Textile waste valorization using submerged filamentous fungal fermentation

Huaimin WANG [a], Guneet KAUR [a,b], Nattha PENSUPA[c], Kristiadi UISAN [a], Chenyu DU [d], Xiaofeng YANG [e], Carol Sze Ki LIN [a,*]

[a] School of Energy and Environment, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong

[b]Sino-Forest Applied Research Centre for Pearl River Delta Environment and Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong

[c] Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand, 65000

[d] School of Applied Sciences, University of Huddersfield, Huddersfield, United Kingdom

[e] Synthetic Biology Center, School of Biology and Biological Engineering, South China University of Technology, Guangdong, China

*Corresponding author: Carol S. K. Lin, School of Energy and Environment, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong

E-mail: carollin@cityu.edu.hk, Tel: +852 3442 7497, Fax: +852 3442 0688

Abstract

Textile waste is one type of municipal solid waste growing rapidly in recent years. In Hong Kong, 306 tonnes of textile waste were produced daily in 2015 and more than 90% of these ended up in landfill. This is the first paper which utilizes textile wastes as substrate for cellulase production via submerged fungal fermentation, subsequently uses produced cellulase in textile waste hydrolysis for recovery of glucose and polyester. *Trichoderma reesei* ATCC 24449 was selected with the highest cellulase activity (18.75 FPU/g) after cultivation using textile blending cotton/polyester 40/60 as substrate. Cellulase production was upscaled in a 5-L bioreactor and the resultant cellulase was used in textile waste hydrolysis. Glucose recovery yield of 41.6% and 44.6% were obtained using fungal cellulase and commercial cellulase, respectively. These results suggest the proposed process has a great potential in treating textile waste and facilitating the recovery of glucose and polyester as value-added products.

Keywords: Cellulase; Hydrolysis; Submerged fungal fermentation; Textile waste; Waste recycling

1. Introduction

The amount of textile waste has increased rapidly in the recent years. The increasing world population inevitably leads to outstripping demand in consumer products such as textiles and apparel (Hu et al., 2018; Pensupa et al., 2017). In Hong Kong, 93.2% of textile waste was disposed to landfill directly, while the remaining 6.8% was either recycled locally or exported for recycling in 2015 (HKEPD, 2017). Major recycling options for textile wastes include second-hand oversea trading and energy recovery by incineration (Ryu et al., 2007; Stanescu et al., 2009). However, large material lost in landfilling or incineration are unavoidable in the currently wasteful, linear system which creates negative impacts on the environment and society. In addition, textile production accounts for significant greenhouse gas emissions. On the other hand, cellulosic material has been intensively investigated in biorefinery to produce biofuels and chemicals (Li et al., 2017; Singhania et al., 2014, 2017). In general, textile waste contains 35-40% of cotton, which is a cellulosic-rich material with high degree of polymerization and crystallinity (Jeihanipour et al., 2010; Shen et al., 2013). In most of the bioprocesses utilizing cotton waste, enzymatic hydrolysis is needed for conversion of cellulose to fermentable sugars (Raj et al., 2009). However, the cost of enzyme remains as one of the main obstacles in commercialization of these processes.

Currently, the majority of commercial cellulase is produced by filamentous fungi via submerged fermentation (SmF) (Singhania et al., 2010). Compared to solid state fermentation, SmF provides a homogeneous environment, continuous oxygen supply and better pH control that can further facilitate cellulase secretion by filamentous fungi (Florencio et al., 2016). In new textiles platform based on the principles of circular economy, textiles and fibres are kept at their highest value during use and re-enter the economy afterwards, never ending up as waste (Morlet et al., 2017). With this in mind, a circular textile recycling initiative could be the one using textile waste as carbon source for SmF with filamentous fungi for cellulase production. Then the cellulase could be recovered for the subsequent hydrolysis of textile waste in order to recover fermentable sugar and the remaining undegradable polyester (PET) fibre.

The present study aims to examine the feasibility for cellulase production using textile waste by SmF, and evaluate the textile hydrolysis performance between fungal cellulase and commercial cellulase. Table 1 shows different types of textile wastes donated by H&M (Hennes & Mauritz, Far East) for this study, and Table 2 shows different cellulase producing fungal strains which were applied in this investigation. Extensive optimization of cellulase production including selection of fungal strain and textile waste, utilization of grinded and pretreated textile, fermentation medium, nitrogen source, effect of Tween 80 and inducer were carried out in this study. Upscale of cellulase production in a 5-L bioreactor was also conducted to produce cellulase for the subsequent textile hydrolysis, and finally the recovery of glucose and polyester for material recycling and reuse allows the establishment of a truly circular platform for the textile industry.

2. Materials and methods

2.1. Strains and media

Aspergillus niger ATCC 201201, *Trichoderma reesei* ATCC 24449 and *Trichoderma longibrachiatum* ATCC 52326 were purchased from the American Type Culture Collection (Rockville, MD, USA). *A. niger* CKB was kindly provided by Prof. Diannan Lu at Tsinghua University, Beijing, China which was isolated from rice straw to digest lignocellulosic material. *A. niger* HDU was a native strain which was kindly provided by Dr. Chenyu Du from University of Huddersfield at the United Kingdom that previously used for textile waste treatment via solid state fermentation (Hu et al., 2018). These three fungi species were often treated as cellulase producer both in research and commercial area (Karray et al., 2016; Leghlimi et al., 2013; Li et al., 2016; Singhania et al., 2017). And also been proven that they have a strong ability to decompose cellulosic materials (Zhao et al., 2018; de Oliveira Gorgulho Silva et al., 2018).

Spore suspensions of these fungal strains were stored at -80 ºC with 30% (w/w) glycerol. Spore suspension was prepared by spending fungal spore culture (around 10 μL) on the surface of potato dextrose agar (PDA) in a petri dish (60 mm × 15 mm), and incubated at 28 ºC for 5 days. After the incubation period, 6 mL of sterilized deionized (DI) water was added to extract spores with gentle scratch using sterilized spatula. After extraction, the spore suspension was aliquoted at 0.5 mL volume per cryogenic tube with spore density of 3×10^7 spores/mL.

All chemicals used in this study were purchased from VWR (PA, USA) and Sigma-Aldrich (MO, USA) except otherwise stated.

