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Optimal Method for Production of Amorphous Cellulose with Increased Enzymatic Digestibility

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ARTICLE INFO	ABSTRACT
	In this paper, a simple and cheap method for producing of amorphous cellulose was studied by treating the initial cellulosic material (MCC and waste paper) with a cold solvent, such as aqueous solution of 7% NaOH/12% Urea, at the various ratios of the solvent to cellulose (v/w) (R). If was found that after treatment of cellulose materials with the solvent at R $\geq$5, a completely amorphous cellulose (AC) is formed. Due to high digestibility, the AC with concentration of 50 g/L is converted to glucose almost completely for 48 h under the action of cellulolytic enzyme CTec-3 with a dose of 30 mg/g solid sample. Such sample can be used as an amorphous standard in the study of crystallinity degree and enzymatic hydrolysis of various types of cellulose and lignocellulose. It was found that enzymatic saccharification is most advantageous to carry out at elevated concentrations of AC, 150 g /L. Due to high cost of MCC, it is preferable to use a cheap cellulose raw material, such as mixed waste paper (MWP), for the commercial production of AC and glucose. The resulting glucose can find application in biotechnology as a promising nutrient for various microorganisms.

1. Introduction

As is known, the conventional method for production of amorphous cellulose (AC) is regeneration from dilute solution (2.3 wt. % or 40 g/L) of microcrystalline cellulose (MCC) in 85% phosphoric acid (PhA) [1,2]. This type of AC, called PhA-Swollen Cellulose (PASC), is characterized by low crystallinity and high saccharification ability under action of cellulolytic enzymes [3,4]. However, the known method for preparation of AC has two main shortcomings. The first problem is low productivity of the process due to use of too dilute cellulose solution, and the second problem is high cost of even commercial grade PhA, estimated at $1 per kg.

The same problems arise if instead of phosphoric acid expensive organic solvents are used, such as ionic liquids (IL) [5], NMMO [6], DMSO/PFA [7], DMSO/DEA/SO$_2$ [8], DMAA/LiCl$_2$ [9], etc. So according to Alibaba catalog, the average price of DMSO, DEA and DMAA is $1.5-2 per kg, NMMO $10-20 per kg and IL $100-150 per kg.

The traditional cellulose solvents, CS$_2$/NaOH system and Cuproxam, used in XX century for production of artificial cellulose fibers and films, are currently prohibited due to high toxicity and environmental hazard.

The cheapest solvent for cellulose is probably aqueous solution of 7% NaOH/12% Urea [10-12], having cost of $0.05 per kg due to low price of commercial chemicals. The problem is that only the dilute 3-4 wt. % solution of

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MCC in this solvent can be obtained, which complicates the regeneration process and reduces the productivity of AC obtaining. The purpose of this study was to optimize the production of AC using the lowest ratio of the solvent to cellulose.

2. Experimental

2.1 Materials and Chemicals

MCC Avicel PH-101 (FMC Co., Princeton, USA) and mixed waste paper (MWP), containing waste of paper towels, paper wipes and blotting/absorbing paper (Amnir Recycling, Hadera, Israel), were used as initial cellulose materials. Sodium hydroxide and urea were supplied from Sigma-Aldrich Co. These chemicals were used to prepare an N/U - solvent, such as aqueous solution containing 7% NaOH and 12% Urea.

2.2 Treatment

Approximately 10 g of initial cellulosic material (MCC powder or 10-15 mm MWP pieces) was placed in a glass beaker cooled with ice/salt mixture having temperature of -15°C. Then, a cold N/U - solvent, was added at the ratio of the solvent to cellulose material (R) from 3 to 10 (v/w) while periodically stirred for 1 h, and after which left overnight in a freezer at -15°C. The treated samples were removed from the freezer and mixed with a 10-fold volume of tap water. The gel-like samples were separated from a liquid medium by means of vacuum Glass filter No1, washed with tap water, neutralized with 1% HCl to pH 6-7 and then washed with distilled water, which was suctioned off under vacuum to obtain 20-25 wt. % solid content.

2.3 Enzymatic Hydrolysis

The initial and treated samples were hydrolyzed with a commercial cellulolytic enzyme Cellic CTec-3 (Novozymes A/S, Bagsvaerd, Denmark). The dose of enzyme was 30 mg per 1 g of solid sample. Hydrolysis of the samples was carried out in 50-mL polypropylene tubes. The samples containing 1 g of the solid matter and 1 ml of 50 mM acetate buffer (pH=4.8) were put into the tubes. Then needed amount of the enzyme was added. Further, an additional amount of the buffer was supplemented to obtain concentration of the cellulosic substrate (C_o) from 50 to 150 g/L. The tubes closed with covers were placed in a shaker incubator at 50°C and shaken for 48 h.

