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Valorisation of textile waste by fungal solid state fermentation: an example of circular waste-based biorefinery

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Abstract

This study investigated the feasibility of using textile waste as feedstock for cellulase production through solid state fermentation. *Aspergillus niger* CKB was selected with the highest cellulase activity (0.43±0.01 FPU g^{-1}) after 7 days of cultivation on pure cotton. Material modification techniques including autoclaving, alkali pretreatment and milling were applied on six types of textiles with various cotton/polyester blending ratios. The results indicated that using autoclaved textile blending cotton/polyester of 80/20 led to the highest cellulase activity (1.18±0.05 FPU g^{-1}) with CMCase, β-glucosidase and avicelase activities of 12.19±0.56 U g^{-1}, 1,731±4.98 U g^{-1} and 2.58±0.07 U g^{-1}, respectively. The fungal cellulase was then extracted and applied to textile waste hydrolysis, in which a sugar recovery yield of 70.2% was obtained. The present study demonstrates a novel circular textile waste-based biorefinery strategy with recovery of glucose and polyester as value-added products.

Keywords: *Aspergillus niger*; cellulose hydrolysis; circular textile; fungal cellulase; solid state fermentation; textile waste recycling

1. Introduction

Disposal and management of textile waste have risen increasing global concerns. Textile waste includes the waste generated from streams of fibre, textile and clothing manufacturing process, commercial service and consumption (Pensupa et al., 2017). The worldwide textile consumption increased from 47 million tonnes to 90 million tonnes in the recent decade (Shui and Plastina, 2013), and it is forecasted to keep rising along with the population growth and general increase of household purchasing power (Statista, 2016). The annual generations of textile waste in China, the United Kingdom and the United States are estimated to be 26.0, 1.7 and 15.1 million tonnes, respectively (SMaRT, 2016; WRAP, 2016; Yang and Yuan, 2016). On global average, 32 kg of textile wastes are discarded per capita each year, of which around 85% end up in landfill (EPA, 2015). Since the post-consumer textile waste is not easily decomposed, accumulation of such waste would lead to infectious diseases, attract pests and spread odors in the environment (Gordon and Hsieh, 2006). According to the evaluation by Waste & Resource Action Programme (UK), 95% of landfilled textile waste is recyclable, whereas only 14 - 15% recycling rate has been achieved at this stage (WRAP, 2012).

Biorefinery is the process to convert biomass to fuels, valuable chemicals and materials (Clark et al., 2006). As an alternative to fossil fuels, renewable biomass source would be a major contributor in the future supply. Cellulose contributes to approximately 35 - 40% of textile waste, which could become a potential feedstock for production of biological products (*e.g.* ethanol and biogas) (Jeihanipour et al., 2010; Shen et al., 2013). Bioconversion of textile waste has been investigated recently through pretreatment and hydrolyzing cellulose to fermentable glucose. The general idea in various pretreatment technologies is to expose cellulosic fibre to cellulase by increasing surface area and removing inhibitors such as sizing agent coated on textile surface. Gholamzad et al. (2014) reported the conversion of polyester-

cotton textile to ethanol via alkaline pretreatment followed by simultaneous saccharification and fermentation. Jeihanipour et al. (2013) examined a high-rate biogas production scheme from post-consumer jeans (100% cotton) through N-methylmorpholine-N-oxide (NMMO) pretreatment and anaerobic digestion, yielding 400 mL methanol g^{-1} volatile solids day^{-1}.

Degradation of highly crystalline structure of cellulose requires synergy of endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) in a complete cellulase system. It was estimated that the cost of cellulase accounts for 10 - 40% of the total production cost in current biorefinery process (Deswal et al., 2011; Johnson, 2016). Therefore, exploring low-cost cellulase producing techniques and substrates is currently under intensive study. Microbial cellulase production using cellulosic residues via submerged fermentation or solid state fermentation have been investigated, and the later has greater advantages as relatively low energy consumption and simple downstream processing (Hölker et al., 2004; Soccol et al. 2017). Fungal cellulase secreted by microorganisms such as *Aspergillus niger* or *Trichoderma reesei* on horticulture waste, agriculture and kitchen waste have been studied, as summarised in Table 1. Whereas cotton-based textile waste has not been utilized as substrate and carbon source in SSF or in cellulase production.

Table 1. Fungal cellulase production by solid state fermentation.

Strain	Substrate	Moisture (%)	Time (day)	FPase activity (FPU g^{-1})	Reference
Aspergillus terreus	Rice straw	86	7	11.0	Narra et al. (2012)
Aspergillus fumigatus SK1	Oil palm trunk	80	7	3.4	Ang et al. (2013)
Trichoderma reesei RUT-C30	Horticultural waste	80	7-8	15.0	Xin and Geng (2010)

Trichoderma reesei RUT-C30	Wheat bran	37	7	3.8	Singhania et al. (2007)
Aspergillus niger P47C3	Soybean bran	60	5	5.6	Delabona et al. (2013)
Aspergillus niger NS-2	Wheat bran	60	4	17.0	Bansal et al. (2012)
Aspergillus niger	Wheat bran	50	3	2.9	Chandra et al. (2007)
Aspergillus niger USM AI 1	Sugarcane bagasse	70	2	2.3	Lee et al. (2010)
Aspergillus sp. SEMCC-3.248	Rice grass	70	5	1.1	Liang et al. (2012)

The present study aims to develop an integrated biorefinery strategy in textile waste valorisation. Cotton-based textile waste was utilized as substrate for fungal cellulase production by solid state fermentation. The cellulase obtained was subsequently applied in textile waste hydrolysis to recover sugar and polyester (PET) for material recycling and reuse. The proposed strategy enable the capture of the embodied value of the PET fibre, which contributes to the transition of a circular textiles industry.

2. Materials and methods

2.1 Textile waste

Different types of textile waste blending of cotton and polyester provided by H&M (Hennes & Mauritz, Far East) were used as raw feedstock in this study. Pure cotton, pure PET and jeans (99% cotton and 1% elastane) were also employed. Each type was classified by component and dyestuff as listed in Table 2. Dyestuff is a category of substances for staining or coloring on fabrics.

Table 2. Textile waste used in this study.

