1026-1265/2002

Effect of Surfactants on Enzymatic Hydrolysis of Cellulosic Fabric

Nahid Hemmatinejad[1], Farzaneh Vahabzadeh[2] and Sohaila S. Kordestani[3]
(1) Department of Textile Engineering; (2) Department of Chemical Engineering;
(3) Department of Medical Engineering, Amir Kabir University of Technology
P.O.Box: 15875-4413 Tehran, I.R. Iran.

Received 17 September 2001; Accepted 24 May 2002

ABSTRACT

The effect(s) of four different types of surfactants on the enzymatic hydrolysis of cellulosic fabric was studied. Amount of reducing sugar produced during the reaction was used as a measure of extent of the hydrolysis. The non-ionic surfactant increased the rate of the reaction by approximately 12% while in the presence of amphoteric, anionic and cationic surfactants the rate of the hydrolysis decreased by 36, 38, and 50%, respectively. The physical properties, such as tensile strength and crease recovery (angle) of the cellulosic fabrics were measured. In the presence of non-ionic surfactant, the tensile strength loss for cellulase treated fabric was increased by 17%. The loss of tensile strength did not change using cationic surfactant while in the presence of amphoteric and anionic surfactants, the strength loss decreased rather insignificantly. All the results obtained from measuring the physical properties of the enzymatic treatment of the cellulosic fabric were in agreement with those from reducing sugar measurments. Moreover, the Michaelis-Menten equation, which has been used in the previous work of this group, was also used in the present study and the maximal velocity, V'_{max}, and the half-saturation constant, K'_m, both were calculated. In order to measure the catalytic specificity of the cellulase on this hydrolytic reaction on the tested fabrics, the ratio of V'_{max}/K'_m, was determined. The catalytic specificity for the fabrics treated with non-ionic surfactant was increased, although this ratio for the fabrics treated with amphoteric, cationic, and anionic surfactants decreased. The performance of these surfactants in relation to this enzymatic reaction was discussed.

Iranian Polymer Journal, 11 (5), 2002, 333-338

Key Words:
cellulase,
enzymatic hydrolysis,
reducing sugar release,
cellulosic fabric,
surfactant.

INTRODUCTION

In recent years trends of interests over the use of enzymes have been increased considerably [1]. In textile industry, producing cotton fabric with soft, smooth surfaces and brightened colours are examples of the successful application of biotechnology in the area of fabric's research [2]. In the practical use of the cellulase treatment of cotton fabrics still there are some problems unsolved such as: the presence of dyes and auxiliaries like surfactants in the reaction mixture of the cotton

(∗)To whom correspondence should be addressed.
E-mail: hemmati@cic.aku.ac.ir

fabrics and their effect(s) on the fabrics substrates [2].

Cellulase as a complex enzyme system is composed of at least three major components which work together uniformly to carry out the enzymatic hydrolysis of cellulose, which is known as the heterogeneous reaction between solid and liquid phase, where the enzyme converts the solid cellulose into the soluble sugars [3].

Extensive works have been done on the subject of cellulase action on cellulose and it is known that the catalytic reaction rate of cellulase is affected by several factors such as pH, temperature, presence of the chemicals along with the substrate [2].

Reproducibility of the cellulase treatment is relatively poor as compared to that of the other chemical and mechanical methods used in the textile processes [2]. This has been considered as one of the biggest disadvantages of the enzymatic treatment, although the use of the enzyme(s) in these processes has some advantages from the viewpoint of energy conservation, pollution control and safety regulations over the environments [2].

Surfactants as the typical auxiliaries are extensively used in the textile industry [4]. Surfactants are among known chemical protein denaturants [5]. Target site of these compounds on the protein structure is hydrophobic domains and/or charged groups of the proteins. Formation of partially unfolded substructures including micelle-like regions are considered as the driving force for this type of denaturation process [5]. Studies on the action of surfactants on cellulase reported in literature show the importance and special character(s) of surfactants on this popular enzyme [5].

