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Enhanced enzymatic hydrolysis of cellulose by partial modification of its chemical structure

H.M. Shaikh [a], M.G. Adsul [b], D.V. Gokhale [b], A.J. Varma [a,*]

[a] Polymer Science & Engineering Division, National Chemical Laboratory, Pune 411008, India
[b] NCIM, Biochemical Sciences Division, National Chemical Laboratory, Pune 411008, India

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2,3-Dibenzylimine cellulose

ABSTRACT

A series of 2,3-dialdehyde celluloses with different degrees of oxidation were used for deriving corresponding dicarboxylate, dicarboxy, and Schiff's base cellulose derivatives. The dialdehyde cellulose was hydrolyzed by cellulase to a lower extent than the starting cellulose, except at high levels of aldehyde content (above 50%). For dicarboxylate and dicarboxy celluloses, the highest level of oxidized NaDCC and DCC hydrolysed up to 70 and 60% respectively which was 3–4 times more than cellulose. The 2,3-dioxime cellulose derivative hydrolyzes only up to 16.3% for the highest level of oxidized dioxime. In the case of 2,3-diethylimine cellulose, all derivatives hydrolyse faster than the native cellulose. Up to 75% hydrolysis was observed for 2,3-diethyimine cellulose-50, 2,3-dipropylimine and 2,3-dibutylimine cellulose. The 2,3-dibenzylimine cellulose hydrolyses a little slower than the alkylimine derivatives. The 2,3-dihydrazone cellulose derivatives with all level of oxidation showed resistance towards enzymatic hydrolysis.

1. Introduction

Cellulose is the most abundant bio-renewable source and holds huge potential as an alternative to fossil fuels for sustainable production of fuels and chemicals (Baratieri, Baggio, Fiori, & Grigiante, 2008). Conversion of lignocellulose into biofuels (particularly ethanol and butanol) and useful chemicals has attracted increasing attention in recent years. Although biofuels possess several advantages over conventional fossil fuels, lignocellulosic biofuel has not yet been produced on a commercial scale because of the low yields and high production cost (Adsul, Singhvi, Gaikaiwari, & Gokhale, 2011). The lignocellulosic biofuel process can be made economical by developing cost-effective pretreatment and hydrolysis strategies. The effective conversion of lignocellulosic to sugars and recovery of the sugars is regarded as holding the key to the success of this technology. Further, hydrolysis of cellulosic materials produces glucose which is a platform chemical to produce a wide array of chemicals which are hitherto only produced from petroleum sources. This explains the ever-increasing interest in developing new methodologies to improve the yields and rates of hydrolysis of cellulosic materials. Regarding techno-commercial feasibility, the process will be commercially viable if the by-product of the hydrolysis, which is a dialdehyde alcohol, can be separated by membranes or other methods to yield an important functional compound with potential to be a platform chemical.

The hydrolysis of cellulose by mineral acids is strongly affected by the acid concentration and temperature and mineral acid hydrolysis yields byproducts that are fermentation inhibitors. However, recoveries of the acid or disposal of the neutralized acid, along with corrosion problems, pose significant challenges to this methodology. If enzymes are to be used for hydrolysis of cellulose, various factors play important roles such as physical properties of the substrate, composition of substrate, crystallinity of cellulose, degree of polymerization, enzyme complex synergy, bulk and pore diffusion, and kinetics (Chang & Holtzapple, 2000; Grethlein, 1985; Hendriks & Zeeman, 2009; Zhang & Lynd, 2004). Other workers have reported factors such as available surface area, particle size, pore size of the substrate and lignin content as limiting the enzymatic hydrolysis of cellulosic materials (Chang & Holtzapple, 2000; Koullas, Christakopoulos, Kekos, Macris, & Koukios, 1992; Laureano-Perez, Teymouri, Alizadeh, & Dale, 2005; Puri, 1984; Thompson, Chen, & Grethlein, 1992).

What then are the ways to enhance hydrolysis of cellulose substrate? We have proposed a new methodology, by which we disrupt the chemical structure of cellulose by various chemical reactions, thereby enabling enzymes to access the anomeric carbon, which

* Corresponding author. Tel.: +91 20 25902191; fax: +91 20 25902618.
E-mail address: aj.varma@ncl.res.in (A.J. Varma).

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is the point of hydrolysis by β-glucosidase and endo-glucanase enzymes. A study of hydrolysis of such cellulose derivatives could throw new light on the specificity of the cellulase enzyme complex. To test this hypothesis we synthesized cellulose derivatives having various functionalities on the cellulose chain. Thus we prepared a series of periodate oxidized cellulose to obtain 2,3-dialdehyde cellulose in which the pyranose ring of cellulose selectively breaks at C-2 and C-3 positions. This 2,3-dialdehyde cellulose was then further modified to sodium salt of 2,3-dicarboxy cellulose, 2,3-dicarboxycellulose and Schiff's bases. The hydrolysis behavior of these derivatives was studied by hydrolytic (cellulase) enzymes. It was observed that depending on the chemical functionality on the cellulose backbone, cellulose may or may not hydrolyze efficiently. Thus, this method could potentially be used to achieve a faster hydrolysis of cellulosic materials with maximum recovery of glucose and other glucose based products.