2.4 Analysis

Structural state of the samples was studied by method of X-ray diffraction [13]. Concentration of glucose (C_g, g/L) in the hydrolyzate was determined by the by HPLC-apparatus of Agilent Technologies 1200 Infinity Series. The Amines HPX-87H column was used. Main conditions of the analysis were temperature 45°C; mobile phase 0.005 M sulfuric acid; flow rate 0.6 ml/min. The hydrolyzate was preliminary filtered through 0.45 μm Nylon filter. Degree of digestibility (DD, %) of samples after enzymatic hydrolysis was calculated by the equation:
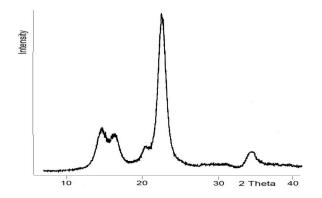
$$DD = 90 \ (C_g/C_o) \tag{1}$$

3. Results and Discussion

X-ray study of the initial MCC Avicel showed that this cellulose type is a crystalline matter having crystallinity degree (DCr) of 75% (Figure 1, Table 1). After processing of MCC with N/U solvent at R =3, the CI allomorph of the initial cellulose is transformed into CII allomorph with a low DCr of 28% (Figure 2). However, when MCC is treated with the solvent at R ≥ 5, a completely amorphous cellulose (AC) is formed. Thus, the minimum R required for preparation of AC after cellulose processing with solvent is about 5. The same minimum R is required to obtain AC by treatment of mixed waste paper (MWP) (Table 1).
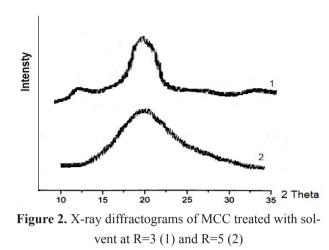
Table 1. Degree of crystallinity (DCr), degree of amorphicity (DAm) and crystalline allomorph (AL) of cellulose samples

Sample	DCr, %	DAm, %	AL
Initial MCC Avicel	75	25	CI
Initial MWP	60	40	CI
MCC treated with solvent at R=3	28	72	CII
MCC treated with solvent at R=5	0	100	no
MCC treated with solvent at R=10	0	100	no
MWP treated with solvent at R=5	0	100	no

Figure 1. X-ray diffractogram of MCC

Figure 2. X-ray diffractograms of MCC treated with solvent at R=3 (1) and R=5 (2)

The X-ray findings are confirmed by the results of enzymatic hydrolysis of cellulose samples. As shown in Figure 3, after enzymatic hydrolysis at cellulose concentration C_o=50 g/L, the degree of digestibility (DD) of initial MCC Avicel is relatively low, with DD=40% only. The enzymatic digestibility of CII sample reaches 80% due to low DCr of this sample, whereas samples of amorphous cellulose, AC1 and AC2, are hydrolyzed almost completely.
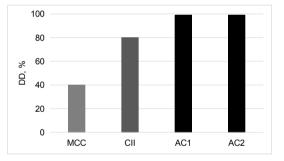

Figure 3. Degree of enzymatic digestibility (DD) of MCC samples at C_o=50 g/L: initial sample (MCC) and samples treated with solvent at R=3 (CII), R=5 (AC$_1$), and R=10 (AC$_2$)

As is known, the crystallinity of cellulose is the main factor impeding the enzymatic hydrolysis [14,15]. Therefore, decrease in DCr or increase in DAm facilitate the enzymatic digestibility of cellulose samples (Figure 4).
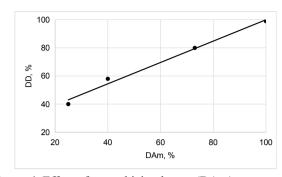

Figure 4. Effect of amorphicity degree (DAm) on enzymatic digestibility (DD) of cellulose samples at C_o= 50 g/L