Moreover, based on the work reported by Woodward et al., cellulases turn over substrate so slowly that it is not always possible for the researchers to select infinitely small enzyme concentration to hydrolyze cellulose [3]. In fact expressing the reaction rate, as the function of substrate concentration could be a very artificial convention when the reaction takes place on namely hydrated solid substrate, where it is not possible to change the concentration of substrate site, such as fabric [6,7]. In many of these types of experiments, it cannot be assumed that use of Michaelis-Menten kinetics is a proper and an applicable method [7].

Therefore, an alternative approach has been suggested by Woodward et al.[3] and by Bailey [7]: it is more suitable to express the reaction rate as a function of other variables such as enzyme concentration. It is shown that for cellulase action in this type of hydrolytic reactions, the reaction rate will increase with the total enzyme concentration, accordingly [6]. It is convenient to define a maximal velocity (V'_{max}) and corresponding arbitrary half-saturation constant (k'_m) and by analogy to Michaelis-Menten relationship, the following equation has been presented by Woodward et al.:

$$V = \frac{V'_{max}[E]}{[E] + k'_m}$$

in fact, this modified form of the Michaelis-Menten equation has been successfully used in the protease treatment of wool fabrics [8].

In order to develop the efficient strategies for utilization of auxiliaries such as surfactants in the field of fabric finishing along with enzyme(s), one should know more about the detailed procedure of this process. In the present work the effect(s) of four different types of surfactants on the enzymatic hydrolysis of cellulosic fabrics has been studied. The results have been discussed in terms of possible mechanism of the tested surfactants on the enzyme action on the fabrics.

EXPERIMENTAL

Materials

Cellulosic fabrics which is used as the substrate in this enzymatic procedure had the following specifications:
Cotton/polyester: 55/45 scoured fabrics. The weight per unit area of the fabric was 120.6 g/m^2 and the yarn count of weft and warp of the fabric were 32 and 28, respectively.
Surfactants as the appropriate derivatives of the various compounds has the following specifications:
- Anionic: Ultravon GPN (ANS) is a poly-containing preparation based on sodium sulphonate and ethoxylated alcohol.
- Non-ionic: Irgazol NA (NNS) is an ethoxylated fatty alcohol.
- Cationic: Tinegal MR(CAS) is n-tetraalkyl ammonium halide.
- Amphoteric: Albegal A(AMS) is alkylamine polyglycol ether.

The surfactants were kindly supplied by Ciba Co.
Cellulase enzyme, which is used in this study, was generously provided by Novo-Nordisk-Tehran office (Celluosoft).

Methods

Cotton fabrics in the appropriate size were prepared (15×15) and 10 g of the fabrics was soaked in 200 mL buffer solution containing the appropriate concentration of the enzyme (0.1, 0.2, 0.5, 1, 2 g of enzyme per liter of the buffer solution). Buffer had the following specification: acetate buffer, 0.02 M, pH 4.5.

Temperature and time of the enzymatic treatments were 50 °C, 30 min, respectively. The experiment was carried out in Linitest dyeing apparatus with 20:1 liquor per g of fabric, agitation in the bath was provided by 10 steel disk. Reducing sugar released upon this hydrolytic reaction was measured at the end of the time interval.

Sugar Determination

The amount of sugar released was determined using dinitrosalicylic acid (DNS) procedure and the standard curve for this spectrophotometric procedure (λ_{max}595nm) was prepared in the range using glucose [9].

Physical Parameters

Some of physical parameters of the fabrics measured in this study were: loss of strength, thickness, and crease recovery (angle).

Loss of strength was measured on an Instron tester T.M.S.M model, England, speed: 5cm/min, gauge length: 20 cm.