2. Methods

2.1. Materials

Cotton linter cellulose was supplied by Reliance Cellulose Products Limited, Secundrabad, India. It contained >95% alpha cellulose. Cellulose from sugarcane bagasse prepared by a proprietary process developed by our laboratory was used (Varma, WO PCT/IN08/00569, 2008).

2.2. Chemicals

Sodium metaperiodate (99.5%), sodium thiosulphate (99.5%), and sodium bicarbonate (99.5%) were a product of S. D. Fine Chemicals, India. Soluble starch (Merck), sodium hydroxide, methanol GR grade, potassium iodide, potassium dichromate, and 3,5-dinitrosalysilic acid were procured from Rankem India, Ltd. and used without further purification.

2.3. Oxidation of cellulose to 2,3-dialdehyde cellulose with different levels of oxidation (Shaikh, 2011)

In a two neck three liter round bottom flask equipped with mechanically rotary shaft, a known weight of cellulose was taken. This flask was wrapped with aluminum foil to prevent exposure to light. To this flask were added known quantities of sodium metaperiodate. Sodium metaperiodate was dissolved slowly in two liters of distilled water and the pH of this solution was measured to be between 3.2 and 3.5. Nitrogen gas was purged in the reaction mixture. The reaction was carried out in the dark under nitrogen atmosphere to avoid decomposition of sodium metaperiodate and photo-oxidation. Temperature of the reaction mixture was maintained between 50 and 55 °C using a water bath for various time intervals, after which the reaction mixture was cooled to room temperature. Oxidized cellulose was filtered off, washed thoroughly with distilled water several times until the filtrate became neutral. The final washing was done by methanol and the product was dried under vacuum at 60 °C and characterized by FTIR, CP/MAS ^{13}C NMR, and TGA. Quantification of sodium metaperiodate consumed in the reaction was carried out by sodium thiosulphate method (Scott, 1939).

2.3.1. General methods for synthesis of sodium 2,3-dicarboxy celluloses (NaDCC)

For synthesis of sodium salt of 2,3-dicarboxy celluloses (NaDCC) with different degrees of oxidation (i.e. ~5%, 15%, 25%, and 50%) the required quantities of dialdehyde cellulose was dispersed in distilled water. Sodium chlorite solution and required amount of acetic acid diluted in distilled water to give pH 3.5–4 was added slowly to dialdehyde cellulose. The temperature of reaction mixture at 25–30 °C and constant stirring was maintained. The start of oxidation was indicated by the formation of yellow coloration and evolution of chlorine dioxide (ClO_2) gas. The reaction was continued for 7 h until there was no further evolution of chlorine dioxide. After this nitrogen gas was bubbled through the reaction mixture to remove the dissolved gases from the reaction. The pH of the reaction mixture was adjusted to 8.5–9 by addition of 10 N NaOH solution. The product was settled at the bottom and was separated by decantation and/or centrifugation. The product was washed several times with distilled water until the filtrate was neutral. It was dried under vacuum at 60 °C and characterized by FTIR CP/MAS ^{13}C NMR and TGA. Sodium detection was done by Energy-dispersive X-ray spectroscopy (EDAX). These products of different level of oxidations are insoluble in common organic solvents. Detailed spectral characterization of these compounds is being published separately in a paper on synthesis and characterization of theses derivatives.

2.3.2. General methods for synthesis of 2,3-dicarboxycelluloses (DCC)

For synthesis of 2,3-dicarboxycelluloses (DCC) with different degree of oxidation (i.e. 5, 15, 25 and 50%), the required quantities of sodium salt of 2,3-dicarboxy cellulose was dispersed in distilled water and 0.2 N HCl was added with stirring till the pH reached 3.5. This solution was kept in a freezer at 1 °C for about half an hour with stirring at regular intervals. After this the aqueous fraction was decanted off and 30 ml distilled water was added and kept at 5 °C for 24. The solution was decanted off and the product washed several time until the filtrate was neutral. It was dried in vacuum at 60 °C and characterized by FTIR, CP/MAS ^{13}C NMR, and TGA. These products of different levels of oxidation are insoluble in common organic solvents. Detailed spectral characterization of these compounds is being published separately in a paper on synthesis and characterization of theses derivatives.

2.3.3. General methods for synthesis of Schiff's bases of 2,3-dialdehyde cellulose

In a 250 ml round bottom flask 100 ml of 0.2 M acetate buffer was taken, pH of this buffer was adjusted between 4.8 and 5.6. To this buffer known amounts of hydrazine, oxime and amines was added slowly with constant stirring. The known quantity of oven dried 2,3-dialdehyde cellulose with various levels of oxidation was added portion wise over a period of 10 min. These reaction mixtures were then stirred from room temperature to 55 °C for different reagents for various time intervals. Reaction mixtures slowly turn faint yellow to pale yellow in colour (with deeper colour being observed for higher oxidation levels of DAC). After a fixed time interval the reaction mixtures were allowed to settle down. The insoluble solid settled at the bottom was separated by centrifugation. The filtrates were drained and solid products were washed several times with distilled water till neutral filtrate was obtained. The products were dried under vacuum at 60 °C for 24 h and characterized by FTIR, CP/MAS ^{13}C NMR, and TGA. Nitrogen contents were determined by elemental analysis as well as by EDAX for low level of nitrogen content. All products obtained from different level of oxidized dialdehyde cellulose are insoluble in common organic solvents. Detailed spectral characterization of these compounds is being published separately in a paper on synthesis and characterization.