Component (w/w %)	Dyestuff
Pure cotton	Reactive dyestuff
Cotton/PET (80/20)	Reactive dyestuff
Cotton/PET (60/40)	Reactive dyestuff
Cotton/PET (40/60)	Reactive dyestuff
Pure PET	Disperse dyestuff
Jeans (cotton 99% and elastane 1%)	Indigo dyestuff

2.2 Microorganisms

Different cellulase producing fungal strains were used in solid state fermentation. *Trichoderma reesei* ATCC 24449 was collected from American Type Culture Collection. *Aspergillus niger* N402 was obtained from Prof. David Archer in the University of Nottingham in the United Kingdom. *Aspergillus niger* CKB and *Rhizomucor variabilis* were obtained from Dr. Diannan Lu at Tsinghua University in China. *Aspergillus oryzae* was isolated from a soy sauce starter by the Amoy Food Ltd., Hong Kong (Leung et al., 2012). *Trichoderma longibrachiatum* was collected from Prof. Colin Webb from The University of Manchester in the United Kingdom. All strains were cultivated on potato dextrose agar (PDA) medium in petri dishes at 28 °C for 7 days. The spores were collected in 30% glycerol solution and stored in -80 °C freezer until use.

2.3 Textile waste modification

The textile waste used in this study were grinded into small pieces (around 0.8×0.8 cm^2), and pretreated by three different modification methods, *i.e.* autoclaved modification, freezing

105 alkali/urea soaking and milling. For autoclaving pretreatment, mineral solution was added to
106 the textile waste fabrics to adjust the desired initial moisture content and the textile waste
107 samples were autoclaved at 121 °C for 15 min. For freezing alkali/urea soaking, textile waste
108 fabrics were mixed with 7 w/v% sodium hydroxide and 12 w/v% urea at -20 °C for 6 h and
109 then washed by deionized water (DI water) flushing to remove chemical residues. Collected
110 textile samples were dried in an oven at 40 °C to constant weight. Lastly for milling
111 modification, textile waste fabrics were milled to fine powder form (< 1 mm) by a laboratory-
112 scale hammer crusher.
113
114 2.4 Solid state fermentation (SSF)
115 Fungal cellulase was produced on textile waste via solid state fermentation (SSF). For each
116 SSF, 2 g (dry weight) of crude or modified textile waste sample was inoculated with 0.3 mL
117 spore suspension (2×10^8 spores mL^{-1}) in a petri dish. The mineral solution consisted of
118 following compositions (g L^{-1}): urea, 0.3; KH_2PO_4, 2; $(NH_4)_2SO_4$, 1.4; $MgSO_4$, 0.3; $CaCl_2$,
119 0.4; $FeSO_4$, 0.005; $MnSO_4$, 0.0016; $ZnSO_4$; 0.0014; $CoCl_2$, 0.002 (Mandels and Weber,
120 1969). Additionally, yeast extract (Angel, China) was supplemented by 2.5 w/w% as nitrogen
121 source. DI water was added to the substrate to adjust the initial moisture content at 65% -
122 85%. The weight of each petri dish (with substrate, medium and inoculum) was measured at
123 the beginning of SSF and DI water was added every day to maintain the weight constant. The
124 pH of the prepared medium was 6.3 - 6.5. SSF was conducted in an incubator at 28 °C for 7 -
125 9 days under static condition. Each condition was repeated in duplication.
126
127 2.5 Enzyme extraction
128 At the end of incubation, fungal enzyme was extracted. For each SSF sample, 2 g of
129 fermented substrate was mixed with 60 mL sodium citrate buffer (50 mM, pH 4.8) in a

130 blender (Ling Yang Frozen Machine Co., Hong Kong) for 10 sec. The mixture was
131 centrifuged at 4°C, 10,000 *g* for 3 min to collect the clear supernatant as crude enzyme
132 solution (Pensupa et al. 2013).
133
134 2.6 Enzyme assay
135 Total cellulase activity and individual cellulase activities were determined in duplicate by the
136 following approaches.
137
138 2.6.1 Total cellulase activity
139 The total cellulase activity was determined by filter paper activity (FPase) according to the
140 standardized NREL Laboratory Analytical Procedure (Adney and Baker, 1996). The assay
141 was carried out by adding 0.5 mL enzyme sample into a test tube containing 1 mL sodium
142 citrate buffer (pH 4.8, 50 mM) and a Whatman No. 1 filter paper strip (1.0×6.0 cm, around
143 50 mg). The mixture was incubated at 50°C for 60 min and the releasing sugar was
144 determined by 3,5-dinitrosalicylic acid (DNS) method (Adney and Baker, 1996). The FPase
145 activity was calculated using Eq. (1) according to Adney and Baker (1996).
146

$$\text{FPase activity (FPU/mL)} = \frac{0.37}{\text{Concentration of enzyme that release 2.0 mg glucose}}$$

147 Eq. (1)
148
149 In terms of the textile substrate, the calculation was modified as Eq. (2) on the basis of dry
150 weight of textile.
151

$$\text{FPase activity (FPU/g)} = \frac{\text{FPase activity (FPU/mL)} \times \text{Total volume of the fungal extract (mL)}}{\text{Dry weight of the textile waste used in SSF (g)}}$$

Eq. (2)

2.6.2 Endoglucanase activity and exoglucanase activity

Endoglucanase and exoglucanase were evaluated by carboxymethyl cellulase (CMCase) and avicelase using the procedure developed by International Union of Pure and Applied Chemistry (IUPAC) (Ghose, 1987). Sodium carboxymethyl cellulase (2 w/v%) and avicel (1 w/v%) were used as testing substrate respectively. CMCase and avicelase activities were measured by mixing 0.5 mL enzyme solution with 0.5 mL substrate at 50 °C water bath for 30 min. The reducing sugar (*i.e.* glucose) liberated was reacted with DNS solution and then quantified by absorbance at 540 nm using a UV spectrophotometer (JENWAY, 7300, UK).

2.6.3 β-Glucosidase

β-Glucosidase assay was carried out with 1 mL *p*-nitrophenyl-β-D-glucopyranoside (pNPG, 2mM, Sigma) as substrate, which was digested by 0.1 mL enzyme solution at 50 °C for 5 min. Then the reaction was stopped by adding 2 mL of sodium carbonate solution (1 M), and the amount of *p*-nitrophenol was determined by a UV spectrophotometer at 405 nm (Herr, 1979).

