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Utilization of Cellulase from *Colocasia esculenta* in Treatment of Cotton Fabric

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Abstract

More than 1 million tons of textile waste has been generated each year out of which only 15% is recycled and the rest is causing environmental pollution. Textile waste is recyclable creating huge opportunities for the researchers to come up with a technique which will benefit the environment. Biological treatment of agricultural, industrial, organic and toxic wastes is rapidly increasing due to both economical and ecological reasons. Peel and Corm of underground carbohydrate rich vegetables (*Colocasia esculenta*, *Amorphophallus paeoniifolius, Ipomoea batatas*, *Solanum tuberosum*, *Raphanus sativus, Daucus carota*) was checked for presence of cellulase activity which was confirmed by CMC assay, Filter paper assay, Congo red plate method. On comparing the activity of different crude extracts, *Colocasia esculenta* and *Daucus carota* peelshowed highest specific activity 1346.81 U/mg (CMCase) and 156.17 U/mg (Filter Paper assay) and *Raphanus Sativus* corm showed highest CMCase and filter paper activity of 296.7 U/mg and 534.11 U/mg respectively. Molecular weight of cellulase isolated from peel of *Colocasia esculenta* is approx. 43000 Dalton. Cellulase activities towards CMC was confirmed by presence of clear bands in Zymogram studies. Change in cotton fabric texture with increased absorption capacity is confirmed by weight loss studies and SEM analysis. Cotton fabric with increased absorption capacity can be combined with the bioreactor technology for the treatment of industrial effluents.

Keywords: Enzymatic treatment; Cellulase: Zymogram, SEM

Introduction

Textile industry is one of the major economic sectors of the world. Production of textile is exponentially increasing due to globalization. According to the estimation provided by Hollins around 80,000 tons of textile waste are generated per year(1). According to Environmental Protection Agency (EPA), Americans generated 17 million tons of textile waste in 2018 which is equals 5.8 % of total municipal waste (2). Textile waste is generated from commercial and industrial uses and categorised into three types: pre-consumer waste generated by retailers, fiber processing and manufacturing; post-consumers waste generated by disposal of used textile as it is damaged or out of fashion(3).More than 50% of the textile waste is recyclable creating huge opportunities for the researchers to come up with a technique which will benefit the environment (4). Conventional method of fabric waste management include composting, landfilling, incineration which causes soil pollution and global warming effects due to production of

harmful gases (5,1). Water contamination occurs due to poor disposal of domestic and industrial wastes is also critical issue in domestic country. According to World Health Organization, around 2 million people drink contaminated water and almost 829 000 deaths occurred by diarrhoea after drinking polluted water(6).

Natural textile fibers are made up of cotton, linen, viscose, protein based like wool and silk which can be degraded by enzymatic hydrolysis(1).Biological treatment of agricultural, industrial, organic and toxic wastes is rapidly increasing due to both economical and ecological reasons (7).Cellulose is the major polymer of natural fibers and the monomer glucose are linked by glycosidic linkage. Cellulase is used for hydrolysis of β-1,4 glycosidic bonds to produce glucose. It is made up of three components endoglucanase (endo-1,4-β-D-glucanase), exoglucanase (exo-1,4-β-D-glucanase), β-glucosidase. Endoglucanase cleaves randomly on the cellulose crystals and produce cello-oligosaccharide, exoglucanase acts on the non-reducing ends of linear chain microcrystal of cellulose and convert them into cellobiose, β-glucosidase acts on the cellobiose and convert it into glucose(8).