Using the above procedures, the following cellulose derivatives were prepared: 2,3-dialdehyde cellulose (5%, 15%, 25%, 50%), sodium 2,3-dicarboxylate cellulose (5%, 15%, 25%, 50%), 2,3-dicarboxy cellulose (5%, 15%, 25%, 50%), 2,3-dioxime cellulose (5%, 15%, 25%, 50%), 2,3-diethylimine cellulose (5%, 15%, 25%, 50%), 2,3-dipropylimine cellulose (5%, 15%, 25%, 50%), 2,3-dibutylimine cellulose (5%, 15%,

25%, 50%), 2,3-benzylimine cellulose (5%, 15%, 25%, 50%), 2,3-dihydrazone cellulose (5%, 15%, 25%, 50%).

2.4. Enzymatic hydrolysis

The hydrolysis of the samples was carried out in 25 ml flask containing 9 ml citrate buffer (pH 4.5, 50 mM) and 1 ml of cellulase enzyme preparation obtained from a mutant of *Penicillium janthinellum* NCIM 1171. The flasks were incubated at 50 °C with shaking at 200 rpm. The samples were analyzed for reducing sugars after 12, 24, 36 and 48 h by dinitrosalicylic acid methods (Fischer & Stein, 1961). 1 ml of enzyme preparation contains 5 units of FPA, 15 units of β-glucosidase and 200 units of CMCase.

2.4.1. Hydrolysis of cellulose (cotton linters) in the presence of physically added reagent (anhydrous hydrazine)

100 mg of cotton linters were hydrolyzed in 25 ml conical flask containing 9 ml of Citrate buffer (pH 4.5, 50 mM), 1 ml enzyme preparation and 7.0 mg and 18 mg of anhydrous NH_2–NH_2 reagent separately. 1 ml of enzyme preparation contains 5 units of FPA, 15 units of β-glucosidase and 200 units of CMCase. The pH was adjusted to 4.5–5.0. The flasks were incubated at 50 °C with shaking. The 1 ml of hydrolyzed samples was removed after suitable time interval for the analysis of reducing sugars.

2.4.2. Hydrolysis of cellulose (cotton linters) in the presence of 2,3-dihydrazone cellulose

50 mg of cotton linters, 50 mg of 2,3-dihydrazone cellulose and a mixture of 25 mg of cotton linters and 25 mg of 2,3-dihydrazone cellulose were hydrolyzed in 25 ml conical flask containing 4.5 ml of citrate buffer (pH 4.5, 50 mM), 0.5 ml enzyme preparation. The pH adjusted to 4.5–5.0. The flasks were incubated at 50 °C with shaking. The 0.5 ml of hydrolyzed samples was removed after suitable time interval for the analysis of reducing sugars. 1 ml of enzyme preparation contains 5 units of FPA, 15 units of β-glucosidase and 200 units of CMCase.

3. Results and discussion

3.1. Enzymatic hydrolysis of cellulose with same level of oxidation (cotton linters)

Complete enzymatic hydrolysis of cellulose to glucose requires a cocktail of endoglucanase, exoglucanse and β-glucosidase enzymes. During the hydrolysis of cellulose, the endoglucanases attack the cellulose polymer chain in a random manner creating new reducing ends. This reaction is followed by the hydrolysis with exoglucanases, which attack the cellulose from either end, forming cellobiose. Finally, the β-glucosidase completes the hydrolytic process through the formation of glucose from cellobiose. It is considered that all three enzymes work in a synergistic manner for hydrolysis of both cellulose and modified cellulose (Mansfield, Mooney, & Saddler, 1999).

In this study we present the results of studies on hydrolysis of pure cellulose and its chemical modifications containing aldehyde, carboxylate, carboxy and schiff bases. These dialdehydes are shown in FTIR the spectra (Fig. 1). The hydrolysis data are presented in Tables 1 and 2.

Table 1 represents the enzymatic hydrolysis behavior of cotton linter cellulose which is oxidized to an extent of 15% of the glucose monomer units to 2,3-dialdehyde cellulose (DAC-15). This oxidized cellulose is then converted to Na salt of dicarboxy cellulose and then to dicarboxy cellulose. In this case it was observed that dialdehyde cellulose appears to hydrolyze at a slightly lower rate than the parent cellulose.

Since oxidation causes decrease in crystallinity of cellulose, it is expected that oxidized cellulose should hydrolyze at a rate faster

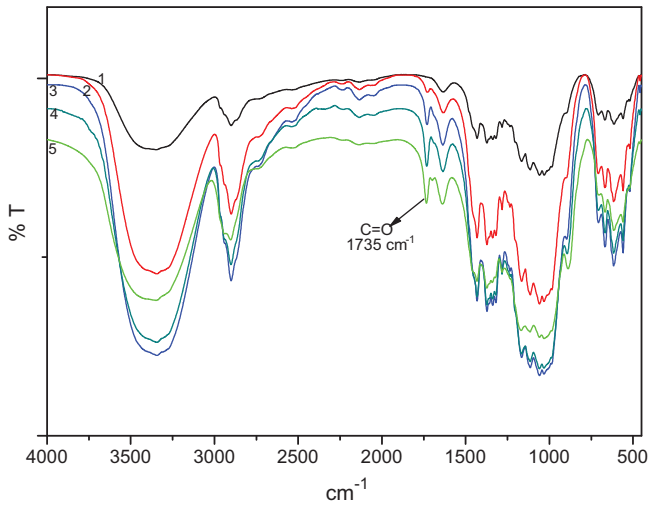

Fig. 1. Overlapping of FTIR spectra of cellulose and 2,3-dialdehyde cellulose (DAC) with various level of oxidation showing carbonyl peak at 1735 cm^{-1} in dialdehyde cellulose (1) Cellulose, (2) DAC-5, (3) DAC-15, (4) DAC-25, and (5) DAC-50 (numbers 5, 15, 25 and 50 denotes the levels of oxidation (%) of 2,3-dialdehyde cellulose).

