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Optimization of Wash Bath Temperature for Effective Biopolishing of Textile Garments

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ABSTRACT

Over the past several decades, various methods have been used by the garment industry to enhance the value of their products. The primary method has been through the use of chemicals. In the t-shirt manufacturing industry, the predominant method for adding value has been biopolishing. Pilling in cotton garments can negatively affect the appearance and durability of the garments. Improving the drapability and increasing the durability of the garments at low cost had gained traction as an alternative to the costly mercerizing process. Cellulase, a naturally occurring product made from microorganisms, has been beneficial for this use. Removing fuzz and making the fabric pill resistant is one of the main applications of microbial cellulase. Identifying an optimum wash bath temperature for biopolishing, and subsequent processing, will provide a strong economical reason for the garment processing industry to adopt this value-adding process.

Keywords: Biopolishing, Cellulase, Cellulose, Textile Garments, Pilling, Enzymatic process

1. Introduction

As work place dress codes become more informal, the days of wearing a full suit to the office are gone. With the rapid growth of tech industries, where work can be done from home or from a coffee shop in addition to in an office, dress etiquette has changed significantly. Informal dress codes have been widely accepted. Today, many companies allow their employees wear to t-shirts to work. This shift has shifted the garment design industry into top gear to generate more colorful combinations appropriate for other environments besides the most informal occasions. This change in culture in regard to clothing did not just provide new opportunities for the t-shirt industry; it also increased the challenges of achieving the unique, fashionable, and value-enhanced products, while remaining competitive in price.

Similar to reductions in operating margins, the dying process should be more economical while producing high value products(Bajaj, 2002). However, the garment industry had been struggling for decades to control pilling, especially in garments knitted with carded yarn. Pilling reduced the quality and appearance of the garment(McCarthy, 1997).

This inadvertently negatively affected the value proposition of the brand's marketability. Prior to the advent of biotechnology in daily manufacturing, controlling pilling was highly problematic. The pilling becomes pronounced with increased processing, such as scouring, dyeing, and similar procedures. However, garment processing is an important process in creating colorful articles of clothing. To achieve beautiful design and colors, the textile processing industry has several processes that have been in practice for decades(Emilia Csiszar, Szakács, & Rusznak, 1998). Using age-old practices had resulted in several inefficient processes and unsatisfactory results in the finishing touches.

Experts are researching ways and means for the use of innovative processes in all facets of garment manufacturing. In garment manufacturing as a whole, the most problematic process is the knitting and coloring of the yarn or fabric(Franks, 2000). In addition to difficulties in achieving the desired colorful results, pilling becomes more pronounced as a result of preprocessing and coloring fabric. The constant mechanical action causes tiny fibers in the yarn to break, creating fluffiness(Teeri, 1997).

Improving the aesthetics of the t-shirt, or other garment, through the process called bioprocessing with the cellulase enzyme is a boon to the industry, especially considering that t-shirts made with natural cotton will pill and fluff to a greater degree(Emilia Csiszar et al., 1998). This gives the garment a fuzzy texture. As the garment undergoes repeated washing and wearing, broken and entangled yarn becomes more pronounced, and the small fluffy fibers lose color with repeated washing. After a time, the garment appears to be old and worn out.

Before the introduction of enzymes for bioprocessing, controlling the pilling effect was performed with chemicals; this caused the fabric lose its tensile strength. After extensive research involving modern biotechnology, microbes were put to use(Franks, 2000). Enzymes were employed in several textile processes. One such process is biopolishing, which by action removes the projecting small fibers from the yarn. This improves the texture and appearance of fabrics. This not only creates a smoother fabric that is resistant to pilling, but also improves softness, luster, and drape(Pedersen & Screws, 1998). In short, biopolished fabrics look better and last longer, without increased pilling, even after repeated washing and wearing.

