A PRATICAL METHOD FOR DETERMINING OF CELLULASE ACTIVITY IN TEXTILE INDUSTRY

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ABSTRACT

Enzymes are increasingly being used in industries for a wide variety of products in an attempt to create a “cleaner” technology and decrease the use of harsh chemicals which are not appropriate regarding environmental aspects. Cellulases are the most successful enzymes used for the textile processing. It is well known that with cellulases cotton, jute, linen, ramie and other cellulose based fiber can be hydrolyzed and as a result, a clean polished surface can be obtained. Moreover, cellulases are also in use denim washing for worn effect and stone wash. It is possible to expand its usages. On the other hand, the activity of these enzymes is not controlled properly during the use in textile mills. Although a lot of measuring methods are available for determination of the enzyme activity, all these methods are not in the same sensitivity and the usage of them is limited owing to their sophisticated procedure and instruments. So these sophisticated methods are generally used in medical science, biochemistry and food industries. However in textile mills the activation of enzymes is not measured due to the lack of a practical test method. In this study a method, useful and easy to apply in textile industry, is tried to present.

INTRODUCTION

Enzymes are increasingly being used in process industries for a wide variety of products in an attempt to create a “cleaner” technology and decrease the use of harsh chemicals that are not environment friendly (1).

Utilization of highly specific enzymes for various textile-processing applications is becoming increasingly popular because of their ability to replace harsh organic/inorganic chemicals currently used by the textile industry. Pollution associated with the textiles industry has traditionally been related to the acids used in desizing of cloths, bleaching chemicals, and the production of dyes. Cellulolytic enzymes have merged quite recently with potential applications in processing of cotton and other cellulosic fabrics. Cellulase enzymes are used for biostoning and fading of jeans, finishing of cotton and cotton-blended fabrics for improved softness and enzymatic removal of fuzz and pills. Some enzymes are very successful textile finishes. However, very little is known about the various processing mechanisms. This is mainly due to the lack of biochemical knowledge of textile chemists and lack of understanding of textile process by biochemists and biotechnologists (1, 2, and 3).

In nature cellulose is degraded by both fungi and bacteria. These organisms produce cellulases that specifically degrade cellulose, yielding shorter chain cellulose

polymers and glucose which are metabolised by these organisms. Typically, the fungal and some bacterial cellulolytic enzyme systems consist of several enzymes acting at the ends (exoglucanases, also called cellobiohydrolases) or in the middle (endoglucanases) of the cellulose chains. Trichoderma reesei is one of the most important industrially used strains for cellulase production. According to current knowledge, its cellulolytic system is composed of two cellobiohydrolases (CBHI and CBHII) and at least six endoglucanases (EGI, EGII, EGIII, EGIV, EGV and EGVI) and two β-glucosidases. The sixth endoglucanase, named EGVI, has been described on the protein level (4).

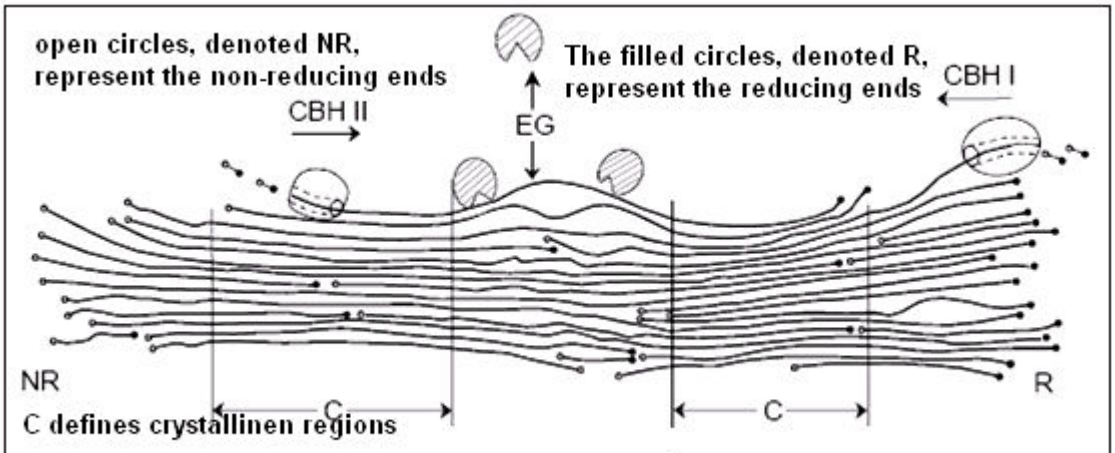


Figure 1: Mechanism for enzymatic hydrolysis of cellulose (4)

The endoglucanases mainly hydrolyze internal bonds in the cellulose polymer, producing new chain ends and thereby causing a considerable decrease in cellulose DP. Exoglucanases initiate the hydrolysis at the chain ends, and do not produce significant amounts of new chain ends on the cellulose surface. CBHII splits cellobiose from the non-reducing and CBH I from the reducing ends of cellulose chains. Cellobiohydrolases can also act on crystalline cellulose without the aid of endoglucanases. β-glucosidases complete the hydrolysis process by catalyzing the hydrolysis of cellobiose to glucose (Figure 1). Efficient overall hydrolysis of crystalline cellulose by cellulases requires the synergistic action of both EGs and CBHs, as reviewed by Teeri and Koivula. Maximum synergism is usually obtained with a large amount of exo-enzyme and a minor amount of endo-enzyme. It is also known that the degree of synergy is dependent on the substrate used (4).