than native cellulose. It appears that in dialdehyde cellulose, the aldehyde substituent hinders the enzymes perhaps due to hydrogen bonding of aldehyde with other hydroxyl in cellulose as well as formation of hemiacetal and hemialdol like structure, in addition to Schiff's base derivatives of oxidized cellulose, as shown in Fig. 2 (Fan, Lewis, & Tapley, 2001).

Similar observation was made by Feng, Hanshu, and Tejirian (2009) who conducted similar studies on cupric ion and hypochlorite oxidized cellulose and observed profound decrease in hydrolysis rate mainly due to interference of functional groups. However, as shown in Table 1. NaDCC-15 (15% oxidized sodium 2,3-dicarboxycellulose) and DCC-15 (15% oxidized 2,3-dicarboxycellulose) the observed hydrolysis rate was significantly greater than the parent DAC-15 and cotton linter cellulose (the hydrolysis profile is shown in Fig. 3). Thus while cellulose and DAC-15 hydrolyzed only to ~20% in 48 h, NaDCC-15 and DCC-15 hydrolysed to an extent of 42–48% in the same time. This enhanced rate could be due to decrease in crystallinity and enhanced binding of the enzymes to the carboxylate/carboxy functional group of NaDCC and DCC.

3.2. Enzymatic hydrolysis of cellulose with same level of oxidation (bagasse derived cellulose)

Similar hydrolysis was conducted on sugarcane bagasse derived cellulose prepared by a proprietary process developed by our laboratory (Varma, WO PCT/IN08/00569, 2008). Bagasse cellulose was also oxidized to ~15% to make 2,3-dialdehyde cellulose and then converted to sodium salt of 2,3-dicarboxy cellulose and 2,3-dicarboxy cellulose. Here also, a similar hydrolytic pattern was seen as for cotton cellulose and its derivatives but the extent of hydrolysis was much greater, going up to >70% for NaDCC-15. The reason for this enhancement can be attributed to lower crystallinity and lower degree of polymerization (DP) of bagasse cellulose as compared to cotton cellulose (the hydrolysis profile is shown in Fig. 4).

3.3. Enzymatic hydrolysis of Schiff's bases of 2,3-dialdehyde cellulose made from cotton linters cellulose

Enzymatic hydrolysis studies were also carried out on Schiff bases of 2,3-dialdehyde cellulose such as oxime, hydrazone and

Table 1
Enzymatic hydrolysis of oxidized cellulose with same level of oxidation (15% oxidation level) using cellulose from cotton linter and bagasse.

Sr. No.	Samples	Hydrolysis (%)			
		12 h	24 h	36 h	48 h
A	Cellulose (cotton linter)	6.6	11.1	16.3	20.5
1	2,3-Dialdehyde cellulose-15	4	6.6	13.8	17
2	Sodium 2,3-dicarboxy cellulose-15	19.5	29	40.5	48
3	2,3-Dicarboxy cellulose-15	17	27	38.6	42
4	2,3-Dioxime cellulose-15	4.2	7.5	10.4	12.3
5	2,3-Diethylimine cellulose-15	16	25.5	30	37.3
6	2,3-Dipropylimine cellulose-15	20	25	28	34.3
7	2,3-Dibutylimine cellulose-15	25	31.8	35.4	38.8
8	2,3-Benzylimine cellulose-15	16.3	27.5	32	36.1
9	2,3-Dihydrazone cellulose-15	N.D.			
B	Cellulose (bagasse derived)	25.3	35	36.5	40.0
1	2,3-Dialdehyde cellulose-15	8.8	11	24.5	30.0
2	Sodium 2,3-dicarboxy cellulose-15	38	50.5	65.5	75.0
3	2,3-Dicarboxy cellulose-15	28.7	39	45.8	60.5

N.D.: not detectable.

imines. Table 2 compiles the details of enzymatic hydrolysis of all cellulosic derivatives.

It was observed that 2,3-dialdehyde cellulose with low levels of oxidation hinders the enzymatic hydrolysis (discussed earlier) but higher level of oxidation (around 50%) hydrolyzes faster that the native cellulose. This could be due extensive molecular weight degradation of this derivative which helps the enzyme for easy access for hydrolysis. Thus 2,3-dialdehyde cellulose-50 (50% oxidation level) hydrolyzes up to 35.7% in 48 h which is more than the cellulose. In the case of sodium 2,3-dicarboxy cellulose hydrolysis was faster than cellulose as well as dialdehyde cellulose as discussed earlier. A similar effect was observed

Table 2
Enzymatic hydrolysis of series of oxidized celluloses with different degrees of (5–50%) of oxidation based on glucose monomer units. All derived from cotton linters.