Cellulase is the third largest industrial enzyme and has wide variety of industrial applications like in textile and detergent, food and beverage, Paper and pulp, Bioethanol and biofuel(9). In textile industry, cellulase is used in the biostoning of denim jeans that gives faded or aged look to the denim, biopolishing of cotton fibers by hydrolyzing the small protrusions of the fibers from the surface of cotton creating smooth and glossy appearance, improves dye absorbance and removes excess dye(10). Cellulases can also be used in the treatment of municipal waste water which contains 40%-50% cellulose, 9–12% hemicelluloses, and 10–15% lignin on a dry-weight basis(11,12). Toilet papers usually generate 23% of the organic waste in the sanitary wastewater which results in the presence of 7.9 K tons of cellulose in the Amsterdam's wastewater recovered by fine mesh(13).Bio-conversion of agricultural waste utilizing rice straw, yam peels, cassava peels, and banana peels into valuable products is the strategy employed successfully to to treat industrial waste water(14).

In the present paper, our main objective was to check for the presence of cellulase activity in the peel and corm extracts of six different underground , carbohydrate rich crops (*Colocasia esculenta*, *Amorphophallus paeoniifolius, Ipomoea batatas*, *Solanum tuberosum*, *Raphanus sativus, Daucus carota*) and check if the isolated cellulase can change the surface properties of the fabric and ultimately degrade cotton fabric,. This paper describes isolation, partial purification, enzyme characterization and its effect on cotton fabric.

Materials and Methods

Material

Colocasia esculenta, Amorphophallus paeoniifolius, Ipomoea batatas, *Solanum tuberosum, Raphanus sativus, Daucus carota* was purchased from Safal at Ghaziabad and was washed in running tap water followed by rinsing with distilled water and air drying .

Extraction of crude enzyme from underground vegetables

Washed and dried *Colocasia esculenta*, *Amorphophallus paeoniifolius, Ipomoea batatas*, *Solanum tuberosum*, *Raphanus sativus, Daucus carota* werediced into small pieces separately after removing the peels from the corms. The corm and peel were weighed and homogenized in ratio of 1:3 gm/L with 0.1 M Citrate buffer, pH 5.0 separately. The homogenate was filtered through three layers of cheese cloth and the filtrate was then centrifuged at 10,000 rpm for 30 min at 4 ºC in a refrigerated centrifuge to obtain a clear supernatant.

Protein assay

Protein concentrations were determined according to the dye binding method of Bradford

using Bovine serum albumin as standard(15).

Determination of cellulase activity in peel and corm of underground vegetables

Enzyme Method

The Dinitrosalicylic acid (DNS) method for the measurement of reducing sugar was used for the determination of cellulase activity(16). CMCase and FPase activity was assayed using a modified method described by (17).The Carboxymethyl-cellulase (CMCase) assay was performed with 1% (w/v) CMC as the substrate dissolved in 0.1 M citrate buffer. The reaction was initiated by the addition of 0.1 mL of sample (peel, corm crude extract and dialysed sample) with 0.9ml of citrate buffer (0.1M, pH 5) and 1ml of 1% CMC were added into the test tubes and incubated at 50 °C for 1 hour. By the addition of 2 mL 3,5- DNS, the reaction was terminated and was then kept for 10 min in a boiling water bath and 1 ml Rochelle salt solution 40% and 6 mL of distilled water was further added. After vortexing 250 µl of each was loaded onto 96 well plate and the absorbance was measured at 540nm. One unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 µmol of glucose equivalent to 1 min. Filter-paper activity (FPase): Briefly, the methods are similar to the CMCase assay method, but the substrate used was Whatman no. 1 filter paper (25mg) soaked in 1 mL 0.1M citrate buffer (pH 5.0).

Congo Red plate method

Presence of yellow clear zone confirms the presence of cellulase activity in congo red plate assay Clear zones was checked for isolated crude extracts of peel and corm of *Colocasia esculenta Amorphophallus paeoniifolius, Ipomoea batatas*, *Solanum tuberosum*, *Raphanus sativus, Daucus carota* was identified from Congo red plate assay described by (18). Agar plates were prepared with 1% Carboxymethyl cellulose and 1% agar dissolved in deionized water. The 100µl of crude enzyme sample was loaded into each well and incubated at 50 °C for 24 hours. After 24 hours, the wells were washed with distilled water and further stained with 0.1% congo red for 30 mins and destained with 1M NaCl until the yellow clear zones were observed