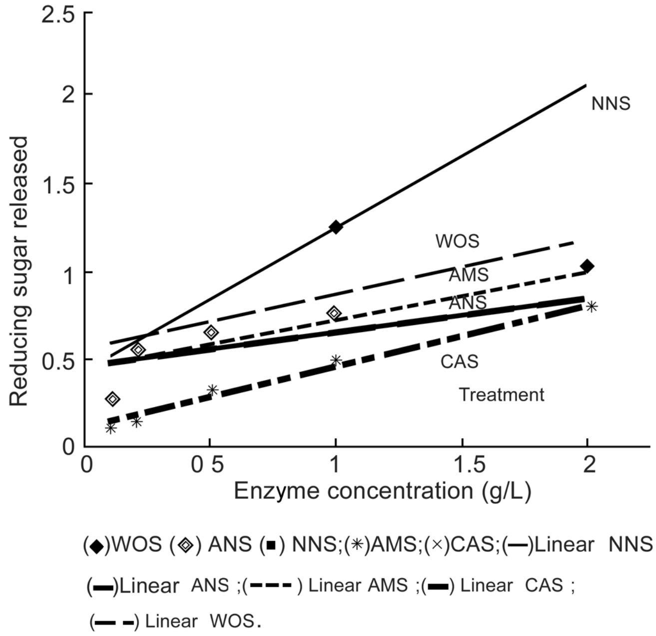
Variation in thickness was measured on Shirley equipment (O.S.K), 240 g/cm^2, England.

Calculation of Kinetic Constants (V'_{max}/k'_m)

Reciprocal Lineweaver-Burk plot is among the most common linear forms in the expressing of Michaelis-Menten equation: [V'_{max} [E]/ k'_m+ [E]] as modified by Bailey [7], and Lineweaver-Burk equation:

$$\frac{1}{V} = \frac{k'_m}{V'_{max}}\left(\frac{1}{[E]}\right) + \frac{1}{V'_{max}} \times V'_{max}$$

V'_{max} is the maximal velocity, V the rate of the reaction at which the velocity is independent of the enzyme concentration. k'_m is the concentration of the enzyme at which the velocity reaches half of the maximal velocity (V'_{max}/2). The catalytic specificity of the enzyme action has been defined as the ratio V'_{max}/k'_m.



(NMS): y=0.8263x + 0.4206 (ANS): y=0.2026x + 0.4426
(WO): y=0.3239x + 0.5409 (CAS): y=0.3511x + 0.0947
(AMS): y=0.2954x + 0.4205

Figure 1. Enzymatic hydrolysis of the cellulosic fabric in presence of the surfactants at different concentrations of the enzyme.

RESULTS AND DISCUSSION

Effects of different kinds of surfactants on the cellulase treatment of cellulosic fabrics were examined. Figure 1 shows the amount of reducing sugar released for each one of the enzymatic treatments. It is shown that the amount of reducing sugar released (SR) in the presence of NNS has been increased by approximately 12% in comparison to a content without surfactant (WOS), while the quantity of SR in the presence of other surfactants namely amphoteric (AMS), anionic (ANS), and cationic (CAS) decreased by 13, 18, 50 percent, respectively.

It is well known that enzymatic reaction involves an adsorption of the enzyme onto substrate to form the enzyme-substrate complex. As it has been mentioned before the adsorption mechanism has a rather special meaning in the cellulase hydrolysis of cellulose. Surfactants, amphiphilic molecules, tend to adsorb onto surfaces therefore, provide a means of surface alteration in the enzymatic reaction of cellulose [10]. This improvement in the reaction rate is reported to be more evident when NNS is used [10]. Moreover, the data obtained by Mizutani and Bertoniere in the recent work show that the use of NNS on the cellulase treatment of cotton fabric enhanced the effect of cellulase [11]. Based on the results and observation from scanning electron microscopy, those authors suggested that there could be a relationship between the enhancement effect obtained and the degree of crystallinity and orderness of the cellulosic fibres [11]. However, according to the work reported by Helle et al. [10], cellulose crystallinity showed to be relatively invariant over extended hyrolysis time. They suggested that crystallinity is considered perhaps, a less important factor than it is originally perceived to be [10].