Sr. No.	Modified 2,3-dialdehyde cellulose	Hydrolysis (%)			
		12 h	24 h	36 h	48 h
1	Cellulose (cotton linter)	6.6	11.1	16.3	20.5
2	2,3-Dialdehyde cellulose-5	2.8	3.2	4.6	5.6
3	2,3-Dialdehyde cellulose-15	4	6.6	13.8	17.0
4	2,3-Dialdehyde cellulose-25	7.2	9.1	13.8	16.6
5	2,3-Dialdehyde cellulose-50	14.5	26.8	32.1	35.7
6	Sodium 2,3-dicarboxy cellulose-5	10.7	20	31.6	35.1
7	Sodium 2,3-dicarboxy cellulose-15	19.5	29	40.5	48.6
8	Sodium 2,3-dicarboxy cellulose-25	25	35.5	48.7	56.4
9	Sodium 2,3-dicarboxy cellulose-50	48	55.6	63.9	72.3
10	2,3-Dicarboxy cellulose-5	8.9	18.2	28	30.3
11	2,3-Dicarboxy cellulose-15	17	27	38.6	42.7
12	2,3-Dicarboxy cellulose-25	20	31.2	42.6	50.4
13	2,3-Dicarboxy cellulose-50	42	50.2	59.9	61.5
14	2,3-Dioxime cellulose-5	3	4.7	6.1	7.8
15	2,3-Dioxime cellulose-15	4.2	7.5	10.4	12.3
16	2,3-Dioxime cellulose-25	4.5	6.3	10.2	14.4
17	2,3-Dioxime cellulose-50	5.5	8.3	13.3	16.3
18	2,3-Diethylimine cellulose-5	15	24.1	28	34.6
19	2,3-Diethylimine cellulose-15	16	25.5	30	37.3
20	2,3-Diethylimine cellulose-25	17.2	25	33.4	40.0
21	2,3-Diethylimine cellulose-50	20	38	60.5	75.3
22	2,3-Dipropylimine cellulose-5	17	24.2	26.3	31.1
23	2,3-Dipropylimine cellulose-15	18	25	28	34.3
24	2,3-Dipropylimine cellulose-25	20.2	25.5	30	35.9
24	2,3-Dipropylimine cellulose-50	25.7	40	45.9	51.5
26	2,3-Dibutylimine cellulose-5	22	28.3	32.6	35.7
27	2,3-Dibutylimine cellulose-15	25	31.8	35.4	38.8
28	2,3-Dibutylimine cellulose-25	28.5	36.6	45.3	50.2
29	2,3-Dibutyllimine cellulose-50	33.3	41	48.7	56.6
30	2,3-Benzylimine cellulose-5	11.3	21	23.1	25.7
31	2,3-Benzylimine cellulose-15	14.0	19.8	24.3	27.2
32	2,3-Benzylimine cellulose-25	15.6	20.4	25.0	30.6
33	2,3-Benzylimine cellulose-50	16.3	22.6	26.7	36.6
34	2,3-Dihydrazone cellulose-5	N.D.			
36	2,3-Dihydrazone cellulose-15				
37	2,3-Dihydrazone cellulose-25				
38	2,3-Dihydrazone cellulose-50				

Note: the number denotes the level of oxidation.
N.D.: not detectable.