Partial purification of Cellulase enzyme from Colocasia esculenta

Colocasia peel (20 g) was weighed and homogenized in ratio of 1:3 (gm/ml) with 100mM Citrate buffer, pH 5.0. After filtering with three-layer cheese cloth the homogenate was centrifuged at 10,000 rpm for 30 min at 4 ºCto obtain a clear supernatant which was precipitated with ammonium sulphate. Ammonium sulphate was slowly added to supernatant to bring 0-60% saturation at 4 ºC with constant stirring and kept for an hour on ice. The precipitated protein was recovered by centrifugation at 10,000 rpm for 20 min at 4 ºC in a refrigerated centrifuge.Supernatant was discarded and precipitate was resuspended in 100mM citrate buffer at pH 5, at ration of 1:1 pellet to buffer. The resuspended0-60% pelletwas then dialyzed against 10mM citrate buffer, pH 5, 4litres with three times buffer change. Sample was estimated for protein and cellulase activity.

Optimization studies for cellulase enzyme

The influence of temperature on the enzyme activity was checked at different temperatures (10°C, 20°C, 40°C, 50°C, 60°C, 80°C) at pH 5.0 for 1 hour using 1% CMC and 25mg filter paper as substrate. Reducing dye release was estimated by DNS method. Formation of reducing sugar was quantified by measuring the colour change at 540nm using microplate reader(19). Enzyme was also assayed at different pH (4-9)with 1% CMC and 25mg filter paper as a substrate. All followed by incubation at 50°C for 1 hour.

Polyacrylamide Gel Electrophoresis and Zymogram

SDS-PAGE is used to determine the molecular weight of purified protein by following

the method described by (20). The partially purified enzyme was loaded on the 5% stacking gel and 10% resolving gel. Electrophoresis was performed at 120V for 2-3 hours. The gel was stained with Coomasive Brilliant Blue dye for 30 mins and destained with 30% methanol and 10% glacial acetic acid. The molecular weight of the enzyme was determined by medium range molecular marker, Merck. For Zymogram analysis, 1% CMC was incorporated in non-denaturing polyacrylamide gel electrophoresis with 10% resolving gel and 5% stacking gel. The gel was run at 120V for 2-3 hours. The gel was washed with Triton 100-X and floated in sodium citrate buffer for 1 hour at room temperature. Further the gel was incubated at 50°C for 2 hours in sodium citrate buffer (pH 5). The gel was stained with 0.1% Congo red and de-stained it with 1 M NaCl until the clear yellow zones were appeared and observed under the white light illuminator (21).

Treatment of cotton fabric

Textile fabric was enzymatically hydrolysed in 0.1M Sodium citrate buffer in 100ml reagent bottle. 6 cotton fabric (each

Table 1: Protein estimation and Cellulase activity from peel of different crops

Sample (Crude extract)	Total protein (mg)	Total activity unit	Specific activity (U/mg)	Total activity unit	Specific activity (U/mg)
PEEL		CMCase		Filter Paper Assay	
Ipomoea batatas	1.45	135.08	204.38	24.10	38.37
Solanum tuberosum	1.60	60.67	101.91	6.42	11.24
Colocasia esculenta	0.317	153.07	1346.81	10.05	84.91
Amorphophallus paeoniifolius	0.75	140.21	826.81	15.72	64.79
Raphanus Sativus	2.4	118.51	146.24	49.57	48.10
Daucus Carota	1.33	61.67	103.9	82.02	156.17