Two different cultivation media were compared in this study: (i) Csiszar medium (Csiszar *et al*. 2007), and (ii) Mandels medium with yeast extract (Mandels & Reese, 1957). The compositions of these two media are listed in Table 2. After preparation, the pH of the medium was adjusted to 5.0 by adding either HCl (3 mol/L) or NaOH (5 mol/L) prior to autoclave. Tween 80 (0.1%) was added when necessary. Sole nitrogen source includes beef extract,)$NH_4(_2SO_4$, yeast extract, peptone, $NaNO_3$, urea and soybean meal which were used at a concentration of 0.5% (w/v). Cellulase inducers include sawdust, molasses, wheat bran and cellobiose were selected for investigation with three different concentrations (0.1%, 0.5% and 1% w/v). Control groups were set up for both nitrogen source and inducer experiment, represented no addition of nitrogen source or inducer in fermentation medium. Seed culture preparation involves the activation of spores on PDA plate to obtain enough spore suspension solution. Spore solution (2 mL) with 10^8 spores/mL was inoculated into 100 mL Mandels medium containing 3% (w/v) glucose. Cultivation was carried out at 28 ºC and 150 rpm for 48 h.

2.2. Textile waste handling and pretreatment

Similar to our previous study, 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 (Hu et al., 2018). 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 1. Textile wastes were grinded into small pieces (around 0.8×0.8 cm^2) using a double shaft shredder (OMS Machinery Co., Ltd., China).

Pretreatment process was conducted by our collaborator Dr. Shao-Yuan Leu in The Hong Kong Polytechnic University. Briefly, grinded textiles were soaked in a mixture of 12% NaOH (w/v) and 7% urea (w/v), and then stored at -20 ºC for 6 h. Later, these samples were thawed and washed with DI water until pH dropped to 7.0 (Gholamzad et al., 2014; Kuo and Lee, 2009).

2.3. Shake flask fermentation

Shake flask fermentation was carried out in a laboratory shaker incubator (innova®42 New Brunswick Scientific). Temperature and shaking speed were controlled at 28 ºC and 150 rpm, respectively. Shake flasks (DURAN® Erlenmeyer flask, 250 mL narrow neck) with 100 mL working volume were used. Seed culture of 10 mL was transferred into 90 mL of fermentation media in shake flask experiment.

2.4. Batch fermentation in 5-L fermentor

Batch fermentation for cellulase production was carried out in a 5-L benchtop fermentor (BioFlo®/CelliGen® 115 New Brunswick) with 3-L working volume.

Temperature, agitation, aeration rate and dissolved oxygen (DO) were controlled at 28 ºC, 300-800 rpm, 5 L/min and 20% respectively. For aeration, compressed air was used. Agitation rate was controlled automatically to maintain DO at 20% saturation value. Inoculation size was 20% (v/v) and 10 g/L textile waste was used as a substrate in SmF. In addition, 10 g/L glucose was supplemented in Mandels medium for fungal cultivation in fermentor.

2.5. Hydrolysis experiment of textile waste

2.5.1 Total cellulase activity

The total cellulase activity was determined by filter paper activity (FPase) according to the standardized NREL Laboratory Analytical Procedure (Adney & Baker, 1996). The assay was carried out by adding 0.5 mL enzyme sample into a test tube containing 1 mL sodium citrate buffer (pH 4.8, 50 mM) and a Whatman No. 1 filter paper strip (1.0 × 6.0 cm, around 50 mg). The mixture was incubated at 50 °C for 60 min and the releasing sugar was determined by 3,5-dinitrosalicylic acid (DNS) method. The FPase activity was calculated using Equation 1 according to Adney and Baker (1996).

$$\text{FPase activity } \left(\frac{\text{FPU}}{\text{mL}}\right) = \frac{0.37}{Concentration\ of\ enzyme\ that\ release\ 2.0mg\ glucose} \quad (1)$$

2.5.2 Textile waste hydrolysis

Pretreated cotton was used as substrate for enzymatic hydrolysis. Fungal cellulase from textile waste fermentation (fermentation filtrate) and commercial cellulase from Novozyme® (Celluclast 1.5L) with a dosage of 25 FPU/g were used in hydrolysis. This is an optimized dosage reported from optimization of hydrolysis of pretreated cotton using Novozymes® cellulase in our earlier study (Hu et al., 2018). Experiment was carried out in 250 mL Duran bottles with 35 mL working volume. The hydrolysis was conducted in duplicate at 50 ºC and 350 rpm for 96 h. Samples were taken at regular time intervals (3 hours interval in first 12 hours, then 24 hours interval from 24th hours till 96 hours) for determination of hydrolysis yield using Equation 2. The dehydration factor (1.111) was set with consideration for addition of water to the cellulosic chains (Goshadrou et al., 2013).

$$\text{Hydrolysis yield (\%)} = \frac{\text{Amount of glucose released (g)}}{\text{Amount of initial cellulose in substrate (g)} \times 1.111} \times 100\% \qquad (2)$$

2.6. Analytical methods

For cellulase activity analysis, hydrolysis temperature was maintained at 50 ± 0.1 ºC using a water bath. Measurement of absorbance at 540 nm was done using a spectrophotometer (JENWAY 7300). Fungal cells were separated from fermentation broth by centrifugation at 10,000 *g* for 3 mins. The supernatant was stored at -80 ºC until analysis. Thawed supernatant was filtered by Nylon membrane filter with

0.22 μm pore size and 13 mm diameter (Jin Teng, China) prior to analysis. Glucose concentration was analyzed using high-performance liquid chromatography (HPLC, Waters, UK) equipped with Aminex HPX-87H column (Bio-Rad, CA, USA). In each analysis, 10 μL sample was injected into the column (60 ºC) and was eluted isocratically with 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. Detection was performed by Refractive Index (RI) detector at 35 ºC and Photodiode Array (PDA) analyzer at 210 nm.

2.7. Microscopic observation and SEM analysis of textile waste substrate

Physical changes of the textile substrate in SmF were detected by Scanning Electron Microscope (SEM). Images of grinded, pretreated and fermented textile wastes were taken at magnifications of 70 and 1200 with voltage 20 kV using a Germany SEM (Carl Zeiss EVO 10).

3. Results and discussion

3.1. Pretreatment of textile waste for fungal cellulase production

3.1.1. Comparison of grinded and pretreated textile wastes for cellulase production

Fig. 1 demonstrates the results of cellulase production using pure cotton, jean, cotton/PET 80/20, cotton/PET 60/40 and cotton/PET 40/60 in both grinded and pretreated textile waste. It showed that pretreated textile wastes dominated by achieving at least 9.5% higher cellulase activity than grinded textile in all five types of textile wastes. Pretreated textile achieved 1.83-fold higher cellulase activity than grinded textile when using cotton/PET 40/60 with *T. reesei* ATCC 24449 in SmF. The reasons for this observation are manifold. Firstly, 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 (Hu et al., 2018). Additionally, pretreatment with alkaline also washed out the coating on textile surface which was conducted primarily to increase textile resistance to the environment (Saxena et al., 1992; Shen et al., 2013). Thus, after pretreatment, the cotton component in textile waste became more accessible to fungal growth and metabolism, thereby resulting in higher cellulase activities.