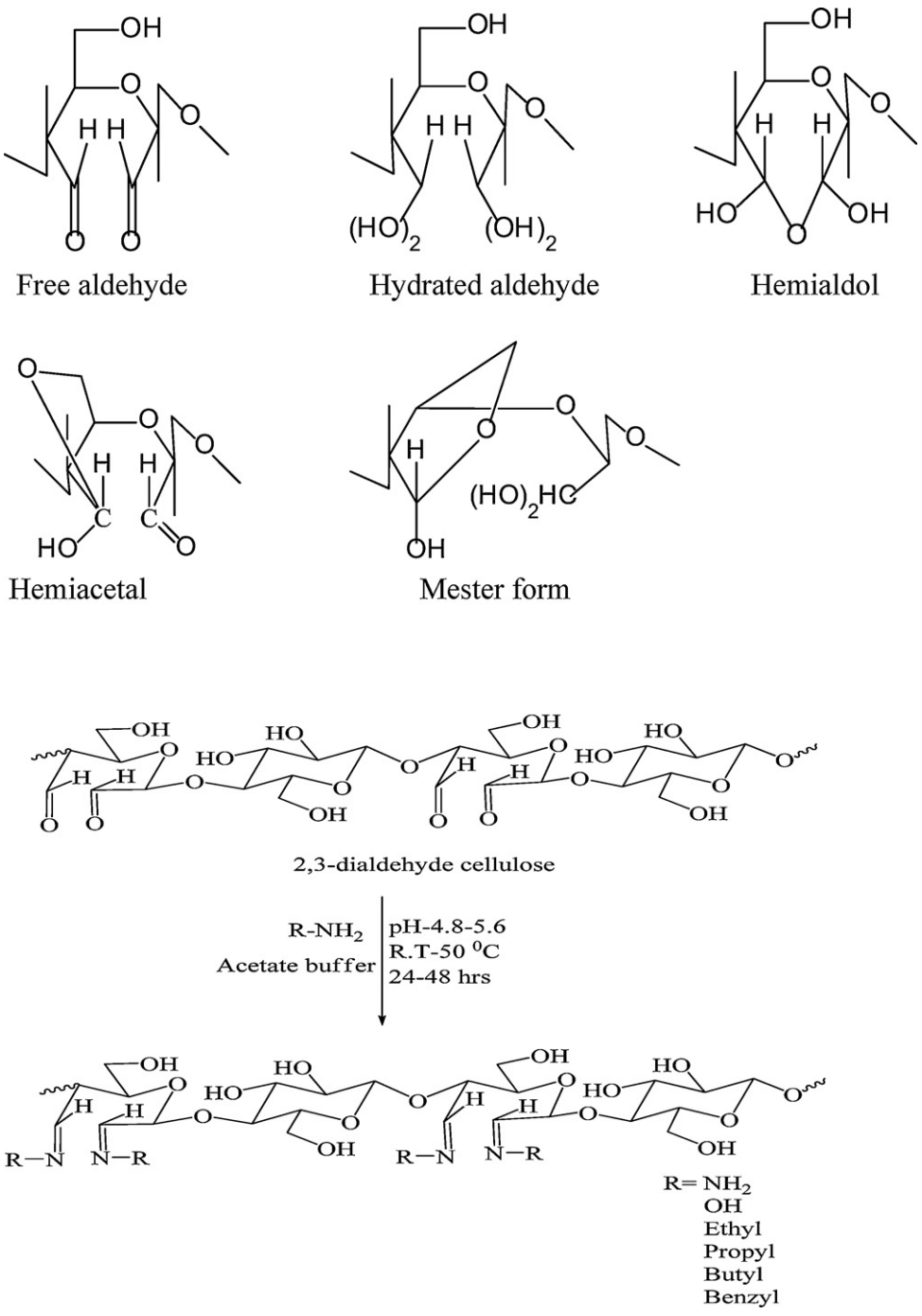

Fig. 2. Structural forms of 2,3-dialdehyde cellulose and Schiff's base obtained from 2,3-dialdehyde cellulose.

for 2,3-dicarboxy cellulose. This enhanced rate could be due to decrease in crystallinity and probably enhanced binding of the enzymes to the carboxylate/carboxy functional groups as well as molecular weight degradation of NaDCC and DCC. Thus highest level of oxidized NaDCC and DCC hydrolyses up to 70 and 60% respectively within 48 h which is 3–4 times more than cellulose.

The oxime derivatives i.e. 2,3-dioxime cellulose derivative hydrolyzes up to 5.5% in initial 12 h and maximum hydrolysis reached was 16.3% in 48 h for the highest level of oxidized dioxime. This is less than the cotton cellulose. This may be due to oxime substituent creating inter- and intra-molecular hydrogen bonds (similar to the dialdehyde cellulose case) which cause the slow enzymatic hydrolysis, even though the crystallinity of this derivative is less than the initial cellulose as shown by WAXRD (Fig. 5).

In the case of 2,3-diethylimine cellulose, all derivatives hydrolyze faster than the native cellulose perhaps due to molecular weight degradation. Up to 75% hydrolysis within 48 h was observed for 2,3-diethyinine cellulose-50. Similar facts were observed for 2,3-dipropylimine and 2,3-dibutylimine cellulose where maximum hydrolysis reaches up to 51.5 and 56.6% respectively for highest level of oxidation. While 2,3-dibenzylimine cellulose hydrolyses little slower than alkylimine derivatives because of steric effect of phenyl ring which may hinder the easy access of enzyme for hydrol-

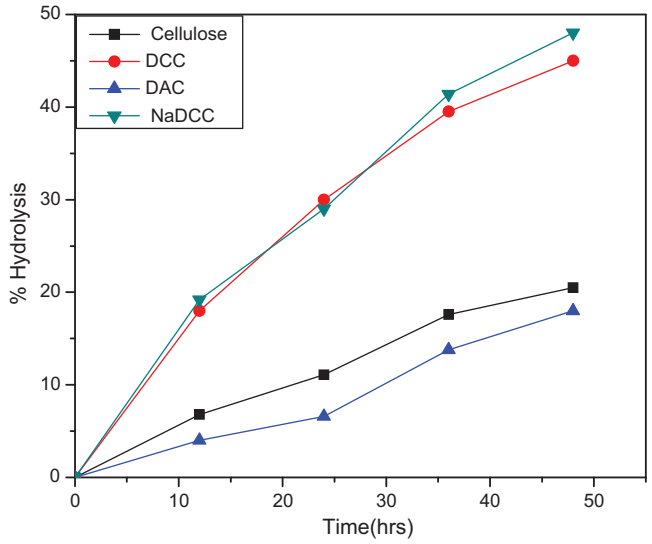

Fig. 3. Enzymatic hydrolysis profile of cellulose (cotton linters), 2,3-dialdehyde cellulose (DAC-15), sodium 2,3-dicarboxy cellulose (NaDCC-15) and 2,3-dicarboxy cellulose (DCC-15), all synthesized from cotton linter cellulose.