2.7 Microscopic observation and SEM analysis of textile waste substrate

The fermented substrate was observed by a microscope (Keyence, VHX-2000) at a magnification of ×300. Physical changes of the textile substrate in SSF was detected by Scanning Electron Microscope (SEM). Images of textile surface before and after SSF were taken at magnifications of ×1,000 and ×3,000, with voltage 20 kV using a Germany SEM (Carl Zeiss EVO 10).

176 2.8 Enzymatic hydrolysis of textile waste

177 The textile waste cotton/PET 80/20 (0.8×0.8 cm^2, modified by freezing alkali/urea soaking)
178 was subjected to enzymatic hydrolysis. Commercial cellulase (Novozyme, Celluclast 1.5 L)
179 and fungal cellulase extracted from SSF were used separately under the same hydrolysis
180 condition: adding textile fabrics in 100 mL of sodium citrate buffer (50 mM, pH 4.8) at
181 0.16% solid-to-liquid ratio, with enzyme dosage of 25 FPU g^{-1} substrate. The hydrolysis was
182 conducted in duplicate at 50 °C and stirred at 350 rpm for 96 h. Samples were taken at
183 regular time interval for determination of hydrolysis yield using Eq. (3). The dehydration
184 factor (1.111) was set with consideration for addition of water to the cellulosic chains
185 (Goshadrou et al., 2013).
186

$$\text{Hydrolysis yield (\%)} = \frac{\text{Amount of glucose released (g)}}{\text{Amount of initial cellulose in substrate (g)} \times 1.111} \times 100\%$$

187 Eq. (3)

188 The amount of glucose was measured by ultra-performance liquid chromatography (UPLC,
189 Waters, UK) using the column Aminex HPX-87H (Bio-Rad, USA) with sulfuric acid (5 mM)
190 as mobile phase.
191

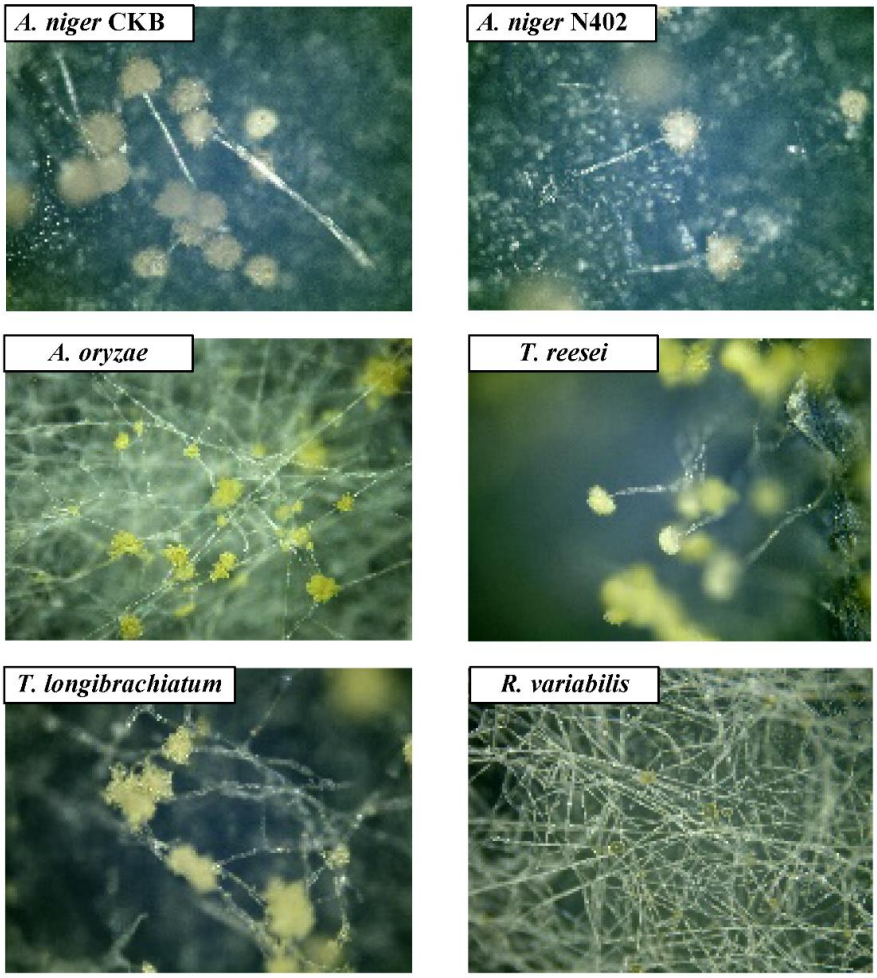
192 **3. Results and discussion**

193 3.1 Selection of fungal strains

194 The combination of fungal strain and substrate in SSF is crucial to the fungal cellulase
195 activity. Various fungi have been investigated in SSF for cellulase production. As listed in
196 Table 1, *Aspergillus* and *Trichoderma* species are two of the most proficient cellulolytic
197 microorganisms, and are widely used in SSF on lignocellulosic substrate such as agricultural
198 and plant biomass with various moisture condition (Yoon et al., 2014). Moisture content is

essential for fungal growth and metabolism in SSF. It has been pointed out that low moisture condition limits the solubility of nutrients while high moisture level could decrease the porosity of substrate and oxygen transfer (Kumar et al., 2011).

In this study, six different fungal strains collected from various sources were incubated on pure cotton fabric to select the most active fungus for cellulase production using textile waste feedstock. For each strain, the SSF was conducted under various initial moisture contents (65%, 70%, 75%, 80% and 85%) at 28 °C for 7 days. As shown in Figure 1, fungal growth and colonization of the six strains on textile substrate were clearly detected by optical microscope. The fungal hyphae and spores could be observed from day 1 and day 2, respectively.