The word *biopolish* indicates the process of polishing fabric using a non-chemical process obtained from the biological source – living microorganisms. Bioprocessing is carried out using a group of enzymes called cellulase(Wadham, 1994). The protein molecules are similar to cellulose, which serves as a substrate. It possesses the ability to degrade cellulose, which is a complex polysaccharide carbohydrate molecule(Bhat, 2000). Cellulose is also the major constituent of the plants. Many soil-borne organisms, including fungi and bacteria, have the ability to degrade the cellulose in the plant.

Cellulase is a systematic enzyme that breaks cellulose down into glucose(E Csiszar, Urbánszki, & Szakacs, 2001). The mode of action of cellulase enzymes on cotton is complex. Cellulose is the major component of cotton. The ease of use and the efficiency of the cellulase enzyme in removing pilling generated a significant interest in this novel product(Gross & Kalra, 2002). The main advantage of the enzymatic processing is that it is more economical, requires less water to process the fabric, and is also eco-friendly.

Chemically, cellulose is a linear polymer composed of D-glucose subunits linked by β-1, 4 glycosidic bonds forming the dimer cellobiose. These form long chains linked together by hydrogen bonds and van der Waals forces. Cellulose is usually present in a crystalline form, and a tiny fraction of the chains forms amorphous cellulose. In the latter conformation, cellulose is more susceptible to enzymatic degradation(Pérez,

Munoz-Dorado, de la Rubia, & Martinez, 2002).

The biopolishing process involves applying the cellulase to the wash bath with fabric; it partially digests excess and protruding yarn fibers, loosening them from the fabric. The resulting fuzz is then removed by the high-speed mechanical agitation of the fabric. For this paper, we studied the efficiency of the cellulase enzyme in removing the pills in different wash bath temperatures.

2. Materials and Methods

2.1 Cellulase Enzyme Preparation:

The cellulase enzyme was prepared using a submerged fermentation process. *Aspergillus niger* was used as a microbial source.

2.2 Microbial Culture: The microorganism *Aspergillus niger* strain, obtained from a sugar cane breeding institute, was cultured and preserved in potato- dextrose agar slants. It was used as the inoculum source. It was grown for five days at 30^0 C, and the culture was stored at 4^0 C for subsequent inoculum preparation.

2.3 Inoculum Preparation: Aspergillus niger grown in a 250 ml Erlenmeyer flask, using a malt media containing malt extract (30g/l), yeast extract (5 g/l). Spores from PDA agar slants were inoculated in a 250 ml flask that contained 50 ml of malt medium. Flasks were incubated at 28^0 C (with a slight variation in the temperature of $\pm$ 1^0 C), and constantly shaken using a rotary shaker operating at 110 rpm. Incubation continued for 48 hours.

2.4 Cellulase Production Media: Cellulose powder medium(Fadel, 2000)(Tolan & Foody, 1999)was used for growth and mass multiplication of the fungal spores. The medium was optimized with grams per liter of the following chemicals: MgSO4. 7H2O - 0.05, K2HPO4-1.0, NaCl - 6.0; (NH4)2SO4 - 1.0; CaCl2 - 0.1; peptone – 0.5, yeast extract - 0.5; glucose - 4.0; cellulose powder - 5.0 was the main carbon source. The pH was adjusted to pH 5.0.

After 48 hours, the culture was transferred from the inoculate media to 500 ml conical flasks containing 50 ml of cellulose powder media at a rate of 5% (v/v). The culture was incubated at 25^0 C for 10 days. The culture was filtered through a 0.45micron filter and a subsequent filtration with 0.2 micron filter. The mycelium free extract was the crude Cellulase enzyme.

2.5 Determination of Cellulase Activity: Unit activity of the crude enzyme was estimated through the use of the Filter Paper Assay method. Standard method was used(Mandels, 1975). Enzymatic activity in the filtered crude lysate was assessed as follows: Whatman filter paper 1 with size 1x 4 cm weighing 40 mg was inserted into a solution made of 0.05 M sodium citrate buffer (pH 5.0). Both the filter paper and the sodium citrate solution were incubated at 50^0 C for one hour. Dinitrosalicylic acid (DNS) method was employed for measuring the released reducing sugar. One unit activity was defined as the amount of enzyme releasing 1µmole of reducing sugar from filter paper in one ml per min. Carboxy methyl Cellulase activity was measured using the reaction mixture that contains one ml of 1% carboxymethyl cellulose dissolved in 0.5 M citrate acetate buffer (pH 5.0). Diluted enzyme was added to the mixture. The solution was incubated at 50° C for one hour.