As it is known, the enzymatic hydrolysis of cellulose involves transport of enzyme molecules and soluble sugars between the solid substrate and bulk reaction solution [10]. Therefore, modification of the surfaces and interfacial properties of the reaction system may have some impacts on this transport [12]. Surfactants lower the surface tension and they may also prevent surface inactivation of cellulase by interfering with the hydrophobic part of the enzyme structure [10].

One of the characters in enzyme inactivation is protein unfolding followed by aggregation of the molecules, which is mainly due to reoccurrence of the hydrophobic interaction (improper structural feature of the integrated form of proteins for these cases)[4].

It is shown that in the enzymatic hydrolysis of cellulose, these types of improper hydrophobic interactions are weakened by the presence of NNS [10]. In addition to these hydrophobic interactions, the binding of small molecules such as surfactants to proteins affects the proteins' structures and may modulate the proteins' function and properties [13]. It is reported elsewhere that NNS lack a denaturing quality as compared to that of denaturing effect(s) of cationic and anionic surfactants [13]. In fact the results obtained in the present study are in the agreement with these findings and comments.

It is postulated that surfactant exerts its improving effect(s) on cellulase action, perhaps by two mechanisms: (1) altering the cellulose's properties, and by the disturbing the structure of cellulose (reduction the degree of crystallinity), making the substrate more accessible to the enzyme, (2) preventing the adsorbed cellulase from becoming inactive [12]. On the other hand, some studies on the cellulase treatment of cotton fabrics showed that ionic surfactants impose some inhibitory effects on the cellulose catalytic reaction [4]. The results obtained by Ueda and Koo show that occurrence of the electrostatic interaction(s) between charged molecules in the treatment solution or even on the surfaces of cotton substrate could lead to the formation of a less active enzyme or enzyme-substrate complex, or both [4]. In that work no specific type of enzyme inhibition was addressed [4].

In the present study, the results obtained from the tensile strength loss (%) of the cellulase treated cellulosic fabics are in agreement with the pattern of the hydrolysis of the fibres by cellulase and that of the amount of the sugar released (Table 1 and Figure 2). Loss of the tensile strength for the cellulase treated cotton fabrics is shown to be increased when NNS is used in the reaction mixture as compared to that of the control treatment (Table1). This does not necessarily mean that the improved action of NNS on the enzyme treated cotton fabrics is coincided on the improved physical

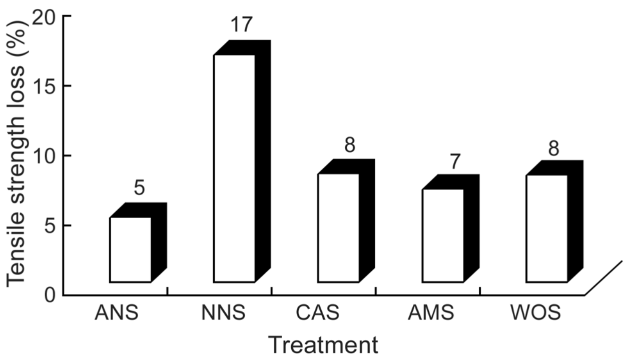


Figure 2. Loss of strength of fabric treated with cellulase in presence or absence of the fixed concentration of different surfactants.

