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Microbial degumming of silk yarn

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Abstract
Silk is the queen of fibers. Degumming with alkaline pH, and high temperature causes degradation of silk fiber. Proteolytic enzyme works under milder condition and cause less damage to the fiber. Various mediums were being used for degumming of silk. Out of which minimal medium was found to be the most effective one with 28% weight loss while conventional methods shows only 24% weight loss. Two isolates *Bacillus subtilis* and *penicillium citrinum* out of four microbes were opted for enzyme production. The efficiency of the enzyme was studied in terms of weight loss, whiteness index, dyed and assessed for color value, texture, feel and luster.

Keywords: Degumming, Minimal Medium, Silk, *Bacillus subtilis* and *penicillium citrinum*

1. Introduction
Degumming is the process of cleavage of peptide bonds of sericin either by hydrolytic or enzymatic methods and its subsequent removal from silk fibroin (Trotman, 1984) [12]. Degumming is a process where sericin is totally removed from the fibroin wall to obtain shine, smoothness and other properties (Freddi *et al.,* 2003) [3]. Raw silk fibers have been traditionally degummed with soap-soda ash (Kato, 1968) [10]. Sericin can also be removed during the degumming process using different process using different types of agents or method such as alkali, acid, foam, synthetic detergents, high pressure, microwave and enzyme (Karmakar, 1999) [9]. Several alkali agents such as sodium hydroxide or sodium carbonate are commonly used for the silk degumming process. However, these alkali treatments impose a relatively harsh irritation on silk fibroin fibers (Yamada *et al.,* 2001; Jiang *et al.,* 2006) [13, 8]. Degumming of silk is traditionally carried out with soap or alkali which was not uniform in quality, the strength loss is high and also the chemicals used cause environmental pollution. It is therefore, desirable to replace them by the ecofriendly alternatives and in this respect, the enzyme play a key role. Enzymatic degumming involves proteolytic degradation of sericin using specific protease which does not attack fibroin (Ninge *et al.,* 2007) [11].
Protease is a preferable degumming agent in terms of complete and uniform removal of sericin, retention of tensile properties, and improvement of surface smoothness, handle, and luster of silk fibers (Gulrajani *et al.,* 1998; Gulrajani *et al.,* 2000a & Gulrajani *et al.,* 2000b) [7, 5, 6]. The enzyme degumming process is effective, economic and eco-friendly. Enzymatic treatment of silk fiber as an alternative of conventional process is now in focus. Alkaline proteases perform better than other proteases (acid and neutral) with respect to uniform sericin removal and improvement of silk quality (Chopra *et al.,* 1996) [1]. Inspite of lower performance and higher cost of enzyme compared to chemical, enzymatic treatment attract the attention of scientists and technologists for the ecofriendly aspect of the process (Duran and Duran, 2000; Gubitz and Cavaco-Paulo, 2001) [2, 4]. Enzymatic degumming process would save the resources in terms of water, energy, and chemicals.

2. Materials and Methods
2.1. Material
2.1.1. Selection of yarn
Raw Mulberry silk yarn of 21-25 denier was obtained from Central Silk Board, Varanasi.

2.1.2. Procurement of Protease producing microbe
Microbial cultures were procured from Division of Plant Pathology, IARI i.e. *Penicillium*

citrinum, Bacillus subtilis, Aspergillus niger and *Aspergillus flavus*

2.2. Methods

2.2.1. Conventional degumming

Soap 10g/l and sodium carbonate 2g/l pH-9.5 was used for degumming of silk yarns.

2.2.2 Cultivation of Fungi and Bacteria for Protease Production

Fungi and Bacteria sourced from IARI, were inoculated respectively on PDA and NA slants and were incubated for 3-4 days at 28 ± 2°C for fungi and 1 -3 days at 37 ± 2°C. These were then inoculated on Minimal medium (MM), Potato dextrose broth (for fungi) and Nutrient broth medium (for bacteria) to look for protease production (Table 2.1) using casein agar plates.