2.6 Sample Fabric Preprocessing and Dyeing: Sample fabric was knitted with 20s count yarn using Relanit 1.6 ER (Mayer & Cie, GmBH &CO) circular knitting machine. A 12 meter knitted fabric (single piece) was chosen randomly from a 60 meter roll. The fabric was taken through the preprocessing steps of scouring and bleaching were performed with following combination of chemicals: 2.50 g/l Sodium Hydroxide, 2.50 g/l Hydrogen peroxide 50 %1.00 g/l Imerol PCLF, 1.00 g/l Sirrix 2UD, 0.80 g/l Stabilizer SOF, (all materials from Clariant) at 98 ◦ c for

60 min, after hot wash, neutral the fabric. The preprocessed fabric was then dyed with reactive dye (Drimaren Black CL – S, Clariant). The process was carried out using the mini-soft TRD (Thies) soft flow dyeing machine.

2.7 *Sample size:* The dyed fabric was then cut into 12 – one feet fabric samples. Each treatment were carried out in triplicate.

2.8 Biopolishing: Two set of fabrics (in triplicate) designated as control was not subjected to cellulase enzyme treatment. Two sets of fabrics (in triplicate) were subjected to cellulase enzyme treatment. The washing of both untreated and treated fabrics was done as per the following protocol: The fabric to liquor ratio was set at 1:15, wing speed was set at 60 rpm, and pH was maintained at 5.5. One set of untreated and enzyme-washed fabrics were subjected to 45^{O} C. Another set of untreated fabric and enzyme-washed fabric was carried out at 55^{O} C. In the enzyme wash bath, cellulase enzyme was added at the concentration of 1% of owg. All of the fabrics were washed for 60 minutes. At the end of the wash, temperature was increased to 70^{O} C to inactivate the enzyme. Following the enzymatic wash, a simple wash was done for 10 minutes, to remove any loose fuzz and pilling from the fabrics.

Pilling was assessed by an experienced panel. The pilling index ranged from severe pilling at 5 and no pilling at 0(Morgado, Cavaco-Paulo, & Rousselle, 2000). All of the 12 fabrics were randomly assessed by the panel. All 12 fabrics were weighed, and the weights were recorded.

Statistical Analysis: All of the data was processed and analyzed using the JMP (SAS) software.

3. Results and Discussion

3.1 Determination of Cellulase Activity: The enzymatic activity assessed indicates that the active enzyme was at the concentration of 4.2 IU/ml.

3.2 *Pilling:* All four fabrics were assessed according to the pilling indexes. Control fabrics that were treated at temperatures of 45^{O} C and 55^{O} C did show a higher pilling index for all three replicates. This is due to the abrasion between fabrics and the dyeing vessel during the dyeing process. As the fabric was knitted with 20s count carded yarn, increased pilling is generally observed after the mechanical action(Siddiqui, Azhar, Rashid, & Rajoka, 1996). In this instance, dyeing could have slightly decreased the carded yarn's pilling tendency.

In comparing fabrics that were subjected to the enzyme treatment with untreated fabrics, significant reduction in pilling was observed in the enzyme-treated fabrics. Irrespective of variations in temperature, pilling rate was no different between the two different treatment temperatures. Cellulase significantly reduced the pilling, through the action of enzymatic cleavage of the crystalline region. Temperature variations neither enhanced nor prevented the active sites' activation throughout the duration of the wash cycle. The figure 2 and 4 shows that the efficiency rate for pill removal was higher when the fabric was treated with the cellulase enzyme, compared to the untreated control. The ANOVA indicates that the mean pilling index is significantly different between the untreated and enzyme treated fabric.