MATERIAL AND METHOD

The procedure for determining enzyme activity is shown in Figure 2. For these studies substrates are viscose and cotton instead of avicel (5). Suprem viscose and cotton knitted fabrics with 153 g/m^2 and 141.5 g/m^2 weight respectively are dyed with Remazol Brilliant Blue R spec. (C.I.Reactive Blue 19) according to Figure 3. In measuring activation of four commercial cellulase enzymes with spectrophotometric method, substrates dyed with RRBR spec. are affected by enzymes (Figure 4). By enzymatic hydrolysis, the dyestuff is set free and at the end of the processes, the absorbance (at 595 nm) of the bath is measured by spectrophotometer (Shimadzu UV-1201). With the aid of the absorbance-concentration graphic (Figure 5) which has

done before, the amount of dyestuff in *mg* is calculated. According to the amount of dyestuff, the activation of the cellulase enzyme is evaluated.

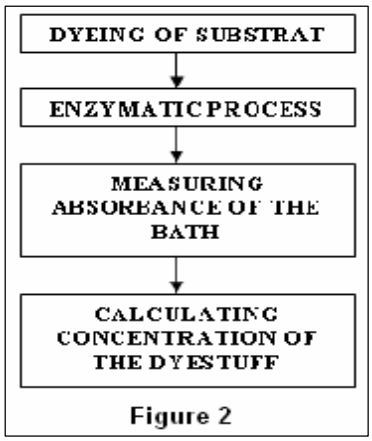
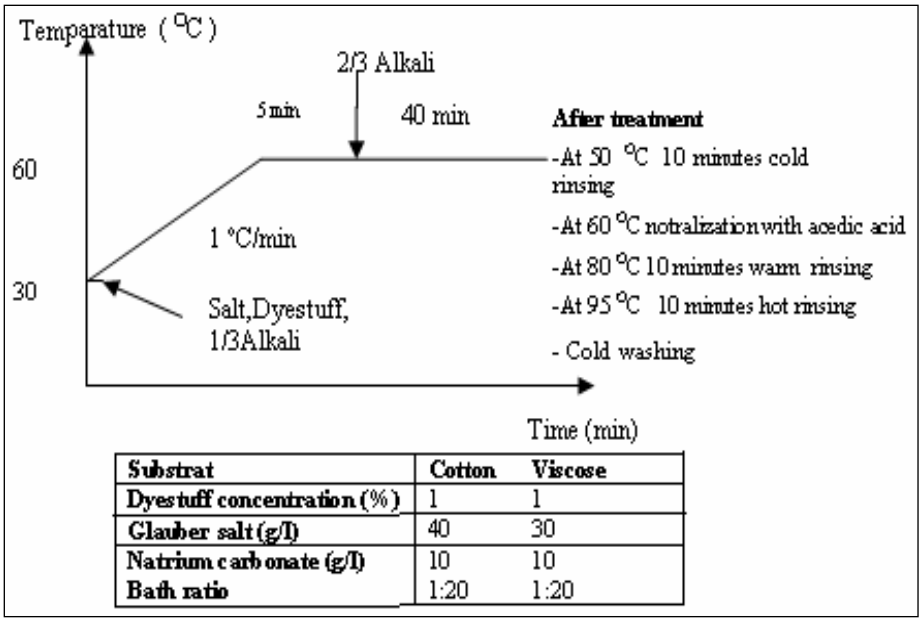


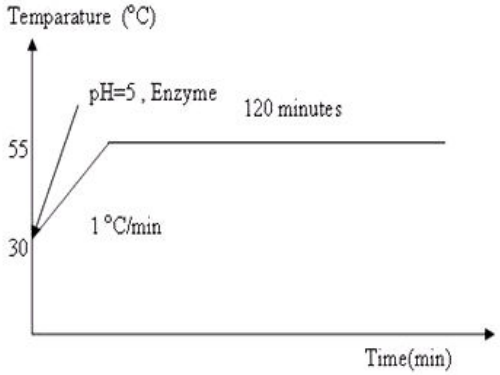
Figure 2



Substrat	Cotton	Viscose
Dyestuff concentration (%)	1	1
Glauber salt (g/l)	40	30
Natrium carbonate (g/l)	10	10
Bath ratio	1:20	1:20

Figure 3: Dyeing Method

Unit description: % 1 RBBR-viscose (-cotton) fabric is treated with 3 ml/l enzymes in two hours. 1 mg dyestuff released into the 1 liter of bath equals to 1 RBBR – viscose unit. If the same processes apply to cotton fabric it means 1 RBBR-cotton unit.