3.1.2. Comparison of textile waste surface morphology using Scanning Electron Microscopy (SEM)

Initial experiments were performed in shake flasks in order to determine the ability of fungal strains to grow on textile waste as substrate. After cultivation, textile waste's surface morphology and fungal growth were observed by SEM. Fig. 2(a), (b), (c) at a magnification of 70 show that the textile structure was partly broken down after freezing soda pretreatment process and single fibers were released after SmF. Fig. 2(d), (e), (f) illustrate the comparison of fiber surface structure after SmF at a higher magnification of 1200. After pretreatment, fiber surface was obviously rougher. In addition, significant break down of fibre surface and coverage of fungal biomass was observed after SmF. These results indicate that pretreatment of textile waste with freezing soda leads to rougher fibers, which facilitates the fibers to be easily accessed by enzymes (Hu et al., 2018). The pretreated textile was further decomposed both in single fibers and blending structure during fermentation due to the biocatalytic reactions from fungal cellulase produced.

3.2. Optimization of substrates and fermentation media

3.2.1. Selection of fungal strains for fungal fermentation of textile wastes

In this study, evaluation of five fungal strains and six types of textile wastes for cellulase production was conducted. Fig. 3 depicts cellulase production of different fungal strains with different types of textile wastes. Different fungal strains exhibited a clear preference on textile waste type in terms of cellulase production capacity. Results from this study showed that for jean, the highest cellulase production of 5.68 FPU/g was achieved when fungal fermentation was conducted using *A. niger* ATCC 201201. Comparatively higher cellulase productions of 6.88 FPU/g, 7.50 FPU/g and 7.51 FPU/g were obtained when pure cotton, cotton/PET 80/20 and cotton/PET 60/40 were used with *A. niger* HDU. It was found that SmF using *T. reesei* ATCC 24449 resulted in the highest level of cellulase activity (6.73 FPU/g) with textile blending containing higher PET content (i.e. cotton/PET 40/60). One of the ultimate aims in the proposed circular textile waste-based biorefinery strategy is to eliminate the textile waste downstream by enabling the close-loop recycling for textiles industry via capturing the embodied value of the PET fibre. Therefore, *T. reesei* ATCC 24449 would be the preferred fungus when using textile waste stream with high portion of PET. The experimental results in this section show that optimal combination of specific fungal strain and textile waste would lead to higher cellulase production in SmF. As shown in Fig. 2, *A. niger* HDU was used for pure cotton, cotton/PET 80/20 and cotton/PET 60/40, while *A. niger* ATCC 201201 was used for jean and *T. reesei* ATCC 24449 was used for cotton/PET 40/60.

The rest of fungal strains examined namely *A. niger* CKB and *T. longibrachiatum* ATCC 52326 resulted in lower cellulase production as compared to others. For *A. niger* CKB, although it produced 6.75 FPU/g cellulase with pretreated cotton/PET 60/40, the amount was still less than that produced by *A. niger* HDU (7.51 FPU/g). *T. longibrachiatum* ATCC 52326 only produced 2.78 FPU/g of cellulase at supreme, which was only 30-50% as compared to other fungal strains examined. Thus, these two strains were not applied in the subsequent experiments. Another important consideration is that pure PET textile waste can neither be decomposed by pretreatment nor utilized in fungal fermentation. This was also observed by almost no cellulase activity resulted from all fungi grown on pure PET as substrate. Therefore, pure PET textile waste was not considered in the subsequent investigation.

3.2.2. Effect of nitrogen source on cellulase production

Cellulase production could be significantly influenced by the effect of nitrogen source (Matkar et al., 2013; Pensupa et al., 2013). In Mandels medium, the nitrogen source consists of a mixture of yeast extract, urea and $)NH_4(_2SO_4$ (Mandels & Reese, 1957). Investigation of the preferred sole nitrogen source using different types of nitrogen sources was conducted in this study. Fig. 4(a) shows that for pretreated jean fermented with *A. niger* ATCC 201201, the use of NH_4NO_3 as nitrogen source resulted in the highest cellulase activity of 5.02 FPU/g, and peptone resulted in the second highest cellulase activity of 4.01 FPU/g. In terms of inorganic source, the cellulase activities achieved by NH_4NO_3 were around 2-fold higher than both $(NH_4)_2SO_4$ and $NaNO_3$. Comparison of cellulase activities with several organic nitrogen sources revealed that the values for urea and soybean meal were similar, which were 2.95 FPU/g and 3.09 FPU/g, respectively. The cellulase activities of these two sources were lower than peptone (4.01 FPU/g), but higher than beef extract and yeast extract. On the other hand, for pretreated cotton/PET 80/20 fermented with *A. niger* HDU, the use of soybean meal as nitrogen source led to the highest cellulase activity of 4.38 FPU/g. Other nitrogen sources produced lower cellulase activities around 2.00 FPU/g. These results indicate that the nitrogen sources presented in Mandels medium were not the optimal nitrogen sources among those examined in textile waste SmF. Therefore, this medium component could be replaced with other types of nitrogen sources (e.g. use of soybean meal for cotton/PET 80/20) to achieve a higher cellulase production when using textile waste as a substrate in SmF.

3.2.3. Effect of Tween 80 on cellulase production

Tween 80 has been reported in several literatures to have controversial effect in cellulase production as well as cellulose hydrolysis (Yang et al., 2011; Zeng et al., 2006). Zeng et al. (2006) reported that Tween 80 has a positive effect on production of amylase, CMCase and xylanase, but exerted a negative effect on protease production. While in hydrolysis of cellulose, Tween 80 shows an obvious improvement of cellulose conversion in higher shaking speed mainly because Tween 80 has a protection effect on adsorbed cellulase (Yang et al., 2011). Tween 80 is a surfactant which can enhance the transportation between cells and broth (Reese & Maguire, 1969). Another effect of adding Tween 80 was enhancing the removal of dyestuffs on textile waste to fermentation (result not shown) and therefore, textile surface would be more accessible by fungal cells, resulting in ease of fungal biomass accumulation. Thus, it is necessary to determine the influence of Tween 80 on SmF with different types of strains as well as different textiles. Fig. 4(b) shows that for pure cotton, cotton/PET 80/20, cotton/PET 60/40 and cotton/PET 40/60, the addition of Tween 80 in culture medium has a negative effect on cellulase production. These four types of textiles were dyed with reactive dyestuff (Table 1). Dönmez (2002) reported that for *Candida tropicalis*, prolonged lag period and decreased cell growth rate occurred when yeast cells accumulated reactive dyes. Nevertheless, *A. niger* SA1 strain was also reported very robust in dyestuffs accumulation and textile wastewater clarification (Ali et al., 2010; Fu & Viraraghavan, 2001). From our observations, reactive dyestuffs were partly washed out from textiles into the medium during autoclaving of fermentation medium. Accumulation of dyestuffs in fungal cells was observed by gradual change of fungal biomass color according to the color of dyes. Interestingly, fungal cells did not show an obvious growth inhibition due to the presence of dyes, as shown by no difference in (fungal) cell dry weight upon fermentation using colored textiles. However, cellulase production was affected negatively by these reactive dyestuffs. Furthermore, the addition of Tween 80 in jean fermentation using *A. niger* ATCC 201201 showed a positive effect of 8.3% increase in cellulase production. A logical hypothesis could be that the indigo dye in jean is usually harvested from plants, and behaves to be less harmful than other reactive dyes in cellulase production. In summary, Tween 80 is not a suitable additive for *A. niger* HDU and *T. reesei* ATCC 24449 strains in SmF using textile with reactive dyestuff, but it would certainly benefit the cellulase production for *A. niger* ATCC 201201 using jean with indigo dye. Therefore, Tween 80 was applied in the subsequent fermentation using jean as substrate.