Emily Fischer previously studied enzyme kinetics, and coined the term *lock and key*, which well characterized the mode of action of the cellulase on the substrate cellulose. As proposed by Medell and Reese, the enzymatic digestion of the substrate, especially cellulose, does not have a rate constant typical of any other enzyme kinetics. However, the hydrolysis itself will form a bell-shaped curve if the pH of the medium varies widely from acidic to alkaline pH(Saddler et al., 2001). During the mode of action, cellulase catalysis is a complex and

multistep process until the cellulose is broken down to glucose residues.

The enzyme washed fabrics showed a considerable reduction in pilling when compared to untreated fabrics. A comparison of the two fabrics that were biopolished with cellulase enzyme at two different temperatures (45^{O} C and 55^{O} C) did not show any significant difference in the pilling index (Figure 6). Both fabrics felt softer. The complex process, involves subsequent and synchronized action of all three enzyme complexes, to achieve the degradation and breakdown of cellulose fibers. This is highly characterized, and earlier studies were conducted at a particular temperature. The mode of action of cellulase is more complex, and the operating environment for enzymes determines the outcome. Enzymatic hydrolysis starts with endoglucanases (EC3.2.1.4). The endoglucanases swell the cellulose fibers, which was identified as critical to the starting point for the enzymatic activity. During the pre-process stage, the scouring process had caused the cotton to become permeable. However, the endoglucanases caused a slight polymerization of the cellulose, since the easily accessible and hydrated region is hydrolyzed, leaving the exposed inner bonds(Macarron et al., 1993).