Fabric Weight	4 gr viscose or cotton fabric
Enzyme (four different commercial cellulose enzyme)	3 ml/l
pH	5
Bath ratio	1:30

Figure 4: Standard enzymatic process

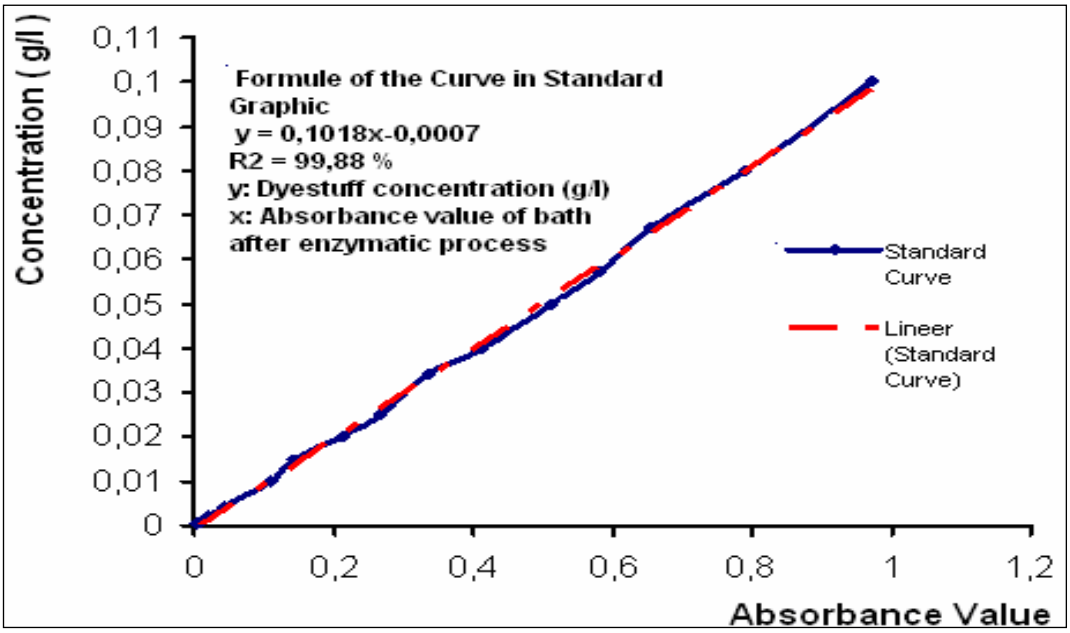


Figure 5: Standard Concentration-Absorbance Graphics

RESULTS AND DISCUSSION

All the enzymes contain about equal amount of protein (0.5 mg/ml) and behave towards cotton and viscose in a same way and order. But activation values are different for cotton and viscose fabrics. Generally enzymes are very effective to viscose except enzyme C. Enzyme C is less effective to cotton than others. Cotton and viscose fabrics are based of cellulose. But cellulases affect them in different way because of different structures of enzymes and celluloses.

Enzyme D > Enzyme B > Enzyme A > Enzyme C (for cotton fabric)
Enzyme D > Enzyme B > Enzyme A > Enzyme C (for viscose fabric)

Table 1. Activities of Enzymes

	SUBSTRATE					
	Viscose			Cotton		
Enzyme	Absorbance Value (595 nm)	Conc. (mg/l)	Activity (RBBR-viscose unit)	Absorbance Value (595 nm)	Conc. (mg/l)	Activity (RBBR-cotton unit)
A	0.128	12.3304	***12.33***	0.062	5.6116	***5.61***
B	0.135	13.043	***13.04***	0.073	6.7314	***6.73***
C	0.021	1.4378	***1.44***	0.037	3.0666	***3.07***
D	0.139	13.4502	***13.45***	0.086	8.0548	***8.05***

CONCLUSION

Nowadays usage of enzymes in textile finishing are very common because of ecological But enzymes are different from conventional chemicals like caustic etc. Enzyme concentration isn't enough to evaluate power of enzyme. Because each of enzymes effects certain substrate, not all chemicals and activation values of enzymes define power of enzymes. It is known that substrates are very important to determine activation of enzymes. Celullose is a substrate for cellulases. But type of cellulose is very important. For example, CMC is a substrate for cellulases. Unfortunately, it doesn't represent cellulose of fibres because of solubility. Some of the methods are difficult or unpractical for textile factories. On the other hand, cotton and viscose are certainly substrate in finishing mill. So the method which is defined above is very practical, useful and exact for textile finishing.

The most important point is that cellulases are more effective to viscose than cotton. This comparison is difficult in case of standart substrates like CMC and avicel.

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