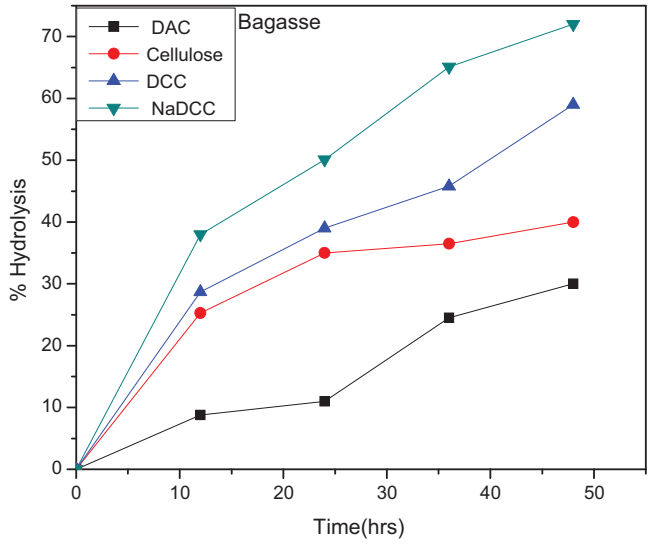

Fig. 4. Enzymatic hydrolysis profile of cellulose (bagasse), 2,3-dialdehyde cellulose (DAC-15), sodium 2,3-dicarboxy cellulose (NaDCC-15) and 2,3-dicarboxy cellulose (DCC-15), all synthesized from bagasse cellulose.

ysis. Thus only 36.6% hydrolysis was observed in 48 h for highest levels of oxidized 2,3-dibenzylimine derivatives.

3.4. Enzymatic hydrolysis of 2,3-dihydrazone cellulose

Interestingly, 2,3-dihydrazone cellulose derivatives with all level of oxidation show resistance towards enzymatic hydrolysis.

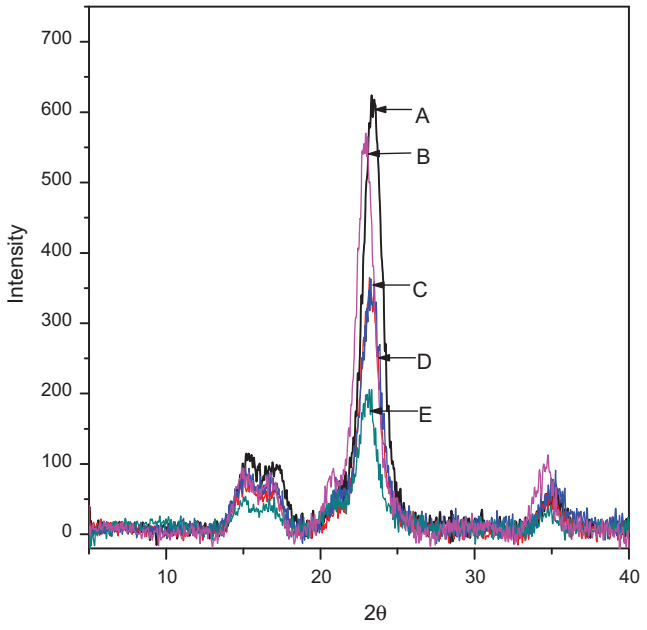

Fig. 5. WAXRD of cellulose and 2,3-dioxime cellulose with various levels of oxidation ((A) Cellulose, (B) 2,3-dioxime cellulose-5, (C) 2,3-dioxime cellulose-15, (D) 2,3-dioxime cellulose-25, (E) 2,3-dioxime cellulose-50, number denotes the level of oxidation).

To confirm this fact, two sets of hydrolysis experiment were carried out. In the first experiment, the reagent (anhydrous hydrazine) from which this derivative was synthesized, was physically mixed with cotton linter to study whether this is due to residual reagent attached physically to the cellulose. In the second set of experiment, 2,3-dihydrazone cellulose was directly added to pure cellulose to study the hydrolysis behavior of cellulose to study whether this derivative hydrolyzes or prevents hydrolysis of pure cellulose. The details of hydrolysis of these experiments are presented in Table 3.

It was observed that cellulose without added reagent hydrolyzes to a maximum of 30.8% in 98 h. However, physically added hydrazine inhibits hydrolysis of cellulose and only ~8% hydrolysis was observed in 98 h. Polymeric 2,3-dihydrazone cellulose (50% modified) also did not hydrolyze significantly, reaching only 7.83% in 98 h. On the other hand, cellulose with added 2,3-dihydrazone cellulose (50:50 mixtures) hydrolyses almost equally with that of control (pure cellulose) indicating that the presence of 2,3-dihydrazone cellulose in the mixture does not affect the hydrolysis of pure cellulose. The physical presence of reagent hydrazine affects the cellulose hydrolysis. The hydrazone derivative of cellulose was also not hydrolyzed. However, the presence of hydrazone cellulose did not affect the hydrolysis of pure cellulose when added to hydrazone cellulose. This shows that the enzyme is not bound to the hydrazone cellulose; in other words, hydrazone cellulose (5–50% hydrazone derivative) is a polymer that resists hydrolysis, an aspect which needs further investigation. This will aid the general studies

Table 3
Enzymatic hydrolysis of cellulose (cotton linter) in the presence of physically added reagent and 2,3-dihydrazone cellulose.

Sr. No.	Substrates	Hydrolysis (%)			
		24 h	48 h	72 h	98 h
1	Cellulose without any reagent (50 mg)	2.96	13.7	16.6	30.80
2	Cellulose (50 mg) + NH_2–NH_2 (7 mg)	3.00	–	–	8.33
3	Cellulose (50 mg) + NH_2–NH_2 (18 mg)	2.16	6.00	6.60	7.66
4	2,3-Dihydrazone cellulose-50 (50 mg)	2.60	5.16	6.33	7.83
5	Cellulose (25 mg) + 2,3-dihydrazone cellulose (25 mg)	5.50	8.90	18.33	31.50

aimed towards pretreatment of cellulosic biomass for hydrolysis of sugars.