Table 2.1: Fermentation conditions used for the growth of Microbes

S.No	Microbes	Temperature	Media	Conditions
1.	*Penicilliun citrinum*	28°C	PD & MM Broth	Static
2.	*Aspergillus niger*	28°C	PD & MM Broth	Static
3.	*Aspergillus flavus*	28°C	PD & MM Broth	Static
4.	*Bacillus subtilis*	37°C	NA& MM Broth	Static

2.2.3 Preparation of culture media

Different broth media (Table 2.2) were prepared and inoculated by the selected microbes to test for the protease production and degumming efficacy.

Table 2.2: Composition of Culture Media

Media		
Medium 1	**Medium 2**	**Medium 3**
Minimal medium	Potato dextrose broth	Nutrient broth
Glucose – 0.5%	Potato dextrose – 250 ml	Peptone- 5g/l
Peptone – 0.5%	Casein – 10 g/l	Beef extract – 3 g/l
$MgSO_4.7H_2O$ – 0.5%	Peptone – 10g/l	Distilled water-1000ml
$K\ H_2PO_4$ – 0.5%	Distilled water-1000ml	pH -7
$FeSO_4\ .7H_2O$ – 0.001 %	pH – 6.5	
pH – 7.5 (bacteria) 6 (fungus)		

2.2.4 Preparation of Casein agar

Casein agar plates (Casein – 1% and Agar – 2%) were commonly used for the initial screening of proteolytic activity. The clear zone of casein agar hydrolysis was an indication of protease production; the isolates were selected on the basis of large zone of clearance. Out of all the media tested, only Minimal medium gave best results and was used for further study.

2.2.5 Procedure for Degumming

Fermented Broth was centrifuged and the cell- free supernatants were used for the degumming of raw silk yarn (Table 2.3). It was carried out in orbital shaker at 50°C for 2 hrs at pH 9. After degumming, the silk yarn was washed with hot water. Deactivation of enzyme was done by boiling the broth and the sample were then washed with hot and cold water. After this, all the samples were dried and the weight loss was taken.

Table 2.3: Standardized recipes for degumming

Recipe	Weight of yarn	Time	pH	Temperature
Culture filtrate– 50 ml	0.50 grams	2 hrs	9	50 °C± 2 °C

As zone of proteolysis on casein agar plates was maximum with the Minimal Medium broth, it was used for further studies.

2.3.6. Weight loss

Weight of yarn – 0.5grams

Weight loss was calculated by

$$\text{Wt loss \%} = \frac{(\text{Initial weight-final weight}) \times 100}{\text{Initial weight}}$$

2.3.7. Dyeing of sample

Samples of silk fibers obtained after degumming processes were dyed using acid Magenta (C.I Number- A. Red 186)

3. Results and Discussions

3.1 Conventional method of degumming

Various chemical methods tested, out of all soap and sodium carbonate was found to be most effective degumming agent resulting in 24% weight loss. Control sample was being made by degumming with soap and sodium carbonate and its Whiteness index and K/S recorded.

Table 3.1: Percentage Weight loss in Mulberry silk yarn after chemical degumming

Treatments	Liquid Ratio	Temp.	Time	Weight After Degumming (Grams)	% Weight Loss
Soap – 10 g/l Sodium carbonate – 2 g/l pH – 9.5	1:30	95°C	20 min	0.38	24

Table 3.2: Whiteness index, K/S and L*, a*, b* values of Mulberry silk yarn after chemical degumming.

Treatments	Whiteness Index	K/S	L*	a*	b*
Raw sample	30.016	13.50	43.332	56.86	-8.634
Soap – 10 g/l Sodium carbonate – 2 g/l pH – 9.5	82.859	38.72	40.95	60.70	-8.77

On comparing whiteness index and K/S value of Mulberry silk yarn degummed with various chemical methods, maximum whiteness Index (82.85) was found to be of yarns degummed with soap and sodium carbonate, indicating it to be an effective degumming method. This is because of hydrolysis of peptide bonds, breakage of Vander Waal forces and salt linkages present in sericin macromolecules. This implies a better dye absorption. The K/S value of soap and sodium carbonate degummed sample was 38.82 indicating a good color Intensity when compared to raw sample i.e. 13.23 (Table 3.2 & 3.3)

Table 3.3: Rating of Texture, luster, feel values of Mulberry silk yarn after chemical degumming.