3.2.4. Effect of inducer on cellulase production

Cellulase induction is a widely applied strategy in commercial cellulase production wherein the inducer functions in regulation of cellulase gene expression (Fekete et al., 2008; Singhania et al., 2017). In this study, sawdust, molasses, wheat bran and cellobiose were selected as the potential inducers. Sawdust and wheat bran are side-products from forestry and food processing industry. They are lignocellulosic biomass with low economic value (Pensupa et al., 2013). Molasses is also a by-product from sugar industry which contains variety of sugars including sucrose, fructose and glucose. On the other hand, cellobiose is a widely used inducer in commercial cellulase production with good induction effect but high price (Kuhad et al., 2016). In this study, cellobiose was assigned as a representative inducer in order to compare the effectiveness of other selected inducers. Inducer addition levels were set as 0.1%, 0.5% and 1% (w/v) to determine their possible effects on cellulase production using textile wastes as substrates (Morikawa et al., 1995; Zhang et al., 2017). Results of inducer addition are shown in Table 2. For *A. niger* ATCC 201201 with jean, the highest cellulase activity was 9.72 FPU/g with addition of 0.1% molasses. For *A. niger* HDU with pure cotton, cotton/PET 80/20 and cotton/PET 60/40, the highest results were obtained at 9.97 FPU/g with 1% cellobiose, 13.10 FPU/g with 1% wheat bran and 9.84 FPU/g with 1% wheat bran, respectively. For *T. reesei* ATCC 24449 with 40/60, 18.75 FPU/g of cellulase was obtained with addition of 1% cellobiose, which was also the highest cellulase activity achieved in all shake flask experiments. These results indicated that molasses efficiently facilitated higher cellulase activity than cellobiose in *A. niger* ATCC 201201 with jean. Wheat bran gave higher cellulase activity than cellobiose in *A. niger* HDU fermentation with both cotton/PET 80/20 and cotton/PET 60/40. However, these by-products are more cost competitive as compared to cellobiose, and therefore are more suitable for use as inducers in cellulase production using textile waste.

3.3. Upscale experiment with 5-L bench-top fermentor

Further efforts on upscaling fungal fermentation were carried out using a 5-L bench-top fermentor with 3-L working volume. Soybean meal was used as nitrogen source because of its good performance in both jean and cotton/PET 80/20, also because it is an inexpensive nitrogen source compare to yeast extract and peptone etc. Tween 80 was added in jean fermentation but not in pure cotton and mixed textile fermentation. For inducers, we added 0.1% molasses for jean, 1% cellobiose for pure cotton and 1% wheat bran for mixed textile (based on table 2, inducers with highest cellulase production were selected).

During the fermentation, rapid fungal growth in the form of white hyphen was generated at around 12 h. Compared with smaller scale shake flask fermentation, the difference of fungal morphology would be attributed to the higher initial glucose concentration and better oxygen supply, which significantly enhanced fungal growth. Fig. 5 depicts the highest cellulase activity of 5.46 FPU/g for jean fermented with *A. niger* ATCC 201201. A similar result was obtained using pure cotton as substrate which produced 5.66 FPU/g cellulase. This was the highest cellulase activity obtained in upscale study, which was significantly lower than the values obtained in shake flask fermentation. It was suspected that the high fungal cell biomass in upscale bioreactor inhibited cellulase production (Singhania et al., 2017). Since textile wastes are usually discarded as mixtures without any source separation into textile types based on their compositions, it was considered worthwhile to investigate the utilization of mixed textiles as substrate for fungal cellulase fermentation. Mixed textile fermentation was carried out using three types of cotton/PET textile blends, with blending ratios of 80/20, 60/40 and 40/60. As expected, the results of cellulase production using mixed textiles were lower as compared to the use of one type of textile waste as sole feedstock. The maximum cellulase activity of 2.88 FPU/g was obtained using *A. niger* HDU in the SmF. Overall, the results of this study shows the possibility of using textile waste as a substrate in submerged cellulase production. Further efforts are needed for optimization of fermentation conditions in upscale fermentation.

3.4. Enzymatic hydrolysis of textile waste

In order to recycle cellulosic component and PET material, the pretreated cotton was hydrolyzed from cellulose to glucose. The fungal cellulase which resulted from SmF (i.e. fungal fermentation filtrate) using mixed textile waste in Section 3.3 with total cellulase activity of 2.88 FPU/g was used as the enzyme source. In comparison, commercial cellulase was also employed under the same hydrolysis condition. The time profile of hydrolysis yield was plotted in Fig. 6. It was observed that from 0 to 12 h, fungal fermentation filtrate presented improved hydrolytic efficiency as compared to commercial cellulase. This could be attributed to relatively higher ratio of endocellulase in fungal fermentation filtrate as compared to those in commercial cellulase, so it could quickly break down the crystalline structure of cellulose and therefore accelerate the rate of hydrolysis in the initial stage (Singhania et al., 2017). However, the hydrolysis yield from commercial cellulase became higher than SmF after 12 h. The rate of hydrolytic reaction was sufficiently fast and hydrolysis reached equilibrium at 24 h for both fungal fermentation filtrate and commercial cellulase. Final hydrolysis yields of 41.6% and 44.6% were resulted by fungal cellulase and commercial cellulase, respectively. These results indicated that a comparable enzymatic effect was obtained using fungal cellulase as compared to commercial cellulase in textile waste hydrolysis. Finally, the PET recovered after hydrolysis could be processed into regenerated PET fiber by melting spinning for reuse in textile applications. As far as what we have achieved, firstly, the feasibility of this biological recycling method has been proved. The raw textiles were first pretreated with physical crush and chemical treatment. Then pretreated textiles were fermented by fungi to produce cellulase. Afterwards, cellulase activity was improved through optimization of fermentation parameters including strain selection, nitrogen source, inducer and surfactant. Cellulase production was used to hydrolyze pretreated cotton and competitive hydrolysis yield was achieved compared to commercial cellulase. However, cellulase yield is still not high even after optimization. Main challenge of this biological method would be the improvement of cellulase activity in submerged fermentation.