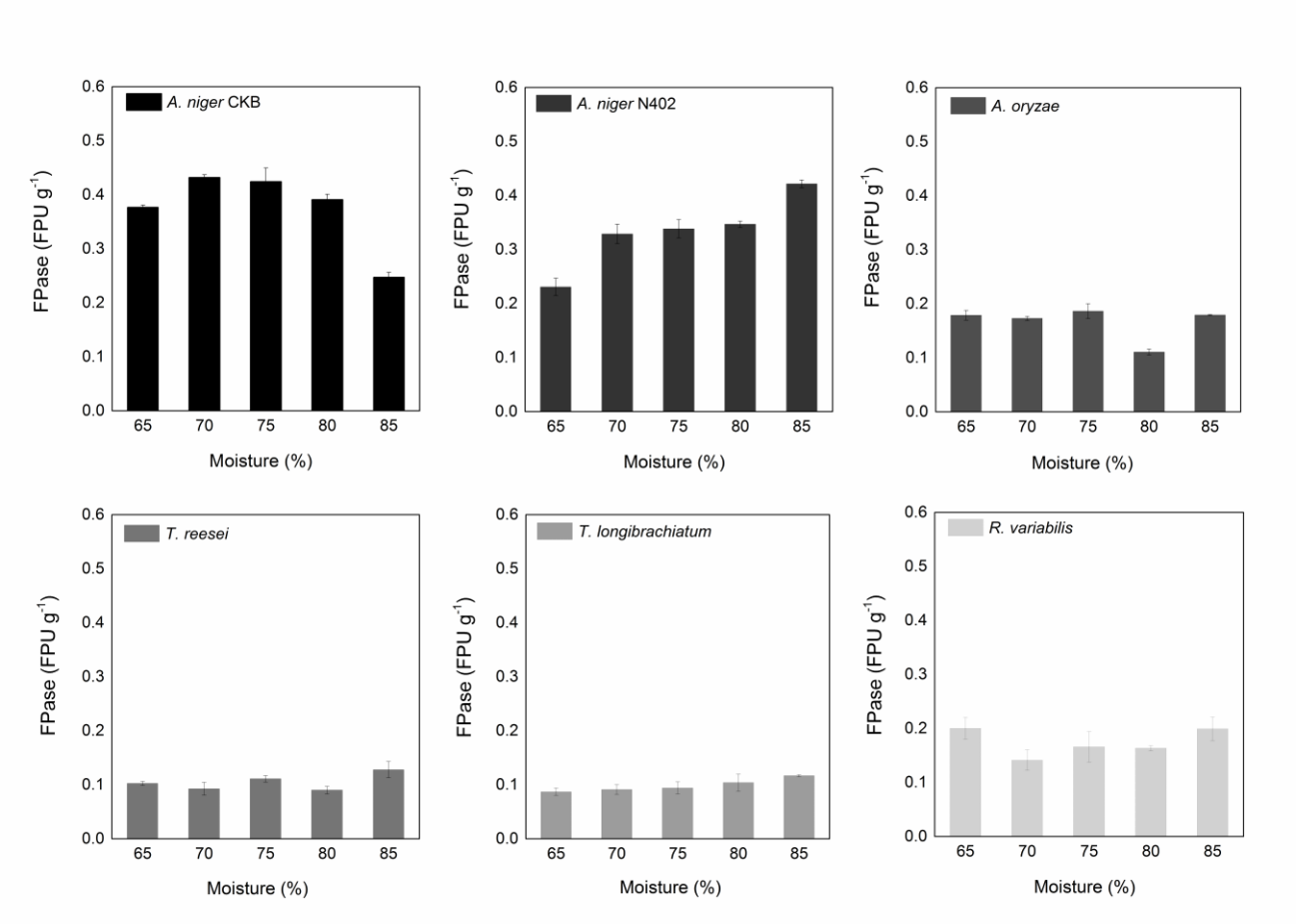
211

212 **Figure 1.** Microscopic pictures of fungal growth on pure cotton fabrics after 7 days of SSF.

213

214 At the end of SSF (*i.e.* day 7), cellulase produced from different strains was extracted and the
215 total cellulase activity (FPase) was analyzed as results presented in Figure 2. It was found that
216 *A. niger* CKB and *A. niger* N402 produced the highest level of FPase activity. In comparison,
217 *Trichoderma* species exhibited poor adaption to textile substrate as indicated by the low
218 cellulase activity. The highest cellulase activity 0.42 - 0.43 FPU g^{-1} was obtained from *A.*
219 *niger* CKB with moisture contents of 70 - 75%. Higher moisture content (*i.e.* over 80%) was
220 not favorable as it reduced the porosity of substrate, thereby decreasing oxygen transfer as a
221 consequence. The result agreed well with similar studies using *A. niger* (Bansal et al., 2012;

222 Delabona et al., 2013). Therefore, *A. niger* CKB incubated at the moisture content of 75%
223 was selected for the subsequent investigation.