Treatments	Texture	Luster	Feel
Raw sample	1	1	1
Soap – 10 g/l Sodium carbonate – 2 g/l pH – 9.5	4	4	4

The luster, texture and feel of control sample was good, while raw sample shows poor luster, texture and feel as can be seen in Table 3.3. This is because raw sample contain a layer of sericin.

3.2 Optimization of broth and fermentation condition for degumming.

In this phase, four protease producing microbes from Division of Plant Pathology, IARI i.e. *Penicillium citrinum, Aspergillus niger, Aspergillus flavus and Bacillus subtilis* were cultured on different media to observe their protease production efficiency.

After 7 days of incubation, the cultures were filtered out and tested for protease production on casein agar plates. The production of zone of clearance around the wells containing these culture filtrates indicated extracellular protease production and hence can be easily distinguished from the non – proteolytic ones. Zone of clearance was observed in all the casein agar plates though maximum size was observed with the culture filtrate obtained from Minimal medium.

On comparing protease activity of different microbes, it was found that *Bacillus subtilis* and *Penicillium citrinum* produced a larger zone of clearance while *Aspergillus niger* and *Aspergillus flavus* produces small zones. So, for further study isolates- *Bacillus subtilis* and *Penicillium citrinum* -forming larger zone were selected.

3.3 Testing different kinds of broth for degumming

This step was progressed sequentially experimenting with one medium after another. These are as follows:-

3.3.1 Minimal medium

Fermented broths were centrifuged and then cell- free supernatants were used for the degumming of raw silk yarn. It was carried out in orbital shaker at 50°C for 2 hrs at pH 9 followed by washing and drying of the yarn. Percent weight loss was taken to find out the extent of degumming.

Table 3.4: Percentage Weight loss in Mulberry silk yarn after Minimal medium degumming.

S.No	Microbes	Liquid Ratio	Temp.	Time	Weight After Degumming (Grams)	% Weight Loss
1.	*Bacillus subtilis*	50ml	50°C	2 hr	0.36	28
2.	*Penicillium citrinum*	50ml	50°C	2 hr	0.37	26
3.	*Aspergillus niger*	50ml	50°C	2 hr	0.43	14
4.	*Aspergillus flavus*	50ml	50°C	2 hr	0.44	12

Degumming with *Bacillus subtilis* and *Penicillium citrinum* culture filtrate gave better weight loss of 28% and 26 % respectively (Table 3.4 and Fig. 3.1) as compared to weight loss observed with culture filtrate of other two microbes (A. *flavus*- 12 % and A. *niger*- 14 %). When compared with control i.e. percent weight loss with chemical degumming (24%) *Bacillus subtilis* and *Penicillium citrinum* culture filtrate produced more weight loss (Fig. 3.1).

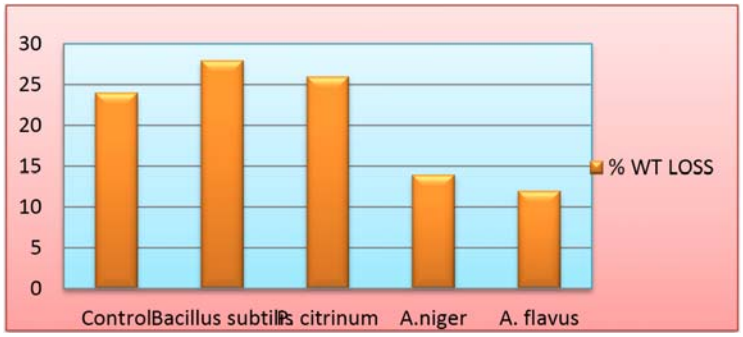

Fig 3.1: Percentage weight loss in Mulberry silk yarn after Minimal medium degumming.