As shown in Fig. 7, we propose a new textile waste lifecycle via biological recycling method. Textile waste is first used as carbon source in submerged fungal fermentation to produce cellulase. Subsequently, the produced cellulase is used for textile waste hydrolysis to obtain glucose-rich hydrolysate and PET fiber. The hydrolysate could be further converted to bioplastics such as poly (lactic acid) and polyhydroxybutyrate via bioconversion, and the remaining PET fiber could be re-spun for new textiles application. The textile waste-based biorefinery approach developed in this study illustrates the effective use of resources via replacement of non-renewable resources with recycled feedstock. At the same time, reduced throughput in the circular textile system by maximising clothing utilisations are key contributors in significantly reducing resource usage.

4. Conclusions

This study developed a novel method for valorization of textile waste using submerged fungal fermentation. Optimization of fermentation media indicated that pretreated textile and Mandels medium are preferred for cellulase production. The highest cellulase activity of 18.75 FPU/g was achieved by *T. reesei* ATCC 24449 with cotton/PET 40/60 based textile and 1% cellobiose addition. Fungal cellulase obtained from SmF resulted in similar hydrolysis yields as commercial cellulase in textile waste hydrolysis. The research outcomes demonstrated practical implementation of circular textile concept via SmF with creation of a new global textiles system whereby textile products could be effectively recycled within the industry. This would enable the shift of the global textiles economy towards a circular economy framework.

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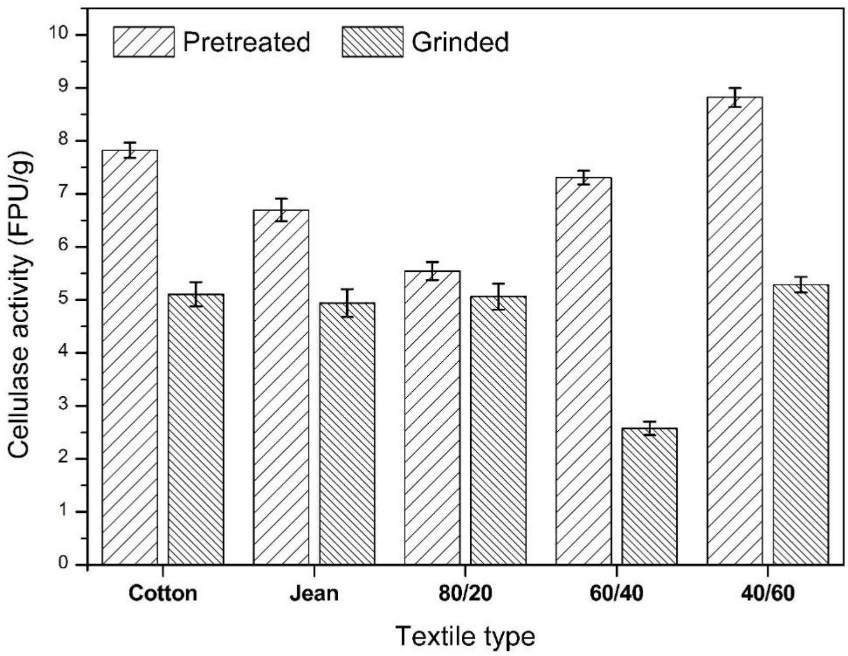


Fig. 1. Cellulase activity achieved with pretreated cotton, jean, cotton/PET 80/20, cotton/PET 60/40, cotton/PET 40/60 and grinded pure cotton, jean, cotton/PET 80/20, cotton/PET 60/40, cotton/PET 40/60.