Table 2: Protein estimation and Cellulase activity from corm of different crops

Sample (Crude extract)	Total protein (mg)	Total activity unit	Specific activity (U/mg)	Total activity unit	Specific activity (U/mg)
CORM		CMCase		Filter Paper Assay	
Ipomoea batatas	4.29	157.85	102.88	272	189.69
Solanum tuberosum	6.81	78.02	23.73	72.28	27.15
Colocasia esculenta	5.58	169.80	153.86	99.22	76.18
Amorphophallus paeoniifolius	4.77	115.66	62.39	43.88	18.80
Raphanus Sativus	1.29	97.62	296.70	263.9	534.11
Daucus Carota	1.99	177.90	228.39	289.45	357.39

25mg) pieces was added to each reagent bottle containing 3ml crude extract of peel with 6ml buffer, 3ml crude extract of corm with 6ml buffer, 1.5 peel extract and 1.5 corm extract with 6 ml buffer respectively. Hydrolysis was performed at 50 °C for different time intervals (1,2,4,24,48,72 hours) to find optimum time interval for treatment of cotton fabric.

Water drop absorption test

The test is performed to check the water absorption capacity for treated sample compared to control samples. 100 µl of water droplet was kept on the treated sample and the time taken to absorb the water drop was noted.

Visualisation of fabric surface

After treatment all the samples were thoroughly washed to remove any impurities and further dried. SEM analysis was done at magnification 5000X.

Results and Discussion

Comparative analysis of different tuber crops

Peel and corm extracts from *Colocasia esculenta, Amorphophallus paeoniifolius, Ipomoea batatas*, *Solanum tuberosum, Raphanus sativus, Daucus carota*was isolated and studied for their cellulase activity. Protein estimation was done using Bradford assay as shown in Table 1 and Table 2. Enzyme activity was estimated by DNS method. On comparing the activity of different crude extracts, *Colocasia esculenta* and *Daucus carota* peel showed highest specific activity 1346.81 U/mg (CMCase) and 156.17 U/mg (Filter Paper assay) and *Raphanus Sativus* corm showed highest CMCase and filter paper activity of 296.7 U/mg and 534.11 U/mg respectively as shown in Figure 1.

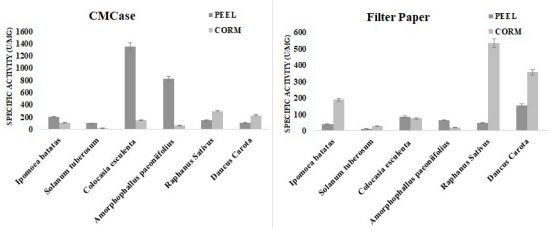

Figure 1: Cellulase activity of different crops a) CMCase b) Filter Paper Assay (Error with 5% value)

Congo Red plate method

Endoglucanase activity of *Colocasia esculenta*, *Amorphophallus paeoniifolius, Ipomoea batatas*, *Solanum tuberosum*, *Raphanus sativus, Daucus carota* was further confirmed by Congo red plate diffusion assay. Clear zones were visualized under white light illumination as shown in Figure 2. Presence of clear yellow zones around the sample well confirmed the presence of CMCase activity.

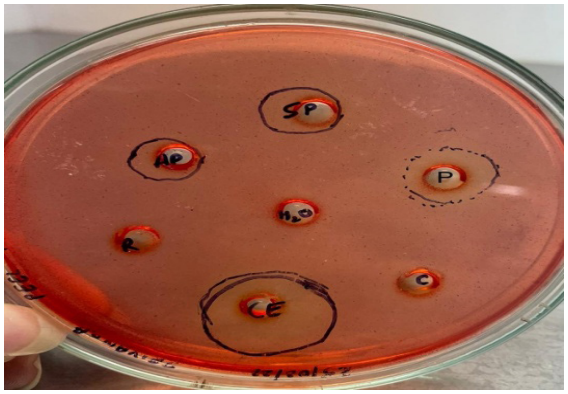

Figure 2: Congo red plate assay: Peel of *Colocasia esculenta* (CE), *Amorphophallus paeoniifolius* (AP), *Ipomoea batatas* (SP), *Solanum tuberosum* (P), *Raphanus sativus* (R), *Daucus carota* (C). Clear yellow zones were observed under white light illuminator