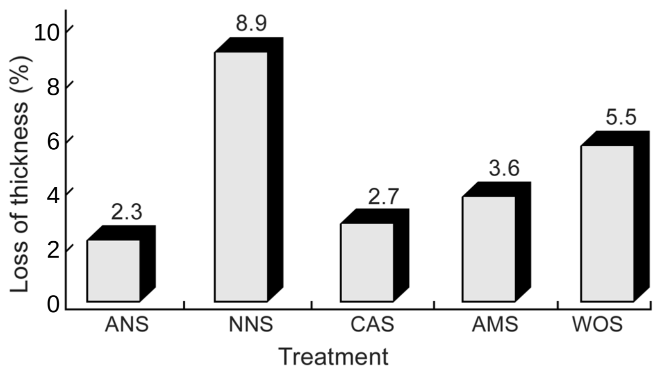


Figure 3. Thickness of the enzymatic treated cellulosic fabrics in presence or absence of the fixed concentration of different surfactants.

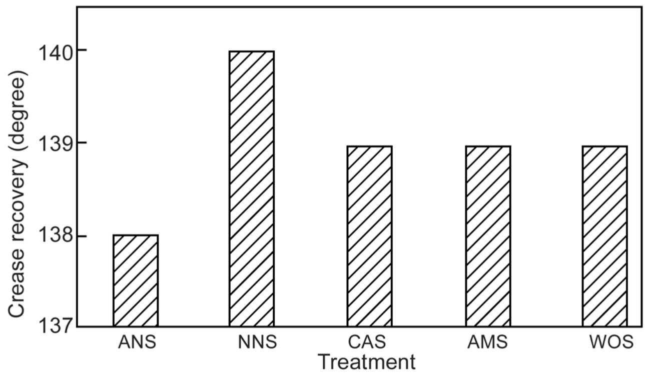


Figure 4. Crease recovery (angle) of treated cellulosic fabrics in presence or absence of surfactant.

proporties of the tested fabrics (Table 1 and Figures 1 and 2). However, results reported by Mizutani and Bertoniere show rather a small decrease in the tensile strength of the cotton fabrics treated in the presence of NNS [11].

Tensile strength loss is considered as one of the major factors in determining the physical properties of treated fabrics. Based on the results obtained in the present study, optimization of the conditions for action of cellulase on cellulosic fabrics in relation to extent of sugar released as well as on the degree of strength loss, both should be investigated. Results obtained from the thickness and crease recovery (angle) measurement of the enzyme treated fabrics are shown in Figures 3 and 4. These results are also in agreement with the results presented in Figure 1 and in Table 1.

In order to express the specificity of an enzyme toward a particular substrate in the reaction mixture, it is possible to obtain the ratio of k_p/k_m (considering the general model of the enzyme kinetics:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_p} E + P$$

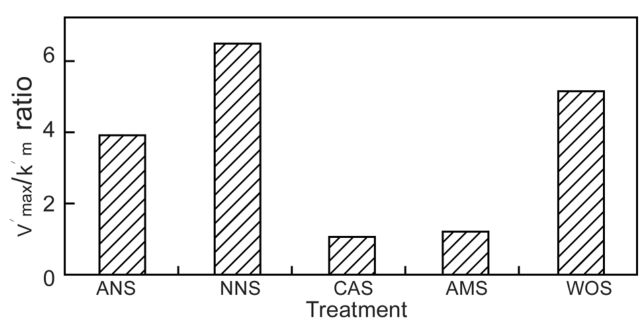


Figure 5. Catalytic specificity of the cellulase hydrolytic reaction for cellulosic fabrics in the presence or absence of four different types of surfactants.

Table 1. Effect of different types of surfactants on the tensile strength loss of the cellulose fabrics treated enzymatically.

Treatment	Tensile strength loss*(%)
WOS	8
CAS	8
AMS	7
ANS	5
NNS	17

(*) Relative to that of untreated sample

Because the maximal velocity of the reaction equals to $k_p \times [E]_{total}$, and total enzyme concentration remains almost constant for the different reaction mixtures, therefore it is a common practice to use the V'_{max}/k'_m ratio instead of k_p/k_m ratio [4]. Figure 5 shows that specificity of cellulase in the presence of NNS is more than that for other surfactants used the enzymatic treatments (the kinetic parameters, V'_{max} and, k'_m were calculated from double reciprocal plot in the same way as reported in previous work on wool fabric [10].