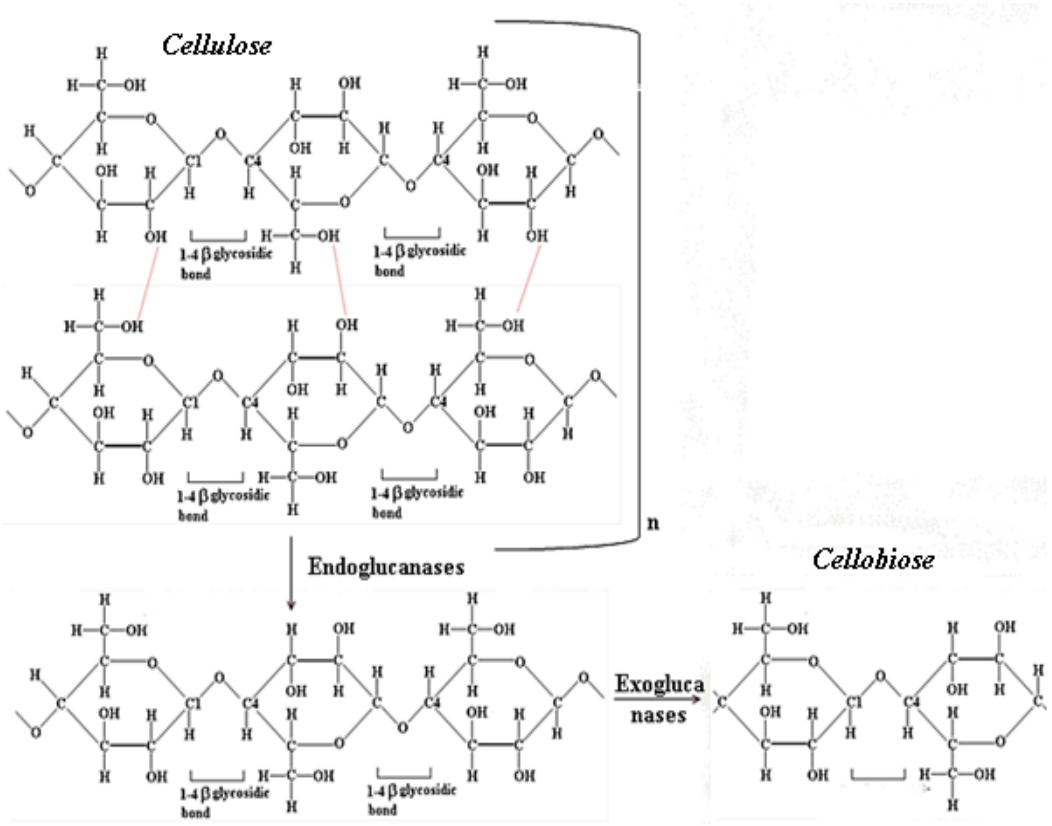

Figure 1. Chemical Action of Cellulase Enzyme in Pill Removal

Endoglucanases facilitate the synergistic action, which is followed by exoglucanases (EC 3.2.1.94). Exoglucanase is a processive enzyme that attacks the crystalline base of the cellulose polysaccharides, releasing the substrate for the β-glycosidase (EC 3.2.1.21). This particular enzyme initiates its action from the end of the polysaccharide chain. This synergistic action of the two enzymes in the cellulase complex may vary with acidic to alkaline pH. Optimizing the washing

condition is critical to achieving the desired outcome(Pedersen & Screws, 1998).

Previous studies have focused on the effects of temperature on the production of the enzyme by several stains of fungi. For processing the fabric with the highest degree of precision, the temperature within the dye vessel is important. Several processes, especially coloring process is highly dependent on the temperature as it can vary the outcome of the shade. For removing pilling and increasing the aesthetic value of the fabric, the action of both the endoglucanase and the exoglucanase is more critical than the β-glycosidase(White & Brown, 1981). The removal of the fuzz needs only the cohesive action of the two glucanases, as shown in Figure 1. Partially digested fuzz will then be destroyed, through the agitation of the dye winch.

Operating temperatures have an effect on the stability of the enzyme, and as well have an impact on the effectiveness of the enzymatic activity. The enzymatic inactivation starts to occur at a particular temperature, given the natural variations in the maintaining the wash bath temperature, the three dimensional structure of the enzyme cellulase can be disrupted at extreme temperatures. The cellulose binding domain (CBD) of the endoglucanase plays a vital role in initiating the hydrolysis. Hydrolysis, which is a result of synergistic action of the cellulase complex, requires an optimal equilibrium in the operating environment. Kinetics of CBD and the catalytic domain of endo and exoglucanase depend on the several factors in the wash bath. A drastic variation in the operating environment could potentially alter the binding, releasing and subsequent movement of the domains from one chain to another hydrolyzing the crystalline region. As previous studies indicated, the temperature dependent activities of the CBD and Catalytic domains are necessary for effective cellulose hydrolysis(Linder & Teeri, 1996). However, considering the scale of the fabric processing maintaining a uniform bath temperature throughout the process is a challenge, and hence optimizing the wash temperature is mandatory for adopting the bioprocessing successfully. Our study indicates the efficiency of the cellulase in hydrolyzing the lose fibers were higher at wash bath temperature of 45^0C to 55^0C compared to the untreated fabric.

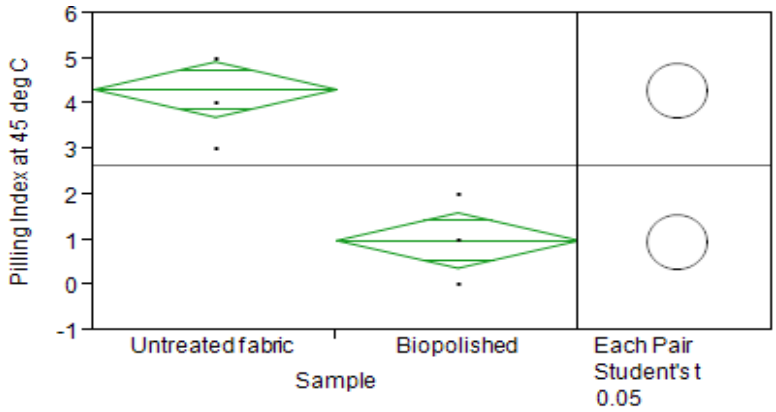

Figure 2. Pilling index at 45^O C indicates that the efficiency of pill removal was significantly greater than it was for untreated fabric. (P value < alpha 0.5)