Optimization studies for cellulase enzyme

The optimal temperature and pH of peel enzyme for CMCase and Filter paper was found to be 50°C and pH 5 respectively. The results have shown that the enzyme was stable at temperatures between 10°C and 80°C. The optimal temperature and pH of corm enzyme for CMCase and Filter paper was found to be 40°Cand pH 6respectively. The results have

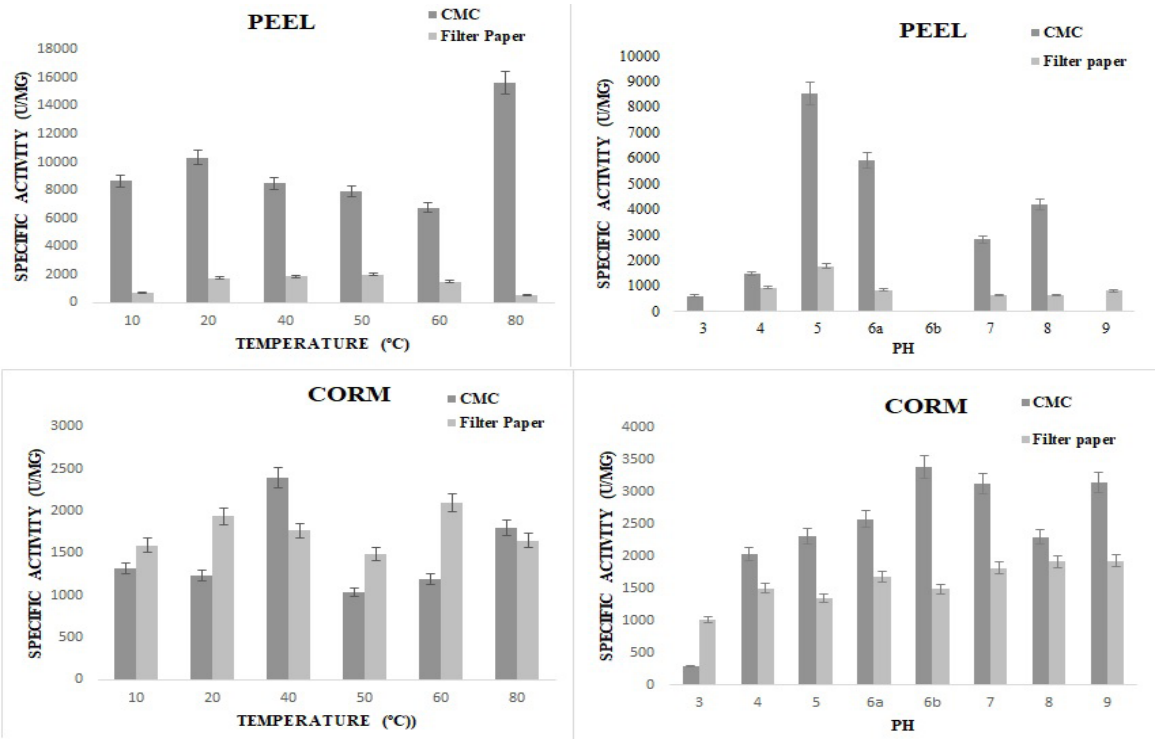

Figure 3: Effects of temperature and pH on cellulase enzyme activity (Error with 5% value)

shown that the enzyme was stable at alkaline pH as shown in Figure 3. Both the enzymes were stable at 80°C.

Partial Purification of cellulase enzyme

The crude enzyme from peelwas isolated from *Colocasia esculenta*(20g) and partial purificationwas done by dialysis of 0-60% ammonium sulphate precipitated sample.The purification fold obtained through Carboxymethyl cellulase assay was 7.7-fold purity where as in Filter paper assay it was 6.8-fold as shown in Table 3.