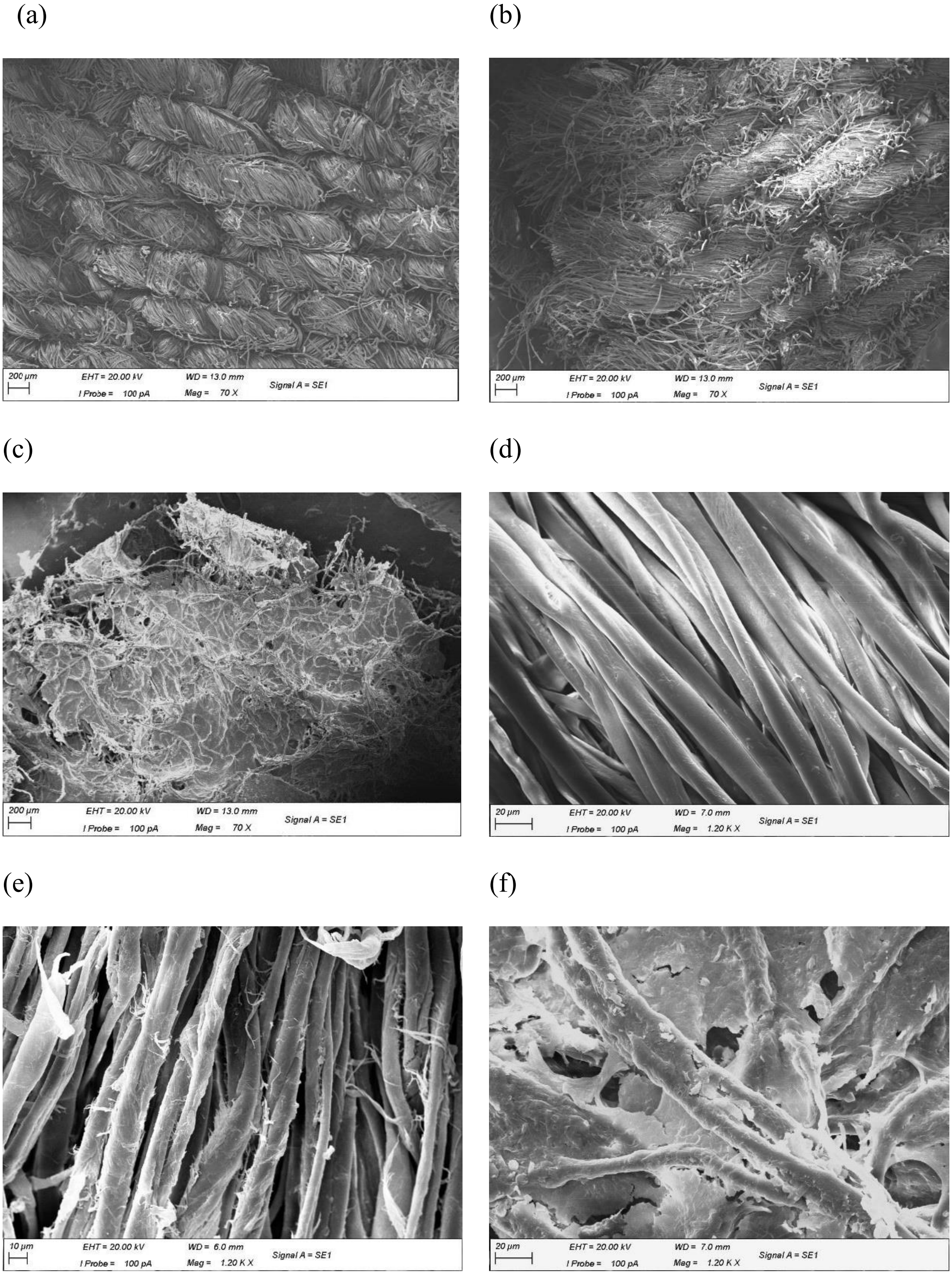


Fig. 2. SEM images of grinded jean, pretreated jean and fermented jean. (a) grinded jean, magnification of 70; (b) pretreated jean, magnification of 70; (c) fermented jean, magnification of 70; (d) grinded jean, magnification of 1,200; (e) pretreated jean, magnification of 1,200; (f) fermented jean, magnification of 1,200.

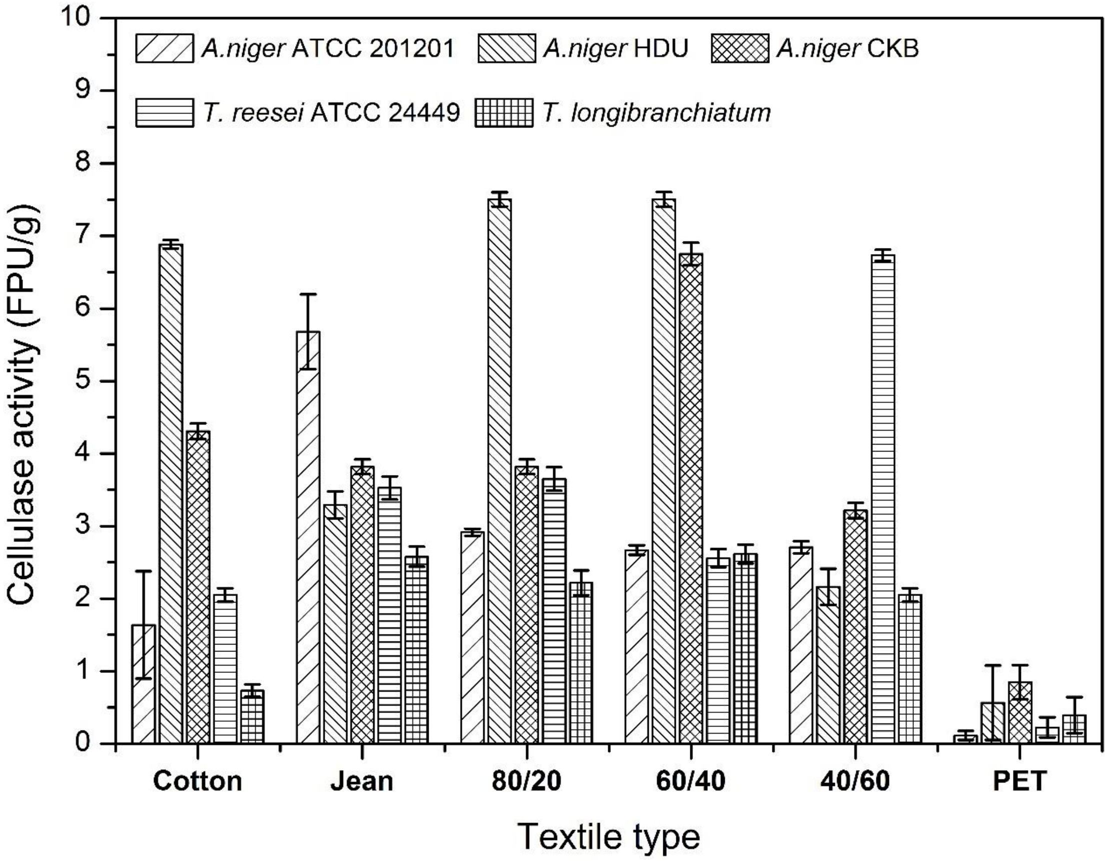
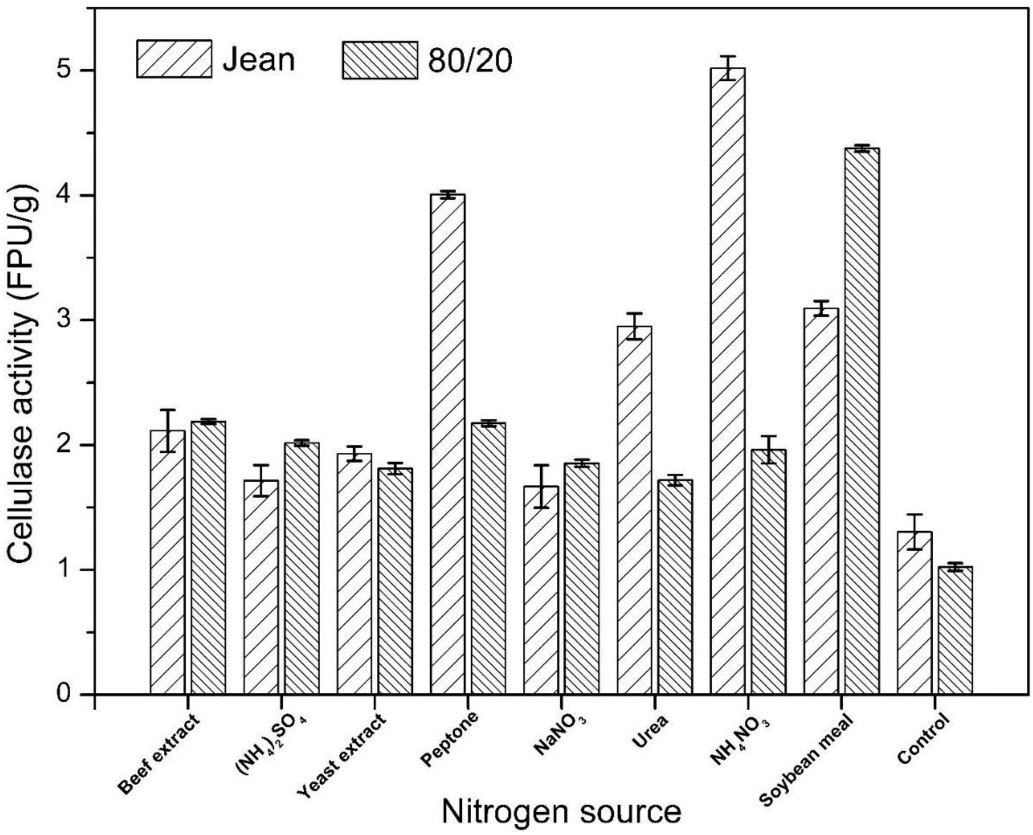