224

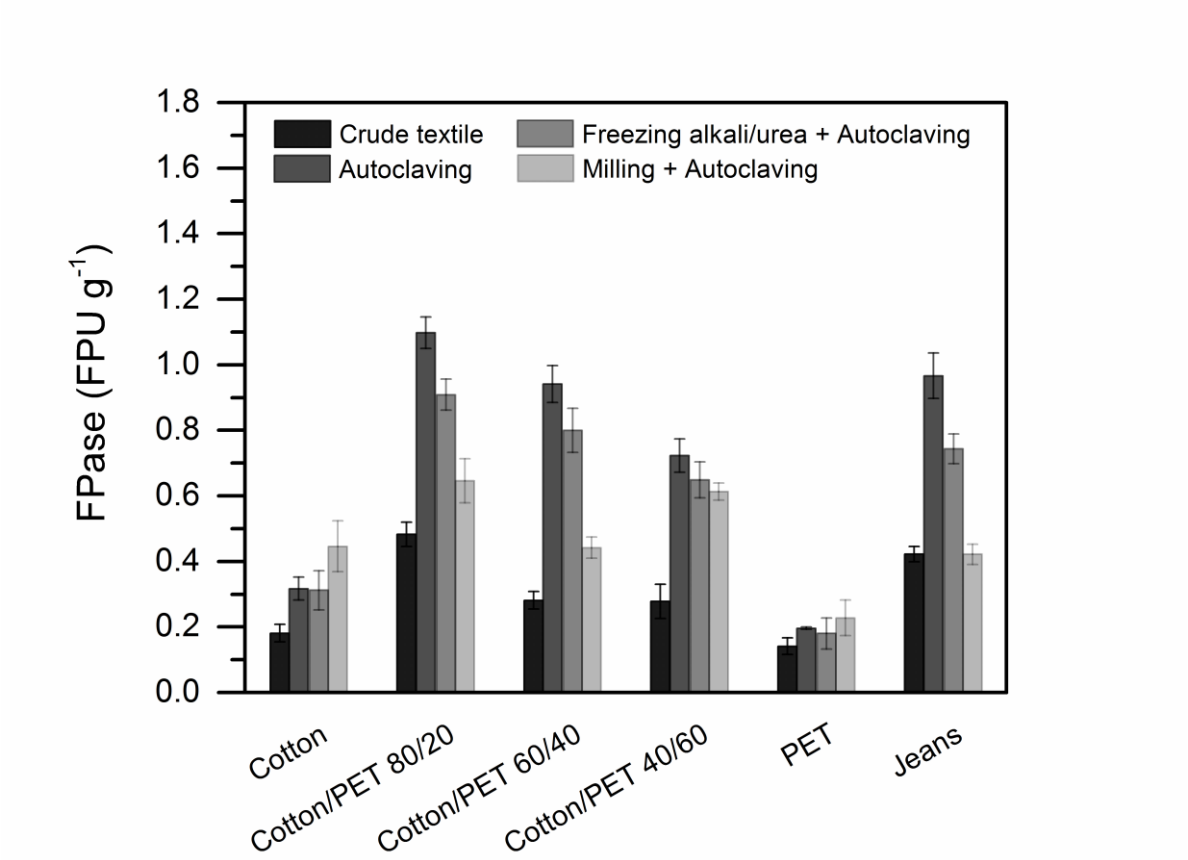
225 **Figure 2.** FPase activities generated by various fungal strains after 7 days of SSF with
226 different moisture conditions.

227

228 3.2 Cellulase production on different types of crude/modified textile waste

229 Cotton is composed of high crystallinity microfiber bundles with glucan. It was reported that
230 the range of the crystallinity indexes of avicel, wood pulp and cotton are 0.50 - 0.60, 0.50 -
231 0.70 and 0.81 - 0.95, respectively (Zhang and Lynd, 2004). Therefore, various pretreatment or
232 modification techniques have been proposed to ease enzyme access to cellulosic fibre and to
233 decrease crystallinity, such as acid/base soaking and ionic liquids treatment (Hong et al.,
234 2012; Shen et al., 2013). Gholamzad et al. (2014) reported that the maximum ethanol

235 production from alkali pretreated textile achieved 70%, largely improving the yield of 36%
236 obtained from crude textile. In this study, six different types of textile waste were used as
237 substrate in SSF (Table 1). Prior to inoculation, the textile was modified by several methods
238 as illustrated in Section 2.3: 1) autoclaving; 2) freezing alkali/urea soaking and autoclaving;
239 3) milling and autoclaving. The crude textile without any pretreatment was employed as a
240 control group. The fungus *A. niger* CKB spore suspension (3×10^7 spores g^{-1} dry fabric) was
241 incubated on textile with initial moisture content of 75%. After 7 days, the total cellulase
242 activities from different substrates were determined and the results are shown in Figure 3.
243
244 Autoclaving is a widely used pretreatment or modified technique applied to substrate for
245 fermentation, although its effect on material morphology is rarely discussed. According to our
246 investigation, the result indicated that for jeans and textile blending of cotton/PET,
247 autoclaving modification significantly improved the cellulase activity by 2 - 3 folds. For
248 instance, the FPase activity from cotton/PET 80/20, cotton/PET 60/40 and jeans increased
249 from 0.48±0.04, 0.28±0.02 and 0.42±0.03 FPU g^{-1} to 1.09±0.05, 0.94±0.06 and 0.96±0.06
250 FPU g^{-1}, respectively with material autoclaved prior to SSF. It could attribute to the textile
251 morphology modification by the mild hydrothermal treatment in autoclave (121 °C, 15 psi),
252 which partially disrupted the substrate in pressurized steaming process and exposed cellulase
253 to the fungus (Yoon et al., 2014).



254

255 **Figure 3.** The effect of different modification techniques on various types of textile substrate

256 used in SSF.

257

258 Freezing alkali/urea pretreatment has been reported as an effective pretreatment to decrease

259 cellulose crystallinity (Mohsenzadeh et al., 2012). As shown in Figure 3, this method indeed

260 contributed to increase cellulase activity. However, the alkali pretreated textile required

261 cleaning by abundant DI water flushing, and its high alkalinity (*i.e.* pH 9-10) would inhibit

262 the fungal growth and cellulase production as compared to those using autoclaved substrate.

263 Similarly, Rahnama et al. (2013) reported that alkali pretreated rice straw generated much

264 lower cellulase activity in comparison with crude substrate. As to the milling modification,

265 the addition of mineral solution agglomerated the fine powder formed textile to semi-wet

266 blocks, which however reduced the contacting area of the substrate and nutrients accessible to

267 fungal enzymes. The situation of SSF on pure cotton and pure PET were different that milling

modified fabrics generated slightly higher cellulase activity. Therefore, autoclaving modification was conducted before SSF in the following investigation on cotton/PET blended material as described in Sections 3.3 - 3.5.

Moreover, it was found that for autoclaved textile blending of cotton/PET, the resultant cellulase activity was of a positive correlation with cotton content (i.e. 40%, 60% or 80%). In other words, higher cotton content led to higher fungal cellulase activity. By contrast, the FPase activity from pure cotton was significantly lower than that from cotton/PET blends, probably due to the limited aerobic condition in firm binding of pure cotton fabrics (as the SEM detection shown in supplementary material). While the surface of cotton/PET blended textile was covered by incompact furs, which provided higher contact area and better oxygen transfer, thereby contributing to fungal growth and metabolism.

Results from this study showed that the highest FPase activity 1.09±0.05 FPU g^{-1} was obtained from the textile cotton/PET 80/20, while the lowest (< 0.20 FPU g^{-1}) was produced on pure PET substrate.

