm

Appendix

(ITP/109/15TP)

Effect of starch on SSF

Moisture: 80%, Temperature: 28 °C, Duration : 9 days, Substrate: Cotton/PET 80/20



Starch as carbon source to support cell growth

Starch	Cellulase activity	REF
0% to 0.5%	1.42 to 1.53 FPU (increase 7.7%)	This study
0% to 0.75%	0.76 to 0.87 FPU (increase 14%)	Liang et al, 2012

(ITP/109/15TP)

Conclusion: insignificant

Effect of different cellulase producing inducers

Moisture: 80%, Temperature: 28 °C, Duration : 9 days, Substrate: Cotton/PET 80/20

Loading ratio (w/w %)	Cellulase activity (FPU g^{-1})				
	Sucrose	Lactose	CMC	Avicel	Filter paper scrap
0	1.54	1.49	1.52	1.53	1.50
0.5	1.52	1.48	1.14	1.52	1.37
1	1.39	1.37	0.81	1.55	1.18
1.5	1.42	1.06	0.98	1.47	1.07
2	1.43	1.17	1.07	1.40	1.28
5	1.27	1.16	1.05	1.42	1.24

↑ 1.3%