Table 3.5: Whiteness index and K/S, L*, a*, b* values of Mulberry silk yarn after Minimal medium degumming.

	Whiteness Index	K/S	L*	a*	b*
Control	82.859	37.616	29.926	56.065	-3.422
Bacillus subtilis	84.721	49.561	22.049	37.696	2.947
P. citrinum	84.68	40.226	21.443	37.81	2.566
A.niger	74.225	38.500	32.360	57.995	2.257
A. flavus	81.563	39.134	21.87	38.134	3.166

Bacillus subtilis showed highest whiteness index i.e. 84.859 followed by *Penicillium citrinum* i.e. 84.68. The whiteness index of proteolytic extract of these two microbes was higher than that of chemical degumming (Table 3.5, Fig. 3.2).

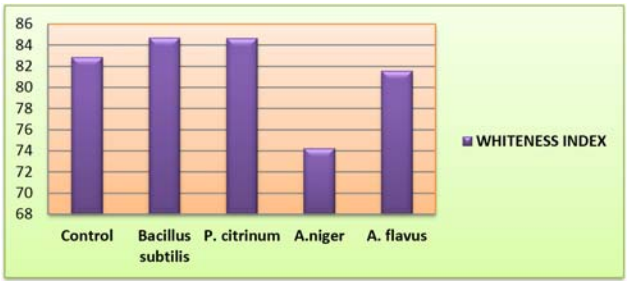

Fig 3.2: Whiteness index values of Mulberry silk yarn after Minimal medium degumming.

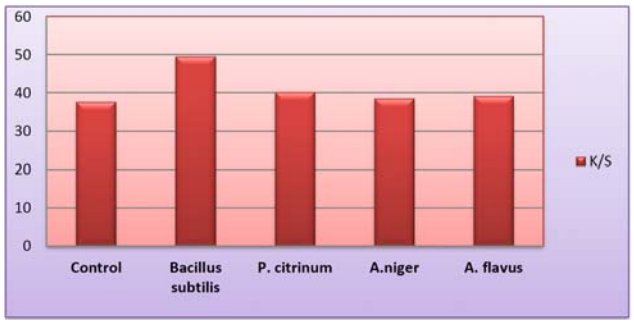

Fig 3.3: K/S values of Mulberry silk yarn after Minimal medium degumming

It was found that *Bacillus subtilis* has higher K/S value than control, hence Bacillus had higher color depth than control (Fig. 3.3). *Penicillium citrinum* also showed good K/S value than all other samples tested. The a* and b* values of *Bacillus subtilis* and *Penicillium citrinum* samples were positive, thus indicating magenta component whereas b* value of control is negative, thus indicating blue component (Table 3.5). The L* value of *A.niger* is highest (32.36), hence it is lightest in shade whereas the L* value of *Penicillium citrinum* is lowest (21.443), thus, it is deepest in shade as shown in Table 3.5.

Table 3. 6: Rating of Texture, Feel and Luster values of Mulberry silk yarn after Minimal medium degumming.

Enzyme	Texture	Feel	Luster
Control	4	4	5
Bacillus subtilis	5	5	5
P. citrinum	5	5	5
A.niger	4	4	4
A. flavus	4	4	4

The luster, texture and feel of *Bacillus subtilis* and *P.citrinum* culture filtrate treated samples was excellent, while control shows excellent luster and very good texture and feel as can be seen in Table 3.6. This is because proteolytic treatment had milder action on fibroin while alkaline soap medium has damaging effect.

3.3.2 Degumming with all media other than minimal medium (Medium 2 and 3)

Either no or very little degumming of silk yarn was taken place with all these media. So no whiteness index and color value was assessed.

4. Conclusion

The results of the study revealed that microbes could be exploited for producing proteolytic enzymes. These enzymes can be used for degumming the silk fibers to make the process more eco-friendly. Enzymatically degummed samples were found to be superior in terms of whiteness, dye uptake, luster, feel, softness and percent weight loss as compared to conventionally degummed samples. This is because proteolytic enzymes work under milder conditions.

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