3.3 Time course and individual cellulase activity

Hydrolysis of cellulose is divided into primary hydrolysis and secondary hydrolysis (Zhang et al., 2006). In primary hydrolysis, chains of cellulose are hydrolyzed by endoglucanase (CMCase) to form short chain ends, which are further fractionated into soluble sugars (*e.g.* cellobiose) via catalytic action by exoglucanase (avicelase). The cellobiose is subsequently hydrolyzed to glucose with the aid of β-glucosidase. In order to achieve the optimal synergistic effect, the investigation on the time courses of total cellulase activity and individual cellulase activities are of prime importance. SSF used autoclaved textile

cotton/PET 80/20 as substrate and after inoculation of *A. niger* CKB (3×10^7 spores g^{-1}), it was incubated at 28 °C with initial moisture content of 75% for 17 days. Figure 4 shows the time profiles of enzyme activities of FPase, CMCase, avicelase and β-glucosidase in the SSF.

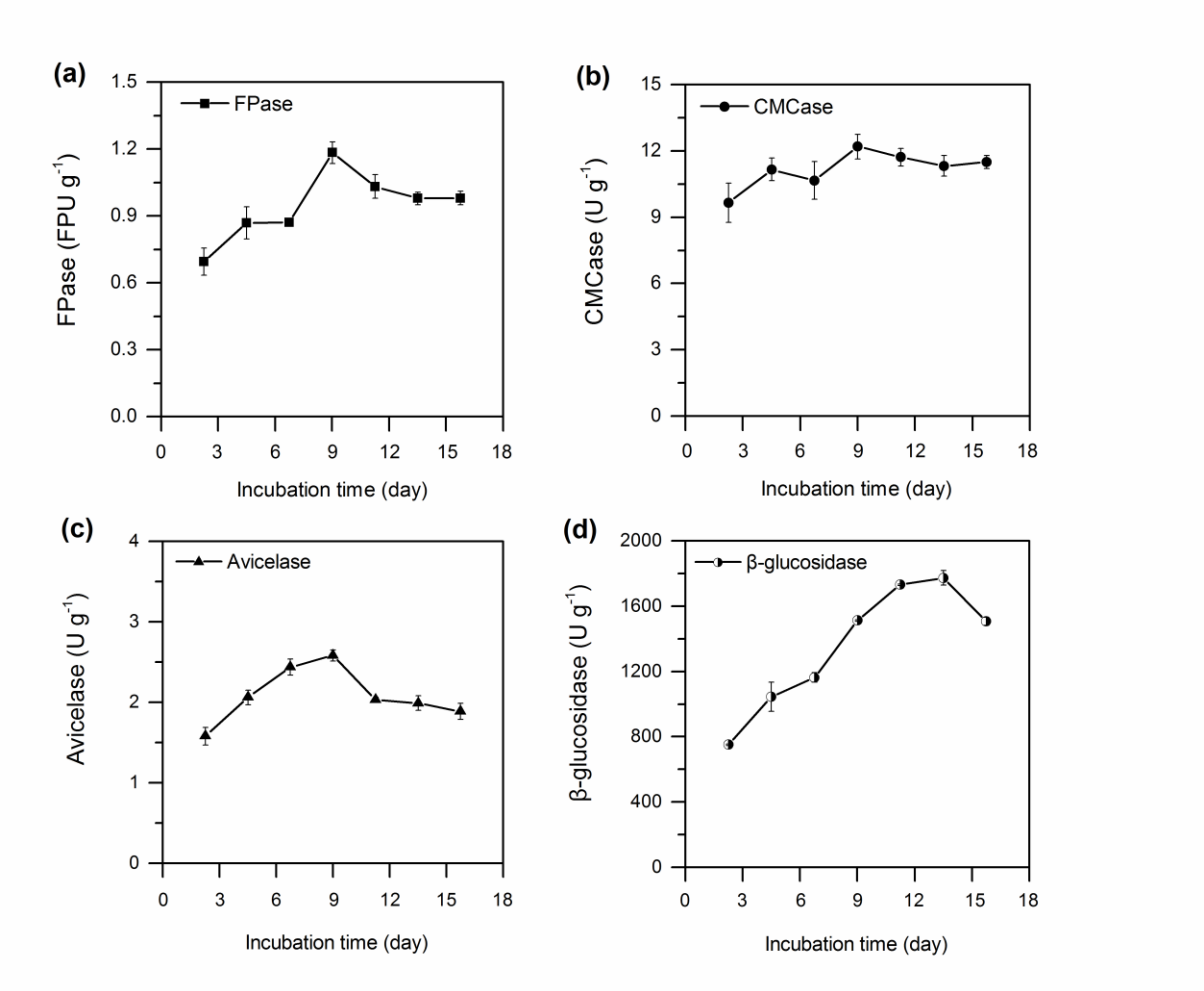


Figure 4. Time courses of enzyme activities of (a) FPase, (b) CMCase, (c) avicelase and (d) β-glucosidase.

The trends of CMCase (Figure 4b) and avicelase (Figure 4c) indicate that enzyme activities reached the maximum of 12.19±0.56 U g^{-1} and 2.58±0.07 U g^{-1} respectively on day 9, and reduced dramatically afterwards. β-Glucosidase exhibited increasing activity as incubation period lasting to day 11 (Figure 4d). The β-glucosidase activity on day 11 (1,731±4.98 U g^{-1}) and day 14 (1,773±30.86 U g^{-1}) were similar, and then it dropped to 1,507±24.92 U g^{-1} on

day 17. Meanwhile notably, after the initial increase in the first 5 days, a slight reduction in CMCase activity was observed on day 7 along with a retardation of β-glucosidase activity. Consequently, the synergistic effect brought total cellulase a short interim lag during day 5-7 before reaching the highest activity of 1.18±0.05 FPU g^{-1} on day 9, then followed by a sharp decrease afterwards (Figure 4a). The result is in agreement with other reported studies that cellulase production peaked within 6-16 days during colonization phase and then decreased in formation of fruiting body (Elisashvili et al., 2009; Montoya et al., 2012). Other explanations for the activity decline occurred on FPase, CMCase and avicelase are attributed to depletion of nutrients after a period of 9 days or denaturation of the enzymes (Xin and Geng, 2010). Based on these above, the incubation period of SSF on textile waste is proposed to 9 days to harvest the highest cellulase activity.