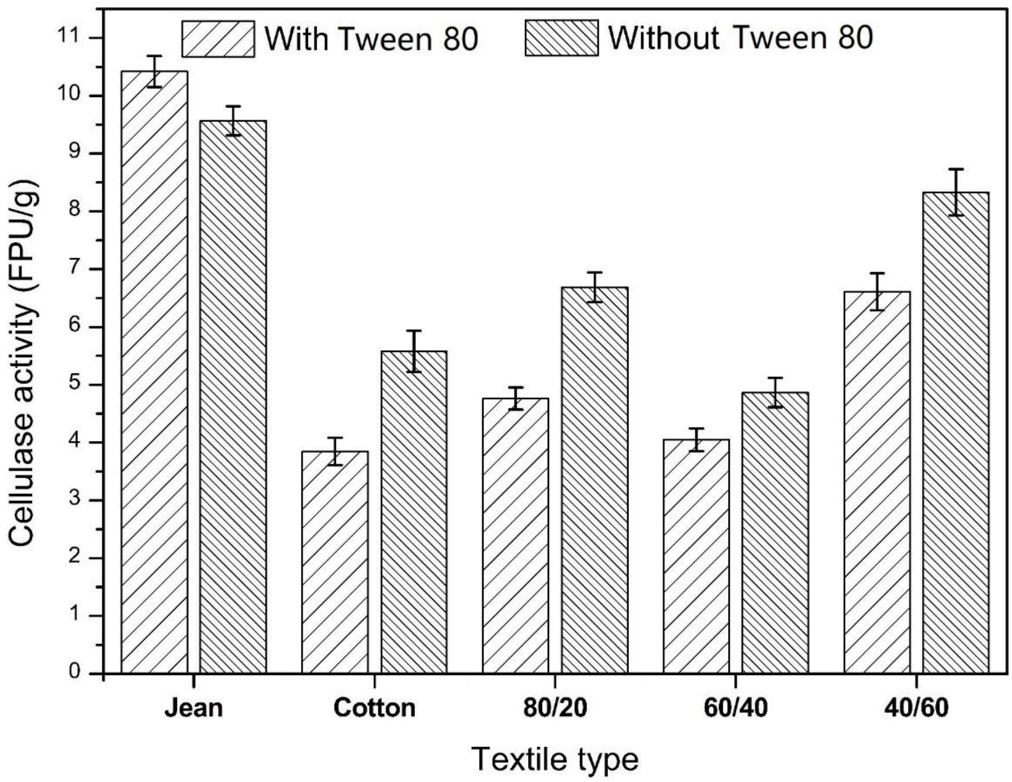
Fig. 3. Compare of cellulase production using *A. niger* ATCC 201201, *A. niger* HDU, *A. niger* CKB, *T. reesei* ATCC 24449, *T. longibrachiatum* ATCC 52326 fermented with grinded pure cotton, jean, cotton/PET 80/20, cotton/PET 60/40, cotton/PET 40/60 and pure PET.

(a)



Jean
80/20
Cellulase activity (FPU/g)
0
1
2
3
4
5
Beef extract
$(NH_4)_2SO_4$
Yeast extract
Peptone
$NaNO_3$
Urea
NH_4NO_3
Soybean meal
Control
Nitrogen source

(b)



With Tween 80
Without Tween 80
Cellulase activity (FPU/g)
0
1
2
3
4
5
6
7
8
9
10
11
Jean
Cotton
80/20
60/40
40/60
Textile type

Fig. 4. Medium optimizations include nitrogenous and Tween 80. (a) Effect of sole nitrogen source in cellulase production on both pretreated jean with *A. niger* ATCC 201201 and pretreated cotton/PET 80/20 with *A. niger* HDU. Nitrogen source loading ratio is 0.5% (w/v) and control group means without nitrogen source addition. (b) Effect of Tween 80 (0.01% w/v) in cellulase production on pretreated jean, pure cotton, cotton/PET 80/20, cotton/PET 60/40 and cotton/PET 40/60.

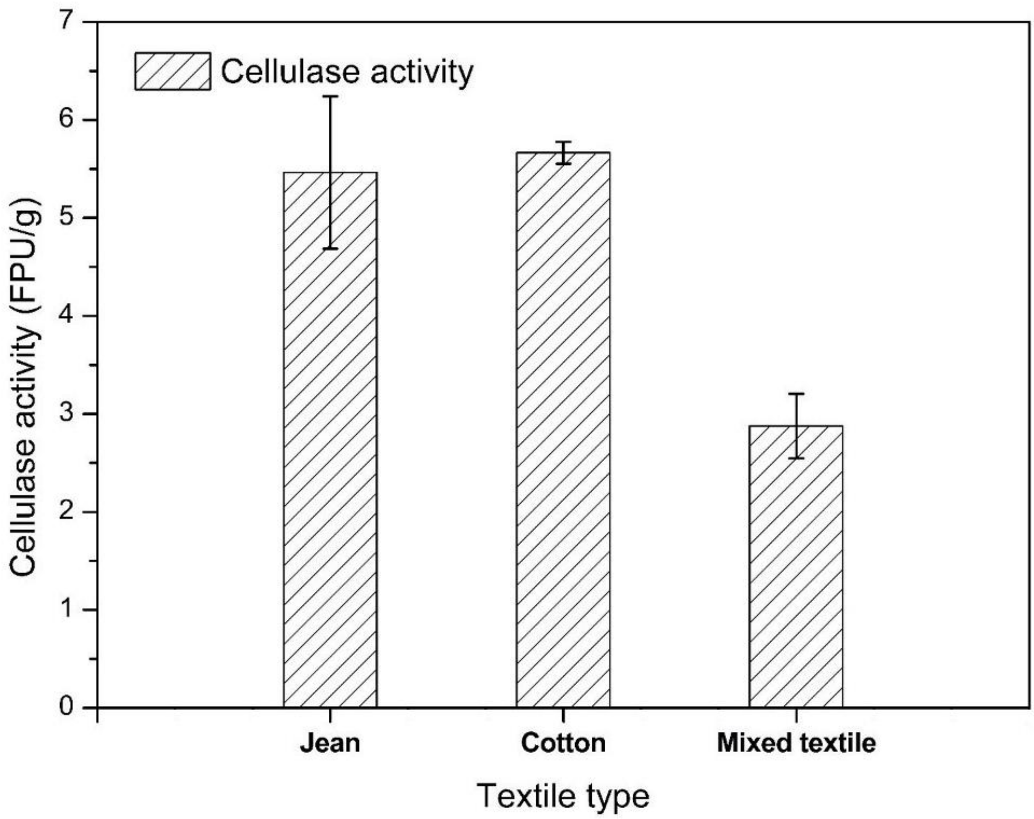


Fig. 5. Upscale fermentation using pure cotton, jean and mixed textile separately in 5-L bioreactor. Mixed textile contains equal amount of pretreated textile waste cotton/PET 80/20, cotton/PET 60/40 and cotton/PET 40/60, respectively.