Table 3: Protein estimation, CMCase activity of partially purified enzyme from Peel of *Colocasia esculenta*

Sample	Total protein (mg)	Total activity unit	Specific activity (U/mg)	Percentage yield (%)	Purification Factor
CMCase Activity					
Crude Extract Peel	4.14	2284.5	50.76	100	1
Ammonium Sulphate (0-60%) Peel	0.78	509.2	391.76	22.29	7.71
FilterPaper Activity					
Crude Extract Peel	4.14	17.18	13.21	100	1
Ammonium Sulphate (0-60%) Peel	0.78	116.96	89.97	680.76	6.8

SDS-Page and Zymogram

SDS-PAGE analysis of partially purified sample was done using polyacrylamide gel electrophoresis and activity staining with 1% of CMC polymerised in native gel. The molecular weight of the enzyme isolated from peel is around 43kDaas shown in Figure 4.

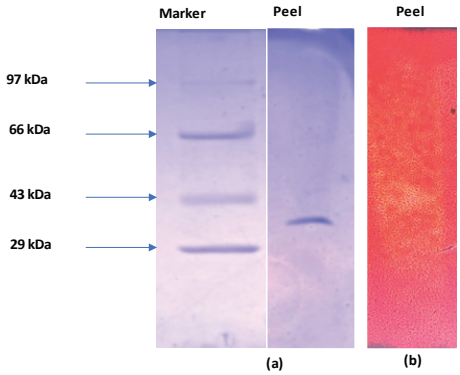

Figure 4: SDS-PAGE (a) with 10% resolving gel and 5% stacking gel; Zymogram analysis (b) non-denaturing polyacrylamide gel electrophoresis with 7% resolving gel and 5% stacking gel of partially purified peel enzymeisolated from *Colocasia esculenta.* Clear band indicates the presence of cellulase activity at 43000kDa.

Treatment of cotton fabric

Samples treated with crudepeel extract, crudecorm extract, and a mixture of peel and corm at 50 ºC showed changes in properties of the fabric. Cotton fabric was treated at different time interval (1,2,4,24,48,72 hours) to analyse the time for degradation and it showed release of glucoseincreased with time shown in Figure 5.Cellulase present in the peel and corm extracts can be used for the degradation of cotton fabric as it showed high filter paper activity. Mixture of peel and corm enzyme showed high glucose release of 151.4 ug. It also affects the weight of the fabric due to cellulose hydrolysis. An average of 5.2% loss is observed in 48 hours. Elaborate individual experiment is needed to confirm this.

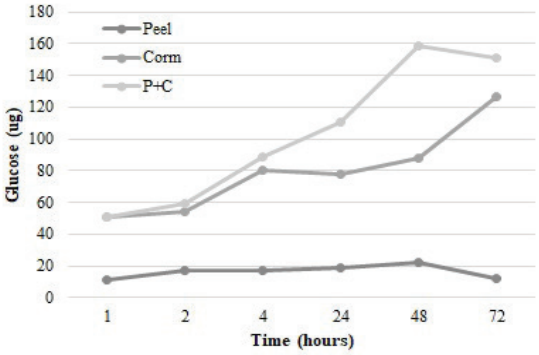

Figure 5: Glucose liberated during degradation of cotton fabric from 1,2,4,24,48,72 hours

Water Absorbency

Cellulose based materials can be used as a filter, adsorbent for dyes, oils, waxes, flocculants present in the industrial wastewater(22). Gallons of waste is generated by oil and gas industry due to spillage, transportation which causes environmental pollution(23). Microporous membrane was first used in World War II and Cellulose acetone membrane was used in reverse osmosis for saline retention and polymer membrane is used to treat contaminated water(24). The water absorption time reduced in treated fabric as compared to the untreated control fabric. Highest absorption is seen in sample treated with corm extract. In peel it took 1.05 seconds, in corm 0.95 seconds, in peel and corm mixture 2 seconds whereas in control it took 1320 seconds as shown in Table 4, Figure 6.