As review by Yoon et al (2014), in most SSF, β-glucosidase usually takes longer incubation time to reach the peak, as compared to CMCase or avicelase. For instance, the CMCase from SSF on wheat bran was harvested on day 11, while β-glucosidase had the best activity on day 15 (Elisashvili et al., 2008). The different peak time of individual enzymes also occurred in this study. Cellulose hydrolysis mechanism is one of the possible reason that primary hydrolysis was firstly carried out by endoglucanase (CMCase) and exoglucanase (avicelase). The subsequently secondary hydrolysis which is catalyzed by increasing β-glucosidase started to dominate in the later phase.

Cellulase production by SSF has been reviewed by several studies such as Yoon et al (2014) and Soccol et al (2017). For a specific comparison of total and individual cellulase activities from bio-wastes, relevant studies in recent years are summarized in Table 3. CMCase and β-glucosidase are the most frequently evaluated individual cellulases, whereas avicelase is

rarely measured. It has been pointed that cellulase system from *A. niger* usually has weak or absent CMCase and avicelase (Yoon et al., 2014). As compared to results from other studies, cellulase produced by *A. niger* CKB from textile waste was a complete system of cellulosic enzymes. Remarkably, β-glucosidase obtained by the proposed circular textile waste-based biorefinery strategy is the highest activity reported worldwide, to date.

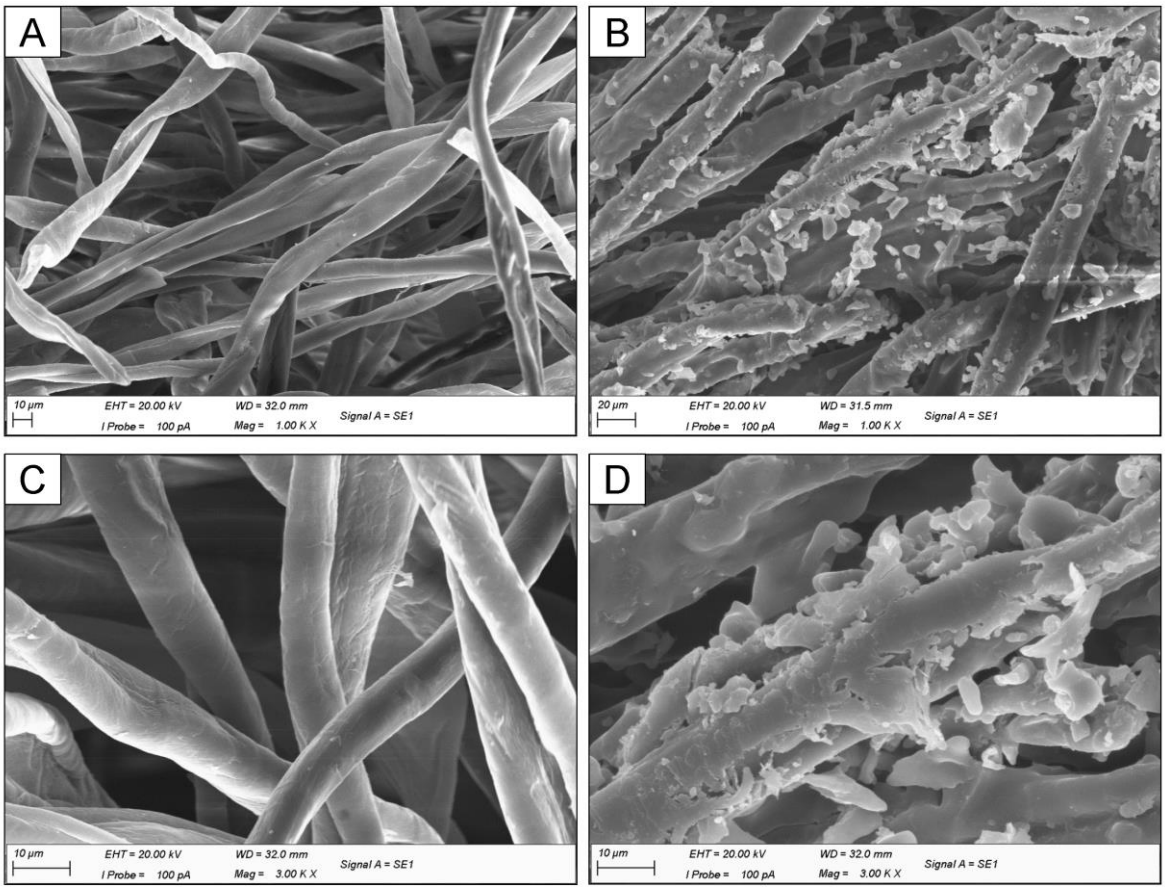
335 Table 3. Individual cellulase activities produced via SSF.

Strain	Substrate	FPase (FPU g^{-1})	CMCase (U g^{-1})	β-glucosidase (U g^{-1})	Avicelase (U g^{-1})	Reference
Aspergillus fumigatus SK1	Oil palm empty fruit bunches	1.6	21.2	22.2	-	Soleimaninanadegani et al. (2014)
Aspergillus fumigatus SK1	Oil palm trunk	3.4	54.3	4.5	-	Ang et al. (2013)
Aspergillus fumigatus P40M2	Agro-industrial residues	5.0	56.6	105.8	-	Delabona et al. (2013)
Trichoderma harzianum SNRS3	Rice straw	6.3	111.3	173.7	-	Rahnama et al. (2013)
Aspergillus niger NS-2	Agricultural and kitchen waste residues	17.0	310.0	33.0	-	Bansal et al. (2012)
Aspergillus terreus	Rice straw	11.0	20.9	4.6	0.5	Narra et al. (2012)
Fomitopsis sp. RCK2010	Wheat straw and rice straw	4.7	84.1	69.1	-	Deswal et al. (2011)
Trichoderma reesei	Horticultural waste	15.0	90.5	61.6	-	Xin and Geng (2010)
Aspergillus niger N402	Wheat straw	24.0	85.5	80.1	19.7	Pensupa et al. (2013)
Aspergillus niger CKB	Textile waste	1.2	12.2	1,731.0	2.6	This study

336 3.4 Scanning electron microscope of textile substrate

337 The fungal growth and morphological change of textile substrate (autoclaved cotton/PET
338 80/20) were detected by Scanning Electron Microscope (SEM). Figure 5 (A) and (B) at
339 magnification of ×1,000 show the textile fibre was well colonized by *A. niger* CKB mycelium
340 and spores after 9 days of SSF. Figure 5 (C) and (D) compare the surface structure before and
341 after SSF at a higher magnification of ×3,000. It could clearly observed that the crystalline
342 structure of original textile was partially disrupted to a rough, unsmooth and rugged status,
343 owing to the digestion of cellulose by fungal enzymes.