Thus, if the AC concentration at enzymatic hydrolysis is 50 g/L, then the enzymatic digestibility of this sample reaches 100% for 48 h at enzyme dose of 30 mg/g solid cellulose. Such sample can be used as an amorphous standard, instead of expensive PASC, in the study of enzymatic hydrolysis of various types of cellulose and lignocellulose.
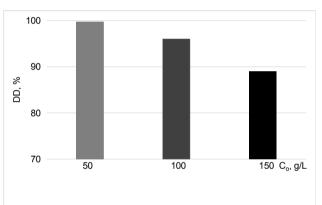

Figure 5. Dependence of enzymatic digestibility (DD) of AC$_1$ on concentration of cellulosic substrate (C_o)
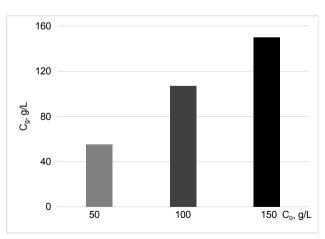

Figure 6. Dependence of glucose concentration (C_g) formed after enzymatic hydrolysis of AC$_1$ on concentration of cellulosic substrate (C_o)

Although an increase in the concentration of AC at enzymatic hydrolysis leads to a decrease in the degree of digestibility (Figure 5), the concentration of obtained sugar (glucose) increases in this case (Figure 6).

Since MCC is an expensive cellulose material (cca $4-5 per kg), it cannot be applied for preparation of AC and glucose on the pilot or industrial scale. For commercial production of AC and its enzymatic saccharification purposes, it is advisable to use a cheap raw material, such as mixed waste paper, which can be supplied by price of $50 per ton. As can be seen from obtained results, AC produced by treatment of MWP with N/U - solvent at R=5 is highly digestible and produces an increased amount of glucose after enzymatic hydrolysis at Co=50 g/L (Figures 7, 8).

Increase in the concentration (C_o) of AC reduces the degree of enzymatic digestibility. On the other hand, increase in Co of AC during enzymatic hydrolysis leads to enhance in concentration of obtained glucose. Thus, to increase the concentration of glucose, it is most advantageous to carry out enzymatic saccharification at an increased concentration of AC, 150 g/L (Figure 8). This conclusion is consistent with literature data [16].
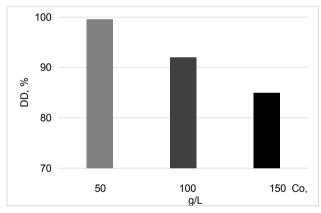

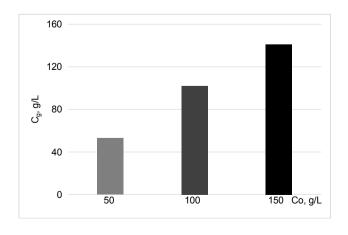**Figure 7.** Dependence of digestibility (DD) of AC from MWP on concentration of cellulosic substrate (C_o)

Figure 8. Dependence of glucose concentration (C_g) formed after enzymatic hydrolysis of AC from MWP on concentration of cellulosic substrate (C_o)

The resulting glucose can find application in biotechnology as a promising nutrient for various microorganisms in the production of ethanol [17]; acetic, lactic and citric acids [18]; feed proteins, yeasts and enzymes [19-21]; bacterial cellulose [22] and polyhydroxyalkanoates (biopols) [23], and other valuable products. The biotechnological application of cellulosic glucose obtained by enzymatic hydrolysis of AC from waste paper, instead of food glucose, can reduce the area of energy crops, decrease the consumption of agrochemicals and water, which ultimately can prevent an increase in food prices and its shortage.

4. Conclusions

The optimal method for producing of amorphous cellulose (AC) was developed by treating the initial cellulosic material with a cold solvent, such as aqueous solution of 7% NaOH/12% Urea, at ratio of the solvent to cellulose (v/w) R=5. Due to high digestibility, the AC with concentration of 50 g/L is converted to glucose almost completely for 48 h under the action of cellulolytic enzyme CTec-3 with a dose of 30 mg/g solid sample. Such sample can be used as an amorphous standard in the study of crystallinity degree and enzymatic digestibility of various types of cellulose and lignocellulose. The decrease in crystallinity or increase in amorphicity of cellulose samples facilitate their enzymatic digestibility. It was found that enzymatic saccharification is most advantageous to carry out at elevated concentrations of AC, 150 g /L. Due to high cost of MCC, it is preferable to use a cheap cellulose raw material, such as mixed waste paper (MWP), for the commercial production of AC and glucose. The resulting glucose can find application in biotechnology as a promising nutrient for various microorganisms.

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