Table 4: Water drop absorption test

Sample	Time (s)
Control	1320
Peel	1.05
Corm	0.95
Peel + Corm	2

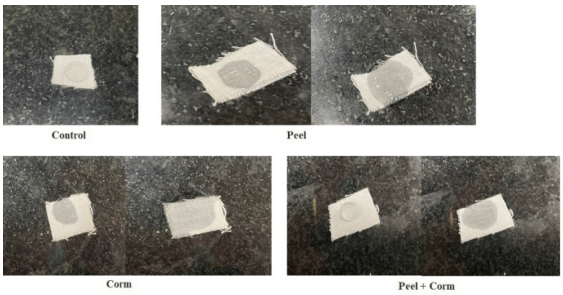

Figure 6: Water Absorbency: Highest absorption is seen in sample treated with corm extract. In peel it took 1.05 seconds, in corm 0.95 seconds, in peel and corm mixture 2 seconds whereas in control it took 1320 seconds

Scanning Electron Microscope Detection

To investigate the changes in surface morphology, SEM analysis was performed for the treated cotton fabric at the magnification of 5000x. The samples were observed after 72 hrs of treatment with peel, corm, and mixture of peel and corm extract at 50 ºC.Maximum structural changes are seen in fabric treated with mixture of peel and corm which showed serrated edges as shown in Figure 7.

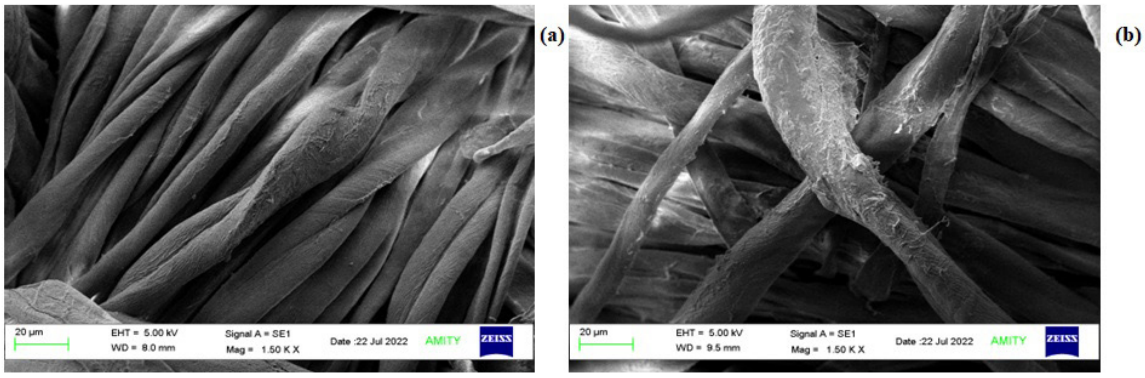

Figure 7: SEM a) control;b) mixture of Peel and Corm. Fabric treated with corm is showing serrated edges

Conclusion

On comparing the activity of different crude extracts, *Colocasia esculenta* peel showed highest specific activity of 1346.8 U/mg (CMCase) and *Raphanus Sativus* corm showed highest filter paper activity of 534.1. U/mg. Cellulase present in the peel and corm extracts of these crops can be used in recycling or complete degradation of waste cotton fabric. Mixture of peel and corm enzyme showed high glucose release of 151.4 ug after 72 hrs of incubation. Structural cotton fabric and its increased absorption capacity is confirmed by weight loss studies (5.2%), increased absorption capacity (approx 1 sec), and SEM analysis which shows presence of serrated edges of treated cotton fabric as compared to control untreated fabric. Cotton fabric with increased absorption capacity can be combined with the bioreactor technology for the treatment of industrial effluents. Further studies related to complete degradation of cotton fabric and increasing the yield of glucose in the elute is in progress Studies related to utilization of material with good absorption property produced by low cost environmentally friendly methods can be utilized for waste water treatment of textile dying industries and petroleum industries.

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