344



345

346 **Figure 5.** SEM of textile substrate (cotton/PET 80/20) before and after SSF (A: textile
347 substrate before SSF, magnification of ×1,000; B: textile substrate after SSF, magnification of
348 ×1,000; C: textile substrate before SSF, magnification of ×3,000; D: textile substrate after
349 SSF, magnification of ×3,000).

350

351 3.5 Enzymatic hydrolysis of textile waste

352 In order to recycle cellulosic component and PET material, the textile waste cotton/PET
353 80/20 was hydrolysed to digest cellulose into glucose. The fungal enzyme (extracted from
354 textile waste SSF in Section 3.3) with total cellulase activity of 1.18 FPU g^{-1} was used as an
355 enzyme source. In comparison, commercial cellulase "Celluclast 1.5 L" from Novozymes®
356 (USA) was also employed under the same hydrolysis condition. With enzyme dosage of 25
357 FPU g^{-1}, corresponding individual cellulase activities from fungal enzyme Celluclast 1.5 L
358 are listed in Table 4. As compared to diluted Celluclast 1.5 L, fungal enzyme contained
359 higher CMCase and β-glucosidase activities, but lower avicelase activity. In hydrolysis,
360 cellulose component was decomposed into soluble sugar (*i.e.* glucose) and was separated
361 with the solid residue (*i.e.* PET) by filtration at the end of hydrolysis. The time profile of
362 hydrolysis yield is plotted in Figure 6. Although from 0 - 48 h, commercial cellulase
363 presented a relatively better efficiency, the final hydrolysis yields from commercial cellulase
364 and fungal cellulase were close after 96 h of hydrolysis. Fungal cellulase produced from SSF
365 contributed to a yield of 70.2% in textile waste hydrolysis, which is comparable to the yield
366 of 77.2% from commercial enzyme product. The relatively lower hydrolysis yield was
367 probably caused by inadequate avicelase in fungal enzyme (Table 4). At last, the PET
368 recovered after hydrolysis has been processed into PET fibre by melting spinning for reuse in
369 textile applications.

370

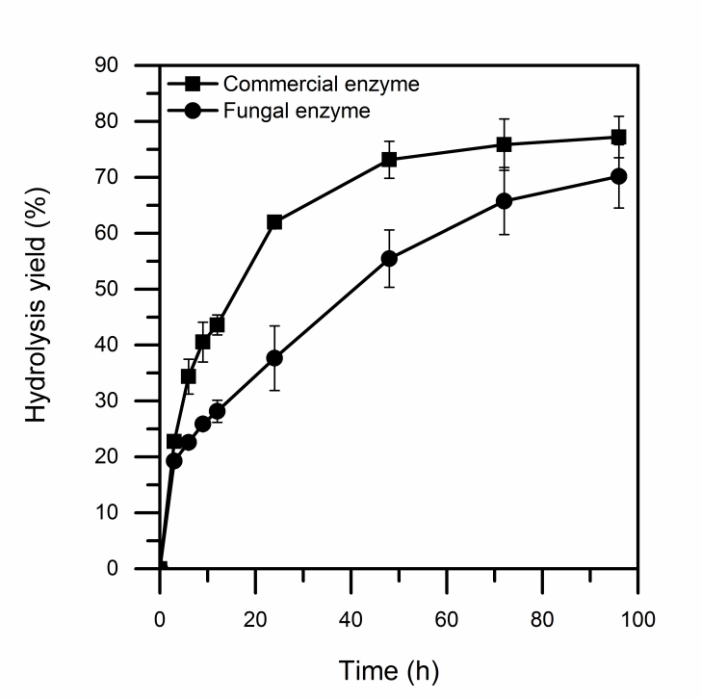
371 Table 4. Enzyme dosages of hydrolysis using fungal celluase or commercial cellulase.

Enzyme dosages	Fungal cellulase (from textile waste)	Diluted Celluclast 1.5L
FPase (FPU g^{-1})	25.0	25.0
CMCase (U g^{-1})	253.9	114.2
Avicelase (U g^{-1})	53.7	118.9

β-glucosidase (U g^{-1})	31,500.0	1,633.3

372

373



374

375 **Figure 6.** Textile hydrolysis by commercial cellulase and fungal cellulase from textile waste.

376

377 Currently, the process optimisation and upscaling of SSF on textile waste and fungal
378 enzymatic hydrolysis of textile waste are under investigation in our group. Fungal cellulase is
379 going to be produced from larger quantities of textile waste using 1 L bioreactor, which
380 would promote the applicability of the proposed method in industry.

381

382 **4. Conclusions**

383 This study developed a novel method for valorisation of textile waste. Cotton/PET based
384 textile was used as substrate in fungal solid state fermentation for cellulase production.
385 *A. niger* CKB was selected as it generated high cellulase activity. Autoclaving was applied to
386 facilitate the fibres to be easily accessed to enzymes. The highest total cellulase activity of

1.18±0.05 FPU g^{-1} was harvested on day 9 with CMCase of 12.19±0.56 U g^{-1}, β-glucosidase of 1,731±4.98 U g^{-1} and avicelase of 2.58±0.07 U g^{-1}. This enzyme product was applied in textile hydrolysis to recover glucose from cellulose with comparable enzymatic effect to commercial cellulase. The research outcomes enable close loop recycling for textiles industry by capturing the embodied value of the PET fibre. The proposed circular textile waste-based biorefinery strategy could eliminate the textile waste downstream. Finally, the incorporation of these processes in future bioeconomy for the production of value-added products will be an important contribution towards the development of closed loop textile-to-textile recycling.

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Supplementary material:

SEM of textile substrate (cotton/PET 80/20 and cotton 100%) at magnification of ×300 (before SSF)