4. Conclusions

Oxidation of cellulose was carried out to in order to develop a series of dialdehyde celluloses having different extents of aldehyde groups on the same cellulose chain. This 2,3-dialdehyde cellulose was further transformed to carboxylate, carboxy and Schiff's bases with different degrees of oxidation. The latter polymers were used for deriving corresponding dicarboxylate, dicarboxy, and Schiff's base cellulose derivatives. Thus, in all thirty seven cellulose derivatives were synthesized. The hydrolysis behavior of these derivatives was studied by cellulase enzymes. It was found that the dialdehyde cellulose hydrolyzed to a lower extent than the starting cellulose, except at high levels of aldehyde content (above 50%) and this was attributed to H-bonding of aldehyde with other hydroxyls in cellulose as well as formation of hemiacetal and hemialdol like structures. For dicarboxylate and dicarboxy celluloses, the highest level of oxidized NaDCC and DCC hydrolyzed up to 70 and 60% respectively within 48 h which was 3–4 times more than cellulose. The oxime derivatives i.e. 2,3-dioxime cellulose derivative hydrolyzes only up to 16.3% in 48 h for the highest level of oxidized dioxime. This is less than the starting cellulose. In the case of 2,3-diethylimine cellulose, all derivatives hydrolyze faster than the native cellulose. Up to 75% hydrolysis within 48 h was observed for 2,3-diethyimine cellulose-50. Similar facts were observed for 2,3-dipropylimine and 2,3-dibutylimine cellulose where maximum hydrolysis reaches up to 51.5 and 56.6% respectively for the highest levels of oxidation. The 2,3-dibenzylimine cellulose hydrolyses a little slower than the alkylimine derivatives. Thus only 36.6% hydrolysis was observed in 48 h for highest level of oxidized 2,3-dibenzylimine derivatives. The 2,3-dihydrazone cellulose derivatives with all level of oxidation show resistance towards enzymatic hydrolysis.

Thus, a strategy based on partially chemically altering the structure of cellulose to achieve a greater extent of hydrolysis of cellulose has been investigated; the results show that this methodology has opened a promising area of research for further research.

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References

Adsul, M. G., Singhvi, M. S., Gaikaiwari, S. A., & Gokhale, D. V. (2011). Development of biocatalysts for production of commodity chemicals from lignocellulosic biomass. *Bioresource Technology*, *102*, 4304–4312.

Baratieri, M., Baggio, P., Fiori, L., & Grigiante, M. (2008). Biomass as an energy source thermodynamic constraints on the performance of the conversion process. *Bioresource Technology*, *99*, 7063–7073.

Chang, V. S., & Holtzapple, M. T. (2000). Fundamental factors affecting enzymatic reactivity. *Applied Biochemistry and Biotechnology*, *84–86*(1–9), 5–37.

Fan, Q. G., Lewis, D. M., & Tapley, K. N. (2001). Characterization of cellulose aldehyde using Fourier transform infrared spectroscopy. *Journal of Applied Polymer Science*, *82*, 1195–1202.

Feng, X., Hanshu, D., & Tejirian, A. (2009). Detrimental effect of cellulose oxidation on cellulose hydrolysis by cellulase. *Enzyme and Microbial Technology*, *45*, 203–209.

Fischer, E. H., & Stein, E. A. (1961). *Biochemical Preparations*, *8*, 27–33.

Grethlein, H. E. (1985). The effect of pore size distribution on the rate of enzymatic hydrolysis of cellulose substrates. *Nature Biotechnology*, *3*, 155–160.

Hendriks, A. T. W., & Zeeman, M. G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, *100*, 10–18.

Koullas, D. P., Christakopoulos, P., Kekos, D., Macris, B. J., & Koukios, E. G. (1992). Correlating the effect of pretreatment on the enzymatic hydrolysis of straw. *Biotechnology and Bioengineering*, *39*, 113–116.

Laureano-Perez, L, Teymouri, F., Alizadeh, H., & Dale, B. E. (2005). Understanding factors that limit enzymatic hydrolysis of biomass. *Applied Biochemistry and Biotechnology*, 1081–1099.

Mansfield, S., Mooney, D., & Saddler, J. N. (1999). Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnology Progress*, *15*, 804.

Puri, V. P. (1984). Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. *Biotechnology and Bioengineering*, *26*, 1219–1222.

Scott, W. W. (1939). (p. 458).(5th ed.). *Standard methods of chemical analysis* New York: D. Van Nostrand Comp.

Shaikh H. M., (2011). Ph.D. thesis, University of Pune, Pune.

Thompson, D. N., Chen, H. C., & Grethlein, H. E. (1992). Comparison of pretreatments methods on the basis of available surface area. *Bioresource Technology*, *39*, 155–163.

Varma, A. J., (2008). *A process for fractionating sugarcane bagasse into high alpha-cellulose pulp, xylan and lignin*. WO PCT/IN08/00569 dated 05/09/2008.

Zhang, Y. H. P., & Lynd, L. R. (2004). Towards an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnology and Bioengineering*, *88*, 797–824.