ANOVA Table (45^O C)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Sample	1	50	50	66.6667	<.0001
Error	16	12	0.75		
C.Total	17	62			

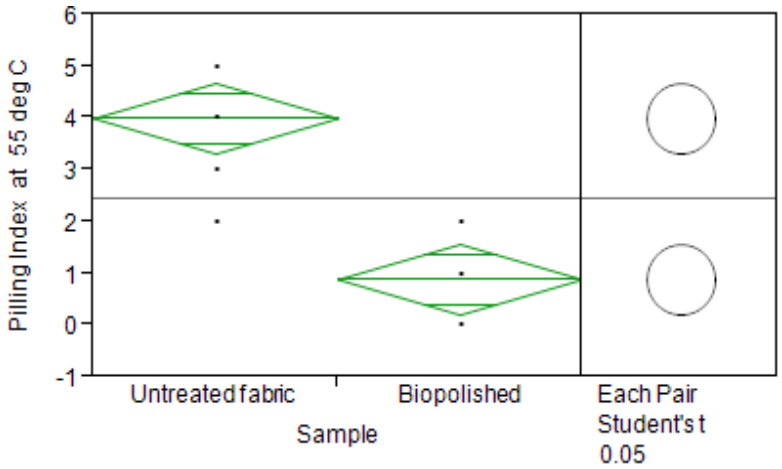

Figure 3. Pilling index at 55^{O} C indicates that the efficiency of pill removal was significantly greater than it was for the untreated fabric. (P value < alpha 0.5)

ANOVA Table (55^{O} C)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Sample	1	43.556	43.556	46.806	<.0001
Error	16	14.889	0.931		
C.Total	17	58.444			

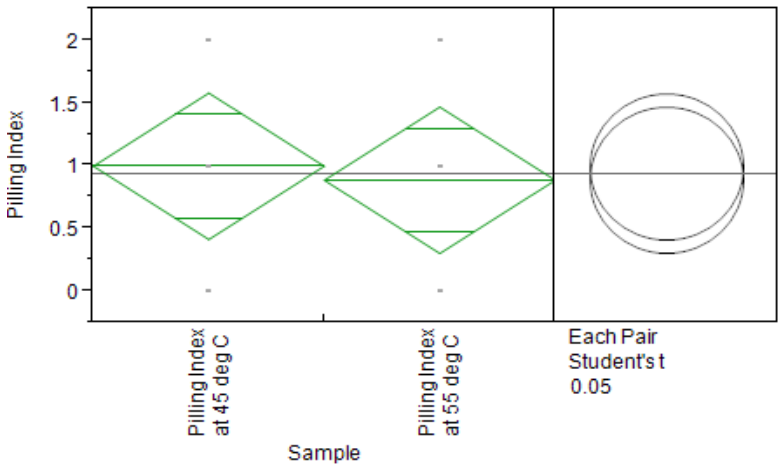

Figure 4. Comparing cellulose wash bath efficiency in regard to pill removal at temperatures of 45^{O} C and 55^{O} C, there is no statistical difference between the two temperatures

ANOVA Table (45^{O} C vs. 55^{O} C)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Sample	1	0.056	0.056	0.082	0.779
Error	16	10.889	0.681		
C.Total	17	10.944			

3.3 Enzymatic Effect on Fabric Weight: The fabric was weighed as soon as the enzymatic process completed and the fabric was dried. All twelve fabrics were weighed. Both of the untreated control fabrics from temperature 45^{O} C and 55^{O} C did not show significant weight loss. Both biopolished fabrics had lower weights compared to the pre-enzyme wash, and the controls. The weight loss can be attributed to the biopolishing effects, which remove fuzz and substantially reduce pilling. However, this study showed that the weight loss did not change, regardless of wash temperature. Optimum temperature is required for the enzyme substrate interaction(Csiszár et al., 2001). Also, the duration of the wash plays an important role in controlling the extent to which the enzyme catalyzes the cellulose hydrolysis(Ying, 1999). In any given dyeing process, fluctuation in the wash temperature is the norm. It is essential to understand the temperature variation's effect on strength and weight loss during the enzymatic process. The weight loss after the fabric biopolishing was greater, compared to the untreated fabric. The significant difference between the enzyme-washed and the untreated fabric shows that the cellulase degraded the cellulose, especially; the loose fibers were readily acted upon.