The catalytic specificity of cellulase in the presence of CAS and AMS decreased significantly, however, this specificity reduction in the presence of ANS was less than that of the two above named surfactants (Figure 5). Possible formation of the attractive force(s) between cellulose with its negatively charged surfaces [7] and the cationic surfactant may be of more importance as compared to the existence of the repulsion force(s) between the cellulose substrate and anionic surfactant.

Presence of the amphoteric surfactant in the reaction mixture may show again the importance of occurrence of the attractive force between the substrate and the positively charged portion of the AMS (Figure 5). Regardless of the occurrence of the forces between the ionic surfactants and cellulose surfaces described above, more works are needed and in fact are underway to explain more precisely the possible inhibitory action of the ionic surfactants on cellulase enzyme.

CONCLUSION

The effects of four different types of surfactants in enzymatic treatment of cellulosic fabrics were studied. Measuring amount of the reducing sugar released during the enzymatic reaction showed that the presence of nonionic surfactant increases the rate of reaction which accompained by decrease in the strength loss, while decreasing the rate of reaction in presence of amphoteric, cationic and anionic may indicate that these surfactant possibly acting as an inhibitor on the action of cellulase.

Therefore, lack of denaturing effect of NNS reported elsewhere [12], was confirmed in the present study. Although cationic, amphoteric and anionic surfactants may have some denaturing effects as mentioned by others.

REFERENCES

1. Cegarra J. "The state of the art in textile biotechnology", *JSDC*, **112,** 326-329 (1996).
2. Koo H., Ueda M., and Wakida T., "Cellulase treatment of cotton fabrics", *Tex. Res. J.*, **64,** 2, 70-74 (1994).
3. Woodward J., Lima M., and Lee E., "The role of cellulase concentration in determining the degree of synergism in the hydrolysis of microcrystalline cellulose", *Biochem J.*, **255,** 895-899 (1988).
4. Ueda M., Koo H., and Wakida T., "Cellulase treatment of cotton fabrics Part II: Inhibitory effect of surfactants on cellulase catalytic reaction", *Tex. Res. J.*, **64,** 10, 615-618 (1994).
5. Bailey J. and Ollis D, *Biochemical Engineering Fundamentals*, 2nd ed., McGraw Hill (1986).
6. Nidetzky B., Steiner W., Hayn M., and Claegssens M., "Cellulose hydrolysis by the cellulases from Trichoderma Reesei: A new model for synergistic interaction", *Biochem. J.*, **298,** 705-710 (1994).
7. Bailey J. and Oills D., "Enzyme kinetics of cellulose hydrolysis", *Biochem J.*, **269,** 1001-1002 (1989).
8. Hemmati N., Kordestani S.S, and Vahabzadeh F., "Enzymatic treatment of wool fabric: Effect of the surfactants", *Iran. Polym. J.*, **10,** 125-132, 2, (2001).
9. Miller L.G., "Use of dinitrosalicylic acid reagent for determination of reducing sugar", *Anal. Chem.*, **31,** 426-428 (1959).
10. Hell S., Duff J.B., and Cooper D., "Effect of surfactants on cellulose hydrolysis", *Biotechnol. and Bioeng.*, **42,** 611-617 (1993).
11. Mizutani C., Bertoniere N., "Influence of surfactants on enzyme treatment of cellulosic fibres", The 5th Asian Textile Conference, Proceeding 2, Kyoto, Japan, 670-672 (1999).
12. George G., Sung L., and Mukul S., "Surface active compounds from microorganism", *Biotechnology,* **10,** 1, 60-64 (1992).
13. Ghobadi S., Safarian S., and Mossavi-Movahedi A.A., "Octyl glucoside induced formation of the molten globule - like state of glutamate dehydrogenase", *J. Biochem,* **130,** 571-677 (2001).