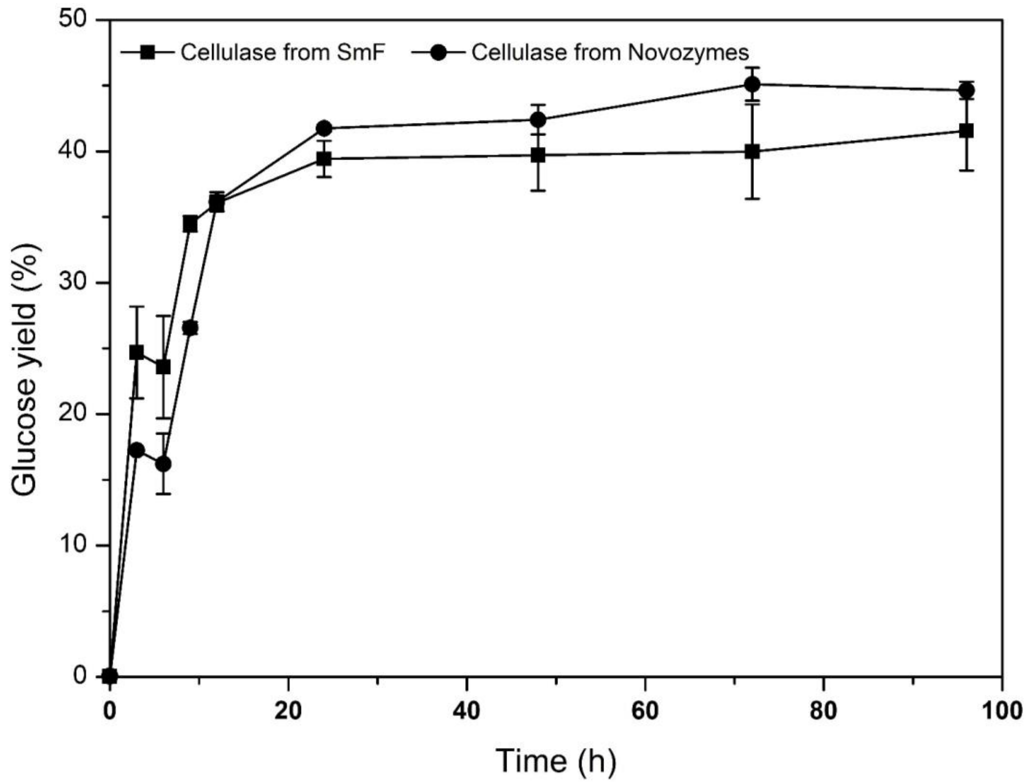


Fig. 6. Textile hydrolysis by commercial cellulase and fungal cellulase from textile waste, with pretreated cotton as substrate.

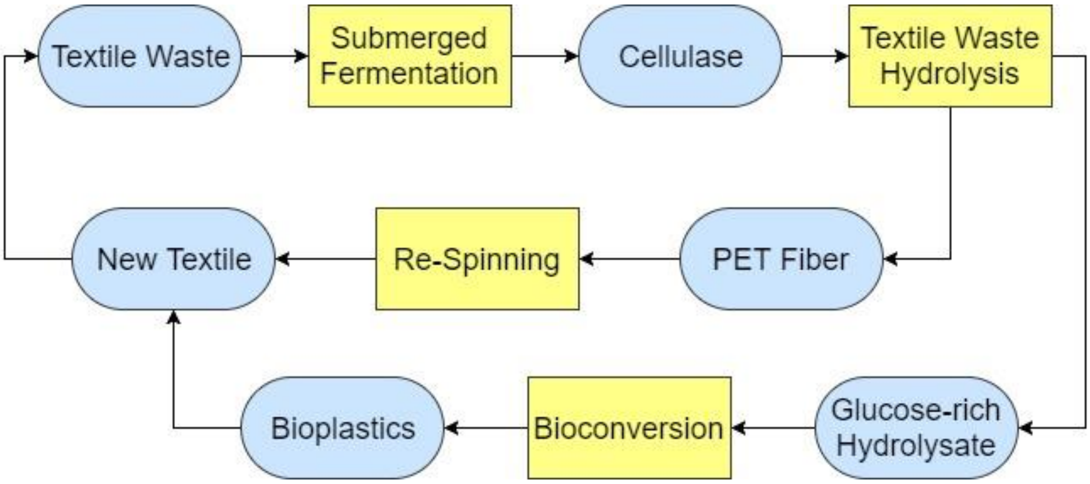


Fig.7. Process flow diagram for biological recycling and regeneration of textile waste.

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

Table 1. Textile wastes used in this study.

Textile	Strain	Inducer type & concentration (w/v) % [a]	Cellulase activity (FPU/g) [a]
Jean	*A. niger* ATCC 201201	Sawdust 1%	5.49 ± 0.23
		Molasses 0.1%	9.72 ± 0.65
		Cellobiose 0.1%	9.04 ± 0.32
		Wheat bran 1%	8.35 ± 1.01
		Control [b]	1.23 ± 0.09
Pure cotton	*A. niger* HDU	Sawdust 0.1%	8.54 ± 0.10
		Molasses 0.1%	7.76 ± 0.17
		Cellobiose 1%	9.97 ± 0.54
		Wheat bran 1%	3.40 ± 0.07
		Control [b]	1.56 ± 0.04
Cotton/ PET (80/20)	*A. niger* HDU	Sawdust 0.1%	3.76 ± 0.98
		Molasses 0.1%	10.83 ± 1.64
		Cellobiose 1%	7.96 ± 1.79
		Wheat bran 1%	13.10 ± 0.50
		Control [b]	0.80 ± 0.12
Cotton/ PET (60/40)	*A. niger* HDU	Sawdust 0.1%	5.69 ± 0.11
		Molasses 1%	9.55 ± 0.64
		Cellobiose 1%	6.66 ± 0.47
		Wheat bran 1%	9.84 ± 0.31

		Control [b]	1.18 ± 0.05
Cotton/ PET (40/60)	*T. reesei* ATCC 24449	Sawdust 0.1%	7.12 ± 0.18
		Molasses 1%	8.61 ± 0.36
		Cellobiose 1%	18.75 ± 0.81
		Wheat bran 1%	8.16 ± 0.24
		Control [b]	1.04 ± 0.07

Table 2. Results of submerged fermentation with addition of inducer.

[a] Values in this table only show the highest cellulase activity and the concentration used for each type of inducer.

[b] Control means no addition of inducer in fermentation medium.