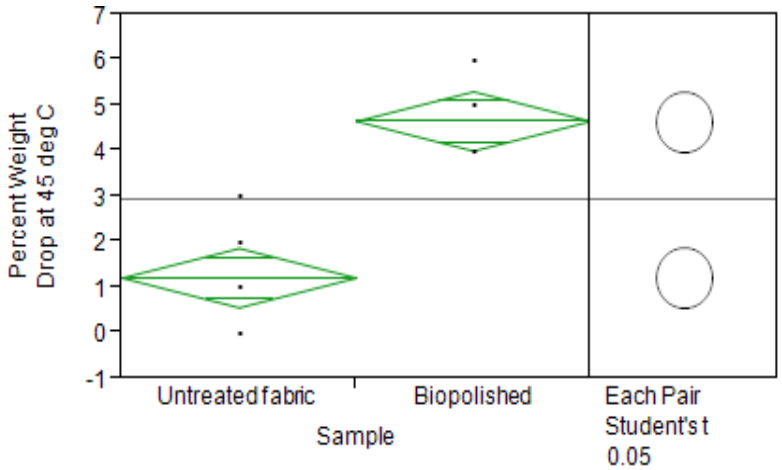

Figure 5. Percentage of weight reduction compared to pretreatment at 45^{O} C: Statistical analysis at alpha 0.5 indicates that weight reduction for biopolished fabric was greater, compared to the untreated fabric. (P value < alpha 0.5)

ANOVA Table

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Sample	1	53.389	53.389	63.016	<.0001
Error	16	13.556	0.847		
C.Total	17	66.944			

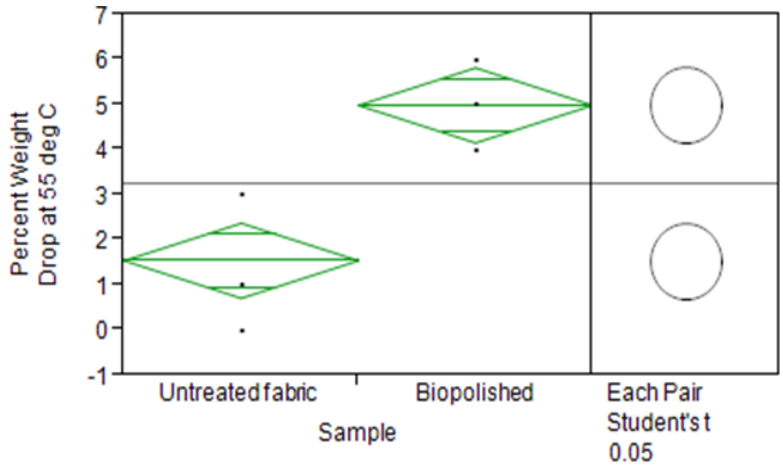

Figure 6. Percentage of weight reduction compared to pretreatment 55^{O} C: Statistical analysis at alpha 0.5 indicates that weight reduction for biopolished fabric was greater, compared to the untreated fabric. (P value < alpha 0.5)

ANOVA Table

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Sample	1	53.389	53.389	38.44	<.0001
Error	16	22.222	1.389		
C.Total	17	75.611			

4. Conclusions

Based on the study, it is concluded that the cellulase enzyme significantly reduces pilling and increases the aesthetic value of knitted garments. It is also evident that differences in wash bath temperature in the range of 45^{0}C to 55^{0}C do not affect the efficiency of the biopolishing. In addition, variations in temperature have no effect on the weight of fabrics treated. However, duration and enzyme concentration could be a significant factor in achieving pill-free
fabric. Having observed no difference in enzyme efficiency in relation to temperature range can be a basis for further study of conducting the enzyme wash in single-pot processing comprised of dyeing and washing. This could make biopolishing more economical for the fabric processing industry.

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